TroubleShooting Guidelines

A Compilation from

The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

Foreword

Tulip Diagnostics (P) Ltd., is one of the largest manufacturer of in-vitro diagnostic reagents and kits in India.

Since its inception in 1988, Tulip Diagnostics has expanded significantly and now has nine manufacturing facilities—7 in Goa and 2 in Uttarkhand—in India. All the manufacturing facilities are ISO 13485 certified and most of the products manufactured are CE certified.

The company's diverse portfolio includes almost all areas of in-vitro diagnostics as Immuno-hematology, Immunology, Immunoturbidimetry, Immunochemistry, Hematology, Clinical biochemistry as well as Microbiology, Disinfection, Bacteriology and products built on these essential platforms including Instrumentation.

The company has created a global presence with exports to more than 88 countries worldwide.

Empowering customers and engaging in customer centric work is what Tulip believes in. Knowledge building and sharing is a medium to empower our esteemed customers towards continuous learning and growth. Knowledge building is essential to keep abreast of all new developments in the medical and diagnostic world.

With this in view, Tulip Diagnostics had launched The CRUX, the bimonthly forum for the laboratorians in the year 2004. The contents of the magazine are divided into 3 sections namely Disease Diagnosis, Troubleshooting, and Interpretation.

This handbook 'Trouble Shooting Guidelines' is a compilation of the Troubleshooting section from all The CRUX publications. We sincerely believe that the contents of this handbook will be of immense use to all our esteemed customers.

Happy Reading!

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SPECIMEN HANDLING

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Blood Collection

The tissue that is tested most often is blood. By and large most diseases would produce some alteration in this liquid connective tissue of the body. Before one tests it, one has to obtain it.

Blood can be obtained from a) the capillaries b) veins or c) arteries.

Procedure common to all sources of blood collection:

1. Explain to the patient what you are going to do.
2. In a warm environment, relax (physically and mentally) and reassure the patient properly. Excessive stress and exercise increase factor VIII, vWFAg and fibrinolysis. Warm the site of puncture. (Can forego this under emergency situations).
3. Identify and sterilize the site of skin puncture with 70% alcohol (like IPA). Let dry.
4. Obtain required quantity of blood, dispense the same in appropriate containers (gently mixing wherever essential) and perform necessary tests as soon as possible. Having collected the sample, apply gentle pressure with sterile gauze with at least three fingers at the site for a time that is longer than the patient's clotting time. Rubbing of the puncture site is strictly contraindicated. Place an adhesive dressing at the puncture point.
5. Exercise all biohazard precautions. Wear disposable plastic or rubber gloves. Do not injure yourself with syringes, needles and lancets. Once used, they should be immediately discarded.

Capillary blood:

1. Use as a last resort only for infants less than one year of age.
2. Use medial or lateral aspects of a pre-warmed heel. Under aseptic precautions puncture to a depth of 2-3 mm with a sterile lancet. Wipe the first drop with a sterile gauge. If necessary squeeze very gently to encourage free flow of blood.
3. Collect into appropriate container, can be a micropipette, capillary tube or a microtainer or a microvette.

Venous blood:

1. All sample collections (except arterial blood) should be from the veins, even in a neonate, if possible.
2. Whenever possible, venous blood samples must be collected without the use of a cuff. Venous occlusion causes haemoconcentration, increase in fibrinolytic activity, platelet release and activation of some of the clotting factors.
3. In the majority of patients, however, light pressure using a tourniquet is required. This should be applied for the shortest possible time (< 1 minute). A sphygmomanometer cuff can be used and inflated to diastolic pressure and the skin over the site can be tapped for a few times.
4. In obese patients veins over the dorsum of the hand can be used after warming it by immersion in warm water. Dry the hand; clench the fist and suitable veins will become apparent. This site tends to bleed readily than other sites, elevate the hand, apply gentle pressure for several minutes and place an adhesive dressing over the puncture site.
5. Loose and rolling veins can be fixed between the thumb and the index finger.
6. Sometimes one may transfix the vein and no blood flows, just retract the needle a bit and blood will start flowing.
7. Loosen the tourniquet when you enter the vein.
8. Draw the piston of the syringe slowly and once adequate blood is collected, dispense the same in appropriate anticoagulated or plain containers.
9. Make sure that the patient has relaxed the fist after the blood has been collected.
10. Venipuncture must be clean and blood from indwelling catheters should not be used.

Ideal site is the antecubital fossa of the arm. Needle gauge should be 21 or larger (this avoids unnecessary frothing and shearing stress).To minimize the affects of contact activation good quality plastic or polypropylene syringes should be used. If glass syringes are used they should be adequately coated with silicon.

Differences between capillary and venous blood: PCV, RBC count and Hb. of capillary blood are slightly greater than in venous blood. TLC and neutrophils are higher by 8%; monocytes are higher by 12– 100% (especially in children). Platelet count is lesser by 9 – 32 % (because of adhesion of platelets to the skin puncture site).

Arterial blood:

Arterial blood is used to measure oxygen and carbon dioxide tension and to measure pH. Arterial punctures are technically more difficult, increased pressure within the arteries often leads to haematoma formation. Reflex arterial spasm restricts blood flow with possible severe effects on circulation. Patients may complain of uneasiness as aching, tenderness, sharp piercing sensation, and cramp.

1. Select the puncture site. Most commonly, the radial artery is used. Before starting, please ensure that the ulnar artery is present (perform Allen's test), if absent, choose another site. Femoral or brachial arteries can also be used. Scalp arteries are used in infants; catheterisation of the umbilical artery is frequently used in neonates for up to 48 hours after birth.
2. Anaesthetise the puncture site, if necessary.
3. Patient should be absolutely calm. Hyperventilation caused by anxiety may significantly alter the blood gas measurements.
4. Prepare the syringe. Wet the barrel and needle or cannula with sterile anticoagulant (usually heparin). Expel excess solution.
5. Record the patient's temperature.
6. Palpate the artery,
7. With the bevel of the needle facing up, puncture the skin 5 to 10 mm distal to the finger which locates the artery. Aim for the artery at a point directly below the finger. Blood rushing to the finger usually forces the plunger back. If not, gently pull back on the plunger. Obtain required amount of blood. Quickly withdraw the needle and syringe. At the same time place a sterile cotton ball or a dry gauze sponge over the puncture site. Apply firm pressure for at least five minutes, or longer if the patient has a longer clotting time. Watch the puncture site for another two minutes to avoid haematoma formation. Expel any air bubbles from the syringe. Remove the needle and cap the syringe with a tight-fitting Luer cap. Mix the specimen with anticoagulant by gentle inversion of the syringe. Immediately transport the specimen on ice to the laboratory.

Allen's test - Compress the radial and ulnar arteries at the wrist until the palm of the hand becomes blanched. Release pressure from the ulnar artery. Observe that the hand becomes flushed. If it does not, it means that the ulnar artery is absent.

Capillary Blood Collection: Best Practices

Skin puncture or capillary blood collection involves puncturing the dermis layer of the skin to access the capillary beds which run through the subcutaneous layer of the skin. Blood obtained via skin puncture is a mixture of undetermined proportions of blood from arterioles, venules, capillaries, plus interstitial and intracellular fluids. The proportion of arterial blood is greater than that of venous blood, due to the increased pressure in the arterioles leading into the capillaries versus the pressure in the venules exiting the capillaries. Warming of the puncture site further "arterializes" the blood and increases blood flow. Capillary blood collection is the preferred method of blood specimen collection for newborns and infants. Clinical Laboratory Standards Institute (CLSI) recommends capillary blood collection via heelstick for infants less than one year of age. For children older than one year, capillary blood collection via fingerstick should be considered, where appropriate.

Capillary blood collection may also be used for adults under certain circumstances including:

- Patients with fragile, superficial or difficult to access veins
- Patients where multiple unsuccessful venipunctures have already been performed, especially if the test(s) requested requires only a small volume of blood
- Patients with burns or scarring in venous blood collection sites
- Extremely obese patients
- Patients requiring frequent blood tests
- Patients receiving IV therapy in either arms or hands
- Patients at risk for serious complications associated with venipuncture, venous thrombosis, or deep venous puncture (e.g. deep vein puncture in infants, thrombophlebitis)
- Patients requiring only one blood test for which a capillary specimen is appropriate
- Patients whose veins are "reserved" for intravenous therapy or chemotherapy
- Point-of-care testing where only a few drops of blood are needed

Capillary blood collection is inappropriate for:

- Severely dehydrated patients
- Patients with poor circulation
- Coagulation studies requiring plasma specimens
- Tests that require large volumes of blood

(i.e. Erythrocyte Sedimentation Rate (ESR) and blood cultures)

It is important to understand that there are differences between some analytes in capillary blood as compared to venous or arterial blood specimens. Glucose, potassium, total protein, and calcium have been reported to show statistically and/or clinically important differences. With the exception of glucose, the concentration of these analytes is lower in capillary blood.

The following tests are commonly performed using capillary blood:

- Point-of-Care testing (POCT, i.e. blood glucose monitoring)
- Complete Blood Count (CBC), haemoglobin & hematocrit (H&H)
- Peripheral Blood Smear (manual slide for white blood cell (WBC) differential)
- Neonatal Blood Gases
- Neonatal Bilirubin
- Neonatal Screening (filter paper or blood spot testing)
- Electrolytes

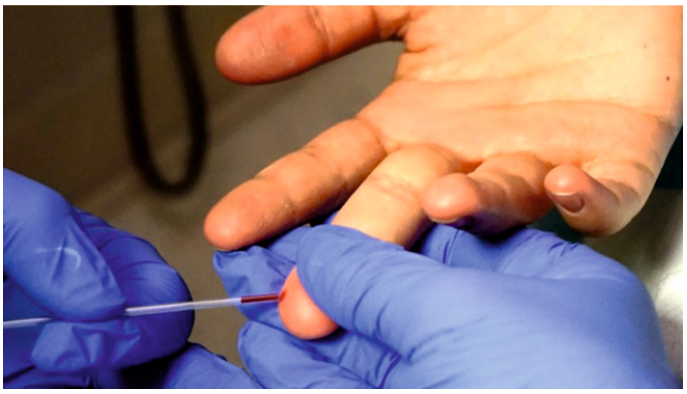
According to CLSI, a skin puncture device should be a sterile, disposable, single-use device with a permanently retractable blade or needle to reduce the possibility of accidental needlestick injuries and reuse. The use of manual lancets or blades without a retractable feature is a violation of OSHA regulations.

General Site Selection

The patient's age, accessibility of the puncture site, and the blood volume required should all be taken into consideration when selecting the skin puncture device type and puncture site. Select a site that is warm, pink and free of any calluses, burns, cuts, scars, bruises, or rashes. The site should not be cyanotic (bluish from lack of oxygen), edematous (swollen), or infected. Avoid skin areas that have evidence of previous punctures or are otherwise compromised.

Fingerstick Site Selection

The recommended site for capillary collection on adults and children over one year of age is the palmar surface of the distal (end) segment of the third (middle) or fourth (ring) finger, ideally of the non-dominant hand. Fingers on the non- dominant hand are generally less calloused. The puncture should be made slightly off center from the central, fleshy portion of the fingertip and if using a blade-type puncture device, perpendicular to the fingerprint whorls. Puncturing along or parallel to the whorls may cause the blood to follow the pattern of the fingerprint, redirecting the flow and making it more difficult to collect. The index finger is often calloused and potentially more sensitive to pain due to additional nerve endings. The thumb also may be calloused and has a pulse, indicating arterial presence, and, therefore, should be avoided. The distance between the skin surface and the bone in the fifth finger also makes it unsuitable for puncture. The side and tip of the finger should be avoided, as the tissue is about half as thick as the central portion of the fingertip.



Heelstick Site Selection

The recommended site for heel punctures is the lateral (outside) or medial (inside) plantar surface of the heel. In small or premature infants, the heel bone (calcaneus) may be no more than 2.0 mm beneath the skin surface and no more than half this distance at the posterior curvature of the heel. Puncturing deeper than 2.0 mm on the plantar surface of the heel of small infants may, therefore, risk bone damage. When using

incision devices, puncturing the heel at a 90° angle to the length of the foot is recommended. Such incisions create a "gap" puncture (one which opens when pressure is applied) and further enhance blood flow.

For infants, punctures must not be performed on:

- The posterior curvature of the heel.
- The central area of an infant's foot (area of the arch).
- Punctures to this area may result in injury to nerves, tendons and cartilage.
- The fingers of a new born or infant less than one year old.
- Earlobes.

Best Practice for Capillary Blood Collection

The following steps should be performed in accordance with the facilities' recommended procedures.

1. Review the test requisition(s).
2. Gather the appropriate supplies (lancing device, gloves, gauze, alcohol, bandages, etc.).
3. Positively identify the patient.
4. Verify diet restrictions (fasting required, etc.) and any latex sensitivity (if products containing latex are being used).
5. Wash hands and put on gloves.

NOTE: *All patient and laboratory specimens are treated as potentially infectious and handled according to "standard precautions". The patient should be sitting or lying down.*

6. Select appropriate puncture site.
7. Warm the puncture site.
8. Clean the puncture site with 70% isopropyl alcohol and allow to air dry. The site must be allowed to air dry in order to provide effective disinfection.
9. Notify older children and adults of the imminent puncture.
10. Puncture the skin with the disposable lancing/incision device.
11. Wipe away the first drop of blood with a dry gauze pad (refer to each point-of-care device manufacturer's instructions).
12. Collect the specimen in the appropriate container, and mix according to the manufacturer's instructions.
13. Seal the specimen container.
14. Apply direct pressure to the wound site with a clean gauze pad and slightly elevate the extremity.
15. Label the specimen container in direct view of the patient or guardian to verify identification, and record time of collection. Label each container individually.
16. Properly dispose of the lancet/incision device in a puncture-resistant disposal container.
17. Properly dispose of any other contaminated materials (gloves, gauze, etc.) in a container approved for their disposal.
18. After removing gloves, wash hands before proceeding to the next patient.

Special Specimen Collection Requirements:

1. Bilirubin samples must be protected from light, both during and after collection, as light breaks down bilirubin. If collecting a capillary specimen from an infant in an incubator, turn off the ultraviolet (UV) light source above the infant during specimen collection. Collect capillary blood specimen quickly to minimize exposure of the blood specimen to light. Use amber collection containers or foil to protect specimens from light. Ensure that the UV light is turned back on before leaving the nursery.
2. For newborn screening (filter paper collection), gently touch the filter paper against the blood drop in a single step to allow a sufficient quantity of blood to soak completely through the paper and fill the preprinted circle. The paper must not be pressed against the puncture site on the heel. If the circle does not fill entirely, wipe the heel and touch a different circle to the blood drop until the circle is completely filled. Blood must soak through the paper within the circle to the other side, and must be applied to one side of the paper only.

Top 10 Keys to Obtaining a High Quality Capillary Blood Specimen

1. Positively Identify the Patient – Positive identification of the patient is the most important step in specimen collection. Patient misidentification can lead to incorrect diagnosis, therapy and treatment. The consequences can be serious, even fatal to the patient.
2. Puncture Site and Lancing/Incision Device Selection –Determine the appropriate puncture site and lancing/incision device for the patient and the tests requested. Using the wrong size lancet/incision device may result in excessive squeezing, prolonged or incomplete collection, poor specimen quality (hemolysis, clotting) and possible redraws, as well as injury to the patient (mainly children).
3. Warming the Puncture Site – Only a limited amount of blood will easily flow from a capillary puncture. Warming the puncture site will increase blood flow up to seven times and is critical for the collection of blood gases and pH specimens. CLSI guidelines recommend warming the skin puncture site for three - five minutes with a moist towel or commercially available warming device at a temperature no greater than 42°C.
4. Cleaning the Puncture Site – Allow the alcohol to air dry. Performing skin puncture through residual alcohol may cause hemolysis and can adversely affect test results. It also may cause additional discomfort for the patient. Do not use povidone-iodine to cleanse the puncture site. Povidone-iodine interferes with bilirubin, uric acid, phosphorus and potassium.
5. Wipe Away the First Drop of Blood – Immediately following skin puncture, platelets aggregate at the puncture site to form a platelet plug, initiating the clotting process. Without wiping away the platelet plug, bleeding may stop prior to completion of the blood collection, resulting in insufficient blood volume and redraws. In addition, the first drop of blood contains tissue fluid, which can cause specimen dilution, hemolysis and clotting.

   NOTE: *For point-of-care testing (i.e. blood glucose monitoring), use of the first drop of blood may be appropriate. Refer to the manufacturer's instructions for use.*

6. Avoid Milking, Scooping or Scraping of the Puncture Site – It is recommended to touch the collector end of the container to the drop of blood. After collecting 2 or 3 drops, the blood will freely flow down the container wall to the bottom of the tube. Excessive squeezing (milking), scooping and scraping may cause hemolysis and/or tissue fluid contamination of the specimen. Using a "scooping"or scraping motion along the surface of the skin can also result in platelet activation, promoting platelet clumping and clotting.
7. Collect Specimen Quickly – Puncturing the skin releases thromboplastin, which activates the coagulation process. Specimens must be collected quickly to minimize the effects of platelet clumping and microclot formation (hematology testing). Specimens also should be collected quickly to avoid exposure to atmospheric air and light (blood gases and bilirubin testing).
8. Fill to the Correct Fill Volume – Fill containers to the recommended fill volume (if indicated). Underfilled containers will have higher concentrations of additives. For K2 EDTA, higher concentrations may cause erroneous results for MCV and red cell indices and cause RBC and WBC morphological artifacts. Consequently, overfilled containers will have lower concentrations of EDTA and may result in clotting.
9. Mix Specimen – Microcollection tubes must be inverted the appropriate number of times to ensure that the blood and anticoagulant are sufficiently mixed. Mixing is essential to prevent the formation of microclots and platelet clumps, which can cause inaccurate or erroneous test results. Small clots can also occlude sample aspiration probes or tubing in laboratory instruments, resulting in instrument downtime and/or additional unscheduled maintenance. While modern analyzers have sophisticated detection systems to recognize platelet clumps, it is still possible for platelet clumps to cause erroneous test results (e.g. platelet count, platelet volume, red cell volume, white cell count). Adequate mixing, both during and after the completion of capillary blood collection, will help minimize these occurrences.
10. Properly Label Specimen – Each tube should be individually labeled at the bedside prior to leaving the area. Mislabeling of the specimen can lead to incorrect diagnosis, therapy and treatment. The consequences can be serious, even fatal, for the patient.

In summary, there truly is an "art" to capillary blood collection. These 10 steps provide guidelines to assist your facility in selecting the appropriate lancing/incision device and puncture site for a successful capillary blood collection—the first time. A high-quality specimen minimizes errors and possible re-draws, while enhancing customer satisfaction and patient care.

Phlebotomy

BLOOD COLLECTION

ROUTINE VENIPUNCTURE AND SPECIMEN HANDLING

VENIPUNCTURE PROCEDURE

The venipuncture procedure is complex, requiring both knowledge and skill to perform. Each phlebotomist generally establishes a routine that is comfortable for her or him. Phlebotomists are considered to have occupational exposure to blood borne pathogens. The performance of routine vascular access procedures by skilled phlebotomists requires, at a minimum, the use of gloves to prevent contact with blood. Laboratory coats or work smocks are not typically needed as personal protective equipment during routine venipuncture, but an employer must assess the workplace to determine whether certain tasks, workplace situations, or employee skill levels may result in an employee's need for laboratory coats or other personal protective equipment to prevent contact with blood. It is an employer's responsibility to provide, clean, repair, replace, and/or dispose of personal protective equipment/clothing. As part of presenting a professional appearance, an institutional dress code may include wearing of a laboratory coat or smock.

Several essential steps are required for every successful collection procedure:

1. Patient comfort. Is the seating comfortable and has the patient been seated for at least 5 minutes to avoid being rushed or confused?
2. Carry out hand hygiene before and after each patient procedure, before putting on and after removing gloves.
3. Identify the patient using two different identifiers, asking open ended questions such as, "What is your name?" and "What is your date of birth?"
4. Assess the patient's physical disposition (i.e. diet, exercise, stress, basal state).
5. Check the requisition form for requested tests, patient information, and any special requirements.
6. Label the collection tubes at the bedside or drawing area.
7. Select a suitable site for venipuncture.
8. Prepare the equipment, the patient and the puncture site.
9. Perform the venipuncture, collecting the sample(s) in the appropriate container(s).
10. Recognize complications associated with the phlebotomy procedure.
11. Assess the need for sample recollection and/or rejection.
12. Promptly send the specimens with the requisition to the laboratory.

ORDER FORM / REQUISITION

A requisition form must accompany each sample submitted to the laboratory. This requisition form must contain the proper information in order to process the specimen. The essential elements of the requisition form are:

- Patient's surname, first name, and middle initial.
- Patient's ID number.
- Patient's date of birth and sex.
- Requesting physician's complete name.
- Source of specimen. This information must be given when requesting microbiology, cytology, fluid analysis, or other testing where analysis and reporting is site specific.
- Date and time of collection.
- Initials of phlebotomist.
- Indicating the test(s) requested.

An example of a simple requisition form with the essential elements is shown below:

LABORATORY SERVICE - UNIVERSITY OF UTAH HOSPITAL

Patient Name: _____

Patient ID: _____

Patient Birthdate: _____ Sex: _____

Source of Specimen: _____

Date Collected: _____ Time: _____ Phleb: _____

Physician: _____ Location: _____

Diagnosis: _____

Tests Requested:

_____ Electrolyte Panel _____ Complete Blood Count

_____ Hepatic Panel _____ Protime / PTT

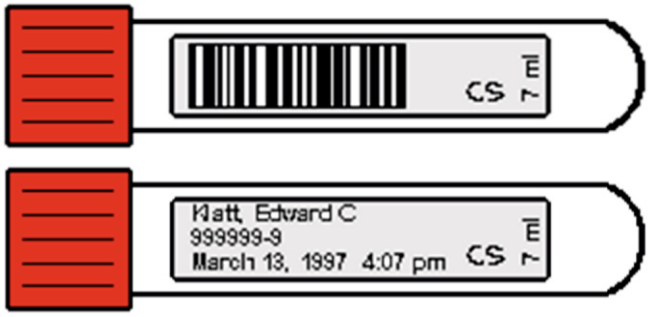
LABELING THE SAMPLE

A properly labeled sample is essential so that the results of the test match the patient. The key elements in labeling are:

- Patient's surname, first and middle.
- Patient's ID number.
- **NOTE:** Both of the above MUST match the same on the requisition form.
- Date, time and initials of the phlebotomist must be on the label of EACH tube.

Automated systems may include labels with bar codes.

Examples of labeled collection tubes are shown below:



EQUIPMENT:

THE FOLLOWING ARE NEEDED FOR ROUTINE VENIPUNCTURE:

- Evacuated Collection Tubes - The tubes are designed to fill with a predetermined volume of blood by vacuum. The rubber stoppers are color coded according to the additive that the tube contains. Various sizes are available. Blood should **NEVER** be poured from one tube to another since the tubes can have different additives or coatings (see illustrations at end).
- Needles - The gauge number indicates the bore size: the larger the gauge number, the smaller the needle bore. Needles are available for evacuated systems and for use with a syringe, single draw or butterfly system.
- Holder/Adapter - use with the evacuated collection system.

- Tourniquet - Wipe off with alcohol and replace frequently.
- Alcohol Wipes - 70% isopropyl alcohol.
- Povidone-iodine wipes/swabs - Used if blood culture is to be drawn.
- Gauze sponges - for application on the site from which the needle is withdrawn.
- Adhesive bandages / tape - protects the venipuncture site after collection.
- Needle disposal unit - needles should NEVER be broken, bent, or recapped. Needles should be placed in a proper disposal unit IMMEDIATELY after their use.
- Gloves - can be made of latex, rubber, vinyl, etc.; worn to protect the patient and the phlebotomist.
- Syringes - may be used in place of the evacuated collection tube for special circumstances.

ORDER OF DRAW

Blood collection tubes must be drawn in a specific order to avoid cross-contamination of additives between tubes. The recommended order of draw for plastic collection tubes is:

1. First - blood culture bottle or tube (yellow or yellow-black top)
2. Second - coagulation tube (light blue top). If just a routine coagulation assay is the only test ordered, then a single light blue top tube may be drawn. If there is a concern regarding contamination by tissue fluids or thromboplastins, then one may draw a non-additive tube first, and then the light blue top tube.
3. Third - non-additive tube (red top)
4. Last draw - additive tubes in this order:
    1. SST (red-gray or gold top). Contains a gel separator and clot activator.
    2. Sodium heparin (dark green top)
    3. PST (light green top). Contains lithium heparin anticoagulant and a gel separator.
    4. EDTA (lavender top)
    5. ACDA or ACDB (pale yellow top). Contains acid citrate dextrose.
    6. Oxalate/fluoride (light gray top)

    NOTE: Tubes with additives must be thoroughly mixed. Erroneous test results may be obtained when the blood is not thoroughly mixed with the additive.

PROCEDURAL ISSUES

PATIENT RELATIONS AND IDENTIFICATION:

The phlebotomist's role requires a professional, courteous, and understanding manner in all contacts with the patient. Greet the patient and identify yourself and indicate the procedure that will take place. Effective communication - both verbal and nonverbal - is essential. Proper patient identification MANDATORY. If an inpatient is able to respond, ask for a full name and always check the armband or bracelet for confirmation. **DO NOT DRAW BLOOD IF THE ARMBAND OR BRACELET IS MISSING.** For an inpatient the nursing staff can be contacted to aid in identification prior to proceeding. An outpatient must provide identification other than the verbal statement of a name. Using the requisition for reference, ask a patient to provide additional information such as a surname or birthdate. A government issued photo identification card such as a driver's license can aid in resolving identification issues. If possible, speak with the patient during the process. The patient who is at ease will be less focused on the procedure. Always thank the patient and excuse yourself courteously when finished.

PATIENT'S BILL OF RIGHTS:

The Patient's Bill of Rights has been adopted by many hospitals as declared by the Joint Commission on Accreditation of Healthcare Organizations (JCAHO). The basic patient rights endorsed by the JCAHO follow in condensed form are given below.

The patient has the right to:

- Impartial access to treatment or accommodations that are available or medically indicated, regardless of race, creed, sex, national origin, or sources of payment for care.
- Considerate, respectful care.
- Confidentiality of all communications and other records pertaining to the patient's care.
- Expect that any discussion or consultation involving the patient's case will be conducted discretely and that individuals not directly involved in the case will not be present without patient permission.
- Expect reasonable safety congruent with the hospital practices and environment.
- Know the identity and professional status of individuals providing service and to know which physician or other practitioner is primarily responsible for his or her care.
- Obtain from the practitioner complete and current information about diagnosis, treatment, and any known prognosis, in terms the patient can reasonably be expected to understand.
- Reasonable informed participation in decisions involving the patient's health care. The patient shall be informed if the hospital proposes to engage in or perform human experimentation or other research/educational profits affecting his or her care or treatment. The patient has the right to refuse participation in such activity.
- Consult a specialist at the patient's own request and expense.
- Refuse treatment to the extent permitted by law.
- Regardless of the source of payment, request and receive an itemized and detailed explanation of the total bill for services rendered in the hospital.
- Be informed of the hospital rules and regulations regarding patient conduct.

VENIPUNCTURE SITE SELECTION:

Although the larger and fuller median cubital and cephalic veins of the arm are used most frequently, the basilic vein on the dorsum of the arm or dorsal hand veins are also acceptable for venipuncture. Foot veins are a last resort because of the higher probability of complications.

Certain areas are to be avoided when choosing a site:

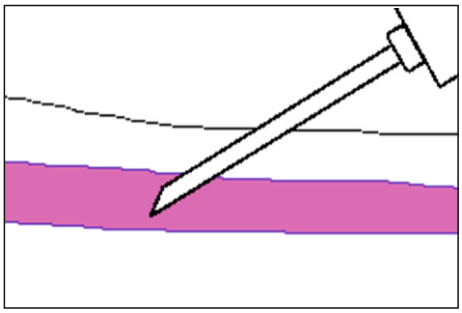
- Extensive scars from burns and surgery - it is difficult to puncture the scar tissue and obtain a specimen.
- The upper extremity on the side of a previous mastectomy - test results may be affected because of lymphedema.
- Hematoma - may cause erroneous test results. If another site is not available, collect the specimen distal to the hematoma.
- Intravenous therapy (IV) / blood transfusions - fluid may dilute the specimen, so collect from the opposite arm if possible. Otherwise, satisfactory samples may be drawn below the IV by following these procedures:
    - Turn off the IV for at least 2 minutes before venipuncture.
    - Apply the tourniquet below the IV site. Select a vein other than the one with the IV.
    - Perform the venipuncture. Draw 5 ml of blood and discard before drawing the specimen tubes for testing.
- Lines - Drawing from an intravenous line may avoid a difficult venipuncture, but introduces problems. The line must be flushed first. When using a syringe inserted into the line, blood must be withdrawn slowly to avoid hemolysis.
- Cannula/fistula/heparin lock - hospitals have special policies regarding these devices. In general, blood should not be drawn from an arm with a fistula or cannula without consulting the attending physician.
- Edematous extremities - tissue fluid accumulation alters test results.

PROCEDURE FOR VEIN SELECTION:

- Palpate and trace the path of veins with the index finger. Arteries pulsate, are most elastic, and have a thick wall. Thrombosed veins lack resilience, feel cord-like, and roll easily.
- If superficial veins are not readily apparent, you can force blood into the vein by massaging the arm from wrist to elbow, tap the site with index and second finger, apply a warm, damp washcloth to the site for 5 minutes, or lower the extremity over the bedside to allow the veins to fill.

PERFORMANCE OF A VENIPUNCTURE:

- Approach the patient in a friendly, calm manner. Provide for their comfort as much as possible, and gain the patient's cooperation.
- Identify the patient correctly.
- Properly fill out appropriate requisition forms, indicating the test(s) ordered.
- Verify the patient's condition. Fasting, dietary restrictions, medications, timing, and medical treatment are all of concern and should be noted on the lab requisition.
- Check for any allergies to antiseptics, adhesives, or latex by observing for armbands and/or by asking the patient.
- Position the patient. The patient should either sit in a chair, lie down or sit up in bed. Hyperextend the patient's arm.
- Apply the tourniquet 3-4 inches above the selected puncture site. Do not place too tightly or leave on more than 2 minutes (and no more than a minute to avoid increasing risk for hemoconcentration). Wait 2 minutes before reapplying the tourniquet.
- The patient should make a fist without pumping the hand.
- Select the venipuncture site.
- Prepare the patient's arm using an alcohol prep. Cleanse in a circular fashion, beginning at the site and working outward. Allow to air dry.
- Grasp the patient's arm firmly using your thumb to draw the skin taut and anchor the vein. The needle should form a 15 to 30 degree angle with the surface of the arm. Swiftly insert the needle through the skin and into the lumen of the vein. Avoid trauma and excessive probing.



- When the last tube to be drawn is filling, remove the tourniquet.
- Remove the needle from the patient's arm using a swift backward motion.
- Press down on the gauze once the needle is out of the arm, applying adequate pressure to avoid formation of a hematoma.
- Dispose of contaminated materials/supplies in designated containers.
- Mix and label all appropriate tubes at the patient bedside.
- Deliver specimens promptly to the laboratory.

PHLEBOTOMY PROCEDURE ILLUSTRATED:

- **Patient identification**
- **Filling out the requisition**
- **Equipment**
- **Apply tourniquet and palpate for vein**
- **Sterilize the site**
- **Insert needle**
- **Drawing the specimen**
- **Releasing the tourniquet**
- **Applying pressure over the vein**
- **Applying bandage**
- **Disposing needle into sharps**
- **Labeling the specimens**

PERFORMANCE OF A FINGERSTICK:

- Follow the procedure as outlined above for greeting and identifying the patient. As always, properly fill out appropriate requisition forms, indicating the test(s) ordered.
- Verify the patient's condition. Fasting, dietary restrictions, medications, timing, and medical treatment are all of concern and should be noted on the lab requisition.
- Position the patient. The patient should either sit in a chair, lie down or sit up in bed. Hyperextend the patient's arm.
- The best locations for fingersticks are the 3rd (middle) and 4th (ring) fingers of the non-dominant hand. Do not use the tip of the finger or the center of the finger. Avoid the side of the finger where there is less soft tissue, where vessels and nerves are located, and where the bone is closer to the surface. The 2nd (index) finger tends to have thicker, callused skin. The fifth finger tends to have less soft tissue overlying the bone. Avoid puncturing a finger that is cold or cyanotic, swollen, scarred, or covered with a rash.
- Using a sterile lancet, make a skin puncture just off the center of the finger pad. The puncture should be made perpendicular to the ridges of the fingerprint so that the drop of blood does not run down the ridges.
- Wipe away the first drop of blood, which tends to contain excess tissue fluid.
- Collect drops of blood into the collection device by gently massaging the finger. Avoid excessive pressure that may squeeze tissue fluid into the drop of blood.
- Cap, rotate and invert the collection device to mix the blood collected.
- Have the patient hold a small gauze pad over the puncture site for a couple of minutes to stop the bleeding.
- Dispose of contaminated materials/supplies in designated containers.
- Label all appropriate tubes at the patient bedside.
- Deliver specimens promptly to the laboratory.

FINGERSTICK PROCEDURE ILLUSTRATED:

- **Equipment**
- **Proper location on finger**
- **Puncture with lancet**
- **Drop of blood**
- **Wipe first drop**
- **Collecting the specimen**
- **Specimen container**

ADDITIONAL CONSIDERATIONS:

To prevent a hematoma:

- Puncture only the uppermost wall of the vein
- Remove the tourniquet before removing the needle
- Use the major superficial veins
- Make sure the needle fully penetrates the upper most wall of the vein. (Partial penetration may allow blood to leak into the soft tissue surrounding the vein by way of the needle bevel)
- Apply pressure to the venipuncture site

To prevent hemolysis (which can interfere with many tests):
- Mix tubes with anticoagulant additives gently 5-10 times
- Avoid drawing blood from a hematoma
- Avoid drawing the plunger back too forcefully, if using a needle and syringe, or too small a needle, and avoid frothing of the sample
- Make sure the venipuncture site is dry
- Avoid a probing, traumatic venipuncture
- Avoid prolonged tourniquet application or fist clenching.

Indwelling Lines or Catheters:
- Potential source of test error
- Most lines are flushed with a solution of heparin to reduce the risk of thrombosis
- Discard a sample at least three times the volume of the line before a specimen is obtained for analysis

Hemoconcentration: An increased concentration of larger molecules and formed elements in the blood may be due to several factors:
- Prolonged tourniquet application (no more than 1 minute)
- Massaging, squeezing, or probing a site
- Long-term IV therapy
- Sclerosed or occluded veins

Prolonged Tourniquet Application:
- Primary effect is hemoconcentration of non-filterable elements (i.e. proteins). The hydrostatic pressure causes some water and filterable elements to leave the extracellular space.
- Significant increases can be found in total protein, aspartate aminotransferase (AST), total lipids, cholesterol, iron
- Affects packed cell volume and other cellular elements
- Hemolysis may occur, with pseudohyperkalemia.

Patient Preparation Factors:
- Therapeutic Drug Monitoring: Different pharmacologic agents have patterns of administration, body distribution, metabolism, and elimination that affect the drug concentration as measured in the blood. Many drugs will have "peak" and "trough" levels that vary according to dosage levels and intervals. Check for timing instructions for drawing the appropriate samples.
- Effects of Exercise: Muscular activity has both transient and longer lasting effects. The creatine kinase (CK), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and platelet count may increase.
- Stress: May cause transient elevation in white blood cells (WBC's) and elevated adrenal hormone values (cortisol and catecholamines). Anxiety that results in hyperventilation may cause acid-base imbalances, and increased lactate.
- Diurnal Rhythms: Diurnal rhythms are body fluid and analyte fluctuations during the day. For example, serum cortisol levels are highest in early morning but are decreased in the afternoon. Serum iron levels tend to drop during the day. You must check the timing of these variations for the desired collection point.
- Posture: Postural changes (supine to sitting etc.) are known to vary lab results of some analytes. Certain larger molecules are not filterable into the tissue, therefore they are more concentrated in the blood. Enzymes, proteins, lipids, iron, and calcium are significantly increased with changes in position.
- Other Factors: Age, gender, and pregnancy have an influence on laboratory testing. Normal reference ranges are often noted according to age.

REASONS FOR CANCELING A LABORATORY TEST

A test that has been ordered may be cancelled due to problems unrelated to drawing the specimen, and these are the most common causes for cancellations:
- Duplicate test request
- Incorrect test ordered
- Test no longer needed

A test may be cancelled due to a technical problem in the specimen collection process:
- Hemolysis of the specimen
- Clotted specimen
- Quantity of specimen not sufficient
- Collection of specimen in incorrect tube
- Contaminated specimen
- Identification of the specimen is suspect
- Delay in transport - specimen too old

SAFETY AND INFECTION CONTROL

Because of contacts with sick patients and their specimens, it is important to follow safety and infection control procedures.

PROTECT YOURSELF

- Practice universal precautions:
  - Wear gloves and a lab coat or gown when handling blood/body fluids.
  - Change gloves after each patient or when contaminated.
  - Wash hands frequently.
  - Dispose of items in appropriate containers.
- Dispose of needles immediately upon removal from the patient's vein. Do not bend, break, recap, or resheath needles to avoid accidental needle puncture or splashing of contents.
- Clean up any blood spills with a disinfectant such as freshly made 10% bleach.
- If you stick yourself with a contaminated needle:
  - Remove your gloves and dispose of them properly.
  - Squeeze puncture site to promote bleeding.
  - Wash the area well with soap and water.
  - Record the patient's name and ID number.
  - Follow institution's guidelines regarding treatment and follow-up.
  - NOTE: The use of prophylactic zidovudine following blood exposure to HIV has shown effectiveness (about 79%) in preventing seroconversion

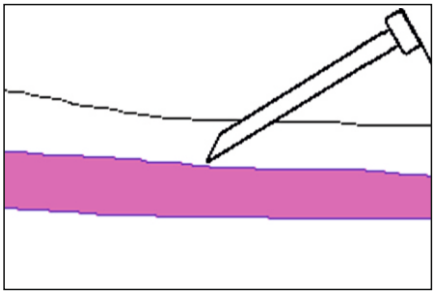
PROTECT THE PATIENT

- Place blood collection equipment away from patients, especially children and psychiatric patients.
- Practice hygiene for the patient's protection. When wearing gloves, change them between each patient and wash your hands frequently. Always wear a clean lab coat or gown.

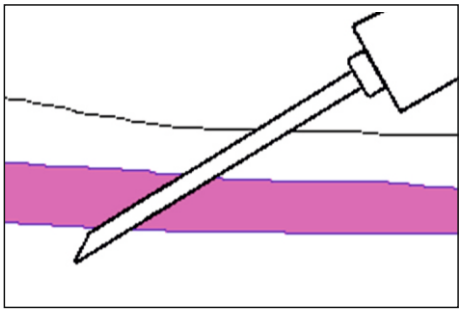
TROUBLESHOOTING GUIDELINES:

IF AN INCOMPLETE COLLECTION OR NO BLOOD IS OBTAINED:

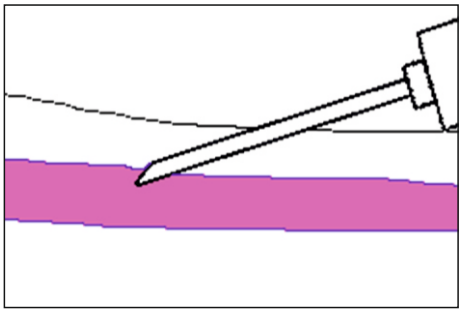
- Change the position of the needle. Move it forward (it may not be in the lumen)



- or move it backward (it may have penetrated too far).



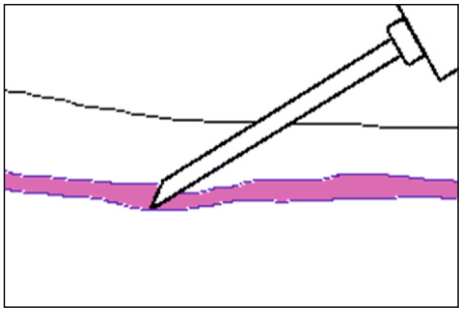
- Adjust the angle (the bevel may be against the vein wall).



- Loosen the tourniquet. It may be obstructing blood flow.
- Try another tube. Use a smaller tube with less vacuum. There may be no vacuum in the tube being used.
- Re-anchor the vein. Veins sometimes roll away from the point of the needle and puncture site.
- Have the patient make a fist and flex the arm, which helps engorge muscles to fill veins.
- Pre-warm the region of the vein to reduce vasoconstriction and increase blood flow.
- Have the patient drink fluids if dehydrated.

IF BLOOD STOPS FLOWING INTO THE TUBE:

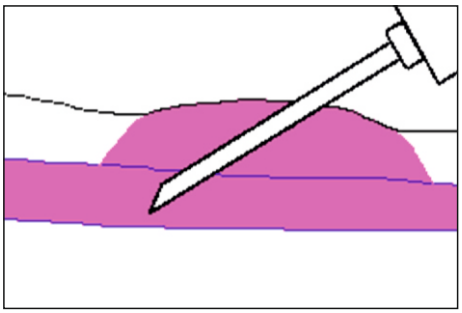
- The vein may have collapsed; resecure the tourniquet to increase venous filling. If this is not successful, remove the needle, take care of the puncture site, and redraw.



- The needle may have pulled out of the vein when switching tubes. Hold equipment firmly and place fingers against patient's arm, using the flange for leverage when withdrawing and inserting tubes.

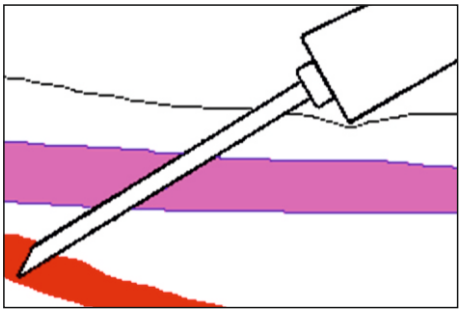
PROBLEMS OTHER THAN AN INCOMPLETE COLLECTION:

- A hematoma forms under the skin adjacent to the puncture site - release the tourniquet immediately and withdraw the needle. Apply firm pressure.



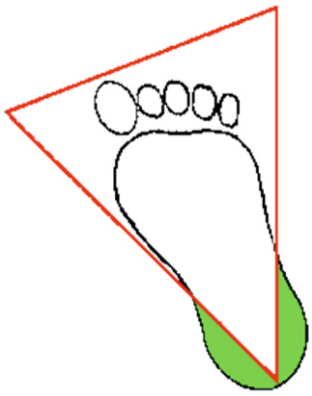
Hematoma formation is a problem in older patients.

- The blood is bright red (arterial) rather than venous. Apply firm pressure for more than 5 minutes.



BLOOD COLLECTION ON BABIES:

- The recommended location for blood collection on a newborn baby or infant is the heel. The diagram below indicates in green the proper area to use for heel punctures for blood collection:



- Prewarming the infant's heel (42 ^{0}C for 3 to 5 minutes) is important to obtain capillary blood gas samples and warming also greatly increases the flow of blood for collection of other specimens. However, do not use too high a temperature warmer, because baby's skin is thin and susceptible to thermal injury.
- Clean the site to be punctured with an alcohol sponge. Dry the cleaned area with a dry cotton sponge. Hold the baby's foot firmly to avoid sudden movement.
- Using a sterile blood lancet, puncture the side of the heel in the appropriate regions shown above in green. Do not use the central portion of the heel because you might injure the underlying bone, which is close to the skin surface. Do not use a previous puncture site. Make the cut across the heelprint lines so that a drop of blood can well up and not run down along the lines.
- Wipe away the first drop of blood with a piece of clean, dry cotton. Since newborns do not often bleed immediately, use gentle pressure to produce a rounded drop of blood. Do not use excessive pressure or heavy massaging because the blood may become diluted with tissue fluid.
- Fill the capillary tube(s) or micro collection device(s) as needed.
- When finished, elevate the heel, place a piece of clean, dry cotton on the puncture site, and hold it in place until the bleeding has stopped.

- Be sure to dispose of the lancet in the appropriate sharps container. Dispose of contaminated materials in appropriate waste receptacles. Remove your gloves and wash your hands.

HEELSTICK PROCEDURE ILLUSTRATED:

- **Heelstick on baby**

PEDIATRIC PHLEBOTOMY:

- Children, particularly under the age of 10, may experience pain and anxiety during the phlebotomy procedure.
- A variety of techniques can be employed to reduce pain and anxiety. Effective methods use distraction. These may include listening to music or a story, watching a video, playing with a toy, having a parent provide distraction with talk or touch, using flash cards, and squeezing a rubber ball. (Uman et al, 2013)

COLLECTION TUBES FOR PHLEBOTOMY

- Collection tubes can vary in size for volume of blood drawn, appropriate to the tests ordered with sample size required, and vary in the kind of additive for anticoagulation, separation of plasma, or preservation of analyte. Larger tube sizes typically provide for collection of samples from 6 to 10 mL.

- Smaller collection tubes for sample sizes of 2 mL or less may be appropriate in situations where a smaller amount blood should be drawn, as in pediatric patients, or to minimize hemolysis during collection, or to avoid insufficient sample volume in the collection tube.

Red Top

ADDITIVE	None
MODE OF ACTION	Blood clots, and the serum is separated by centrifugation
USES	Chemistries, Immunology and Serology, Blood Bank (Crossmatch)

Gold Top

ADDITIVE	None
MODE OF ACTION	Serum separator tube (SST) contains a gel at the bottom to separate blood from serum on centrifugation
USES	Chemistries, Immunology and Serology

Light Green Top

ADDITIVE	Plasma Separating Tube (PST) with Lithium heparin
MODE OF ACTION	Anticoagulates with lithium heparin; Plasma is separated with PST gel at the bottom of the tube
USES	Chemistries

Purple Top

ADDITIVE	EDTA
MODE OF ACTION	Forms calcium salts to remove calcium
USES	Hematology (CBC) and Blood Bank (Crossmatch); requires **full draw** - invert 8 times to prevent clotting and platelet clumping

Light Blue Top

ADDITIVE	Sodium citrate
MODE OF ACTION	Forms calcium salts to remove calcium
USES	Coagulation tests (protime and prothrombin time), **full draw** required

Green Top

ADDITIVE	Sodium heparin or lithium heparin
MODE OF ACTION	Inactivates thrombin and thromboplastin
USES	For lithium level, use sodium heparin For ammonia level, use sodium or lithium heparin

Dark Blue Top

ADDITIVE	EDTA-
MODE OF ACTION	Tube is designed to contain no contaminating metals
USES	Trace element testing (zinc, copper, lead, mercury) and toxicology

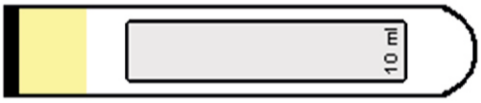
Light Gray Top

ADDITIVE	Sodium fluoride and potassium oxalate
MODE OF ACTION	Antiglycolytic agent preserves glucose up to 5 days
USES	Glucoses, requires **full draw** (may cause hemolysis if short draw)

Yellow Top

ADDITIVE	ACD (acid-citrate-dextrose)
MODE OF ACTION	Complement inactivation
USES	HLA tissue typing, paternity testing, DNA studies

Yellow - Black Top



ADDITIVE	Broth mixture
MODE OF ACTION	Preserves viability of microorganisms
USES	Microbiology - aerobes, anaerobes, fungi

Black Top

ADDITIVE	Sodium citrate (buffered)
MODE OF ACTION	Forms calcium salts to remove calcium
USES	Westergren Sedimentation Rate; requires **full draw**

Orange Top

ADDITIVE	Thrombin
MODE OF ACTION	Quickly clots blood
USES	STAT serum chemistries

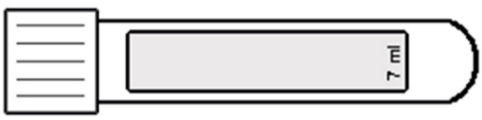
Light Brown Top

ADDITIVE	Sodium heparin
MODE OF ACTION	Inactivates thrombin and thromboplastin; contains virtually no lead
USES	Serum lead determination

Pink Top

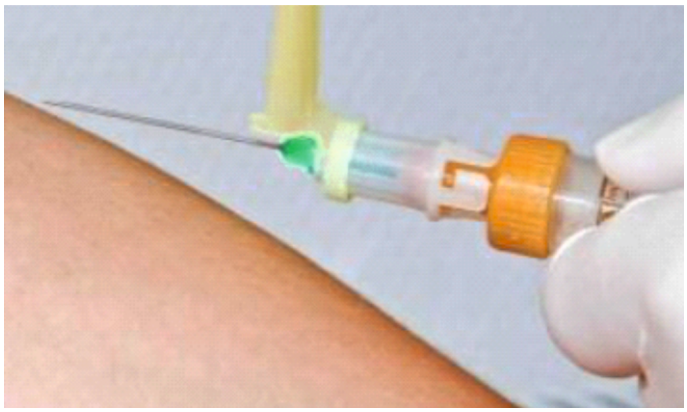
ADDITIVE	Potassium EDTA
MODE OF ACTION	Forms calcium salts
USES	Immunohematology

White Top



ADDITIVE	Potassium EDTA
MODE OF ACTION	Forms calcium salts
USES	Molecular/PCR and bDNA testing

Haemolysis: A Major Challenge in the Lab



Haemolysis is defined as the release of haemoglobin and other intracellular components from erythrocytes, thrombocytes and leukocytes into the extracellular fluid i.e. the plasma or serum, following damage or disruption of cell membranes. Haemolysed specimen is the most common reason for rejection of specimens in the lab. Studies have shown that out of the total specimens received in the lab, 3.3% are found to be haemolysed, and out of the total rejected; 40-70% has been identified to be due to haemolysis. The American Society of Clinical Pathology has established a 2%, or lower, benchmark for haemolysis rates amongst laboratory blood samples. Haemolysis may occur either *in vivo* or *in vitro*, and is a most undesirable condition that influences the accuracy and reliability of laboratory testing. It has been observed that 3.2% of all detectable haemolysis is *in vivo*. *In vivo* haemolysis can originate from hereditary, acquired and iatrogenic conditions, such as autoimmune haemolytic anaemia, severe infections, intravascular disseminated coagulation, haemoglobinopathies, drug transfusion reactions etc. *In vivo* haemolysis does not depend on the techniques of the healthcare provider and is virtually unavoidable and cannot be resolved. *In vitro* haemolysis occurs more often and is caused by improper sample drawing, improper sample processing and improper sample transport (*see figure 1*).

Figure 1. In Vitro Hemolysis

IMPROPER SAMPLE DRAWING
Improper selection of the venepuncture site
Prolonged tourniquet time
Continuous clenching of the fist
Improper drying of the alcohol at venepuncture site
Needle guage
Tube under filling
Syringe Transfer
Catheter IV collection
Traumatic draw
Capillary collection

SPECIMEN PROCESSING
Excessive mixing or vigorous shaking of the tube
Not allowing clotting for sufficient time
Applicator sticks
Time delay before centrifugation
Centrifuge conditions, Speed, time and temperature

TRANSPORT
Mechanical trauma drug transport
Temperature humidity at the time of transport
Length, speed and number of times the sample is transported.

IMPROPER SAMPLE DRAWING

One of the most important reasons for haemolysis is improper selection of the venipuncture site. Drawing blood from distal arm rather than anticubital fossa can result in haemolysis. A study done by Edward et al clearly shows that the anticubital fossa may be more favourable than the distal arm because of the faster flow of blood due increased diameter of the vein and reduced resistance. Difficulty to locate easy venous access, small or fragile veins (alternate sites to the anticubital area, such as small hand veins, are fragile and easily traumatised) unsatisfactory attempts, and missing veins can all lead to haemolysis. An improper venipuncture is indicated by a slow blood flow due to the lumen of the needle not centred in the vein, meaning the bevel opening of the needle is partly in the vein and partly in the vein wall, leading to an increasing aspiration force and rupture of the RBC. The Clinical and Laboratory Standards Institute (CLSI) recommends the use of a tourniquet for localising suitable veins for $\leqslant$ 60 sec. Prolonged tourniquet time, or continuous clenching the fist, results in rupture of the RBC. Venous stasis greater than one minute affects potassium, calcium and albumin by 2.8%, 1.6% and 3.5% respectively, and venous stasis greater than three minutes affects potassium, calcium and albumin by 4.8%, 3.6% and 8.6% respectively. Cleaning the vein puncture site with alcohol and not allowing the site to air dry results in transfer of alcohol from the skin to the blood specimen resulting in haemolysis. The choice of needle gauge size is very important. The use of large bore needles may result in a much faster and more forceful flow of blood through the needles resulting in haemolysis. Using a smaller gauge needle results in a large vacuum force applied to the blood and may cause sheer stress on the RBC, causing them to rupture. The ideal gauge of needle for venipuncture is usually 20-22. Haemolysis from excessive aspiration force is relatively frequent, mainly in cases of small or superficial veins. Pulling the syringe plunger back too fast or forcefully can result in haemolysis. Forcefully expelling the blood from the syringe into the blood tubes can also rupture the RBC. A study done by Edward et al shows that under-filling tubes with blood will cause improper blood-to-additive ratios (especially EDTA), resulting in haemolysis. Haemolysis can be avoided by filling the tubes to the required level. When blood is drawn from a peripheral IV catheter, a higher incidence of haemolysis occurs due to frothing of the blood from a loose connection of the blood collection assemblies.

SPECIMEN PROCESSING

Excessive mixing or shaking of the blood after collection, not allowing to clot for recommended amount of time (30 mins), use of applicator sticks to dislodge the fibrin, prolonged contact of serum or plasma with cells, failure to separate serum from red cells within 60 minutes of venipuncture, exposure to excessive heat or cold, can all cause RBC rupture and haemolysis. Centrifugation at too high a speed frequently compromises the integrity of the blood samples. Re-centrifugation (re-spin) of tubes with gel separators (the gel barrier may open and allow any supernatant that has been in contact with erythrocytes, to mix the supernatant previously above the separator).

TRANSPORTATION

Mechanical trauma during transport may occur, resulting in haemolysis. Variable factors such as temperature, humidity, length, speed, and number of times the specimen is transported, as well as the number of angles or turns the system uses also affects the integrity of the sample. Placing ice or frozen gel packs directly on tubes of blood can result in haemolysis.

HAEMOLYSIS INDEX/SERUM INDEX

The index aids in evaluating sample integrity by determining the level of haemoglobin in serum or plasma. It improves the quality of reported results, in almost no time, with minimal cost and also improved handling of paediatric samples.

The extent of haemolysis can be detected in two ways:

1. Visual haemolysis detection
2. Automated Serum Index.

Visual haemolysis detection Visually, haemolysis is defined as free haemoglobin concentration >30-50mg/dL conferring detectable pink/red hue to serum or plasma. It becomes clearly visible in specimens containing as low as 0.5% lysed erythrocytes. It is based on comparing patient samples with photographs of samples containing various concentrations of haemoglobin. Drawbacks of the visualising method are that it is unreliable since it may over and underestimate the actual prevalence of haemolysed serum specimens (i.e., trained observers are unable to accurately rank the degree of interference in serum). Elevated concentration of bilirubin may further impair the ability to detect haemolysis by visual inspection and therefore lead to serious underestimation of haemolysis in neonatal samples, where elevated bilirubin concentration is commonplace.

Automated Serum Index This is by directly measuring haemoglobin concentration photometrically followed by comparing it with the serum index values for haemolysis that have been determined by the instrument vendor. (These values represent the levels at which the haemoglobin significantly interferes with the analyte testing.) The advantage of an Automated Serum Index is its consistency, reproducibility and improvement in detection of haemolysis.

Notable examples of tests affected by hemolysis are found in the table below.

Fig. 2 : Effect of Hemolysis on some Clinical Biochemistry and Hematology Test Results

Degree of change in analyte	Test result increased by hemolysis	Test result decreased by hemolysis	Test result increased or decreased by hemolysis
Slight change	Phosphate, Total Protein, Albumin, Magnesium, Calcium, Alkaline Phosphatase	Haptoglobin, Bilirubin	
Noticeable change	Alanine Amino Transferase, Creatinine Kinase, Iron, Coagulation tests		
Significant change	Potassium, Lactate Dehydrogenase, Aspartate Amino Transferase	Troponin T	Hemoglobin, Red Blood Cells, MCHC, Platelet Count

Note: If the specimen is grossly hemolyzed, a recollected specimen will be requested. If the recollected specimen is also grossly hemolyzed, it will be processed and a comment added.

INFLUENCE OF HAEMOLYSIS ON ROUTINE CLINICAL TESTING

- Leakage of haemoglobin and other intracellular components into the surrounding fluid may induces false elevation of some analytes or dilution effect. If the analyte in question is present in a higher concentration in blood cells than in plasma, then the analytical value will be increased. Conversely, if the concentration in blood cells is lower, the plasma becomes diluted, and the analytical result is too low. Caraway reported that erythrocytes contain 160-fold as much lactate dehydrogenase, 68-fold as much acid phosphatase, 40-fold as much aspartate aminotransferase, and 6.7-fold as much alanine aminotransferase as does plasma
- Haemoglobin absorbs light strongly at 415, 540 and 570nm. Haemolysis therefore increases absorption in this wavelength range and causes apparent increase in the concentration of analytes measured in this range
- In addition to haemoglobin, erythrocytes also contain proteins, enzymes, lipids and carbohydrates and many of these may also interact or compete with the assay reagents. e.g. free haemoglobin with its pseudo-peroxidase activity interferes in the bilirubin procedure by inhibiting the diazonium colour formation and thereby resulting in low values. Increased concentration of CK is most likely due to analytical interference, due to release of intracellular adenylate kinase, which is not completely inhibited under operating conditions
- Release of intracellular and thromboplastic substances from either leukocytes or platelets, results in prolongations in prothrombin time and dimerised plasmin fragments D(D-dimer) whereas shortening of activated partial thromboplastin time and decrease in fibrinogen values.

OVERCOMING THE CHALLENGE OF HAEMOLYSIS

There should be proper laboratory guidelines and recommendations for the management of haemolysed samples. Proper training and knowledge of the factors that can influence laboratory results, along with appropriate training of the phlebotomists, are essential prerequisites to minimise errors. Every laboratory personnel should be trained properly. Standardised blood collection and handling procedures should be followed. Collection from a haematoma site and prolonged tourniquet time should be avoided. Equipment and connections that may lead to turbulent blood flow leading to haemolysis should be avoided. Vigorous mixing of the specimens after collection should be prevented and appropriate conditions of temperature and humidity should be maintained. Standardised practices for sample transportation and storage should be observed. The blood specimens should be centrifuged within a suitable time of collection, with appropriate conditions of centrifugation (force, spin, time and temperature), supernatant (serum or plasma) timely separated from the blood cells unless the primary tube is provided with a gel separator. There has always been a debate as to whether we should or should not process the haemolysed samples. Basically when a haemolysed sample reaches the laboratory, we can; **1.** Reject the sample for analysis and ask for re-collection **2.** Perform the analysis and report the results with a comment **3.** When a haemolysed sample is referred to the laboratory, the personnel should always ask for new sample(s). In case new sample(s) cannot be obtained, it is the responsibility of the laboratory specialist to communicate the problem to the concerned physician and seek for the best solution for the best of the patient care. It is pointed out by the authors of the recommendations that it is always better not to report the result rather than producing spurious data on unsuitable samples.

Blood Samples Transportation

This protocol is for human clinical blood specimens collected for analysis and to be transported. Collect specimens and package them for shipment as described below unless otherwise directed.

REQUIRED SPECIMENS

Collect the specimens defined below. Blood should be collected only by trained personnel using aseptic methods and working under the direction of a qualified, licensed practitioner. Standard venipuncture blood collection and handling procedures (CLSI Guideline H3 and H18) should be followed.

SPECIMEN COLLECTION

Collect a whole blood specimen into the type of blood tube as directed. The tube will be one of those listed below. Collect the specimen by full-draw, vacuum-fill only (**unopened**) directly into the blood tube. Do not collect by a syringe draw and transfer. Plastic (PET) tubes are preferred. Lavender-top (EDTA) tube. Mix by inverting 8-10 times. Gray-top (oxalate/fluoride) tube. Mix by inverting 8-10 times. Green-top (heparin) tube. Mix by inverting 8-10 times.

EVIDENTIARY REQUIREMENTS

Apply evidence tape and the tape applicator's initials to each specimen container and secondary packaging as described below. **Evidence tape-** Place a single, unbroken strip of waterproof, tamper-evident forensic evidence tape on the container being careful not to cover the specimen ID label. Begin by sticking the tape to the specimen container at one side, somewhat below the top, then apply it over the top, and finally bring it down and stick it to the opposite side of the container. Also apply evidence tape to the blank containers. **Initialing -** The individual applying the evidence tape must write their initials in indelible ink on the tape and container so their initials are approximately ½ on the tape and ½ on the container. Apply tape to the secondary container and initial in the same manner. **Chain-of-custody -** A chain of custody form must be completed for the specimens. Chain of custody forms do not need to be transported with specimens. Each entity/organization handling the specimens is responsible for the specimens only during the time that they have control of the specimens. Each entity/organization receiving the specimens must sign-off on the chain of custody form of the entity/organization relinquishing the specimens to close that chain. When receiving specimens, each new entity/organization must begin their own chain of custody and have the entity/organization relinquishing the specimens sign their chain of custody to start the chain and indicate that they have transferred the specimens. When specimens are transferred between entities/organizations, each entity/organization retains their chain of custody forms. NOTE: When the individual relinquishing the specimens (relinquisher) and the individual receiving the specimens (receiver) are not together at the time of specimen transfer, the relinquisher will document on their chain of custody that the receiver is Courier Tracking Number or have the individual transporting the specimens sign the chain of custody to indicate that they have taken control of the specimens. Likewise, when the receiver receives the specimens, they will document on their chain of custody that the relinquisher is Courier Tracking Number or the have the individual transporting the specimens sign the chain of custody.

STORAGE REQUIREMENTS

Specimens must be refrigerated within 30 minutes of collection and maintained at refrigeration temperature (4°C) continuously. Do not freeze blood specimens. **BLANKS:** Tube blanks for measuring background contamination must be included with each shipment of specimens. For each lot number of tubes used for collection, please provide two (2) unopened tubes.

DOCUMENTATION AND LABELING

Label specimens with labels generated by your facility. Follow your facility's procedures for proper sample labeling. Do not include any personal identifiers on the specimen containers. Inclusion of the collector's initials, and date and time of collection will allow law enforcement officials to trace the specimen back to the collector should there be a need to have the collector testify as to the specimen collection. Maintain a list of names with corresponding sample identification numbers or bar codes at the collection site to enable results to be reported to the patients. Prepare an itemized shipping list for each secondary package.

PACKAGING SPECIMENS FOR TESTING

Follow all current regulations in the packaging and shipping of specimens. Pack and ship these samples as DIAGNOSTIC SPECIMENS. Packaging for diagnostic specimens requires triple packaging, consisting of a leak-proof primary receptacle (the tube), a leak-proof secondary packaging that meets 49CFR173.199(b), and an outer packaging. Use styrofoam-insulated corrugated fiberboard outer packaging containers. Specimens must be maintained at refrigeration temperature (4°C) during transportation. **Secondary packaging:** Primary receptacles must be packed in secondary packaging so they will not break, be punctured, or leak their contents into the secondary packaging under normal handling conditions. Fragile primary receptacles packaged together must be individually wrapped or separated so they are not in physical contact. If more than one type of blood tube specimen is collected package them into separate secondary containers. Separate each tube of blood collected from other tubes (e.g., using a gridded box or a foam rack) or wrap tubes to prevent contact between tubes. To facilitate processing upon receipt, package blood tubes so that similar tubes are packaged together (e.g., all purple-tops together) and not mixed (i.e., purple-tops and green/gray-tops in the same package) in the secondary packaging. Place absorbent material between the primary receptacle and the secondary packaging. Use enough absorbent material to absorb the entire contents of primary receptacles. Place tubes in secondary packages. A variety of secondary packages may be used, for example, a gridded box wrapped with absorbent material and sealed inside a rigid leak-proof container or a two-part system of a plastic bag and envelope. Secondary containers must be marked with a Biohazard warning label or be completely red in color. Wrap a single continuous piece of evidence tape around the secondary container and initial, in indelible ink, ½ on the tape and ½ on the packaging. **Outer packaging:** Use Styrofoam-insulated corrugated fiberboard containers. Outer packaging may not exceed a 4 L (1 gallon) capacity. Place additional absorbent material in the bottom of the outer container for cushioning and to absorb condensation from the cold packs. Add a layer of frozen cold packs. Do not use ice. Place secondary containers on top of the cold packs. Both purple-top and gray/green-top containing secondary packages may be packaged in the same outer packaging. Place additional cold packs or absorbent material between the secondary containers to reduce their movement within the outer container. Place a layer of frozen cold packs on top of the secondary containers. Place a complete, itemized list of contents (with sample

identification numbers) in a plastic zippered bag on top of the secondary packaging before closing the Styrofoam lid. Place the completed chain-of-custody forms in a plastic zippered bag on top of the Styrofoam lid. Affix labels and markings on the top of the packaging to increase the probability the package will be kept in the upright position. Clearly label the package with "From" sender's address and "To" recipient's address. Ensure that two (2) upwards-pointing orientation arrows are located on two opposite sides of the outer container. Place a label on the outer container that indicates the proper name, "DIAGNOSTIC SPECIMENS" adjacent to the shipper's address that appears on the package.

CRITERIA FOR SPECIMEN REJECTION

Specimens may be rejected if any of the following conditions are evident upon receipt: Specimen tube is broken or leaking. Specimen has warmed above 8°C. Primary receptacle not sealed with evidence tape. Evidence tape has been tampered with. Itemized list of specimens is not included.

Specimen Preparation

ALL POTENTIALLY INFECTIOUS MATERIAL SHOULD BE HANDLED, LABELED AND TRANSPORTED ACCORDINGLY.

It is essential that the following instructions be followed exactly to assure delivery of a specimen that is adequate for testing. All specimens must be properly identified by indicating the patient's name on every tube or container. The test request form has to be completed and has to include the time and date of the specimen collection, as well as the signature of the Physician requesting the patient's tests.

CHEMISTRY, HEMATOLOGY AND MISCELLANEOUS

Blood: When whole blood is requested, obtain the full amount into a vacuum tube. Lavender, Gray, Green, and Blue Top tubes contain different anticoagulants that inhibit blood coagulation. When drawing these specimens, immediately invert the tube 10-12 times. Do not shake the tube as this can cause Hemolysis or else dispense required quantity of blood into appropriate non-vacuumed vials and gently mix several times.

Serum: Obtain sufficient blood to yield the required volume of Serum. A plain Red Top tube or Red/Mottled top Barrier tube (Corvac, SST, etc.) should be used. When drawing these specimens, immediately invert the tube 5 times. Allow the blood to clot for about 30 minutes and centrifuge for 15 minutes to separate the serum. If a Barrier tube is used, no other manipulations are required. Make sure that the gel has formed a thick, solid, intact barrier between the serum and the clotted cells. If the gel trails into the bottom of the tube, re-centrifuge the tube for another 10 minutes. If a plain Red Top tube or an ordinary bulb is used, transfer the serum with a pipette to a Transfer tube. It is important to avoid hemolysis. Serum in contact with red cells will produce erroneously high Potassium, LDH, and SGOT results and erroneous low Glucose results. Red top tubes for blood banking specimens should not be centrifuged.

Plasma: Treat the specimen as in blood (above)

Urine:

Urinalysis: To adequately test urine specimens the sample should be collected in a tube with a stabilizing chemical present. The tube provided contains a yellow "pop off" cap and a "Stabilur" tablet (or any other stabilizing substance) which preserves the formed elements such as red cells, white cells, casts and epithelial cells. For urinalysis, use a paper cup and transfer about 10 mL of urine to the stopped tube. If no stabilizing substance is used test the urine sample immediately or within an hour at best.

Urine Chemistry: Most assays require a 24 hour collection that should contain boric acid, hydrochloric acid, or sodium carbonate as a preservative. Some analysis require a urine specimen without any additive. Refer to the specific test in this Compendium for specific test details. Instruct the patient to discard the first urine voided upon arising in the morning and thereafter save all urine specimens in the 24 hour container, including the first morning voiding of the following day. Fluid intake during the 24 hour period should be restricted as much as possible. Measure the 24 hour volume and record it on the container and the test request form. Keep the specimen refrigerated until picked up by the laboratory.

Urine, Drugs of Abuse (DAU): For routine DAU testing, submit a specimen in a blue "pop-off" capped tube.

Urine Culture: Collect the urine into a yellow-label screw capped vial (Boricon). It is not necessary to urinate directly into the vial. It is satisfactory to urinate into a paper cup (non-sterile) and to immediately pour the specimen into the Boricon. Refrigerate the specimen as soon as possible. Otherwise sample may be collected in a sterile urine culture container, if delay is expected, refrigerate (not freeze) the specimen.

Frozen Specimens: Certain tests must be submitted frozen because of the stability of the analyte being tested. Keep all frozen specimens separate from the routine tests and submit a separate test request form. As soon as possible separate the serum or plasma and transfer to a plastic transfer tube. Place the specimen in the office freezer and keep until it is solid. Notify the laboratory Logistics Department as soon as possible that you have a frozen specimen for pick up.

PLEASE STORE YOUR SPECIMEN IN THE REFRIGERATOR OR FREEZER UNTIL PICK UP, UNLESS SPECIFICALLY INSTRUCTED TO DO OTHERWISE. Your driver will pick up specimens from the nurse or receptionist at your office or from a box outside your door if after hours.

Cytology: Use Cytology requisition form for all Cytology specimens. Relevant clinical information should be written down in the space provided.

Directions for making Direct Smears:

1. Write patient's name with lead pencil on frosted end of clean slide
2. Spread material evenly over slide
3. Fix immediately with cytology spray fixative from a distance of 10 - 12 inches until liquid droplets form
4. Allow slides to dry before sending out in designated slide holders.

Directions for sending Fluids (Collected or Aspirated):

1. Write patient's name on container.
2. All fluids including gastric washings, pleural and peritoneal (ascitic) fluids, have to be placed in a container with an equal volume of 50% ethyl alcohol.
3. Send fluid immediately in securely closed containers.

NOTE: A sputum specimen will be considered unsatisfactory for diagnosis if no pigmented macrophages (dust cells) are present.

Aspiration Biopsy by Fine Needle (FNA):

1. Use the form for Non-Gyn Cytology for all FNA requests. Relevant information and clinical data should be written down as requested, in the space provided.
2. Solid masses: Do direct smears and spray with Cytology spray fixative immediately
3. Fluids: Add directly to fixative supplied in special container.

Method For Obtaining An Optimum Fine Needle Aspiration Specimen:

A high percentage of smears are difficult, and sometimes impossible, to accurately diagnose. This difficulty is primarily due to poorly preserved cellular material or a lack of adequate cellular material. Poorly preserved material is usually due to a delay in fixing the smears or spraying them too closely with the fixative and freezing the material. A lack of adequate cells is generally the result of a hypocellular cystic fluid spread too thinly over the slide

Bio-Reference recommends the following procedure

Fine needle Aspiration Technique

Local anesthesia is not necessary.

1. Clean the skin overlaying the mass with an antiseptic
2. After the needle has entered the mass, retract the plunger to create a vacuum in the syringe
3. Move the needle back and forth several times in the lesion as the material is being sucked into the needle by negative pressure
4. The cell sample should remain in the needle and should not be visible in the syringe barrel
5. Before withdrawing the needle from the lesion, the suction must be released to avoid aspiration of the material into the syringe barrel.

Slide Preparation

6. The needle is quickly detached from the syringe and the plunger is

retracted to allow air to fill the syringe
7. Reattach the needle and eject its contents forcefully onto the slides by pushing down on the plunge
8. With the two slide method, place the slides together and gently pull the slides apart with one continuous motion
9. IMMEDIATELY PLACE THE SLIDE(S) IN 50 ML TUBE CONTAINING 95% ALCOHOL to achieve preservation of material
10. After slides are prepared, the syringe is thoroughly rinsed in a separate 50 mL tube of 95% alcohol. Both tubes are then sent along with slides and/or cyst fluid to the laboratory where the specimen is processed using the cytocentrifuge. Using this procedure, even the most hypocellular specimens will generally show a yield of cells which is adequate for diagnosis.

Thin Prep Pap Test Broom-Like Device Protocol

1. Obtain an adequate sampling from the cervix using a broom-like device. Insert the central bristles of the broom into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix. Push gently, and rotate the broom in a clockwise direction five times
2. Rinse the broom into the Preservative Solution vial by pushing the broom into the bottom of the vial 10 times, forcing the bristles apart. As a final step, swirl the broom vigorously to further release material. Discard the collection device
3. Tighten the cap so that the torque line on the cap passes the torque line on the vial.
4. Record the patient's name and ID number on the vial. Record the patient information and medical history on the cytology requisition form.
5. Place the vial and requisition in a specimen bag for transport to the laboratory.

Directions for Obtaining a Routine Cervical Smear

Do not use lubricating gel. **Do** not use Q-tip to obtain endocervical cells; consider using endocervical brush or a specially designed wooden/ plastic spatula. **Do** not obtain during menstruation. **Obtain** a direct scrape of the cervix, preferably at the junction between exocervix and endocervix; consider adding a vaginal pool sample to the cervical sample in women over 40 years of age. **Follow** directions for making Direct Smears.

Note: In order to comply with the standards established internationally, the following guidelines should be followed. The pap smear will be reported as unsatisfactory with the following conditions: **Smears** unlabeled. **Scant** cellularity**. Poor** fixation or preservation. Cells are obscured by inflammation, foreign material or blood. **Not** representative of anatomic site. **Slides** broken beyond repair.
A Gyn pap smear will be considered less than optimal when there is no endocervical component present.

Directions for Obtaining a Vaginal Smear for Hormonal Evaluation (Maturation Index)

Do not use lubricating gel. **Obtain** a lateral vaginal wall scrape. **Follow** directions for making Direct Smears.

Directions for Obtaining Gastrointestinal Tract Washings

Collect fasting specimen and put in labeled container. **Inject** 300 mL of normal saline rapidly. **Aspirate** as much as possible of the injected saline and place in labelled container. **If** possible, repeat steps 2. and 3. with patient in different positions. **Specimens** may be pooled or collected separately in containers of 95% alcohol. **Send** immediately to lab.

Directions for Obtaining Urine for Cytology

Specimen can be randomly collected anytime. **Female** patients should be instructed to wash their genitalia with soap and water prior to collection.
Void directly into container with 50% alcohol. **Send** immediately to the laboratory in securely closed container.

Note: INCLUDE PATIENT AGE AND PERTINENT CLINICAL DATA ON THE REQUEST FORM. If there are any questions about specimen collection or if you need to order the container of fixative, call the Laboratory's Cytology Department.

Pathology, Anatomic (Biopsies and Surgical Specimens)

General Instructions: Use Surgical Pathology requisition form for all biopsies and surgical specimens. Relevant clinical information should be written down in the space provided. **Write** patient's name on specimen container. **Place** all tissues immediately in 10% buffered formalin at ten times the volume of the specimen. Specimen containers with 10% formalin are provided by the laboratory. **Send** immediately to the laboratory in securely closed container. For additional information, please contact the laboratory.

Collection and Transport of Microbiology Specimens:

Correct specimen collection and transport of clinical specimens to the laboratory are extremely important for rapid and accurate identification of significant microorganisms from patient samples. **Please send separate test requisitions for each culture.**

General Consideration for Collection and Transport: Use sterile technique and transport to the laboratory as soon as possible. **Close** collection containers securely to prevent leaking of sample during transport. These specimens are biohazards. Whenever possible obtain specimens prior to the administration of antibiotics. **Do** not use expired tubes or media for specimen collection. **Please** write the patient's name on each specimen container. **Send** specimens in one of the following transport systems: **Swabs** with transport media (culturettes): eye, ear, nose, stool, strep screen, throat, wounds- give site. (Hold at room temperature or refrigerate). **Non sterile** containers: sputum. **Sterile** containers: body fluids (except blood and urine, see below). **Special** transport systems: **Blood** - 2, 20 mL vacutainer tubes with Supplemented Peptone Broth (or any other system). **G.C. Specimens** - Urethral discharge or any source: JEMBEC plates with bag. **Urine** - Container with preservative (boric acid) "Boricon" or even ordinary sterile urine culture container could do. **Parasitology** - Special collection kits with Formalin and PVA.

Body Fluid Culture (For PD fluid see Peritoneal Fluid)

Pleural, pericardial, and synovial fluids must be aspirated aseptically. The body site should be disinfected with an iodophor/povidone prior to aspiration. Use sterile technique. Inoculate into sterile tube or container or blood culture media.

Blood Culture

Disinfect body site with iodophor/povidone prior to venipuncture. Use sterile technique. Inoculate 2 vacutainer tubes containing 18 mL Supplemented Peptone Broth (SPB) - 2 mL draw. disinfect top of tubes with alcohol prior to inoculation. Two sets from separate venipuncture sites recommended. If appropriate Hartleys' broth and Glucose broth may suffice.

Eye, Ear

Collect specimen with a culturette swab. After collection, place swab back into plastic tube. REFRIGERATE OR LEAVE AT ROOM TEMPERATURE.

Fluid
See Body Fluid or Peritoneal Fluid

Environmental (Water and Dialysate) Cultures
See separate instructions for use of Millipore Sampler

Genital Culture
Collect these specimens using a Culturette swab. The swab may be used to culture urethral exudate or inflammation of the vaginal area. While these specimens are not optimal for gonococcal isolation, the diagnosis of vaginitis or urethritis may be made by the recovery of other pathogens. Swabs must be stored at room temperature until transported to the laboratory. If Gonorrhoeae is suspected, do mention that the purpose of the investigation is for Gonorrhea Culture.

Gonorrhea Cultures
JEMBEC (or any other system) plates are provided for the isolation of Neisseria gonorrhoeae from rectal, pharyngeal, and genital sites. JEMBEC plates contain antibiotics to allow the isolation of N. gonorrhoeae from these heavily contaminated areas and must be stored at refrigerated temperatures before use. However, it is important that they be at room temperature at the time of inoculation because cold temperatures inhibit growth of gonococci. Exudate is obtained with a sterile swab and immediately inoculated on the JEMBEC agar. Urethral discharge may be collected with a swab which is then immediately streaked onto the agar surface. A CO_2 atmosphere is necessary to recover neisseria gonorrhoeae. This may be performed by placing a CO_2 generating tablet in the small well present in the plate. The plate is then sealed in a plastic bag. No water is needed to activate the tablet. ONCE SEALED, THE BAG SHOULD BE STORED AT ROOM TEMPERATURE UNTIL TRANSPORTED TO THE LABORATORY.

Gonorrhea/Chlamydia DNA Probe
Specimen collection swabs and transport media are supplied by the laboratory. Remove excess mucus from the cervical os and surrounding mucosa using one of the swabs provided; discard this swab. **Insert** the second swab from the collection kit 1.0 - 1.5 cm into the endocervical canal. **Rotate** the swab 30 seconds in the endocervical canal to ensure adequate sampling. **Withdraw** the swab, avoiding any contact with the vaginal mucosa. **Insert** this swab into the Gen-Probe transport tube, snap off the shaft at the score-line, cap the tube, and store at 2 - 25°C until tested.

Mycology (Yeast)
Cultures for yeast can be submitted on a Culturette. For fluids and sputum, best results are obtained by submitting the entire specimen. If dermatophytes are suspected, the specimen should be submitted in a dry sterile tube.

Nasal Culture
A Culturette swab is gently inserted through the nose to the posterior nasopharynx where it is gently rotated. It should remain in this position for several seconds. The withdrawal should be slow to minimize irritation. Place the inoculated swab into the sterile plastic tube and crush the ampule.

Parasitology Specimens
Stools for Ova and Parasites should be shipped in Ova and Parasite kits- 5 gm minimum of stool in EACH of the paired vials. CLEAR tape preparation or pinworm paddle is appropriate for submission of specimens for pinworm examination. Submit intact parasites (insects or worms) in 70% alcohol.

Peritoneal Fluid or Dialysate Culture
Disinfect bag's injection sampling port. Use sterile collection technique. Inoculate 3 mL each into four vacutainer tubes with SPB (Supplemented Peptone Broth). Disinfect top of tubes with alcohol prior to inoculation.

Sputum Culture
Instruct the patient to obtain material from a deep cough which is expectorated into a sterile container. Sputum containers are best suited for this collection. The volume of specimen need not be large (3 mL). Once collected, sputum should be refrigerated until transport. Be sure that the cap is tightly sealed on the container once the specimen is collected. A leaky container is a biohazard.

Stool Culture
Use Culturette swab. Obtain pea-size feces on swab and place in culturette.

Throat swab Culture
Use Culturette swab to obtain all types of throat specimens. Rub the sterile swab firmly over the back of the throat (posterior pharynx), both tonsils or tonsillar fossa, and any area of inflammation. Once the specimen is collected, the swab should be replaced in the plastic tube and the ampule at the base crushed to wet the swab.

Throat, Group A Strep Screen
Use Culturette to obtain specimen for Group A Strep only. Results take one day versus 2 - 3 days for complete culture.

Urine Culture Screen
Collect a "clean-catch" mid stream urine into a paper cup. Immediately transfer to Boricon tube with Bacteriostatic preservative. A first morning specimen is preferable and should be refrigerated until pick-up.

Note: It is not necessary to collect a specimen for culture in a sterile cup if the urine is immediately transferred to the Boricon tube.

Wound Culture
A superficial wound culture should be collected with a Culturette swab. After collection, place swab back into plastic tube, and refrigerate or leave at room temperature until transport. If the lesion is not open, a sterile needle and syringe should be employed to remove material. the culturette may be inoculated with this sample.

Important
All test requests are to be signed by the ordering physician and the *time and date* of the specimen collection are to be clearly added to the request form.

Note: Reference Ranges are method dependent and may change if a methodology changes. Check the final report for all reference ranges.

Specimen Quality

Hemolysis
Some analytes may be reported erroneously if the serum is not promptly removed from the clot, or if the barrier tube is not centrifuged after the clot has formed.
Major discrepancies are low glucose, high potassium and LD. Additionally, if hemolysis takes place during initial processing and venipuncture, or if prolonged contact with the clot takes place, elevation in cholesterol, creatinine, iron, phosphorus, calcium and most enzymes will be found. Hemolyzed hematological specimens are unsuitable for testing.

Quantity Not Sufficient (QNS)
Most hematology tests require that a full tube of blood be obtained. This is because there is a defined quantity of anticoagulant in each tube and the ratio of this to the blood volume has to be exact to ensure quality results. Particularly important are blue-top tubes used for Blood Coagulation tests. For prothrombin time, activated partial thromboplastin time and fibrinogen determinations exactly 4.5 mL of blood must be obtained (a full tube). The ratio is 1 part of 3.2% buffered sodium citrate added to 9 parts of blood. For CBCs a "short draw" lavender tube will result in red cell crenation, reduced MCV and hematocrit, and possible changes in leukocyte morphology, platelets and total leukocyte counts.

Clotted Specimens
All hematological testing utilizes anticoagulated blood.
For blood counts, a lavender top tube containing the anticoagulant EDTA is required. All specimens should be collected and the tube filled to the limit of the vacuum. Clotted samples, either macroscopic or microscopic in nature, cannot be processed for CBC testing, as such results will produce false leukopenia, lower red cell counts, and aberrant red cell indices.As the equipment used to test blood counts incorporates a clot detector, it is occasionally possible that specimens that appear macroscopically normal will have small microscopic clots that are detected which will produce incorrect results. Similarly, small clots found in blue top tubes (for coagulation tests) will result in falsely prolonged test results.

Icteric Specimens
If the specimen is deeply icteric, falsely elevated cholesterol results may be obtained. It also interferes with usual creatinine estimations.

Lipemic Specimens
Lipemia can falsely elevate ALT and AST. Additionally, it can indicate that the patient did not adequately fast for 12-18 hours before having the specimen collected. In this situation, glucose and triglycerides will be elevated.

Decreased Bilirubin
Bilirubin is photodegradable. Prolonged exposure of the specimen to bright light will produce depressed results.

Decreased CO_2 Levels
Carbon dioxide levels are decreased if the specimen is not tested promptly. CO_2 escapes from red cells in vitro, at a rate proportional to time. This can be minimized by keeping the stopper on the tube and by refrigeration.

Poor Cell Preservation
Blood cells, particularly leukocytes become fragile and can be distorted morphologically if the specimen is older than 24 hours. In such situations, a reliable differential white cell count cannot be performed.

Old Specimens
Blood specimens older than 24 hours cannot be adequately tested for some analytes. Particularly sensitive are most Hematology tests including Blood Coagulation procedures.

Specimen Form Submission

Request Forms

Use individual requisition forms available from the laboratory. All test requests require a physician's written order to process a specimen. Follow the collection instructions for each type of specimen.

Patient Identification

All patients from whom clinical specimens are obtained must be positively identified, utilizing at least two unique identifiers prior to specimen collection. Positive identification is the responsibility of the person collecting the sample.

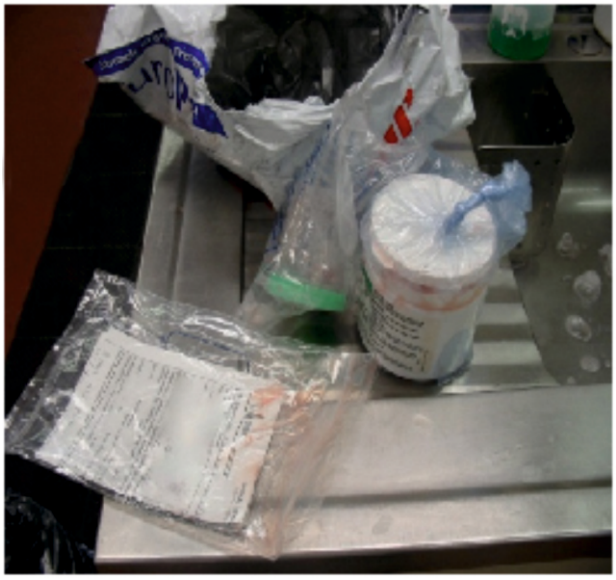
- Required Information
- All specimens must be labeled.

Specimen Labeling: The following information must be legibly recorded on a label affixed in an irreversible fashion to the specimen container:

- Patient's full name (not a nickname)
- Medical Record Number or other unique identifier (ID)
- Date and, if appropriate, time when specimen was obtained
- Specimen source
- Signature/ initials of collector
- The label should be affixed directly to the specimen container and not the bag
- Bar-coded pre-printed labels with accession numbers generated by an information system may be used
- Place the labeled specimen in the provided leak proof sealed plastic biohazard bag
- Place the matching requisition in the outside pouch of the bag.

Transport specimens promptly: See specific test for temperature requirements. The date and signature/ initials of the collector must be recorded after the specimen has been collected and after verifying that the patient name and ID on the label agrees with that on the test requisition. This is the single most important factor in preventing errors in patient specimen identification.

Use of a request form wrapped around the container is not acceptable as a specimen label. Specimens will not be accepted if the information on the specimen label does not match the information on the accompanying requisition.



Required Information on the Requisition Form

On all requests forms, the following information is required

- Patient's name & address
- Patient's gender
- Date of birth
- The last six digits of the patient's social security number or other unique identifier (ID#) wherever mandatory legally and applicable
- Date and if appropriate, time of collection
- Test/s requested
- Type or source of the specimen
- Requesting physician/ or Client Number
- Clinical information if requested
- All applicable medical necessity codes (ICD-9)
- Complete billing and insurance information wherever indicated.

Providing additional relevant information may be important in alerting the laboratory of the need for special handling or specimen work-up. Tests sent to reference laboratories must have patient history information. The need for such information is indicated on the test request form.

Reliability and Value of Test Results

The reliability and value of test results depends on numerous factors. Improper collection, transport, or processing of a specimen can decrease the quality of patient care or result in unnecessary additional testing or treatment. Laboratory personnel cannot label specimens nor complete requisition forms (source, time of collection, patient's name), which they have not collected. Mislabeled (specimen label does not match requisition) or unlabeled specimens should be rejected.

Nongynecological Cytology Practice Guidelines

Quality Control and Quality Assurance Practices

Quality control (QC) and quality assurance (QA) can be considered as the first two levels, respectively, in the hierarchical stages of quality. Quality control is defined as a system for verifying and maintaining a desired level of quality using operational techniques for an individual test or process. Quality control activities span the testing process, from pre-analytic (specimen collection and processing) through analytic (interpretive) and post-analytic (receipt of the report and analysis of results) phases. Quality assurance (QA) is defined as systematic monitoring of quality control results and quality practice parameters to assure that all systems are functioning in a manner appropriate to excellence in health care delivery. Quality assurance is a coordinated system designed to detect, control and prevent the occurrence of errors and, ultimately, to further a clinician's ability to appropriately care for his or her patient. The third stage, quality system, consists of the comprehensive and coordinated efforts to meet quality objectives including the organizational structure and resources. Quality management, the fourth stage includes the first three and also the cost of quality. The hierarchy culminates with total quality management, which is management centered on quality, and aimed at long-term success through customer (patient, physician and payer) satisfaction. A number of quality control and quality assurance measures for cytopathology have been specified by the Clinical Laboratory Improvement Amendments of 1988. All QC and QA processes must be described and documented in the laboratory. **Pre-analytical Quality Control:** Each laboratory must perform and maintain records of routine quality control relating to specimen collection, receipt and preparation. Most of these activities are required by lab accreditation agencies and include such things as: Preparation and distribution of clinical specimen collection and handling instructions, Assurance of properly labeled specimens, Use of a requisition that provides space for all pertinent demographic and clinical data, Accessioning and assignment of a unique specimen identifier, Criteria for specimen rejection, Review of stain quality and maintenance of stain quality records, Procedure for preventing nongynecological specimen cross contamination, Microscope and instrument maintenance, Instrument calibration records.

Analytical Quality Control

Screening of Nongynecological Cytology Specimens: Federal regulations require that the individual examining a cytology specimen be a qualified cytotechnologist or pathologist in a certified laboratory. These individuals may examine up to 100 slides (gynecological and nongynecological) per 24 hours (average 12.5 slides/ hour) and in not less than eight hours. This number is not a performance target but a maximum allowed by law. Pathologists are limited by this ceiling when they perform primary screening. This includes nongynecological slides that have not been previously screened. Each laboratory must establish individual workload limits for each cytotechnologist. The Technical Supervisor of the laboratory must review these limits every six months and re-assess using lab defined performance standards. The record of slides reviewed by the primary screening cytotechnologist or pathologist must be documented and retrievable for inspectors during the retention period as prescribed. Cytotechnologists and pathologists must also maintain work logs for any primary screening site (in cases of multiple site employment), again, for the applicable retention period. As discussed later, all specimens must be reported using descriptive nomenclature; use of a numerical reporting system alone is unacceptable. **Review and Reporting of Nongynecological Cases:** All nongynecological specimens must be referred to a pathologist for final interpretation and final report. Discordance between pathologist and cytotechnologist interpretation, if the cases are screened prior to pathologist examination, can be used as a basis for identifying areas for continuing education. Peer review is often included in a quality assurance program. Multiple people may review difficult or interesting cases for educational and interpretive purposes. Laboratories may require a second pathologist opinion for specific diagnoses and/or type of specimen. See below for variability of documenting intralaboratory consultations. Seeking the opinion of an outside consultant may be considered for unusually difficult cases with significant clinical implications. Documentation of all reviews is essential for quality assurance monitoring. **Rescreening of Negative Cases:** Quality control rescreening of nongynecological cases is usually not required by accreditation agencies. However, re-examination of a subset of cases by a second pathologist prior to release of the final report may be incorporated into the anatomic pathology quality assurance program. The re-examined cases may be randomly chosen or may be selected based on volume and complexity of workload and cytopathology resources.

Post-analytical Quality Control

Cytological-Histological Correlation and Clinical Follow Up: The laboratory must make an effort to correlate nongynecological cytopathology findings with histology and clinical findings. This can be for all specimens or for a focused subset of specimens. It is suggested that if significant disparities exist they should be reconciled. Cytological-histological correlation can be an educational tool used to refine methods of evaluation for both cytology and tissue specimens. The correlation process should be documented in the laboratory quality assurance program. If a nongynecological cytology specimen is collected concurrently with a tissue specimen, cytological-histological correlation is best performed prospectively. Ideally, the cytology and tissue reports should each refer to the other with integration of the correlation statement into either report. Reporting cytological-histological discordance may be helpful in directing further patient management. If an abnormal or nondiagnostic nongynecological cytology result is subsequently followed with tissue sampling, and retrospective correlation is performed, then the result of the correlation should be documented. If histological material is not available, the laboratory may attempt to obtain patient follow-up by sending a letter to the ordering physician requesting this information. **Retrospective Reviews:** There are no federal or accrediting agency requirements for retrospective review of nongynecological cytology specimens. In certain clinical situations, review of previously examined specimens may affect current patient care by determining subsequent management protocols. Retrospective comparison of specimens from multiple body sites within a relatively short time span may be required for clinical staging, or comparison of a current specimen with one from the remote past may distinguish a metastasis from a second primary neoplasm. Amended reports are not indicated in these situations. Results of the review can be incorporated in the current cytology or tissue report or in a separate document. Retrospective reviews are subject to the biasing effect of knowledge of outcome, and this fact should be kept in mind during any such review. **Measures of Performance:** Nongynecological cytology can be both a screening test and a diagnostic procedure depending upon the clinical circumstances and specimen examined. Nongynecological cytology is limited (as are all laboratory tests) by both

false positive (FP) and false negative (FN) results. As a screening test, a false positive is defined as a "positive" test result for a patient who does not have an abnormality. As a diagnostic procedure, a false positive could be defined as a malignant interpretation when in fact the patient has a benign neoplasm or perhaps as the presence of any neoplasm when the condition is reactive or inflammatory. Since "positive" results are variably defined in the medical literature, a standard definition for a false positive nongynecological cytology specimen does not exist. A false negative is defined in this document as a negative or nondiagnostic nongynecological cytology result in a patient with an abnormality or lesion. False negative results may be a consequence of (a.) Sampling variance, (b.) Laboratory interpretation, or (c.) General limitations of the method. Sampling false-negatives occur when diagnostic cellular and noncellular material is not collected or is not transferred to the slide. A laboratory interpretive false negative is one in which diagnostic material is present on the slide, but is not identified during slide examination or is misinterpreted as to its significance. The false negative rate is the sum of lesions missed in sampling plus the false negative proportion (FNP.) The FNP is the measure of the laboratory component of false negative results and is defined as the number of false negative reports divided by the total number of patients sampled who have an abnormality (False Negative Proportion = False Negative reports/True Positive reports + False Negative reports). FNP = FN/TP + FN. The value of determining the FNP for a laboratory is widely acknowledged; however, precise calculation of the FNP requires 100% accurate determination of the true diagnosis. For nongynecological cytology this requires exhaustive cyto-histologic and clinical correlation, which is impractical. Q-Probes studies provide a comprehensive resource for comparative laboratory data and performance benchmarks. These data are a good starting point for laboratory self-assessment since operational definitions, laboratory methods and statistical analyses are specified.

Proper Handling of Breast Cancer Specimen

Assessing Hormone Receptor Status:

Breast cancer is one of the first malignancies for which the use of targeted therapy has become a routine and life-extending practice. For more than 3 decades, management of breast cancer has largely been determined by the measurement of the estrogen receptor (ER), primarily because of the substantial benefit that endocrine therapy provides for patients with ER-positive but not ER-negative tumors. Large overviews of randomized clinical trials have confirmed that women with ER-negative invasive breast cancers do not derive benefit from endocrine treatments. The clinical significance of this biomarker has rendered the assessment of the ER status of primary invasive breast cancer mandatory. Progesterone receptor (PgR), a product of the interaction of estrogen with ER, is also commonly measured but has value mostly as a prognostic marker. In the early 1990s, immunohistochemical (IHC) testing of ER and PgR receptor-specific antibodies was developed for use on sections of frozen tissue; subsequent development of new antigen retrieval methods and the development of new antibodies allowed ER testing to be performed on formalin-fixed and paraffin-embedded material, as well. Despite significant experience with ER and PgR assessments in most laboratories, confirmation studies associated with recent breast cancer clinical trials have shown that there is a 5%-10% false-negative rate and a 5% false-positive rate when institutional results are compared with central laboratory determinations. Although some of this discrepancy is attributable to differences in threshold values, the percentages of error are nevertheless very alarming. In addition, significantly discrepant results in ER testing have been uncovered in several Canadian provinces, where variations in tissue handling, testing methods, training, and staffing were all implicated.

Determining HER2 Overexpression:

The human epidermal growth factor receptor-2 gene ERBB2 (commonly referred to as HER2), which is amplified in approximately 15%-20% of breast cancers, is also a significant biomarker in breast cancer. Gene amplification results in overexpression of a breast cancer tumor cell surface receptor protein, which possesses tyrosine kinase activity and potentiates tumor cell growth. HER2 protein overexpression and HER2 gene amplification are associated with poor clinical outcomes in patients with breast cancer. However, in the 1990s, trastuzumab, a new human monoclonal antibody that targeted this protein, was developed. When used in HER2-overexpressing metastatic breast cancer, trastuzumab, either alone or added to chemotherapy, has been found in clinical trials to reduce the risk for disease recurrence by 50% and the risk for mortality by 30%.It is also highly effective in the adjuvant treatment of early-stage breast cancer. Recent results with a small-molecule HER1/HER2 tyrosine kinase inhibitor, lapatinib, demonstrate that the oral agent, added to capecitabine, improves clinical outcomes in patients with advanced disease. HER2 overexpression determined by IHC testing is now accepted as a strong predictive marker for clinical benefit in both the metastatic and adjuvant settings when drugs targeting the HER2 receptor protein are used. The trials that provided evidence of benefit with these therapies, however, also showed a false-positive rate of 15%-18% in institutionally performed assays relative to those done in a central laboratory. There was also a significant false-negative rate of up to 5%.

Specimen Handling:

Many of the factors leading to poor test accuracy are related to specimen handling before the actual test is performed. Data are emerging on the impact of this variation on testing results. To understand the contribution of specimen handling to breast cancer testing inaccuracy, researchers conducted a retrospective trial involving review of 5077 patient records from 1999 to 2003. It was found a significant discrepancy in the rate of ER-negative tumors when those removed Sunday through Thursday were compared with those removed on the weekend (Friday/Saturday). The testing was performed in a central laboratory with standardized processes and 3 trained pathologists. Although it was concluded that variable specimen handling explained the results, the exact cause of the variation was not known. Researchers therefore conducted a prospective trial comparing facilities that recorded time of tumor removal and time into formalin (test group) with those that did not record those times. It was found that the mean time from removal of tumor to specimen fixation in the test group was 18 minutes. The PgR- (but not the ER-) negative rate was significantly lower in facilities that did not record the time, suggesting that merely documenting the specimen handling is an effective intervention for controlling variation. Frequency of ER-negative test results on breast cancer specimens by hospital and by specimen handling group. *Designates the hospital where the tests were performed. Number of samples included was 5077. Other compelling data about the impact of variable fixation practices come from researchers in far eastern countries where, until recently, all breast cancer was considered ER negative, based on results obtained after prolonged specimen handling. Researchers have published data establishing that the ER-positive rate for breast cancer tumors in far eastern women is identical to that observed in western women when tissue is properly collected and fixed promptly. Previous observations about the lack of ER positivity was attributed to delays in appropriate fixation of tumor specimens.

Warm and Cold Ischemia Time:

The interval from the interruption of the tumor blood supply to the initiation of tissue fixation is widely accepted as an important variable in the analysis of labile macromolecules such as proteins, RNA, and DNA from clinical tissue samples. Warm ischemia time is the time from the interruption of the blood supply to the tumor by the surgeon to the excision of the tissue specimen; cold ischemia time is the time from excision of the tumor to the initiation of tissue fixation. Numerous studies and articles have discussed the progressive loss of activity of these labile molecules following the surgical interruption of blood flow, which leads to tissue ischemia, acidosis, and enzymatic degradation. Although there are data about the impact of variation in cold ischemic time on protein expression in tissues, the contribution of the warm ischemic interval to this macromolecular degradation is currently under study. The standardization of the cold ischemia time is an important step to help ensure that differences in levels of protein expression for clinically relevant targets such as ER are biologically meaningful and are not artifacts related to the manner in which the tissue was handled.

Optimal Fixation Processes:

Timing and documentation. The breast tumor specimen should be fixed quickly in an adequate volume of fixative (optimally 10-fold greater than the volume of the specimen). The time of tissue collection (defined as the time that the tissue is removed from the surgical field) and the time the tissue is placed in fixative both must be recorded on the tissue specimen requisition to document the cold ischemic time. Ideally, when pathologists and surgeons communicate effectively with OR staff, the appropriate recording process (Table given below) becomes routine.

Table. Pathology Responsibilities for Documenting Fixation Intervals

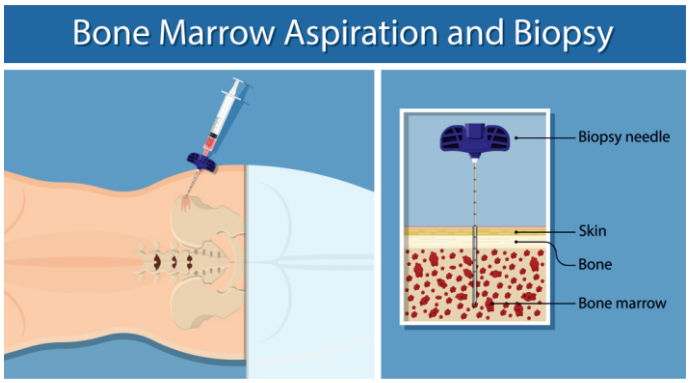
Grossing Room:	Ensure adequate documentation of specimen removal, fixation start, and duration of fixation times. Include specimen removal and fixation start and fixation duration times as part of the dictation.
Transcription:	Ensure that times are included as part of the dictation. Transcribe times to be included in pathology report.
Pathologist:	Ensure times are documented and included as part of the pathology report. Inquire about cases with missing fixation times and ensure proper documentation. Evaluate test results taking fixation interval documentation into account.

Fixation techniques. It is also critical that specimens be adequately fixed in 10% neutral buffered formalin (NBF). To ensure proper fixation, specimens must be promptly examined and sectioned by the pathologist so that fixative will penetrate all the areas of tumor to be microscopically examined. In situations where excision specimens are obtained remotely from the gross examination laboratory, the pathologists should work with operating suite personnel to ensure that the sample is bisected through the tumor and promptly placed in NBF before transport. The time to insertion of tumor sample into fixative, as well as the time of tumor removal from the patient, should be noted on the specimen requisition by the personnel in the surgical suite. Although less optimal than immediate gross examination of the fresh sample by the pathologist, this process is preferable to storage of the sample in the refrigerator unfixed or in fixative without sectioning. Only 10% NBF should be used as the fixative for breast tissue specimens; higher or lower concentrations of NBF are not acceptable. This recommendation is based on published literature regarding the expected or characteristic immunoreactivity of NBF for ER, PgR, and HER2 in breast cancer, which has been accrued over many years and has been clinically validated with patient outcomes in numerous clinical trials. In addition, the US Food and Drug Administration (FDA) approval for assay kits analyzing ER, PgR, and HER2 explicitly states that formalin fixation should be used and that FDA approval for the kits is not applicable if an alternative fixative is used. If the laboratory uses a formalin alternative for fixation, the assay must be validated against tissue fixed in NBF to assure that the alternative fixative yields identical results. The laboratory director assumes responsibility for the validity of assay results when nonstandard procedures are followed. Breast tissue specimens must be fixed in 10% NBF for no less than 6 hours and no more than 72 hours before processing. Formalin, which is aqueous, completely dissolved formaldehyde, penetrates tissue at a rate of approximately 1 mm/hr. Breast excision samples must be incised in a timely fashion to initiate formalin fixation throughout the tissue; fixation does not begin until formaldehyde has penetrated into the tissue. However, permeation of tissue by formalin is not the same as the chemical reaction of fixation, which involves protein cross-linking by formaldehyde. Chemical fixation requires time, with the rate-limiting step being the equilibrium between formaldehyde and methylene glycol in solution, which is time dependent and can be measured in hours ("clock reaction"). Although complete tissue fixation usually requires 24 hours, published studies have documented that breast samples require a minimum of 6-8 hours of formalin fixation to obtain consistent IHC assay results for ER and HER2. Underfixation of breast tissue may lead to false-negative ER results and false-positive HER2 results. In these situations, the tissue is actually fixed in 100% ethanol, which is used to dehydrate the specimens after fixation. Overfixation is likely to be less problematic than underfixation but potentially could also lead to false-negative results due to excessive protein cross-linking by formaldehyde. Standard antigen retrieval protocols are optimized for 24 hours of fixation time. These recommendations apply also to needle biopsy specimens and cytology specimens.

Improving the Process:

Although the new guidelines for ER, PgR, and HER2 specify ideal processes and conditions, both OR and pathology personnel will have to cooperate to implement these practices and make them routine. OR staff, encouraged by surgeons, must routinely record the time of specimen removal from the patient. Samples must be promptly transported to the gross examination room of the pathology laboratory so that they may be promptly examined and fixed. This process change must be explained and enforced by the pathologist. Both pathologists and surgeons must be alert to the potential for nonroutine handling of specimens on Friday afternoons and Saturdays, or at remote locations, and should be vigilant in following up on processes used under these circumstances. Armed with adequate information about the specimen handling conditions for each breast cancer sample, the pathologist who observes an unexpected ER, PgR, or HER2 result will be able to do some problem solving to discover potential reasons for that unusual finding and guide the surgeon and patient correctly. Such unexpected findings might include ER-negative and/or PgR-negative and/or HER2-positive results on low-grade breast cancer specimens, or HER2-negative results on high-grade ER-negative or ER-positive breast cancer specimens. Targeted therapies are highly effective when applied in appropriate patients. Without accurate testing, patients whose tumors will not respond may nevertheless face treatment with expensive and potentially toxic drugs, whereas patients whose lives may be prolonged by effective treatment may be denied those options. In the early days of ER measurement, virtually all US laboratories froze and shipped breast cancer samples to properly qualified laboratories that performed these assays in a controlled manner. It is not unreasonable to expect the same type of uniform compliance with conditions required for today's breast cancer specimen analyses of ER, PgR, and HER2 status. This reality must be kept in mind as healthcare providers face the challenges of altering "business as usual" to comply with new requirements and conditions. Patients will be the ultimate beneficiaries of these changes.

Bone Marrow Aspiration / Biopsy



Overview

The procedure known as trepanning, or trephination, of bone is the oldest surgical practice that continues to have clinical relevance in modern times. The method dates as far back as the Neolithic period and initially entailed the drilling of cranial bones as a form of medical intervention for headaches and mental illnesses. However it was not until 1905, when the Italian physician Pianese reported bone marrow infiltration by the parasite *Leishmania*, that this procedure was applied toward clinical evaluation. In the present day, inspection of the bone marrow is considered one of the most valuable diagnostic tools to evaluate hematologic disorders. Indications have included the diagnosis, staging, and therapeutic monitoring for lymphoproliferative disorders such as chronic lymphocytic leukemia (CLL), Hodgkin and Non-Hodgkin lymphoma, hairy cell leukemia, myeloproliferative disorders, and multiple myeloma. Furthermore, evaluation of cytopenia, thrombocytosis, leukocytosis, anemia, and iron status can be performed. The application of bone marrow analysis has grown to incorporate other, nonhematologic, conditions. For example, in the investigation for fever of unknown origin (FUO), specifically in those patients with autoimmune deficiency syndrome (AIDS), the marrow may reveal the presence of microorganisms, such as tuberculosis, *Mycobacterium avium intracellulare* (MAI) infections, histoplasmosis, leishmaniasis, and other disseminated fungal infections. Furthermore, the diagnosis of storage diseases (e.g. Niemann-Pick disease and Gaucher disease), as well as the assessment for metastatic carcinoma and granulomatous diseases (e.g. sarcoidosis) can be performed. Bone marrow analysis can also be performed in patients with idiopathic thrombocytopenia purpura (ITP), incidental elevated serum paraprotein levels, iron deficiency anemia, B_{12} / folate deficiency, polycythemia vera, or infectious mononucleosis; but these conditions are more appropriately diagnosed by routine laboratory. Bone marrow consists of stem cells, which are large, "primitive," undifferentiated cells supported by fibrous tissue called stroma. There are 2 main types of stem cells and, therefore, the bone marrow consists of 2 types of cellular tissue. One type of stem cell is involved in producing blood cells and the other is involved in producing stromal cells, which are responsible for the supporting stroma. Sampling of the marrow consists of either aspiration of the cellular component and/or acquirement of tissue fragments. Aspiration of the marrow, as shown below, has been primarily utilized for cytologic assessment, with analysis directed toward morphology and obtainment of a differential cell count. Further sampling allows for material to be directed toward other ancillary test such as cytogenetics, molecular studies, microbiologic cultures, immunohistochemistry, and flow cytometry. Biopsies, on the other hand, allow for studies of the marrow's overall cellularity, detection of focal lesions, and extent of infiltration by various pathologic entities.

For patient education information, visit eMedicine's Osteoporosis and Bone Health Center and Cancer Screening Center, as well as Bone Marrow Biopsy.

Preliminary Assessment

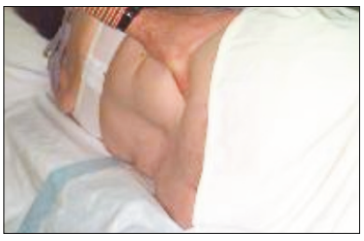
An initial review of the patient's clinical background is necessary to determine whether a bone marrow evaluation is warranted.

Medical history: Travel history: exposure to parasites (leishmaniasis), fungi (histoplasmosis, *Cryptococcus*), mycobacteria. Immune compromise or immune deficiency status: This may contribute to a high infection risk, such as in patients with human immunodeficiency virus (HIV) infection, underlying autoimmune deficiency (e.g. Wiskott Aldrich Syndrome), and/or the use of immunosuppressive agents. Risk of bone fragility: Previous surgeries, chemotherapy, and radiation therapy can increase the risk of bone fragility, as well as pathologic processes that may contribute to bone resorption (e.g. osteoporosis, multiple myeloma). Previous diagnosis of malignancies: These are a risk for metastasis to bone. Glycogen storage diseases. Risk for hematologic anomalies: Contributing factors include a patient's nutrition status, alcoholism, medications, and history of a coagulation factor deficiency. Allergies: Testing and/or knowledge of a patient's allergy status are preventive measures to the potential allergens exposed during bone marrow sampling, such latex, anesthetics (e.g. lidocaine), antiseptics (e.g. povidone-iodine).

Clinical presentation: Perform a thorough physical examination to assess the patient for signs of malignancy, infections, lesions associated with hemorrhagic injury, as well as disorders of hemostasis and coagulation. Laboratory tests should initially include complete blood cell (CBC) counts, a reticulocyte count, peripheral blood smears, prothrombin time/international normalized ratio (PT/INR), and activated partial thromboplastin time (aPTT). Other studies take into account the clinical presentation and may consist of the following: serum iron studies, serum ferritin study, vitamin B_{12} and folate levels, erythrocyte sedimentation rate (ESR), serum protein electrophoresis, platelet function studies, coagulation mixing study, fibrin D-dimers, serum fibrinogen levels, serum bilirubin levels, and radiographs. Obtain informed patient consent that provides procedural information and potential complications (e.g. hemorrhage, infections, pain). This will minimize any apprehension that the patient may have.

Collection Site

The safe and preferred sites for bone marrow aspiration and/or biopsy are described below. **Aspiration and biopsy:** Posterior superior iliac crest: This is the most commonly employed site for reasons of safety, a decreased risk of pain, and accessibility. The posterior superior iliac crest site is localized to the central crest area. See the image below.



Patient position (posterior superior iliac crest).

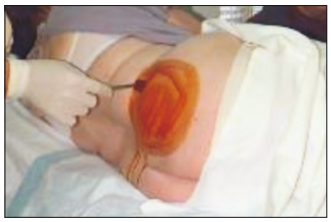
Anterior superior iliac crest: This is an alternative site when the posterior iliac crest is unapproachable or not available due to infection, injury, or morbid obesity. The anterior superior iliac crest site is localized to the

center prominence, under the lip of the crest. This location is generally not preferred due to the dense cortical layer, which makes obtaining samples more difficult and smaller in size, as well as creates a risk for an increased painful event. **Aspiration only:** The sternum is sampled only as a last resort in those older than 12 years and in those who are morbidly obese, but it should be avoided in highly agitated patients. To decrease the risk of penetrating the underlying soft-tissue organs, the sternal site is limited to a region that spans between the second and third intercostal spaces. The tibia is sampled only for infants younger than 1 year, and the procedure is conducted under general anesthesia. This site is localized to the proximal anteromedial surface, below the tibial tubercle. The tibial location is not utilized in older patients because the marrow cellularity is not consistent.

Procedure

A marrow biopsy of the posterior/anterior iliac crest is generally performed before aspiration sampling due to the fact the biopsy technique induces elevated thromboplastic substances. The consequence of this is a reduction in the effectiveness of an aspiration sampling. However, as many clinical requests are for an aspiration sample only, this technique is described first.

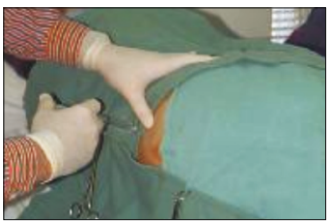
Aspiration: The patient is placed in the lateral decubitus position, with the top leg flexed and the lower leg straight. Palpate the iliac crest, and mark the preferred sampling site with a pen. Aseptic technique is employed, including sterile gloves and gown. The site is prepared with an antiseptic (e.g. povidone-iodine or chlorhexidine gluconate), scrubbed, and draped, exposing only the site to be sampled. See the images below.



Skin preparation.

Site preparation.

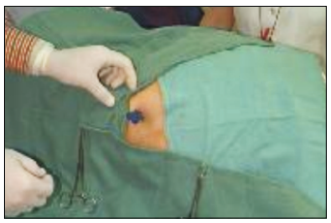
The skin and the underlying tissue to the periosteum are infiltrated with a local anesthetic (e.g. approximately 10 mL of 1% Xylocaine [lidocaine]). A 10-mL syringe with a 25-gauge needle is used to inject an initial 0.5 mL directly under the skin, raising a wheal. A 22-gauge needle is used to penetrate deeper into the subcutaneous tissue and the underlying periosteum, an area roughly 1 cm in diameter.



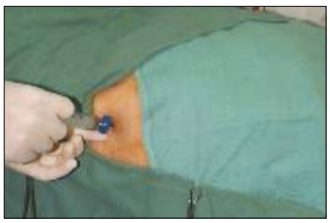
Local anesthetic injection.

Adequacy of the anesthesia is tested by gently prodding the periosteum with the tip of the needle and questioning the patient for any painful sensation. It is important to be aware of changes in the patient's comfort level throughout the procedure to not only decrease the patient's anxiety level, but to minimize movements that may affect the efficacy of the procedure. Having a family member present may help to alleviate the patient's anxiety. To ensure sufficient pain control is being managed well, the person performing the procedure should talk to the patient, discuss the steps taken throughout the process, and listen to the manner as well as the content of the patient's response.

A skin incision is made with a small surgical blade, through which the bone marrow aspiration needle, with a stylet locked in place, is inserted.



Aspiration needle placement.



Bone marrow aspiration.

Once the needle contacts the bone, it is advanced by slowly rotating clockwise and counterclockwise until the cortical bone is penetrated and the marrow cavity is entered. Contact with the marrow cavity is usually noted by a sudden reduction in pressure. The depth of the penetration should not extend beyond an initial 1 cm.

Once within the marrow cavity, the stylet is removed. Using a 20 mL syringe, approximately 0.3 mL of bone marrow is aspirated. A volume greater than 0.3 mL may dilute the sample with peripheral blood and thus is not recommended. The material collected for bone marrow slides is generally not mixed with an anticoagulant, and it is processed immediately by a technologist; this avoids any cellular morphologic artifacts. If there is to be a delay in slide preparation, place the sample in an EDTA (ethylenediaminetetraacetic acid) anticoagulant-containing tube, preferably a pediatric-sized tube to avoid exposure to excess anticoagulant.

If additional marrow is needed for ancillary studies, subsequent specimens are obtained by attaching a separate syringe, collecting 5 mL at a time. The samples are then transferred into an anticoagulant-containing tube that is appropriate to the requested study: heparin for cytogenetic analysis; either heparin or EDTA for immunophenotyping; formalin for a Cytoblock preparation; and, glutaraldehyde for ultrastructural examination.

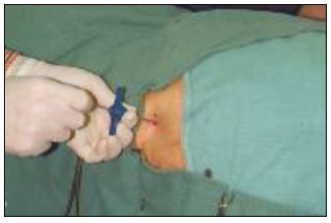
The marrow needle is removed, and pressure is applied to the aspiration site with gauze until any bleeding has stopped (see Postprocedure Care).

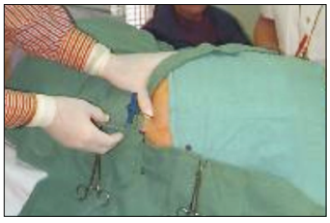
Once the aspiration is completed, the specimen is processed by the hematopathology technician.

Bone marrow biopsy: Any of several needle models can be utilized; however, the Jamshidi needle is considered the most popular. This disposable needle is tapered at the distal end to help retain the specimen for improved extraction.

Patient preparation is to be followed in the manner previously described for bone marrow aspiration.

The needle, with stylet locked in place, is held with the palm and index finger and repositioned so that a new insertion site is created for biopsy sampling. Once the needle touches the bone surface, the stylet is removed.

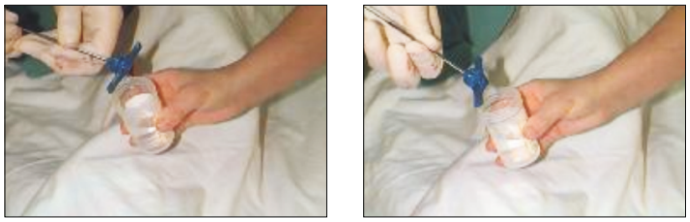




Bone marrow biopsy. Jamshidi needle placement.

Using firm pressure, slowly rotate the needle in an alternating clockwise-counterclockwise motion, and advance it into the bone marrow cavity to obtain an adequate bone marrow specimen measuring approximately 1.6-3 cm in length.

Rotate the needle along its axis to help loosen the sample, pull back approximately 2-3 mm, and advance the needle again slightly, at a different angle, to help secure the specimen.
Following this procedure, slowly pull the needle out, while rotating in an alternating clockwise and counterclockwise motion.
Remove the specimen from the needle and introduce a probe through the distal cutting end. If the aspirate was unsuccessful (i.e., a "dry tap"), the core biopsy may be used to make touch preparations (see Slide Preparation). This must be performed before placing the specimen in formalin.
Place the specimen in formalin solution for histologic processing.



Bone marrow biopsy specimen.

Bone marrow biopsy specimen in fixative solution.

The marrow needle is removed, and pressure is applied to the site with gauze until any bleeding has stopped (see Postprocedure Care).

The Sternum

Note: With this site, only aspiration is to be performed, and it is only to be performed on adolescent and adult patient populations.
The second to third intercostal level of the sternum is palpated, and the selected sample site is marked with a pen. Note: The area chosen should be to one side of the midline as the marrow cellularity is considered to be diminished at that location.
The designated area is prepared with an antiseptic scrub and draped.
Aseptic technique is employed, including sterile gloves and gown.
Local anesthetic is used to infiltrate from the skin to the periosteum.
After small cut is made in the skin with a surgical blade, the aspiration needle with the stylet locked in place, is inserted until the needle touches the bone.
With the same technique described in the above section (see Procedure: Posterior/Anterior Iliac Crest), advance the needle into the marrow cavity, obtain the specimen, and remove the needle. Note: Unlike other sites, the attached guard is not to be removed; rather, it is adjusted to allow for the maximum depth of needle penetration to 0.5 cm. This prevents needle slippage that can result in injury to the underlying mediastinal organs.
Core biopsies are not to be performed from the sternum.

Unilateral Versus Bilateral Iliac Crest Biopsy

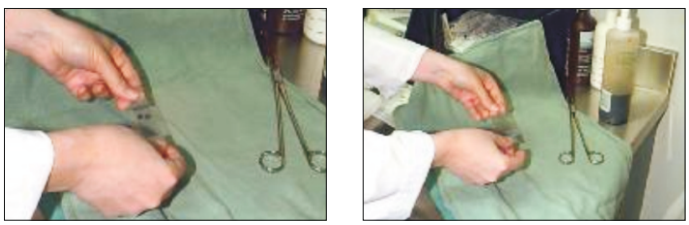
Controversy exists in the application of bilateral iliac biopsies. However, recent studies have indicated that this technique increases the probability of detecting focal lesions, such as in the case of carcinoma and lymphoma staging, where 11-16% of cases may be missed with unilateral biopsies. Wang et al reported an improvement in identifying bone malignancy in the following pathologic cases: Hodgkin disease by 19.5%, sarcomas by 14%, carcinomas by 11.5%, and non-Hodgkin lymphoma by 4.6%. Unilateral iliac sampling was considered sufficient in patients diagnosed with multiple myeloma, chronic myeloproliferative disorders, and myelodysplastic syndromes.

Postprocedure Care

After the procedure, firm pressure is applied for 5 minutes to several layers of sterile gauze placed over the wound site. Remove residual antiseptic to avoid further skin irritation by the solution. If hemorrhage from the wound persists, then place the patient in the supine position, with gauze over the wound site, so that consistent pressure can be applied for a minimum of 30 minutes. Rarely, bleeding may be present; if that is the case, consider placing a pressure dressing, again with the patient in a supine position, for an additional 1 hour. The patient is to be discharged with orders that the wound dressing is to be maintained in a dry state for 48 hours. The wound site is to be checked frequently, and if persistent bleeding or worsening pain occurs, these findings are to be reported to the clinician's office.

Slide Preparation

This stage in bone marrow preparation should be performed by trained personnel, such as a hematopathology technician. Thin-spread preparations of aspiration-collected samples, placed onto glass slides, can be prepared in numerous ways, all of which have the aim to retain and evaluate marrow particles. These spicules of fat droplets (not prominently seen in pediatric cases) and fragmented bone are likely to have adherent cellular material and thus be a target for morphologic evaluation. An aspirate smear is the most simplistic of the methods, similar in presentation as a peripheral blood smear. A drop of the acquired specimen is placed 1 cm from the edge that opposes the frosted "labeled" end and, with a second glass slide placed at a 30º angle, the sample is pushed toward the opposing side in one rapid smooth stroke. Excess sample can be removed by tilting the glass slide onto gauze or pipetting the extraneous fluid. Squash preparations are prepared on glass slides by placing marrow particles on a slide and pressing the particles with another slide. These preparations are used to better observe cellular interactions as the architecture of the marrow unit is preserved. The cover slip method produces samples that have been concentrated more than the squash preparation. The aspirate particles are selected from a petri dish and directly placed onto a glass cover slip. In a manner similar to the squash method, a second cover slip is gently applied to crush the sample. Each cover slip is then stained individually. Thus, enhanced removal of contaminating peripheral blood is performed, again with retention of the marrow unit architecture. At times, biopsy touch prints are useful, especially if the aspirate is dry and the only sample available is the bone marrow biopsy. In touch preparations, the hematopathology technician gently touches the tissue fragment onto a glass slide; this can provide morphologic details similar to that of an aspirate. Marrow particles can be collected in aggregate as a clot and processed in a similar manner to that of tissue. The solid component is concentrated by placing the specimen in a finely meshed bag that retains the tissue fragments, but which allows excess fluid to escape.



Bone marrow aspiration and biopsy slide preparation.

Bone marrow aspiration and biopsy slides before staining.

Standard stains used for the initial evaluation include Wright or May-Grunwald-Giemsa staining which enhance cytologic detail. Other special stains can be utilized for various purposes such as Prussian blue for iron in cases of suspected hemosiderosis or for the ringed sideroblasts of myelodysplastic syndromes. Myeloperoxidase, Sudan Black B, and leukocyte alkaline phosphatase are used in the categorization of acute myeloid leukemias. Periodic acid-Schiff (PAS) stain enhances depiction of cells that are implicated in glycogen storage diseases.

Morbidity/Mortality

In 2002 the British Society of Haematology initiated an annual survey to assess the various types and incidence of bone marrow biopsy adverse events.Bain summarized results of a 7-year (1995 to 2001) retrospective study and identified 26 adverse events among approximately 54,890 biopsies, with an overall annual incidence of 0.05%. The most common side effects in order of decreasing frequency were the following: hemorrhage, needle breakage, and infections. Risk factors for hemorrhage included concurrent anticoagulation therapy or underlying myeloproliferative/ myelodysplastic syndrome, in which platelet function was affected. Two cases were fatal and were attributed to sepsis and massive hemorrhage. Four years later, a prospective study by Bain revealed 15 adverse events in a single year, with an overall incidence of 0.07%, not significantly different from the previous study's results. However, although hemorrhage was still considered the most commonly encountered side effect, this study revealed that pain, anaphylactic reaction, and fractures were prominent secondary consequences. Two fatality cases, attributed to laceration of blood vessels, were reported from 20,323 bone marrow aspiration and biopsy procedures.

Special Concerns

General anesthesia is required for pediatric cases, some sternal bone marrow sampling cases, and in those patients who are highly anxious. Sternal bone marrow aspiration has a higher risk of complications than other sites due to the delicate bone structure (approximately 1 cm thick in adults). Penetration of the underlying mediastinal organs can result in mediastinitis, pulmonary embolism, pneumothorax, cardiac tamponade, and cardiac tissue injury. For these reasons, biopsies are not to be performed from the sternum. Awareness of anatomic variations and pathologies that may affect bone density (e.g. osteoporosis, multiple myeloma) can prevent further complications and injuries. Thrombocytopenia is not a contraindication to bone marrow aspiration and biopsy. Corrective action is required for coagulation disorders before bone marrow sampling. Application of sterile techniques is required in the prevention of infections. Dry tap, or the lack of specimen obtainment during the aspiration sampling process, is most commonly due to technical problems such as misalignment of the needle. Other conditions that should be considered and may contribute to the decision of obtaining a biopsy are recent radiation therapy exposure, aplastic anemia, myelofibrosis, or bone infiltrative neoplasm. Knowing that tissue shrinkage can occur at an approximate rate of 25% after processing, the desired biopsy sampling size should initially be greater than 1.5 cm, preferably 2-3 cm in length (pediatric samples may be as small as 0.5 cm). Such a size will allow for the evaluation of 5 or 6 intertrabecular spaces, which is considered sufficient sampling for a diagnosis. Rarely, chronic pain may occur at the site of bone marrow sampling, thus requiring further clinical management.

Medical-Legal Pitfalls

Failure to prevent, recognize, or initiate rapid response to excessive bleeding or, rarely, to an anaphylaxis anesthetic event during the bone marrow sampling procedure . Failure to use proper safety techniques, such as having a guard device to prevent needle slippage, specifically during sternal aspiration. Failure to identify complications in sampling an iliac crest that results in penetration of the underlying gastrointestinal tract as well as blood vessels—the latter which runs the risk of the development of massive retroperitoneal hemorrhage and gluteal compartment syndrome.

Pleural Effusion Workup

Pleural Fluid Culture and Cytology

Cultures of infected pleural fluids yield positive results in approximately 60% of cases. This occurs even less often for anaerobic organisms. Diagnostic yields, particularly for anaerobic pathogens, may be increased by directly culturing pleural fluid into blood culture bottles.

Malignancy is suspected in patients with known cancer or with lymphocytic, exudative effusions, especially when bloody. Direct tumor involvement of the pleura is diagnosed most easily by performing pleural fluid cytology. Heparinized samples (1 mL of 1:1000 heparin per 50 mL of pleural fluid) should be submitted for analysis if the pleural fluid is bloody and they should be refrigerate if samples will not be processed within one hour. The reported diagnostic yields in cytology vary from 60-90%, depending on the extent of pleural involvement and the type of primary malignancy. Cytology findings are positive in 58% of effusions related to mesothelioma. The sensitivity of cytology is not highly related to the volume of pleural fluid tested. Sending more than 50-60 mL of pleural fluid for cytology does not increase the yield of direct cytospin analysis, and volumes of approximately 150 mL are sufficient when both cytospin and cell block preparations are analyzed. Tumor markers, such as carcinoembryonic antigen, Leu-1, and mucin, are suggestive of malignant effusions (especially adenocarcinoma) when pleural fluid values are very high. However, because of low sensitivity, they are not helpful if the values are normal or only modestly increased.

Tuberculous pleuritis

Suspect tuberculous pleuritis in patients with a history of exposure or a positive PPD finding and in patients with lymphocytic exudative effusions, especially if less than 5% mesothelial cells are detected on differential cell counts. Because most tuberculous pleural effusions probably result from a hypersensitivity reaction to the Mycobacterium rather than from microbial invasion of the pleura, acid-fast bacillus stains of pleural fluid are rarely diagnostic (< 10% of cases). Pleural fluid cultures grow *M. tuberculosis* in less than 65% of cases. In contrast, the combination of histology and culture of pleural tissue obtained by pleural biopsy increases the diagnostic yield for TB to 90%. Adenosine deaminase (ADA) activity of greater than 43 U/mL in pleural fluid supports the diagnosis of tuberculous pleuritis. However, the test has a sensitivity of only 78%. Therefore, pleural ADA values of less than 43-50 U/mL do not exclude the diagnosis of TB pleuritis. Interferon-gamma concentrations of greater than 140 pg/mL in pleural fluid also support the diagnosis of tuberculous pleuritis. Unfortunately, this test is not routinely available.

Additional Laboratory Tests

Additional specialized tests are warranted when specific etiologies are suspected. Measure pleural fluid amylase levels if a pancreatic origin or ruptured esophagus is suspected or if a unilateral, left-sided pleural effusion remains undiagnosed after initial testing. Of note, increased pleural fluid amylase can also be seen with malignancy. An additional assay of amylase isoenzymes can help distinguish a pancreatic source (diagnosed by elevated pleural fluid pancreatic isoenzymes) from other etiologies. Measure triglyceride and cholesterol levels in milky pleural fluids when chylothorax or pseudochylothorax is suspected. Consider immunologic studies, including pleural fluid antinuclear antibody and rheumatoid factor, when collagen-vascular diseases are suspected.

CT Scanning and Ultrasonography

A study involving 41 consecutive patients with hepatic hydrothorax indicated that hepatic hydrothorax virtually always presents with ascites that can be revealed by ultrasonography or computed tomography (CT) scanning. Point of care bedside ultrasonography has become the standard of care in many facilities. Chest CT scanning with contrast should be performed in all patients with an undiagnosed pleural effusion, if it has not previously been performed, to detect thickened pleura or signs of invasion of underlying or adjacent structures. The two diagnostic imperatives in this situation are pulmonary embolism and tuberculous pleuritis. In both cases, the pleural effusion is a harbinger of potential future morbidity. In contrast, a short delay in diagnosing metastatic malignancy to the pleural space has less impact on future clinical outcomes. CT angiography should be ordered if pulmonary embolism is strongly suggested.

Chest Radiography

Effusions of more than 175 mL are usually apparent as blunting of the costophrenic angle on upright posteroanterior chest radiographs. On supine chest radiographs, which are commonly used in the intensive care setting, moderate to large pleural effusions may appear as a homogenous increase in density spread over the lower lung fields. Apparent elevation of the hemidiaphragm, lateral displacement of the dome of the diaphragm, or increased distance between the apparent left hemidiaphragm and the gastric air bubble suggests subpulmonic effusions. Lateral decubitus films more reliably detect smaller pleural effusions. Layering of an effusion on lateral decubitus films defines a freely flowing effusion and, if the layering fluid is 1 cm thick, indicates an effusion of greater than 200 mL that is amenable to thoracentesis. Failure of an effusion to layer on lateral decubitus films indicates the presence of loculated pleural fluid or some other etiology causing the increased pleural density. Note that decubitus films are almost never performed in those institutions with bedside ultrasonography

Diagnostic Thoracentesis

A diagnostic thoracentesis should be performed if the etiology of the effusion is unclear or if the presumed cause of the effusion does not respond to therapy as expected. Pleural effusions do not require thoracentesis if they are too small to safely aspirate or, in clinically stable patients, if their presence can be explained by underlying congestive heart failure (especially bilateral effusions) or by recent thoracic or abdominal surgery. Depending on the clinician's experience, a pulmonologist or interventional radiologist can be consulted for assistance with high-risk diagnostic thoracentesis.

Contraindications

Relative contraindications to diagnostic thoracentesis include a small volume of fluid (< 1 cm thickness on a lateral decubitus film), bleeding diathesis or systemic anticoagulation, mechanical ventilation, and cutaneous disease over the proposed puncture site. Reversal of coagulopathy or thrombocytopenia may not be necessary as long as the procedure is performed under ultrasound guidance by an experienced operator. Mechanical ventilation with positive end-expiratory pressure does not increase the risk of pneumothorax after thoracentesis, but it increases the likelihood of severe complications (tension pneumothorax or persistent bronchopleural fistula) if the lung is punctured. An uncooperative patient is an absolute contraindication for this procedure.

Complications

Complications of diagnostic thoracentesis include pain at the puncture site, cutaneous or internal bleeding from laceration of an intercostal artery or spleen/liver puncture, pneumothorax, empyema, reexpansion pulmonary edema, malignant seeding of the thoracentesis tract, and

adverse reactions to anesthetics used in the procedure. Pneumothorax complicates approximately 6% of thoracenteses but requires treatment with a chest tube drainage of the pleural space in less than 2% of cases. The use of needles larger than 20 gauge increases the risk of a pneumothorax complicating the thoracentesis. In addition, significant chronic obstructive or fibrotic lung disease increases the risk of a symptomatic pneumothorax complicating the thoracentesis.

Procedure

In patients with large, freely flowing effusions and no relative contraindications to thoracentesis, diagnostic thoracentesis can usually be performed safely, with the puncture site initially chosen based on the chest radiograph and located 1-2 rib interspaces below the level of dullness to percussion on physical examination. In other situations, ultrasonography or chest CT scanning should be used to guide thoracentesis. Ultrasonography guidance at bedside significantly increases the likelihood of obtaining pleural fluid and reduces the risk of pneumothorax. A postprocedure chest film may not be needed, but it is always a good practice to look for ultrasonic evidence of a pneumothorax. The presence of lung sliding would confirm the absence of a pneumothorax. After the site is disinfected with chlorhexidine (preferred) or povidone/iodine (no longer recommended) solution and sterile drapes are placed, anesthetize the skin, periosteum, and parietal pleura with 1% lidocaine through a 25-gauge needle. If pleural fluid is not obtained with the shorter 25-gauge needle, continue anesthetizing with a 1.5-inch, 22-gauge needle. For patients with larger amounts of subcutaneous tissue, a 3.5-inch, 22-gauge spinal needle with inner stylet removed can be used to anesthetize the deeper tissues and to aspirate pleural fluid. Confirm the correct location for thoracentesis by aspirating pleural fluid through the 25- or 22-gauge needle before introducing larger-bore thoracentesis needles or catheters. If pleural fluid is not easily aspirated, stop the procedure and use ultrasonography or chest CT scanning to guide thoracentesis. While there is no consensus amount for a diagnostic thoracentesis, a minimum of 20 mL would be enough for basic analysis and culture. Most of these procedures remove less than 100 mL of fluid. When possible, patients should sit upright for thoracentesis. Patients should not lean forward, because this causes pleural fluid to move to the anterior costophrenic space and increases the risk of puncture of the liver or spleen. For debilitated and ventilated patients who cannot sit upright, obtain pleural fluid by puncturing over the eighth rib at the midaxillar to posterior axillary line. To avoid puncturing liver or spleen, the needle should not be inserted below the ninth rib. In such patients, imaging may be required to guide thoracentesis. Supplemental oxygen is often administered during thoracentesis to offset hypoxemia produced by changes in ventilation-perfusion relationships as fluid is removed and to facilitate reabsorption of pleural air if pneumothorax complicates the procedure. The frequency of complications from thoracentesis may be lower when a more experienced clinician performs the procedure and when ultrasonographic guidance is used. Consequently, a skilled and experienced clinician should perform thoracentesis in patients who have a higher risk of complications or relative contraindications for thoracentesis and in patients who cannot sit upright. Postprocedure expiratory chest radiographs to exclude pneumothorax are not needed in asymptomatic patients after uncomplicated procedures (single needle pass without aspiration of air). However, postprocedure inspiratory chest radiographs are recommended to establish a new baseline for patients likely to have recurrent symptomatic effusions.

Idiopathic Exudative Effusions

Despite evaluations with repeated diagnostic thoracenteses, approximately 20% of exudative effusions remain undiagnosed. Clues to the diagnosis that may have been overlooked include (1) occupational exposure to asbestos 10-20 years earlier, which may suggest benign asbestos effusion; (2) medication exposure to nitrofurantoin, amiodarone, or medications associated with a drug-induced lupus syndrome; and (3) hepatic hydrothorax unrecognized in a patient with minimal or undetectable ascites. Among patients with undiagnosed pleural effusions after the primary evaluation, those who meet all 6 of the following clinical parameters are predicted to have a benign course, and no further evaluation is necessary:

- Patients are clinically stable
- Patients do not have weight loss
- The results of the purified protein derivative (PPD) test, used in detecting tuberculous pleural effusion, are negative and the pleural adenosine deaminase (ADA) value, also used in diagnosing tuberculous pleural effusion, is less than 43 U/mL
- The patient does not have a fever
- The pleural fluid differential blood cell count has less than 95% lymphocytes
- The effusion occupies less than 50% of the hemithorax

For other patients with undiagnosed exudative effusions, approximately 20% have a specific etiology determined, including malignancy. For such patients, weigh the benefits and risks of pursuing a diagnostic strategy that will involve using progressively more invasive procedures, given the low likelihood of finding a curable etiology. Note the following:

- Bronchoscopy - Consider only if a patient has parenchymal abnormalities or hemoptysis
- Surgical approaches to the diagnosis of pleural effusions - Includes video-assisted thoracoscopy (pleuroscopy) and open thoracotomy, allows direct visualization and biopsy of the pleura for diagnosis of exudative effusions, which reveals an etiology in 92% of effusions that remain undiagnosed after a medical evaluation, with an operative mortality of less than 0.5%
- Medical thoracoscopy - Where available, may be diagnostic and therapeutic; complete drainage of the effusion and talc sclerosis can be performed at the time of the procedure

Note that in most medical centers, surgical exploration using thoracoscopy or thoracotomy entails the risks of general anesthesia and is probably warranted only in patients who are symptomatic and anxious for a (potentially incurable) diagnosis.

Biopsy

Pleural biopsy should be considered, only if TB or malignancy is suggested. Medical thoracoscopy with the patient under conscious sedation and local anesthesia has emerged as a diagnostic tool to directly visualize and take a biopsy specimen from the parietal pleura in cases of undiagnosed exudative effusions. As an alternative, closed-needle pleural biopsy is a blind technique that can be performed at the patient's bedside. Medical thoracoscopy has a higher diagnostic yield for malignancy. Closed-needle pleural biopsy findings aid in diagnosis of only 7-12% of malignant effusions when cytology findings alone are negative. However, the yield of closed-needle pleural biopsy (histology plus culture) is as high as thoracoscopy for tuberculous pleuritis and is a useful alternative procedure for this diagnosis when available. A randomized comparison of medical thoracoscopy with CT scan–guided cutting-needle pleural biopsy (CT-CNPB), found no statistically significant difference in diagnostic sensitivity between these two approaches. The study included 124 patients with exudative pleural effusion who could not be diagnosed by cytologic analysis. These researchers recommended using CT-ANPB as the primary diagnostic procedure in patients with pleural thickening or lesions observed on CT scans, and using medical thoracoscopy in patients whose CT scans

demonstrate only pleural fluid, as well as in those who may have benign pleural pathologies other than TB.

Pleural Effusion Treatment & Management

Approach Considerations

Transudative effusions are managed by treating the underlying medical disorder. However, regardless of whether transudative or exudative, large, refractory pleural effusions causing severe respiratory symptoms can be drained to provide symptomatic relief. The management of exudative effusions depends on the underlying etiology of the effusion. Pneumonia, malignancy, and TB cause most exudative pleural effusions, with the remainder typically deemed idiopathic. Complicated parapneumonic effusions and empyemas should be drained to prevent development of fibrosing pleuritis. Malignant effusions are usually drained to palliate symptoms and may require pleurodesis to prevent recurrence. Medications cause only a small proportion of all pleural effusions and are associated with exudative pleural effusions. However, early recognition of this iatrogenic cause of pleural effusion avoids unnecessary additional diagnostic procedures and leads to definitive therapy, which is discontinuation of the medication. Implicated drugs include medications that cause drug-induced lupus syndrome (eg, procainamide, hydralazine, and quinidine), nitrofurantoin, dantrolene, methysergide, procarbazine, and methotrexate. A meta-analysis and systemic review of 19 observational studies determined that pleural effusion drainage in patients on mechanical ventilation is safe and appears to improve oxygenation. No data supported or refuted claims of beneficial effects on clinical outcomes, such as duration of ventilation or length of stay.

Parapneumonic effusions

Of the common causes for exudative pleural effusions, parapneumonic effusions have the highest diagnostic priority. Even in the face of antibiotic therapy, infected pleural effusions can rapidly coagulate and organize to form fibrous peels that might require surgical decortication. Therefore, quickly assess pleural fluid characteristics predictive of a complicated course to identify parapneumonic effusions that require urgent tube drainage. These are observed more commonly in indolent anaerobic pneumonias than in typical community-acquired pneumonia.

Indications for urgent drainage of parapneumonic effusions include (1) frankly purulent fluid, (2) a pleural fluid pH of less than 7.0-7.1, (3) loculated effusions, and (4) bacteria on Gram stain or culture. Patients with parapneumonic effusions who do not meet the criteria for immediate tube drainage should improve clinically within one week with appropriate antibiotic treatment.

LAB ERRORS & BIOSAFETY

TroubleShooting Guidelines

A Compilation from

The Crux
BIMONTHLY FORUM FOR THE LABORATARIANS

Large Scale Lab Errors

Extensive use of automation in the clinical laboratory creates the potential for systematic errors that affect a large number of patient results before the error is discovered. When a large-scale testing error is found, the approaches recommended for responding to individual medical mishaps are often inadequate. This report uses 2 case studies to illustrate some of the unique challenges facing laboratory managers confronted with a large-scale testing error. 9 distinct constituencies have been identified that may be impacted by large-scale testing errors, each of which requires laboratory management's thoughtful and timely attention.

Introduction

In October 2008, Quest Diagnostics (QD) initiated what may have been the largest recall of clinical test results, due to a systematic laboratory error that potentially affected more than 300,000 vitamin D values. According to a company spokesperson, 7% of vitamin D tests performed by QD from early 2007 to mid 2008 were impacted by incorrect calibrators used at 4 of 7 QD testing facilities. Because of the controversial usefulness of vitamin D testing in patient management, it was not clear whether any patients were harmed as a result of testing inaccuracy. Although the QD recall and retesting program received national publicity, in our experience large-scale testing errors involve many (possibly most) testing facilities. Laboratories with the most highly developed quality management systems may, ironically, be most adept at identifying systematic errors that pass undetected at less capable facilities. Much has been written about how to respond to errors in health care. Professional societies have advocated discussion of a serious error with the affected patient and the assumption of responsibility. One hospital accrediting organization, The protocols require use of a specific investigatory technique called "root cause analysis" to examine "sentinel events" associated with certain types of serious errors and to address latent causes that can lead to recurrence of problems. The extensive use of automation and other mass-production techniques in the clinical laboratory creates the potential for systematic errors to affect a large number of patient results before the error is discovered. The term "large-scale testing error" is used to mean an error that impacts a large number of clinical laboratory results because of a defect in a system used for high-volume testing. Large-scale testing errors differ from the type of errors that most commonly occur in other health care settings. Most health care errors are one-of-a-kind or rare events that require some individual caregiver's cognitive failure—a slip, lapse, or mistake—usually in concert with other factors. When a large-scale testing error is found, the approaches recommended for responding to individual medical mishaps are often inadequate. Patients potentially impacted by a large-scale testing error may not be immediately identifiable or easily contacted and often number in the thousands. The clinical impact of the error is likely to be incompletely understood at the time the error is discovered, clouding decisions about whether and how to notify caregivers and patients. Finally, large-scale testing errors are likely to be ongoing at the time a laboratory problem is first detected, with additional errors being produced every day. Post discovery investigatory techniques used to study isolated errors, such as root cause analysis, are generally conducted over the course of several weeks during what is assumed to be a safe window in which the error in question is unlikely to recur. When large-scale testing errors are discovered in the laboratory, the potential for additional ongoing damage must be addressed immediately, often before root causes of the error are fully illuminated. This report uses 2 case studies to illustrate some of the unique challenges facing laboratory managers confronted with a large-scale testing error. We use these cases as a springboard for discussing 9 distinct constituencies that may be impacted by large-scale testing errors, each of which requires laboratory management's thoughtful and timely attention.

Table 1.

Constituency	Responsibility
Patients about to undergo testing	Prevent additional errors
Patients who have undergone testing	Assess risk of adverse consequences; notify and correct results or offer retesting as appropriate
Caregivers and other customers	Assess risk of adverse consequences; notify and correct results or offer retesting as appropriate
Payers	Reverse charges for results known or likely to be inaccurate
Regulators and accreditors	Notify of incident as appropriate or required; cooperate in investigation and industry notification as required
General public	Proactive communication of any ongoing risk to public health and safety
Vendors and suppliers	Notify of incident; cooperate in investigation of cause as required
Laboratory owners	Notify of incident; assist with vulnerability assessment and communication
Laboratory workers	Notify of incident; define authorized communication channels for responding to incident; assess risk to laboratory staff and follow up as appropriate

Example 1. Incorrect International Normalized Ratio Results

In 2007, during a certification inspection of Laboratory X, an inspector attempted to verify the laboratory's international normalized ratio (INR) calculation using the laboratory's measured prothrombin time (PT), mean normal PT, and the labeled International Sensitivity Index (ISI) of the manufactured prothrombin reagent. The INR value manually calculated by the inspector was found to be 8% greater than the INR reported by the analyzer that had measured the PT. Manually calculated INR values for 2 more PTs were also greater than the INR reported by the analyzer. The inspector cited the laboratory for a deficiency, and the analyzer in question was immediately taken out of service while the problem was investigated. Investigation the day after the inspection

revealed that the analyzer had been calibrated 2 months before the inspection. Although the reagent ISI had been correctly entered into the coagulometer at the time of calibration, examination of calibration records showed that an additional "adjustment ratio" had not been calculated and entered into the analyzer, as required by a technical bulletin issued by the instrument manufacturer. Instead, the adjustment ratio that had been calculated and entered during an earlier calibration remained in the instrument. The laboratory's written recalibration procedure included instructions to enter the adjustment ratio, but this step had been omitted during the most recent calibration. The technologist who performed the calibration was experienced with coagulation testing and calibration but recently had been transferred from another laboratory within the health care system that used a different model coagulometer produced by the same manufacturer. The model with which the technologist was familiar did not require entry of the adjustment ratio. As a result of the omission during calibration, all INR values calculated by the coagulometer since the recalibration were 8% lower than what they would have been if the correct ratio adjustment factor had been entered during the calibration procedure. No proficiency testing events had occurred during the 2 months between the coagulometer recalibration and the inspection of Laboratory X. On the day after the inspection, the analyzer was recalibrated using the appropriate adjustment ratio. Ten samples were run on the analyzer, the INR was manually calculated for each sample, and all reported results were in agreement with manual calculations. The instrument was then placed back in clinical service. Laboratory X served a large outpatient facility but no inpatients. The laboratory was cited for a total of 6 deficiencies as a result of the inspection. The other 5 deficiencies concerned process issues that did not directly impact the accuracy of test results. The laboratory medical director was aware of all 6 citations and tasked the laboratory technical director with documenting corrective action that had been taken in relation to each deficiency. Three weeks after the inspection, a representative from the Laboratory Accreditation Program called the technical director and inquired into the specific steps that the laboratory had taken to notify caregivers about erroneous INR results. After receiving the call, the laboratory's medical and technical directors organized a "look-back" program to address erroneous results that had been released. A search program was developed using the ad hoc query language that formed part of Laboratory X's laboratory information system. The search revealed that 1,620 INR values had been reported by the coagulometer since its last calibration. Each of the 1,620 reported INR results were presumed to be 8% lower than the value that would have been reported had the instrument calibration been performed correctly. A computer program was then written to generate letters to each physician who had ordered an implicated assay. Each physician was informed of the names of impacted patients, the incorrectly reported INR value, and the correct value. To facilitate follow-up, patients and their INR results were divided into 3 groups: (1) patients with values that were reported as subtherapeutic (INR < 1.5) but that were actually therapeutic (INR between 1.5 and 3); (2) patients with values that were reported as therapeutic but that were actually supratherapeutic (INR >3); and (3) patients with values that when corrected did not cross from one category to another. Of the implicated results, 93% fell into this third group (no change in category). Caregivers were given lists of their patients who fell into each category. The risk management department of the hospital system associated with the laboratory was contacted, as was executive management of the hospital. Six days elapsed before the risk management department cleared for release a letter from the laboratory describing the nature and cause of the problem. This letter was sent to all physicians who had ordered 1 of the 1,620 assays, along with a list of corrected results. Retesting at no charge was offered to all impacted patients, and physicians were encouraged to contact the laboratory if they believed any patient had experienced an adverse event as a result of the error. No communications about adverse events were received. Because letters informing physicians about the erroneous test results were released approximately 5 weeks after the error was discovered, many patients involved by the incident had already been retested as part of routine monitoring of oral anticoagulant therapy. Approximately 20 patients and their caregivers availed themselves of the offer for follow-up testing at no charge. All charges related to the 1,620 incorrectly reported test results were reversed. A sentinel event investigation was initiated that identified the use of multiple models of coagulometers within the health care system as a causal factor for the incident, and the model that required manual entry of the ratio adjustment was replaced approximately 6 months after the sentinel event investigation concluded.

CASE 2: INCORRECT HIV SCREENING RESULTS

In 2006, a technologist at Laboratory Y was reviewing HIV1 and HIV2 enzyme-linked immunosorbent assay screening results and noted that the laboratory had reported a negative result 2 days previously for a patient who had tested positive several weeks earlier. The technologist brought the matter to her supervisor who informed Laboratory Y's medical and technical directors. The medical director had the patient's negative specimen retested twice, and on both retests, the result was positive. As a matter of policy, the laboratory tested 3 levels of controls with each run, and review of control results showed expected levels of absorbance on the day the incorrect negative patient result had been obtained. Laboratory Y's medical director had the negative patient result corrected to positive and called the director of the laboratory that had referred the specimen to Laboratory Y. Until the cause of the problem could be determined and corrected, the medical director of Laboratory Y also instructed testing personnel to have all HIV specimens tested twice before results were released and to release patient results only if both tests produced the same result. The decision to test all specimens twice was made on the day the issue was first noted, as was correction of the erroneous result. The laboratory used an automated system for testing antibodies to HIV1 and HIV2 that had been cleared by the appropriate authorities (**AA**) under the review paradigm. An understanding of the likely cause of the error developed during the next 2 weeks as additional clues were discovered. Two days after the initial error, a technologist noticed that one of the patient wells in the HIV microtiter tray contained less fluid than the other wells. The instrument was stopped, and examination of the tray revealed that patient specimen had not been dispensed into the well in question by the instrument's automated pipettor but had been properly dispensed into all of the other wells in the tray. The batch was discarded, and testing was resumed (again in duplicate). The technologist was instructed to watch the instrument as it divided the patient specimen to ensure that specimen was dispensed into each reaction well. The service representative from the instrument manufacturer was summoned to investigate the issue. The representative told Laboratory Y's section manager that technologists should check for bubbles in pipette tips and check each pipette tip to make sure it had a hole in the end. Neither of these instructions was in the **AA** cleared product insert. During the next week 1 additional "nondispense event" was observed, in which the automated dispensing arm did not dispense patient specimen into a test well. Visual examination of the disposable pipette tip revealed no bubble or obvious defects. The director of product service at the instrument manufacturer was contacted. The instrument manufacturer committed to cooperate in investigating the problem and offered to pay for all reagents associated with retesting until the problem was resolved. Based on the nondispense events that had been observed, Laboratory Y's management estimated that approximately 1 in 400 specimens was not being properly

dispensed into test wells. A nondispense event had the potential to produce a false-negative result, but not a false-positive result, and would only cause incorrect results in truly positive specimens. Because 99% of specimens tested by Laboratory Y were negative, management estimated that approximately (1 in 400 tests) × (1 in 100 specimens) or 1 in 40,000 test results reported by the laboratory would be incorrect. Because the date on which erratic dispensing started was unknown, management considered all results produced on the test instrument to be potentially impacted. Approximately 30,000 specimens had been tested since the instrument was first placed in service, 8 months before the index error became known. The manufacturer of the HIV test system denied receiving other reports of nondispense events. Nevertheless, the director of Laboratory Y reported the problem to the **AA** Office of In Vitro Diagnostic Device Evaluation and Safety and posted the issue on the **AA**s Web-based voluntary reporting system. Laboratory Y was not contacted by the **AA** in follow-up. Laboratory Y was a reference laboratory that received specimens from other clinical laboratories. The director of Laboratory Y determined that a look-back and retesting program should be instituted to notify caregivers and patients about the small but real possibility of false-negative HIV test results. In implementing the look-back program, a number of issues surfaced: (1) As a reference facility, Laboratory Y did not have the addresses of tested patients and, in a number of cases, did not know the names of ordering physicians. Laboratory Y offered to assist client laboratories in notifying ordering physicians and their patients and to defray the costs of printing self-addressed patient letters that could be mailed to ordering physicians. (2) Although it was clear that ordering physicians needed to be informed that the problem potentially impacted HIV test results, it was not clear whether patient letters should include the name of the test because a misunderstanding might cause patients anxiety out of proportion to actual risk. (3) Some people were of the opinion that patients needed to be informed directly, irrespective of the wishes of the patients' ordering physicians. Others believed that the risk of harm (1 in 40,000 results) was too low to require direct patient communication and that communication with the patients' caregiver was adequate. The question of whether retesting should be recommended or simply offered was also debated. (4) There was controversy about whether the name of the instrument manufacturer should be included in letters to referring laboratories or physicians. (5) A special process needed to be created to facilitate no-charge retesting. (6) Debate ensued about whether laboratory charges should be reversed, given the low risk of error in any given specimen. (7) It was not clear whether caregiver notification letters should be signed by the director of Laboratory Y or the director of the referring laboratory or be unsigned. The risk management department at several hospitals that referred specimens to Laboratory Y contacted Laboratory Y management for information and updates. Some risk management departments prepared press releases, but no statements were released to the press. When 2 subsequent nondispense events were detected, Laboratory Y revised its risk estimate and calculated that the risk of an incorrect result might be as high as 1 in 10,000 tests. One hospital risk management department wanted Laboratory Y to send follow-up letters to caregivers with the revised risk estimate, since a risk of approximately 1 in 40,000 tests had been previously reported. Three months after the incident, Laboratory Y replaced its HIV test system with another system that was programmed to detect dispensing failures. The manufacturer of the original test system paid for all reagent expenses associated with testing and part of the look-back and notification expenses and subsequently modified its own HIV test system to automatically detect dispensing failures. Six months after the incident, only 1 patient who had initially tested negative subsequently tested positive. This patient had initially tested negative on 5 occasions, was known to be engaging in high-risk behavior, and was being tested on a regular basis because of his risk profile. The patient was considered to have seroconverted and not to have been subject to a false-negative result.

DISCUSSION

Laboratories use a portfolio of "controls" or "good laboratory practices" to prevent errors and ensure the integrity of laboratory processes and the accuracy of test results. These practices range from contemporaneous quality control testing to periodic employee competency assessment and from preproduction validation of new test methods to external proficiency testing and on-site laboratory inspection. Some controls used in laboratories are prescribed by legislation, regulation, or health care accrediting agencies (eg, CAP etc), others are adopted by laboratory management in response to local imperatives or perceived vulnerabilities. An example of the latter might include the decision by laboratory management to double check the output of a printer that has shown variable function in the past. Although the application of good laboratory practices undoubtedly reduces the frequency of mistakes or "nonconformances," laboratory errors nevertheless occur. As is the case in other branches of medicine, most laboratory errors do not impact patient outcomes. As case 1 illustrates, only a small fraction of laboratory errors affect management of a patient's condition, and only a subset of these errors cause injury to patients. Nevertheless, the potential to injure patients is implicit in most laboratory errors, and for that reason every laboratory error deserves some sort of investigation and response. When automated testing produces laboratory errors on a large scale, the risk of adverse patient impact is increased. Large-scale laboratory errors vary considerably with respect to their cause and potential consequences. The 2 case reports presented in the article concern errors related to execution of calibration procedures and instrument design. The cause of other systematic errors known to us have ranged from defects in reagent quality, variation in manufacture of blood collection tubes, and defects in automated flow cytometry gating software or computer screens used to collect gestational age information for prenatal testing. The diverse causes and consequences of large-scale testing errors make us hesitant to recommend a standard "cookie cutter" approach to error recovery. Incidents that involve blood specimens and chronic diseases offer the opportunity for retesting. Incidents involving irreplaceable specimens or testing that occurs in acute high-stakes settings reduce recovery options.

Reporting Critical Values in Laboratory Practice

Among the most important functions of a pathology or laboratory medicine service is the clear, accurate, and rapid communication of critical test results (critical values) to patient care providers. Pathologists and laboratory professionals are often confronted with many obstacles in the reporting of such critical values, including establishing clinically relevant criteria for critical values, resolving difficulties in locating an ordering provider when a critical value is obtained, and ensuring that the provider understands the severity and implications of a critical result when he or she has questions.

Case Scenario:

A patient with atrial fibrillation who is receiving warfarin has an afternoon cardiology appointment for routine care and anticoagulant monitoring. A basic metabolic profile and prothrombin time are ordered. The specimen is transported to the laboratory by courier, and laboratory testing is completed at 7:30 PM. All values are within normal limits except for an elevated potassium level (K^+) of 6.9 mEq/L (6.9 mmol/L; reference range, 3.2–5.2 mEq/L [3.2-5.2 mmol/L]) and a prothrombin time of 64.7 seconds (reference range, 11.1-13.2 seconds), corresponding to an international normalized ratio of 7.4. These results qualify as *critical values* by your clinical laboratory policy, and the laboratory technologist attempts to contact the ordering clinician by telephone. Calls to the physician's office are not forwarded to an answering service or covering clinician, but rather directed to an office answering machine. The ordering physician does not respond to pages or telephone calls made to the contact numbers listed in the hospital telephone directory or laboratory information system (LIS). The laboratory technologist contacts the on-call pathology resident and asks for assistance.

Questions:

(1) What are laboratory critical values? (2) What are the requirements for critical value reporting? (3) How should clinical laboratories establish critical value lists and determine appropriate thresholds? (4) Who should make and receive critical value notifications? (5) How might critical value reporting be improved? (6) What are the responsibilities of pathologists and laboratory directors in the critical value process?

Background:

Lundberg first outlined the fundamental components of critical value reporting in a *Medical Laboratory Observer* article, describing *critical laboratory values* as "values which reflect pathophysiological derangements at such variance with normal as to be life threatening if therapy is not instituted immediately." They have more recently been described as "laboratory results that indicate a life-threatening situation for the patient. Because of their critical nature, urgent notification of a critical value to the appropriate healthcare professional is necessary." Alternative terms for critical values include *critical results*, *panic values*, and *alert values*. The term *panic values* carries a suggestion of emotional stress and runs against the thoughtful and organized process of communicating important information clearly. Its use is therefore discouraged. Laboratories are required by numerous regulatory agencies to develop and put into practice critical value policies. Although the content of these policies varies according to institutional needs, the core components are often quite similar. This article begins by describing the usual current regulatory requirements for critical value reporting. This information will be followed by a detailed analysis of the fundamental components of critical value notification. Aspects of critical value reporting that have been evaluated in the literature are emphasized, as are current technological advances that may change the way in which critical value reporting takes place.

Establishing a Critical Values List:

Although there are many regulations specifying that laboratories must define and communicate critical values, it may seem surprising that regulations where they exist do not state which laboratory tests require critical value limits and notification. Indeed, individual clinical laboratories face unique challenges that reflect institutional organization, clinical demand, patient population, instrumentation, and staffing. Such variations have hindered the development of universal standards for critical value reporting across laboratories. The idea of a universal critical value list is appealing to many laboratorians and clinicians. For example, many clinicians would likely consider a sodium (Na^+) level of 168 mEq/L (168 mmol/L) a "critical" value regardless of which laboratory performs the test. Indeed, the practice of assigning the laboratory director responsibility for creating and refining the critical value list has led to similar overall inclusion of tests between laboratories without there being a universal mandate or requirement. As an example, virtually all laboratories include Na^+ on their critical value list precisely because it is important for patient care. Furthermore, not communicating a critically elevated Na^+ level could have medicolegal ramifications if an adverse clinical outcome occurred. Defining (and then mandating) a universal set of thresholds for tests, however, would be a daunting task given the scarcity of outcomes-based data on critical value thresholds. Inherent variability in assay-specific reference intervals between institutions is also a complicating factor. An individual laboratory director can account for this variability by defining critical ranges consistent with his or her own assays and instrumentation. How should a laboratory determine which tests to include on a critical value list? Moreover, how should the critical high and low thresholds be established? While ultimately, this determination is the responsibility of the laboratory director, it should be made in communication with the clinicians who use laboratory services, as well as with a medical review board of the institution, if applicable. This task may include meeting with relevant physicians, medical and surgical section chiefs, hospital administrators, and/or nurse managers to discuss critical value policies and to determine if there are any tests that should be included (or omitted) and whether any thresholds should be adjusted according to clinical needs. Not every laboratory test should have critical values associated with it. Critical value lists are, by nature, limited to not hinder the clinical effectiveness of notification. Critical lists that are too inclusive (or that have critical value thresholds that require excessive notification) place an unnecessary burden on laboratory staff. Such lists annoy clinicians, foster a negative attitude toward important laboratory services, and, most important, provide uncertain additional benefit to patient care. At the other extreme, lists that are too exclusive (or with thresholds that are too high or low) might not prevent adverse clinical outcomes, as a delay in the recognition of life-threatening laboratory results by clinicians can be disastrous. A balance must be achieved. The best place to start when establishing or modifying critical value lists is by comparison with previously published lists, practice parameters, and consensus documents because these sources have been refined with the benefit of time, institutional comparison, and clinical performance. Several published studies from CAP (Q-Probes and Q-Tracks) have compared critical value reporting across hundreds of institutions and are a valuable resource for critical value policy assessment. Although most published critical value lists do not include blood bank testing, we have included a

number of transfusion medicine–related scenarios that may benefit from rapid communication and discussion with a responsible clinician. It should be noted that most published reports focus on critical value notifications in general laboratory testing. Finally, many institutions place their laboratory policies (including critical value lists) online, facilitating comparison of lists between peer laboratories.

Critical Value Notification Procedures:

The initial step in the critical value communication process involves identification of an abnormal result by someone in the laboratory. For automated assays, the instrument, middleware, or LIS will notify the laboratory staff (usually the performing technologist) of the critical value. Laboratory policies must clearly indicate whether the assay should be verified and/or repeated before reporting and, if so, within what time frame. Repeat testing is not feasible in many circumstances (e.g. blood culture results), and ongoing improvements in laboratory assays may decrease the clinical usefulness of routine repeat testing before reporting. This is a topic of continued clinical interest and debate. The Patient Safety Goals state that laboratory procedures must indicate "by whom and to whom" critical results are reported, as well as "the acceptable length of time between the availability and reporting of critical results." Documentation is required. The CAP checklist specifies what information must be documented during critical value notifications, including "date, time, responsible laboratory individual, [and] person notified." Laboratory personnel who perform the actual tests are currently responsible for making the vast majority of critical value notifications. It is recommended that critical value notifications should be made by one of the "team members" involved in performing the procedure. Laboratories face an ever-increasing dilemma in critical value notification-the overall volume of laboratory testing is increasing, but a continued shortage in the number of laboratory professionals means that fewer people are expected to do more. Shifting the task of critical value notification away from laboratory technologists may be inevitable at many institutions. Indeed, a few hospitals have implemented the use of automated notification systems for critical value reporting. It is advisable that critical values transmitted from the LIS to a hospital clinical information system trigger the generation of text messages directed to the responsible clinician's mobile phone and computer. If the clinician does not confirm receipt in the clinical information system within 60 minutes, results are communicated by telephone. This approach improves the speed of communication and allowed for full electronic documentation of critical value reporting. Elsewhere, an automated paging system was developed for critical value notification. In that program, critical values transmitted from the LIS generate a page containing the patient name, medical record number, collection time, critical result, and reference range. The clinician must confirm receipt of the critical value by dialing a phone number listed in the message. If the clinician does not respond within 10 minutes (or rejects the notification), the call is escalated to a trained group of operators who proceed with telephone notification. Implementation of that system increased documentation of critical value receipt by physicians and decreased the median time for notification. Several other studies have also evaluated the role of automated paging systems in critical value reporting. It should be emphasized that automated solutions should allow for an *escalation policy* to ensure communication of critical results when clinicians do not acknowledge receipt. Laboratory contact information should also be available so that clinicians with additional questions can ask a laboratory professional or medical director as appropriate. Patient privacy requirements should also be considered with automated solutions because data conceivably might be transmitted and stored on nonencrypted devices. Finally, device compatibility with alphanumeric characters (particularly units) and character limits should also be evaluated because an inaccurate or incomplete notification could lead to medical error and adverse clinical outcome. To whom should critical values be reported? The results are to be conveyed to the "responsible licensed caregiver." These could be a "physician (or other clinical personnel responsible for patient care)" and the "appropriate clinical individual." CLIA refers to "the individual or entity requesting the test and, if applicable, the individual responsible for using the test results". "Who can receive critical values?" for the inpatient and outpatient settings. As expected, answers from virtually all facilities included any licensed caregiver, ordering physician, on-call physician, or resident. The "authorized agent" approach to critical value notification (calling someone whom a licensed caregiver specifies can receive critical value notifications but is not necessarily capable or authorized to act on them independently) should be discouraged. Many facilities allow for reporting of critical values directly to licensed nurses, who are then responsible for conveying these results to ordering and/or covering physicians. According to most regulatory agencies, this would also be an acceptable practice as long as there is documentation that the critical value was then conveyed by the nurse to the ordering physician and/or licensed caregiver. As an alternative, some hospital networks have adopted a policy of reporting all critical values generated from the outpatient setting during "off" hours to a hospital emergency department (ED) or triage center. For example, the standard operating procedure at many hospitals is to report critical results to an ED attending physician, who can then decide whether to act on these results. This approach works well for hospitals, particularly because of the extensive electronic medical record (EMR) available to all clinicians. Such a call reporting system may be of limited benefit, however, at institutions with less robust EMRs. Results would still need to be conveyed to the responsible clinician for long-term management.

Escalation Policies:

What should a laboratory do when a technologist is not able to reach a responsible clinician with the critical value? In these circumstances, abandoning the call entirely is almost never an acceptable solution. An escalation policy or a fail-safe mechanism can be beneficial in such circumstances. An *escalation policy* would direct the laboratory technologist to contact a supervisor, pathology resident, and/or medical director to assist in critical value notification. This policy allows the technologist to refocus on the important task of laboratory testing, and it transfers the responsibility for notification to people who may have greater access to an inpatient or outpatient EMR and who can put the finding in a broader clinical context. In our experience, the pathology and laboratory medicine residents are usually able to contact a covering physician and convey these critical results. Verification of clinician notification should be subsequently conveyed back to the laboratory technologist (and entered into the LIS) to comply with CAP requirements. A *fail-safe mechanism* (or *safety net*) can also be used in cases in which notification continues to be unsuccessful. For example, if a "physician representing the laboratory" determines that immediate care of the patient may be required, the laboratory result and patient information might be conveyed to a physician in the ED to contact the patient directly. An approach to dealing with unreachable clinicians was recently proposed in a 2010 article. The report included algorithms for the inpatient and outpatient settings. After trying to contact the ordering provider, the algorithms include attempting to identify and contact the patient's primary care provider. If still unsuccessful, a chief of service or chief of staff would ultimately be notified. This system has an added benefit of bringing the issue of critical value reporting to the attention of a hospital or departmental administrator who might not otherwise be

aware of problems associated with the process. However, as with other systems in which the ordering provider is not the physician ultimately receiving the call, this process involves clinicians who may know very little about a patient's medical history and the potential ramifications of the critical value. It is advocated for active involvement of a pathology resident, an attending pathologist, and/or a medical director in difficult-to-convey critical value calls. It has been the experience that this involvement usually opens avenues (e.g. investigations via the EMR) that are not readily available to bench technologists. Such interventions can ultimately result in more rapid communication to a clinician familiar with the patient involved.

Repeat Critical Values:

Another common problem in critical value reporting is how a laboratory should handle *repeat critical values*, or subsequent critical values for a given assay on the same patient (but subsequent specimen). Approximately 70% of surveyed laboratories have a policy on repeat critical values. For those that do not, one is strongly recommended because it will clarify laboratory technologist responsibility and establish consistency in performance. There are only 3 options: (1) Call only the first critical value. (2) Call each critical value. (3) Call critical values once per interval of time. As clinicians become quickly annoyed by repetitive calls for critical values, and as such calls may have diminishing value over time, some advocate using interval criteria (for example, calling once every 24 hours). Determining whether critical results meet interval criteria might add additional tasks to laboratory technologists, although LIS or middleware-based rules can be used to perform comparisons automatically. Others have suggested that interval calling is appropriate for only select analytes. Of note, one study demonstrated that lower rates of undocumented critical value results in the medical record were associated with policies that require calling *all* critical results. Such an approach in high-volume laboratories, however, can be exceedingly burdensome to technologists and clinical staff, and there are minimal data to argue the clinical benefit of one approach vs the other. Critical value lists and procedures should include not just critical ranges but also the frequency of when to call for each given test. A laboratory may determine that some tests should be called with each critical value, while others (such as a markedly elevated blood urine nitrogen level) may be called using an interval approach. The laboratory policy should also clarify how to handle critical value notification after a subsequent normal result during the same interval (e.g. 8 AM, critical high; 9 AM, normal; then 10 AM, *critical high*). In most policies, if a normal test result occurs after a critical result, a subsequent critical result is considered new and would be called again. The laboratory's policy should be clear for such scenarios.

Critical Value Audits:

The importance of using critical value data to better understand laboratory process and preanalytic error cannot be overemphasized. For example, one program identified a specimen transport issue that led to falsely elevated K^+ results in some patients. Changing the transport requirements decreased the number of critical high K^+ results. This change not only enhanced the quality of the laboratory's performance but also eased the burden of unnecessary critical value calls. Analysis of critical value limits can also be used to estimate the impact on call frequency that would result from changing threshold requirements. Analysis can reveal differences in critical value patterns by patient location (e.g. falling hematocrit values on surgical services vs low K^+ values on medical services). This information could be used, for example, in the evaluation of new point-of-care programs. Others have used critical values analysis in studies of adverse events and clinical activity at discrete hospital locations. Critical value audits provide tremendous information on laboratory processes, and they are a great starting point for quality improvement initiatives.

Sterility (Infection Control) Maintenance in Relation to Diagnostic Laboratories

HAND WASHING

Infection control tips on hand washing: Hand washing is the single most important procedure for preventing transmission of infections. In contact with body secretions, excretions, Health care workers hands can carry bacteria, viruses, and fungi that may be potentially infectious. Hand washing antiseptic liquid soap (HITMAX™) is recommended. After contact with blood and or body substances, mucous membranes, soiled linen waste, or contaminated equipment. Immediately after removing gloves. Gloves may become perforated and bacteria can multiply rapidly on gloved hands. Before and after performing clean or sterile procedures. Between tasks at different body sites on the same patient (to prevent cross contamination). Between patients contact, and when other wise indicated to avoid transfer of microorganisms to other patients or environments. Before starting your shift duty and after completion of your duty. Before and after, drinking, smoking, applying cosmetics or preparing food. After personal use of toilet.

How to perform hand washing: Remove jewelery (rings, bracelets) and watches. Rinse hands and wrists under water. Apply antiseptic liquid soap (HITMAX™). Using friction, wash hands for at least 10 to 15 seconds cleaning between fingers, nail beds, palms, back of hands, wrists and forearms. If hands are visibly soiled, more time may be required. If there is no assistant to close the tap, cover the tap with fresh tissue paper and gently close. Taking care to see that the hand does not come in contact with unsterile tap. Hand washing technique charts are displayed near sinks and can be followed. Additional alcohol-based solutions with residual activity (STERIMAX™/ TRIOSEPT™) (HICC approved hand rub) are recommended for use in setting where hand-washing facilities are inadequate or inaccessible and hands are not visibly soiled. If these solutions are used as a substitute for hand washing, hand washing with antiseptic liquid soap and water should be performed as soon as possible after procedure.

Universal / Standard Precautions: Wash hands with antiseptic liquid soap and water immediately, if it become contaminated with blood or body fluids. Wash hands routinely before and after contact with a patient and after take off the gloves. Apply standard precautions to all patients regardless of their diagnosis, and to all contaminated equipment and materials. Use judgement in determining when protective barriers are necessary. Wear examination gloves when the hands are likely to be in contact with blood or body fluids, mucous membranes, skin that has open cuts or sores, or contaminated items or surfaces. Wear a protective gown (wear on) or apron when you are likely to soil the cloths with blood or body fluids. Use caution when handling contaminated sharps. Dispose them off immediately after use in a puncture resistant container. Avoid recapping needles. Use a one-handed recapping technique when absolutely necessary. Wear examination gloves whenever handling laboratory specimens and tubes of blood. While performing procedures, use technique that minimize the splashing or spraying of body fluids. Use protective eyewear and mask as needed. Use ambu bag with facemask when giving CPR (cardiac pulmonary resuscitation). Clean up spills of blood or body fluid promptly using gloves, a towel and a disinfectant (1% sodium hypochlorite or SURFAX™). Place soiled linen in a soiled linen room and take it to its final place of disposal Clean, disinfect or sterilize contaminated equipment between uses and before sending equipment for repairs. It is mandatory for all health care workers to take Hepatitis B vaccination. Report any needle stick injury or blood or body fluid exposures promptly to the infection control team.

How to handle a bloodspill? Wear gloves. Cover the spill with cotton cloth or newspaper or any other absorbent material. Pour 1% sodium hypochlorite or SURFAX™ solution over the spill. Wipe spilled area after 30 minutes. Discard soiled material in yellow colour waste bag. Clean / mop the area with hard surface disinfectant like MICROLYSE™. Discard gloves into red colour waste bag. Wash hands with antiseptic liquid soap and water.

Precautions while handling sharps: Use caution when handling all sharps. Do not bend, break or manipulate sharps by hand. Place disposable sharps in puncture resistant container immediately after use. Do not recap or remove needles from a syringe by hand unless no alternative exists. Use one handed recap technique if you must recap a needle. Use a device instead of your hand to pick up or remove contaminated needles. Use a mechanical device (dustpans) to clean up broken glassware. Avoid passing sharps from person to person.

WASTE MANAGEMENT

Purpose: Dispose biomedical waste as per the guidelines. **Responsibility:** Laboratory staff. **Equipment:** Plastic containers. Plastic liners (bags) of four different colours (yellow, red, white and black) and a variety of sizes (big, medium and small) for collection of different types of infected and non infected wastes, a sealing mechanism at the neck of the bag (tie). Gloves. Puncture proof can. Waste collection trolleys. **Procedure:** All hospital waste should be segregated at source by the generator in colour coded plastic bags. Disposal of infected waste – all infected waste is collected in a container with a yellow colour plastic liner (bag). When it is ¾ full seal and send it to the utility room for collection. Disposal of infected plastics and rubber - collected in a container with red colour plastic bag. When it is ¾ full seal and send it to the utility room for collection. Disposal of non-infective plastics, rubber and glass - collected in a container with white colour plastic bag. When ¾ full seal and send it to the utility room for collection. Disposal of non-infective general waste – in black plastic liners (bags) kept in every wastepaper bin. It is removed from the waste bins, collected in large bags and sent to the utility room for collection. The same person replaces fresh liners immediately (if required). Disposal of sharps – needles, syringes, must be placed in a puncture proof container. If syringe and needle cannot be disengaged, both are discarded together into the container. When the container ¾ full will be closed and send it to the utility room for collection. All the categories of waste are weighed and entered into the statement of bio-medical waste format at the generation area and also at the handover to the outsourced agency. This is entered into a computer. Disposal of infected liquid – urine, sputum and other body fluids of all patients are flushed down into the toilet flush. All staff must wear appropriate gloves and protective clothing when handling infected waste (linen, materials or equipment) and strict hand washing procedures must be followed after each contact with patients or infected materials. All new employees should receive mandatory training on handling waste.

VENIPUNCTURE

Venipuncture involves piercing a vein with a needle and collecting blood in a syringe or evacuated tube. **Equipment:** Tourniquet, gloves, syringe or vacutainer tube, vacutainer needle and adapter, alcohol swab, label, laboratory requisition form. **Procedure:** Wash your hands with antiseptic liquid soap (HITMAX™) thoroughly and wear gloves to prevent cross-

contamination. Explain the procedure to the patient. If the patient is on bed rest, ask him to lie supine, with his head slightly elevated and his arms at his sides. Ask the ambulatory patient to sit in a chair and support his arm securely on an armrest or table. Assess the patient's veins to determine the best puncture site. Observe the skin for the vein's blue color, or palpate the vein for a firm rebound sensation. Tie a tourniquet 2" (5cm) proximal to the area chosen. Then ask him to close his fist as you insert the needle and to open it again when needle is in place. Clean the Venipuncture site with an alcohol sponge. Wipe in a circular motion and allow the skin to dry before performing Venipuncture. Immobilize the vein by pressing just below the Venipuncture site with your thumb and drawing the skin taut. Position the needle holder or syringe with the needle bevel up and the shaft parallel to the path of the vein and at a 30-degree angle to the arm. If you are using a syringe, venous blood will appear in the hub; with draw the blood slowly, pulling the plunger of the syringe gently to create steady suction until you obtain the required sample. Pulling the plunger too forcibly may collapse the vein. If you are using a needle holder and evacuated tube, a drop of blood will appear just inside the needle holder. Grasp the holder securely to stabilize it in the vein, and push down on the collection tube until the needle punctures the rubber stopper. Blood will flow into the tube automatically. Remove the tourniquet as soon as blood flows adequately. After you have drawn the sample, apply gentle pressure to the puncture site for 2 or 3 minutes or until bleeding stops. If you have used a syringe, transfer the sample to a collection tube. Note: Avoid using veins in the patient's legs for venipuncture, if possible, because this increases the risk of thrombophlebitis. **Documentation:** Record the date, time and site of venipuncture; name of the test; the time the sample was sent to the laboratory.

FUMIGATION

With excellent housekeeping techniques routine fumigation is not required. However, most often only average cleaning is possible. In such circumstances fumigation can be done monthly and after epidemics. Fumigation should be done with eco-friendly fumigant like SILVICIDE™. Formalin should be avoided since it is carcinogenic. Doors, windows, walls and floors must be scrubbed thoroughly with HICC approved alkaline disinfectant (pH 8 to 9%) like MICROLYSE™ and water. Procedure: Doors, windows, walls and floors must be scrubbed thoroughly with MICROLYSE™ and water. All central oxygen and suction lines should be shut off. Ventilator outlets, air conditioner vents and gaps in doors and windows should be sealed airtight. All movable equipment and furniture should be removed and disinfected. Use a hydrogen peroxide + silver nitrate-based fumigant (SILVICIDE™) for fumigation. A fogger machine that can generate fumes at 5-15 microns particle size should be used for fumigation. **Fumigation procedure:** First calculate the area to be fogged (length x breadth x height in cubic feet). According to area, calculate the required quantity of disinfectant solution & Demineralised/ distilled water. Put your personal protective equipment like gloves, mask, lab coat. Take a measuring cylinder. Measure the required quantity of disinfectant solution & pour into the tank of the fogger. Again take the measuring cylinder & measure the required quantity of DM water, pour into the tank of the fogger. Gently shake the tank in clockwise direction. With switch in OFF position, plug the cord into power outlet of the proper voltage. Place the fogger at a corner of the room with nozzle direction pointed in desired position. Before switching ON ensure that intake air filter is in its place. Then switch ON the machine, fogger will generate the fog. Fogger machine should generate fine spray (5-15 microns) of the liquid & if a thick spray like comes out of the fogger, then adjust the knob (black color) to get a fine mist. Once you put ON the machine, come out of the room & lock the room. The room should be air-tight for maximum effect. After the said time open the door & go inside & put OFF the machine. Take the machine out & again close the room for 1 hour (no one is supposed to go inside for 1 hour which is the recommended contact time). After 1 hour the room is ready for use.

Managing exposure to blood or potentially infectious body fluids: Parental (needlestick) exposure to HIV infection is 0.3% risk of transmission of HIV. This is because of the low concentration of virus in the blood of infected patients. The risk in the case of HBV infected specimen in similar situations is 5-30 %.

Immediate care: For needle- stick injury: Briefly induce bleeding from wound. Wash for 10 minutes with HITMAX™ and water. For non- intact skin exposure: Wash with antiseptic soap and water or antiseptic like ZYTALL™/SAVINOX™/NUSEPT™. For mucosal exposure (e.g. Splash into eyes): Irrigate copiously by running a pint of normal saline over 10 minutes, the eye being held open the another person.

Reporting: All sharps injury (break of skin with any sharp instrument such as hypodermic needle previously used on a patient) and mucosal exposure (blood or body fluids coming into contact with eyes, mouth etc.) should be reported to the Hospital infection control chairman / nurse , immediately following exposure. All blood and body fluids with visible blood are considered infectious. Other body fluids may be potentially infectious and must be evaluated on case - to - case basis.

Protocol for Managing Exposure to Blood or Potentially Infectious Body Fluids in the Laboratory

Parenteral (needlestick) exposure to HIV infection is 0.3% risk of transmission of HIV. This is because of the low concentration of virus in the blood of infected patients. The risk in the case of HBV infected specimen in similar situations is 5-30 %.

Immediate care

For needle- stick injury: Briefly induce bleeding from wound. Wash for 10 minutes with soap and water, or a disinfectant. For non- intact skin exposure: Wash with soap and water or antiseptic. For mucosal exposure (e.g. Splash into eyes): Irrigate copiously by running a pint of normal saline over 10 minutes, the eye being held open the another person.

Reporting

All sharps injury (break of skin with any sharp instrument such as hypodermic needle previously used on a patient) and mucosal exposure (blood or body fluids coming into contact with eyes, mouth etc.) should be reported to the Consultant Pathologist/ Microbiologist and then to the Consultant Physician. All blood and body fluids with visible blood are considered infectious. Other body fluids may be potentially infectious and must be evaluated on case - to - case basis.

Management

Assessing the risk of transmission of HBV/ or HIV infection: For All exposure the following investigations need to be done: Index patient should be checked for the following: HIV antibody, HBsAg, HCV antibody. Health care worker: After obtaining consent, blood of the health care worker is checked for: HBsAg, HIV, Anti HBs antibodies. The blood samples for the investigations listed above are sent for rapid testing.

If the index case is HBsAg positive:

HCW	ACTION
HBsAg	
Antibodies >100 MIU	Reassure
Antibodies negative or <10 MIU	First of HBV vaccine and HBV immuno- globulin(0.6ml/kg-IM)
Antibody between 10- 100 MIU	Booster dose of vaccine
HBsAg Positive	Counselling

Follow up: Staff asked to come back for HBsAg testing at 3& 6 months and for completion of vaccination.

If the index case is HBsAg negative:

HCW	ACTION
HBsAg negative	
Antibodies >100 MIU	Reassure
Antibody negative or <100MIU	Vaccination (full or booster as required)
HBsAg positive	Counselling

If the index case is HIV negative or the index case is unknown:

Do not start chemoprophylaxis; consult the HICC chairman. The HCW is offered HIV antibody testing 0,1,3,6,12 months.

If the index case is HIV positive and HCW is HIV negative, the protocol given below is followed.

- For Indian setting all HIV positive index patients are to be considered as highly infectious.
- Chemoprophylaxis is best when started within 1-2 hours following exposure. The cut off period for chemoprophylaxis is 72 hours following exposure.
- The following investigations are to be done while starting chemoprophylaxis. Do not delay starting chemoprophylaxis for the sake of these investigations.
- Hemoglobin estimation, Platelet count, Reticulocyte count, WBC-total & differential counts, Serum creatinine, Liver function test, Random blood sugar.

Categorization of exposures with recommended prophylaxis

Use three drugs (Zidovudine + Lamuvudine + Indinavir) for All percutaneous injuries with contaminated sharps, Mucous membrane/ non-intact skin exposure with large volumes of body fluid for long duration.

Use 2 drugs (Zidovudine + Lamuvidine) for mucous membrane/ non-intact skin exposure with small volume body fluid for short duration.

If in doubt, start on 3 drug immediately and consult a senior person as early as possible.

Drug Regimen

3 drugs – Azidothymidine (Zidovudine) 200 mg thrice daily, Lamuvidine 150 mg twice daily and Indinavir 800 mg every 8 hours. If Indinavir is not available, Nelfinavir 750 mg three times is to be used.

2 drugs – Zidovudine and Lamuvidine.

Total duration 4 weeks for both.

If the index patient is already on anti-retroviral treatment with 1 drug, add 2 new drugs for the staff. The pharmacy will stock all 3 drugs used in chemoprophylaxis at all times.

Follow up of HCW

The HCW should be tested for HIV antibodies after 6 weeks, 3 months and 6 months following the exposure, irrespective of the HIV status of the index patient.

Counselling

Counselling of the HCW is performed when necessary.

How to handle a bloodspill?

- Wear gloves.
- Cover the spill with cotton cloth or newspaper or any other absorbent material.
- Pour 1% sodium hypochlorite solution over the spill.
- Wipe spilled area after 30 minutes.
- Discard soiled material in yellow colour waste bag.
- Clean / mop the area with detergent.
- Discard gloves into red colour waste bag.
- Wash hands with soap and water.

Standard Operating Procedures for a Medical Microbiology Lab

The medical microbiology laboratory helps health care providers discover and treat infections. As a result, the laboratory workers perform testing on potentially infectious materials and some very dangerous microbes. So it is essential that all laboratory personnel follow a standard operating procedure (SOP) which outlines every aspect of the work to be done in the lab.

Safety:

First and foremost in the microbiology lab should be safety. Any good set of standard operating procedures must include information on standard precautions. Standard precautions involve the use of personal protective equipment (like gloves and gowns) and techniques (like handling of body fluids) to minimize the possibility of contamination or infection. For example, the SOP could read, "Use of gloves to handle culture dishes is mandatory. Only open and process samples under the laminar flow hood."

Samples:

Laboratory results are only as good as the samples. If the samples are of bad quality, then the results will be invalid or not helpful in the treatment of the patient. The SOP should include what samples are acceptable for the different tests done in the microbiology lab. This includes describing how the sample should have been collected, how soon they should be delivered to the lab and what kind of special preparations should be done to them once they arrive. For example, the SOP could read, "Catheterized urine is the best specimen for a urine culture, followed by clean caught urine. Bagged samples or randomly collected samples should be avoided. All urine samples are to be processed within an hour of collection."

Testing:

The SOP must include the procedures for performing any test that is done in the lab. This includes a step-by-step description of how the tests are performed and what the expected results are. The SOP should also describe any quality control testing performed along with other tests. Quality control testing is essential to ensure the validity of the results. An example of this would be a description of the strep throat screen: "Insert the sample swab into the test tube and add the reagents per the package insert of the testing kit. Wait 5 minutes. Remove the swab while allowing as much of the remaining solution to stay in the tube. Insert the testing strip and wait 3 minutes. After 3 minutes, read the results. Make sure that a result was noted in the quality control strip. Results are unacceptable if the quality control strip did not yield the expected results."

Reporting:

Finally, the SOP must include the appropriate format of the final report that is going to be sent out to the provider who ordered the test. The report must include the testing results, normal (expected results), and any interpretation of the results that is necessary. If any of the test results are deemed to be "critical," the SOP must address how those critical results will be reported and to whom. For example, "All positive results on a cerebrospinal fluid sample must be reported to the attending physician or the physician on call immediately. Do not leave a message with clerical staff and do not finalize the report until you note who received the critical message and what time that message was delivered."

Biosafety

Introduction

The purpose of this article is to be a resource for information, guidelines, policies, and procedures that will enable and encourage those working in the laboratory environment to work safely and reduce or eliminate the potential for exposure to biological, chemical and radioactive materials hazard. The goal of the laboratory safety is to minimize the risk of injury and illness to laboratory workers by ensuring they have the training, information, support and equipment needed. The manual promotes safe and practical laboratory procedures, included laboratory biosafety, laboratory biosecurity, microbiological risk assessment, laboratory biosafety levels, information on the use of personal protective equipment, laboratory animal facilities, laboratories equipment, laboratory techniques, hazard communication and packing of infectious substances, biosafety and biotechnology, the proper use of disinfection and sterilization, the use and storage of chemicals and radioactive materials, biosafety officer and biosafety committee and the proper methods of waste disposal. Finally, emphasis must be placed on the practices and procedures used by trained laboratory staff. Since "no biosafety cabinet or other facility or procedure alone guarantees safety unless the users operate safe techniques based on informed understanding." It is the responsibility of everyone, including managers and laboratory workers, to use the information available in these manual and to perform their work in a safe and secure manner.

Laboratory Biosafety

'Laboratory biosafety' is the term used to describe the containment principles, technologies and practices that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release. A laboratory biosafety goal is to ensure that hazardous materials will be handled and disposed of in such a way that people, other living organisms, and the environment are protected from harm. Safety awareness must be a part of everyone's habits, and can only be achieved if all senior and responsible staff has a sincere, visible, and continuing interest in preventing injuries and occupational illnesses.

Manual of Laboratory Safety

Laboratory facilities are designated as: Basic Biosafety Level 1 (BSL-1), Basic Biosafety Level 2 (BSL-2), Containment Biosafety Level 3 (BSL-3) and Maximum containment Biosafety Level 4 (BSL-4).

Biosafety Level 1

Practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans. *Bacillus subtilis, Naegleria gruberi*, infectious canine hepatitis virus, and exempt organisms. BSL-1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for handwashing.

Biosafety Level 2

Practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. Hepatitis B virus, HIV, the *Salmonella*, and *Toxoplasma* are representative of microorganisms to this containment level. BSL-2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a BSC or safety centrifuge cups. Personal protective equipment should be used as appropriate, such as splash shields, face protection, gowns, and gloves. Secondary barriers, such as hand washing sinks and waste decontamination facilities, must be available to reduce potential environmental contamination.

Biosafety Level 3

Practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. *Mycobacterium tuberculosis,* St. Louis encephalitis virus, and *Coxiella burnetii* are representative of the microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols. At BSL-3, more emphasis is placed on: Primary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations should be performed in a BSC or other enclosed equipment, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and ventilation requirements that minimize the release of infectious aerosols from the laboratory.

Biosafety Level 4

Practices, safety equipment, and facility design and construction are applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Agents with a close or identical antigenic relationship to BSL-4 agents also should be handled at this level. When sufficient data are obtained, work with these agents may continue at this level or at a lower level. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at BSL-4. Biosafety level designations are based on a composite of the design features, construction, containment facilities, equipment, practices and operational procedures required for working with agents from the various risk groups. The primary hazards to personnel working with BSL-4 agents are respiratory exposure to infectious aerosols, mucous membrane or broken skin exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals, pose a high-risk of exposure and infection to laboratory personnel, the community, and the environment. The assignment of an agent to a biosafety level for laboratory work must be based on a risk assessment. Such an assessment will take the risk group as well as other factors into consideration in establishing the appropriate biosafety level. For example, an agent that is assigned to Risk Group 2 may generally require Biosafety Level 2 facilities, equipment, practices and procedures for safe conduct of work. However, if particular experiments require the generation of highconcentration aerosols, then Biosafety Level 3 may be more appropriate to provide the necessary degree of

safety, since it ensures superior containment of aerosols in the laboratory workplace. The biosafety level assigned for the specific work to be done is therefore driven by professional judgment based on a risk assessment, rather than by automatic assignment of a laboratory biosafety level according to the particular risk group designation of the pathogenic agent to be used.

Classification of infective microorganisms by risk group:

Risk Group 1 (no or low individual and community risk): A microorganism that is unlikely to cause human or animal disease.

Risk Group 2 (moderate individual risk, low community risk): A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.

Risk Group 3 (high individual risk, low community risk): A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.

Risk Group 4 (high individual and community risk): A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available. Effective biosafety practices are the very foundation of "laboratory biosecurity" activities**.** In the absence of careful implementation, various aspects of biosafety may conflict with laboratory biosecurity. For example, controls that reduce unauthorized access might also hinder an emergency response by fire or rescue personnel. Mechanisms need to be established that allow entry by emergency responders but ensure uninterrupted and constant laboratory biosecurity, control and accountability. Signage may also represent a potential conflict between biosafety and laboratory biosecurity. In the past, biohazard signs placed on laboratory doors identified the biological agents present in the laboratory. However, as a laboratory biosecurity measure to better protection it is recommended certain information on biohazard signs to the laboratory biosafety level, the name and telephone number of the responsible investigator, and emergency contact information.

Microbiological risk-assessment

The backbone of the practice of biosafety is risk-assessment. While there are many tools available to assist in the assessment of risk for a given procedure or experiment, the most important component is professional judgment. Risk assessments should be performed by the individuals most familiar with the specific characteristics of the organisms being considered for use, the equipment and procedures to be employed, animal models that may be used, and the containment equipment and facilities available. The laboratory director or principal investigator is responsible for ensuring that adequate and timely risk assessments are performed, and for working closely with the institution's safety committee and biosafety personnel to ensure that appropriate equipment and facilities are available to support the work being considered. Once performed, risk assessments should be reviewed routinely and revised when necessary, taking into consideration the acquisition of new data having a bearing on the degree of risk and other relevant new information from the scientific literature. One of the most helpful tools available for performing a microbiological risk assessment is the listing of risk groups for microbiological agents. However, simple reference to the risk grouping for a particular agent is insufficient in the conduct of a risk assessment. Other factors that should be considered, as appropriate, include: (1) Pathogenicity of the agent and infectious dose. (2) Natural route of infection. (3) Other routes of infection, resulting from laboratory manipulations (parenteral, airborne, ingestion). (4) Stability of the agent in the environment.

Prevention of MRSA Infections

FAQs For the Workplace

Can you get MRSA from someone at work?

MRSA is transmitted most frequently by direct skin-to-skin contact or contact with shared items or surfaces that have come into contact with someone else's infection (e.g., towels, used bandages).

MRSA skin infections can occur anywhere. However, some settings have factors that make it easier for MRSA to be transmitted. These factors, referred to as the **5 C's**, are as follows: **C**rowding, frequent skin-to-skin **C**ontact, **C**ompromised skin (i.e., cuts or abrasions), **C**ontaminated items and surfaces, and lack of **C**leanliness. Locations where the **5 C's** are common include schools, dormitories, military barracks, households, correctional facilities, and daycare centers.

If you have MRSA, can you go to work?

Unless directed by a healthcare provider, workers with MRSA infections should not be routinely excluded from going to work.

- Exclusion from work should be reserved for those with wound drainage ("pus") that cannot be covered and contained with a clean, dry bandage and for those who cannot maintain good hygiene practices.
- Workers with active infections should be excluded from activities where skin-to-skin contact with the affected skin area is likely to occur until their infections are healed.

What should you do if you think you have a staph or MRSA infection?

See your healthcare provider/ physician or surgeon and follow your healthcare provider's advice about returning to work.

If you have staph, or a MRSA skin infection, what can you do to prevent the spread of MRSA at work and at home?

You can prevent spreading staph or MRSA skin infections to others by following these steps:

- **Cover your wound.** Keep areas of the skin affected by MRSA covered. Keep wounds that are draining or have pus covered with clean, dry bandages. Follow your healthcare provider's instructions on proper care of the wound. Pus from infected wounds can contain staph and MRSA, so keeping the infection covered will help prevent the spread to others. Bandages or tape can be discarded with the regular trash.
- **Clean your hands.** You, your family, and others in close contact should wash their hands frequently with soap and warm water or use an alcohol-based hand sanitizer, especially after changing the bandage or touching the infected wound.
- **Do not share personal items.** Avoid sharing personal items such as uniforms, personal protective equipment, clothing, towels, washcloths or razors that may have had contact with the infected wound or bandage.
- **Talk to your doctor.** Tell any healthcare providers who treat you that you have or had a staph or MRSA skin infection.

What should you do if you suspect that your uniform, clothing, personal protective equipment or workstation have become contaminated with MRSA?

- Wash uniforms, clothing, sheets and towels that become soiled with water and laundry detergent. Drying clothes in a hot dryer, rather than air-drying, also helps kill bacteria in clothes. Use a dryer to dry clothes completely.
- Cleaning contaminated equipment and surfaces with detergent-based cleaners or Environmental Protection Agency (EPA)-registered disinfectants is effective at removing MRSA from the environment. Because cleaners and disinfectants can be irritating and exposure has been associated with health problems such as asthma, it is important to read the instruction labels on all cleaners to make sure they are used safely and appropriately. Where disinfection is concerned, more is not necessarily better.

What can employers do to prevent the spread of staph or MRSA at the workplace?

- Place importance on worker safety and health protection in the workplace
- Ensure the availability of adequate facilities and supplies that encourage workers to practice good hygiene
- Ensure that routine housekeeping in the workplace is followed
- Ensure that contaminated equipment and surfaces are cleaned with detergent-based cleaners or Environmental Protection Agency (EPA)-registered disinfectants.

Other FAQs About MRSA

Transmission and Risk:

Who gets staph or MRSA infections?

Approximately 25% to 30% of the population is colonized (when bacteria are present, but not causing an infection) in the nose with staph bacteria. Staph infections, including MRSA, occur most frequently among persons in hospitals and healthcare facilities (such as nursing homes and dialysis centers) who have weakened immune systems. These healthcare-associated staph infections include surgical wound infections, urinary tract infections, bloodstream infections, and pneumonia.

How common are staph and MRSA infections?

Staph bacteria are one of the most common causes of skin infection in the developed countries and are a common cause of pneumonia, surgical wound infections, and bloodstream infections. The majority of MRSA infections occur among patients in hospitals or other healthcare settings; however, it is becoming more common in the community setting. Data from a prospective study in 2003, suggests that 12% of clinical MRSA infections are community-associated, but this varies by geographic region and population.

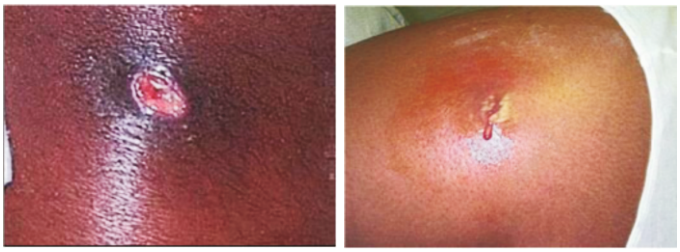
Signs and Symptoms:

What does a staph or MRSA infection look like?

Staph bacteria, including MRSA, can cause skin infections that may look like a pimple or boil and can be red, swollen, painful, or have pus or other drainage. More serious infections may cause pneumonia, bloodstream infections, or surgical wound infections.

Staphylococcus aureus, often referred to simply as "staph," is a type of bacteria commonly carried on the skin or in the nose of healthy people. Sometimes, staph can cause an infection. Staph bacteria are one of the most common causes of skin infections in the United States. Most of these skin infections are minor (such as pustules and boils) and can be treated without antibiotics. However, staph bacteria also can cause serious infections (such as surgical wound infections, bacteremia /

septicemia, and pneumonia).
Methicillin-resistant *Staphylococcus aureus* (MRSA) refers to types of staph that are resistant to a type of antibiotic methicillin. MRSA is often resistant to other antibiotics, as well. While 25% to 30% of the population is colonized with staph (meaning that bacteria are present, but not causing an infection with staph), approximately 1% is colonized with MRSA.
Staph infections, including MRSA, occur most frequently among persons in hospitals and healthcare facilities (such as nursing homes and dialysis centers) who have weakened immune systems. These healthcare-associated staph infections include surgical wound infections, urinary tract infections, bacteremia / septicemia, and pneumonia.
Staph and MRSA can also cause illness in persons outside of hospitals and healthcare facilities. MRSA infections that are acquired by persons who **have not** been recently (within the past year) hospitalized or had a medical procedure (such as dialysis, surgery, catheters) are known as community-associated MRSA infections. Staph or MRSA infections in the community are usually manifested as skin infections that look like pimples or boils and occur in otherwise healthy people.



Usual presentation of MRSA infections

Prevention:

How can I prevent staph or MRSA skin infections?

Practice good hygiene:

- Keep your hands clean by washing thoroughly with soap and water or using an alcohol-based hand sanitizer.
- Keep cuts and scrapes clean and covered with a bandage until healed.
- Avoid contact with other people's wounds or bandages.
- Avoid sharing personal items such as uniforms and personal protective equipment.

Treatment:

Are staph and MRSA infections treatable?

Yes. Many staph skin infections may be treated by draining the abscess or boil and may not require antibiotics. Drainage of skin boils or abscesses should only be done by a healthcare provider.
However, some staph and MRSA infections are treated with antibiotics. If you are given an antibiotic, take all of the doses, even if the infection is getting better, unless your doctor tells you to stop taking it. Do not share antibiotics with other people or save unfinished antibiotics to use at another time.
If after visiting your healthcare provider the infection is not getting better after a few days, contact them again. If other people you know or live with get the same infection tell them to go to their healthcare provider.

Standard Precautions

1) Hand Hygiene

Perform hand hygiene after touching blood, body fluids, secretions, excretions, and contaminated items, whether or not gloves are worn. Perform hand hygiene immediately after gloves are removed, between patient contacts, and when otherwise indicated to avoid transfer of microorganisms to other patients or environments. When hands are visibly soiled with blood or other body fluids, wash hands with soap and water. It may be necessary to perform hand hygiene between tasks and procedures on the same patient to prevent cross-contamination of different body sites.

2) Gloving

Wear gloves (clean non-sterile gloves are adequate) when it can be reasonably anticipated that contact with blood or other potentially infectious materials, mucous membranes, non intact skin, or potentially contaminated intact skin (e.g., of a patient incontinent of stool or urine) could occur. Remove gloves after contact with a patient and/or the surrounding environment (including medical equipment) using proper technique to prevent hand contamination. Do not wear the same pair of gloves for the care of more than one patient. Do not wash gloves for the purpose of reuse since this practice has been associated with transmission of pathogens.

3) Mouth, nose, eye protection

Use PPE to protect the mucous membranes of the eyes, nose and mouth during procedures and patient-care activities that are likely to generate splashes or sprays of blood, body fluids, secretions and excretions. Select masks, goggles, face shields, and combinations of each according to the need anticipated by the task performed.

4) Gowning

Wear a gown, that is appropriate to the task, to protect skin and prevent soiling or contamination of clothing during procedures and patient-care activities when contact with blood, body fluids, secretions, or excretions is anticipated.

5) Appropriate device handling of patient care equipment and instruments/devices

Handle used patient-care equipment soiled with blood, body fluids, secretions, and excretions in a manner that prevents skin and mucous membrane exposures, contamination of clothing, and transfer of microorganisms to other patients and environments. Ensure that reusable equipment is not used for the care of another patient until it has been appropriately cleaned and reprocessed and that single-use items are properly discarded.
Clean and disinfect surfaces that are likely to be contaminated with pathogens, including those that are in close proximity to the patient (e.g., bed rails, over bed tables) and frequently-touched surfaces in the patient care environment (e.g., door knobs, surfaces in and surrounding toilets in patients' rooms) on a more frequent schedule compared to that for other surfaces (e.g., horizontal surfaces in waiting rooms).

6) Appropriate handling of laundry

Handle, transport, and process used linen to avoid contamination of air, surfaces and persons.

Contact Precautions

1) Patient placement

In Patient placement in hospitals and LTCFs, When single-patient rooms are available, assign priority for these rooms to patients with known or suspected MRSA colonization or infection. Give highest priority to those patients who have conditions that may facilitate transmission, e.g., uncontained secretions or excretions. When single-patient rooms are not available, cohort patients with the same MRSA in the same room or patient-care area. When cohorting patients with the same MRSA is not possible, place MRSA patients in rooms with patients who are at low risk

for acquisition of MRSA and associated adverse outcomes from infection and are likely to have short lengths of stay. In general, in all types of healthcare facilities it is best to place patients requiring Contact Precautions in a single patient room.

2) Gloving

Wear gloves whenever touching the patient's intact skin or surfaces and articles in close proximity to the patient (e.g., medical equipment, bed rails). Don gloves upon entry into the room or cubicle.

3) Gowning

Don gown upon entry into the room or cubicle. Remove gown and observe hand hygiene before leaving the patient-care environment. After gown removal, ensure that clothing and skin do not contact potentially contaminated environmental surfaces that could result in possible transfer of microorganism to other patients or environmental surfaces.

4) Patient transport

In acute care hospitals and long-term care and other residential settings, limit transport and movement of patients outside of the room to medically-necessary purposes. When transport or movement in any healthcare setting is necessary, ensure that infected or colonized areas of the patient's body are contained and covered. Remove and dispose of contaminated PPE and perform hand hygiene prior to transporting patients on Contact Precautions. Don clean PPE to handle the patient at the transport destination.

5) Patient-care equipment and instuments/devices

In acute care hospitals and long-term care and other residential settings, use disposable noncritical patient-care equipment (e.g., blood pressure cuffs) or implement patient-dedicated use of such equipment. If common use of equipment for multiple patients is unavoidable, clean and disinfect such equipment before use on another patient. In home care settings limit the amount of non-disposable patient-care equipment brought into the home of patients on Contact Precautions. Whenever possible, leave patient-care equipment in the home until discharge from home care services. If noncritical patient-care equipment (e.g., stethoscope) cannot remain in the home, clean and disinfect items before taking them from the home using a low - to intermediate-level disinfectant. Alternatively, place contaminated reusable items in a plastic bag for transport.

6) Environmental measures

Ensure that rooms of patients on Contact Precautions are prioritized for frequent cleaning and disinfection (e.g., at least daily) with a focus on frequently-touched surfaces (e.g., bed rails, overbed table, bedside commode, lavatory surfaces in patient bathrooms, doorknobs) and equipment in the immediate vicinity of the patient.

7) Discontinuation of Contact Precautions

No recommendation can be made regarding when to discontinue Contact Precautions.

Universal Guidelines in an Influenza like illness Pandemic Scenario (like covid 19)

General Guidance

This guidance is to address the general workflow safety concerns of laboratory personnel during the COVID-19 pandemic. All laboratories should perform site- and activity-specific risk assessments to determine the most appropriate safety measures to implement for particular circumstances. In addition, facilities should adhere to local policies and procedures as well as all applicable federal, state, and local regulations and public health guidelines.

Risk assessments should include the following considerations:

- Analyze the number of people that the laboratory space can realistically and safely accommodate while maintaining
- Assess the flow of personnel traffic. Where possible, design one-way paths for staff to walk through the laboratory space.
- Assess procedures for cleaning and sanitizing commonly shared equipment and areas—for example, counters, benchtops, and desks—to ensure clean surfaces and equipment for all users.
- Review emergency communication and operational plans, including how to protect staff at higher risk for severe illness from COVID-19.

Every institution should have a COVID-19 health and safety plan to protect employees. This plan should be shared with all staff. Ideally, this plan would:

- Instruct sick employees to stay home and not return to work until the criteria to discontinue home isolation are met, in consultation with healthcare providers and state and local health departments.
- Provide information on whom employees should contact if they become sick.
- Implement flexible sick leave and supportive policies and practices. If sick leave is not offered to some or all employees, the institution should consider implementing emergency sick leave policies.
- Designate someone to be responsible for responding to employees' COVID-19 concerns. Employees should know who this person is and how to contact this person at all times.
- Provide employees with accurate information about COVID-19, how it spreads, and the risk of exposure.
- Reinforce training on proper handwashing practices and other routine infection control precautions to help prevent the spread of many diseases, including COVID-19.

Ensure that employees have access to personal protective equipment (PPE); disinfectant products that meet EPA's criteria for use against SARS-CoV-2 external icon; and soap, clean running water, and drying materials for handwashing, or alcohol-based hand sanitizers that contain at least 60% ethanol or 70% isopropanol.

Social Distancing

To the extent possible, adhere to social distancing recommendations by adjusting staff schedules, adding additional shifts, or implementing non-overlapping teams to minimize personnel contact. Identify laboratory tasks and activities that can be performed with reduced or no face-to-face interactions. Examples include limiting the number of laboratory meetings that occur and, when possible, using remote collaboration tools (such as video and phone conferencing), even for those who work in the same location or building. To the extent possible, reconfigure workspaces and locations of shared equipment to reduce crowding. Create one-directional paths and workflows. Declutter workspaces and dispose of unnecessary items to help with reconfiguration. If reconfiguration is not possible, consider placing barriers (plexiglass, partition, plastic, etc.) between computer workstations, desks, or equipment that position staff six feet apart from each other. Minimize personnel traffic and interactions by limiting visits from vendors and other external partners; engage with them virtually whenever possible.

Face Coverings

To help slow the spread of COVID-19, CDC recommends wearing face coverings in settings where social distancing measures are challenging to maintain, like office spaces, computer workstations, and break rooms. In general, laboratory employees should wear a face covering in laboratory spaces that do not have requirements for respiratory PPE and where other social distancing measures are difficult to maintain. Any face covering that is worn inside a laboratory area where personnel work with potentially infectious material should subsequently not be worn outside of that laboratory area. Laboratory PPE are critical supplies, and employees should refrain from removing them from the laboratory for general use. Site- and activity-specific risk assessments, as well as available resources, should determine where specific facial protection, such as disposable masks, should be used.

These face coverings should not be used in place of recommended personal protective equipment (PPE).

- Face coverings are not intended to protect those who wear them and are not considered PPE.
- All staff should follow established PPE requirements for working in laboratory spaces.

Wash hands before putting on face coverings and minimize the removal while in the laboratory. The guidance below describes how to remove a face covering and replace it with a clean face covering:

- Take off the face covering carefully.
- Be careful not to touch eyes, nose, or mouth when removing a face covering.
- Untie the strings behind the head or stretch the ear loops.
- Handle only by the ear loops or ties.
- Place reusable cloth face coverings in a bag and close the bag until it can be washed.
    1. Cloth face coverings should be washed frequently.
    2. Staff are responsible for maintaining and cleaning their cloth face coverings.
- Wash hands immediately after removing.

Depending on the facility's design or configuration, additional physical barriers, such as a face shield, plexiglass, partition, or plastic barriers, may be needed to achieve social distancing goals.

Personal Hygiene and Disinfection

As more workers return to the laboratory, extra measures may be needed to ensure a clean and appropriate environment. Reevaluate current protocols for cleaning, use of PPE, and handwashing. High-touch locations and equipment with a high frequency of handling and contact present a higher probability of contamination in the work area and should be disinfected frequently. Increasing the number of available cleaning supplies and distributing them throughout the laboratory can encourage staff to more frequently clean surfaces and equipment. Use visual reminders, such as posters displayed throughout the laboratory environment, common areas, and restrooms, to emphasize the importance of hand hygiene and to encourage frequent handwashing. Hands should be washed regularly with soap and water for at least 20 seconds. An alcohol-based hand sanitizer containing at least 60% ethanol or 70% isopropanol can be used when soap and water are not available.

Universal Work Precautions (UWP) for Lab Personnel (Especially in relation to HIV transmission)

Health care personnel (HCP) can acquire certain illnesses beyond those acquired by all others who live and work in our society, by virtue of their profession. HCPs are at risk of acquiring any of the whole gamut of infections from patients/ specimens, which may be viral, bacterial, parasitic or fungal.

However this risk due to occupational exposure can be minimized if not obliterated altogether, if we follow universal work precautions. Today with WHO estimates of above 4 million HIV positive persons in India, there is an urgent need to review UWP. Besides HIV, there is the very real danger of acquiring Hepatitis B and Hepatitis C in exactly the same way as HIV and could also be fatal. Hepatitis B is 100 times more infectious than HIV. Besides Hepatitis B is also far more prevalent in India in comparison to HIV with estimated carriers being between 30 and 40 million, a considerable number being infectious. However fortunately effective vaccination is available for Hepatitis B therefore it is strongly recommended for all levels of health care workers. Much of the contamination in the laboratory occurs as a result of penetrating injuries caused by sharp objects and the spilling and splashing of specimen materials.

Components of UWP

1. Hand-washing
2. Barrier precautions (Mask, cap, plastic apron and protection of feet)
3. Careful handling of all kinds of sharps and needles
4. Effective disinfection
5. Sterilization
6. Correct disposal of different kinds of wastes generated in a health care facility.

Guidelines of Basic Practices and Procedures

- Prevention of puncture wounds, cuts and abrasions and protection of existing wounds skin lesions, conjunctiva and mucosal surfaces.
- Application of simple protective measures designed to prevent contamination of the person and his/her clothing.
- Good basic hygiene practices, including regular hand washing.
- Control of surface contamination by containment and disinfection procedures.

Biosafety Regulations for Laboratory Procedures

- Safe disposal of contaminated waste.
- Wear gloves when handling infectious materials or where there is a possibility of exposure to blood and other body fluids. All laboratories that work with material that is potentially infected with HIV require a generous supply of good quality gloves.
- Discard gloves whenever they are thought to have become contaminated or perforated, wash your hands and put on new gloves. Alternatively where there are economic constraints wash gloved hands whenever they get contaminated with blood/body fluids before collecting further samples.
- Do not touch your eye, nose, or other exposed membranes or skin with your gloved hands.

Biosafety Regulations for Laboratory Procedures

- Sharps, reusable blades, cystoscopy instruments, endoscopy instruments use CIDEX (2% Glutaraldehyde) or 5% Korsolex. Disinfection in 30 minutes.
- Use autoclaving for other reusable items (e.g. needle holders, gowns etc.).
- Wherever autoclaving is not possible boiling must be for 30 minutes at the least.

Waste Disposal

Divide waste into 3 parts at source.

i) Household type non-infectious waste
   - Not to be decontaminated.
   - To be disposed off as such.

ii) Infected Sharp Waste Disposables (needles/ surgical instruments)
   - Place in puncture proof container containing disinfectant (1% bleach prepared every morning).
   - Final disposal

iii) Infected non-sharp waste
   - Is to be decontaminated.
   - Placed in disinfectant 5-10% bleach as the case maybe, (left over blood, tissues etc)

Final Disposal After Infection

- Purchase of needle destroyer if resources permit.
- Incineration of all infected waste.
- Deep burial in controlled landfill sites (protected from all sides)
- Shredding of disposable plastic-ware waste.

Post Exposure Care

Minor bleed with percutaneous inoculation, open skin wound breached skin, exposed mucous membrane.

First Aid

- Allow to bleed by squeezing.
- Wash with water
- Antiseptic

Report

Employee identification date, time with place of accident.
Circumstances around accident.
Action taken.

Initial Consultation

Easy access to medical advise with counseling. Consult physician for AZT prophylaxis regime if medication available.

Laboratory Testing

After consent with counselling within 2 weeks, 5 weeks, 12 weeks, 24 weeks.

Clinical Follow up

For fever, pharyngitis, rash, malaise, lymphadenopathy, myalgia, arthralgia within 6 months.

Safety Precautions

- Do not leave the workplace or walk around the laboratory while wearing gloves.
- Wash hands with soap and water immediately after any contamination and after work is, finished. If gloves are worn, wash

your hands with soap and water after removing the gloves. This is a vital and simple precaution that is often overlooked.

- Wear a laboratory gown or uniform when in the laboratory. Wrap around gowns are preferable. Remove this protective clothing before leaving the laboratory.
- When work with material that is potentially infected with HIV is in progress, close the Laboratory door and restrict access to the laboratory. The door should have a sign: **BIOHAZARD NO ADMITTANCE**
- Keep the lab clean, neat and free from extraneous materials and equipment.
- Disinfect work surfaces when procedures are completed at the end of each working day.
- An effective all purpose disinfectant is a hypochlorite solution with a concentration of at least 0.1% available Chlorine (1 g/liter, 1000 ppm)
- Whenever possible, avoid using needles and other sharp instruments. Place used needles syringes and other sharp instruments and objects in a puncture resistant container. Do not recap - used needles and do not reuse needles from syringes for disposal.
- Never pipette by mouth.
- Perform all technical procedures in a way that minimizes the risk of creating aerosols, droplets, splashes or spills.
- Use a biosafety cabinet while working- on aerosolizing specimen. Do not eat, drink, smoke, apply cosmetics or store food or personal items in the laboratory.
- Make sure that there is an effective insect and rodent control programme.

If laboratory personnel have lesions on hand and feet then

a. If superficial he or she should wear protective dressing and wear gloves over it.
b. If wound is deep or raw then the concerned person should not handle samples till the wound heals.

If there is a pregnant health care worker then in view of the occupational risk to the woman and the developing fetus, on compassionate grounds, where possible she should be involved in clerical tasks or stay away from work for the duration of her pregnancy.

Containing Spills

- Cover the spill immediately with absorbent material to avoid aerosolization.
- Soak the material by pouring disinfectant on it.
- Leave the area for 30 minutes.
- Mop with more adsorbent material after wearing gown, mask and gloves.
- Place material in appropriate bin, for disposal (autoclaving or incineration).

QA & QC

TroubleShooting Guidelines

A Compilation from

The Crux
BIMONTHLY FORUM FOR THE LABORATARIANS

POCT Quality Assurance (QA)

Quality Assurance is a vast subject and is of fundamental importance to every laboratory department. It could be argued that QA involvement in POCT is of even greater significance, since laboratory testing is being performed by non-laboratory professionals whose training in quality issues may have been less rigorous than that given to laboratory professionals. This argument presents a strong case for mandatory competency. Quality Assurance is an overview and examination of a complete system, from approaching the patient with the intention of obtaining a sample to looking at the subsequent result report from the laboratory or POCT analyser. Large parts of Quality Assurance involve Quality Control (QC), correlations and External Quality Assessment (EQA). Without successful QC results, correct patient results cannot be assumed. Although not covered in the table below, attention must be given to analyser maintenance schedules, hardware and software replacements and upgrades, service records, annual preventative maintenance reports, complete documentation records and quality control records. A good approach is to divide the QA process into three phases, Pre-Analytical, Analytical and Post-Analytical.

All staff who operate POCT equipment should have an awareness of and be responsible for:

- identifying the patient correctly
- the quality of the patient's sample to be analysed
- ensuring the analyser or procedure is calibrated correctly
- analysing any required Quality Control (QC) samples
- regular analyser or procedure maintenance
- documentation of all QC results, patient results, maintenance records, troubleshooting records, error messages
- their current competency requirements for all relevant POCT processes

Pre-analytical	Analytical	Post-Analytical
patient ID confirmation	analyser operation	correct sample disposal
sample quality	operator and patient ID entry	cleanliness and tidiness
aseptic technique	sample mixing and preparation before analysis	examination and interpretation of results
correct collection tubes	CO-Ox haemoglobin measurements	notification of abnormal results
order of draw	correct analysis technique	inclusion in patient record
capillary samples	analyser maintenance	audit trails
sample labelling	calibrations	competency records
sample transport	quality control troubleshooting	proficiency testing

Pre-Analytical Technique

Patient ID confirmation

To confirm the ID of a patient, two points of ID must be cited. The acceptable choices are in descending order of importance: NHI number, e.g., XYZ9876; hospital temporary allocated number; surname and given name; date of birth.

Sample quality

Regardless of whether the puncture site be a heel, digit, ear lobe or elbow, it is important for the sake of a good sample that the puncture site be:

- clean
- dry
- healthy
- ***not*** from the drip arm
- free from contaminants, e.g., saliva, sugar (from sweets, drinks, etc)
- alcohol free (from sterilizing swabs)

Other types of samples, like CSF, urine, stool, drain fluids, wound swabs, skin scrapings, etc must also be collected in as careful, aseptic manner as possible. The quality of the sample determines the quality of the results.

Aseptic technique

- Always wash your hands before approaching the patient
- Wear gloves (and wash your hands between patients, even if wearing gloves).
- Wear prescribed protective clothing if dealing with infectious patients.
- Prepare the puncture site as described above.
- Always wash your hands after leaving the patient.

Correct collection tubes

Although Phlebotomy staff collect most patient samples via venepuncture, it is important for clinical staff to know which collection tubes are appropriate for which blood test.

The order of draw is also very important.

From first to last, in sequence: blood cultures, red top, SST, blue, green, lavendar, gray. The reason for this sequence is to minimise tube contamination from additives and anticoagulants. Sterilise the entry point of the blood culture tubes before inoculating them.

These colour codes relate to Becton-Dickinson brand products, whether for macro-collecting (venepuncture) or micro-collecting (capillary) samples:

Blood culture tubes	Blood cultures
Red top (Plain tube- no additives) SST(serum separator tube)	Some Biochemistry, Serology, Virology, crossmatching.
Dark blue (chemically clean)	Trace Metal tests
Light blue top (citrate)	Haemostasis tests
Green top (Lithium heparin)	Routine Biochemistry tests
Lavendar or purple top (EDTA)	Haematology; blood lead testing, crossmatching
Gray top (fluoride)	Glucose, lactate

Capillary blood gas samples

Capillary blood gases need to be collected into special balanced-heparin blood gas capillary collection tubes. Always make sure your sample is WELL MIXED. Try not to expose a blood gas capillary sample to ambient air as gas partial pressures in the sample may change. Mix your capillary blood gas sample by holding the tube horizontally and rolling it back and forth between finger and thumb. Don't slosh it back and forth along the tube. Do not use pO_2 readings- capillary blood collects are exposed to too much ambient oxygen. In addition, peripheral perfusion is too variable for consistent pO_2 readings.

Sample labeling

All collection tubes and request forms must be labelled clearly with the patient's ID sticker. When this is not available, their IP No./Op no. or temporary hospital number, surname and date of birth must be written on the tube(s). The request form must state clearly which tests are required and include the same patient information as the sample collection tubes. The request form should also include the date and time of sample

collection as well as a clear indication of the requesting doctor and location of the patient.

Sample transport

A long delay can occur when samples sent to the laboratory for analysis are delivered tardily. This may be for a variety of reasons- a delay in pickup from the ward or delivery forgotten *en route* to the lab. A delay of several hours may result in lowered blood glucose, raised potassium, phosphate and CK- all a result of cellular metabolism or perhaps, haemolysis. If a blood gas is delayed more than 30 minutes, the results may be useless. Many hospitals now utilise a pneumatic tube delivery system which solves the transport delay times. However, tube breakdowns can occur, for a variety of reasons, human, electronic or mechanical. Point of care testing obviates any need for sample transport to the laboratory.

Analytical Technique

Analyser operation; operator and patient ID entry

Please refer to the relevant operator's manual for analyser operation. All clinical staff operating POCT analysers should have a current competency which includes instructions on how to operate POCT equipment. All POC Tests must be able to be traced or audited. This means that print outs, transcriptions or electronic results must contain the POCT operator's ID (or initials at the very least) and the patient's ID. The date and time of analysis and sample type must also be noted on the results.

Sample mixing and preparation

After collecting your venous sample, gently invert the collection tubes at least five times, to thoroughly mix the anticoagulant in the tube with the blood. If a blood gas sample, at the blood gas analyser, mix the arterial or capillary blood gas sample by holding the tube horizontally and rotating it between fingers and thumb. Alternate this action with gentle inversions of the syringe. There must be ***no air bubbles*** present in the syringe or capillary sample. Try not to slosh the blood back and forth along the length of the capillary tube. Keep the blood/air contact to a minimum. Remove caps and any metal flea or mixer before aspirating the sample.

If you are analysing an arterial blood gas sample for CO-Oximetry:

i.e., (haemoglobins and oxygen saturation parameters), it is vitally important that the sample be well-mixed. Gently rotate and invert the blood gas syringe for *at least 30 seconds* before aspirating the sample. The blood must be as homogenous as if it were still in the patient. (The reason for this is in the method of analysis of the haemoglobins- a sample of blood is electronically haemolysed and the intensity of the clear red solution of blood is measured at certain wavelengths).

Correct analysis technique

Always follow the manufacturer's guidelines on sample analysis. Clear simple instructions should be given in the Operator's Manual near the analyser. Failure to analyse the sample properly will lead to erroneous quality control and patient results.

Analyser maintenance

Where an analyser is in use, correct maintenance schedules must be maintained. Always fulfill any daily maintenance criteria required and sign and date the maintenance logs. Note any consumable replacements, e.g., reagents or calibrators and also make note of any changes in lot numbers. **Any change in the status quo of the analyser, like reagent changes, cartridge or test strip lot number changes, etc, MUST be followed by a successful calibration and after that, a successful quality control sample result.**

Calibration

All analytical procedures, whether POCT or laboratory-based in nature must be calibrated before use. Calibrating a procedure means that you are defining a starting point from which all other results flow. A quality control (QC) sample tests the correctness of the calibration. Many people confuse these two concepts. Most blood gas analysers self-calibrate every 30 minutes. If analyser conditions have not altered between timed calibrations, no QC is necessary. Manual POC testing that does not involve analysers may not need to be calibrated: a visually interpreted urine test strip is an example of this. Wherever possible though, a quality control sample should always be analysed daily before patient testing begins, or if the conditions of analysis change, as mentioned above.

Quality Controls

Quality Control (QC) samples and patient samples should be analysed using identical techniques and under identical conditions. The only difference is that the value(s) of the QC sample are known, whereas the patient 's values are not. If the correct QC samples are obtained, we can say with confidence that the last calibration was successful, the analyser or process is functioning correctly and that the patient's results will therefore be correct.

If you have not analysed a QC sample and obtained acceptable results, you cannot assume that the patient results will be correct.

Troubleshooting

Each POCT analyser is accompanied by a Users' Manual, compiled by the POCT Coordinator. Within this Manual will be a rudimentary section on troubleshooting. Often a problem that seems insurmountable can be easily resolved by restarting the analyser- turn it off, wait 10 seconds and turn it on again. On the other hand, a blood clot can be tricky to remove from a blood gas analyser. An Error Log can be found in each Manual. Please take the time to document any problems that occur. This is important, as a documented history of problems can be very useful for warranty or replacement purposes. It is also **a Certification requirement** that all errors be documented and that a complete audit trail exists for each entry, including resolution of the error or problem. If you have a problem that you cannot resolve or you have insufficient time to repair the problem, please contact the POCT Coordinator who is there to be used as a resource and a source of assistance.

Post-Analytical Technique

Correct sample disposal; cleanliness and tidiness

All samples must be discarded into Medical Waste containers. Any body fluid-contaminated tissues or other waste must also be discarded into Medical Waste containers.

DO NOT throw any body fluid-contaminated waste into regular paper waste containers.

All sharps- needles and so forth must be discarded into a Sharps Container.

Always clean up any spills - use Surfax or sodium hypochlorite based disinfectant - to ensure the analyser and surrounds are maintained in a clean and tidy condition. Always leave the POCT analyser or place of work in the same condition as you would expect to find it.

Examination, interpretation, notification , inclusion.

- All POCT operators should have an awareness of the meaning of the results they generate. The results should be examined with respect to units of measurement, reference intervals, meaning of abnormal results, implication to the patient and so on.
- Abnormal whole blood results that are not expected or do not fit with the clinical picture of the patient should be confirmed before being reported. Use a new sample- it is possible that the initial sample is contaminated in some way- tissue fluid, drip arm, clots, etc. If the

result is still abnormal, send a *venous sample* to the laboratory for plasma analysis. (There are several shortcomings to whole blood analysis).

- Please take note and document ANY ERROR CODES generated and how the results may be affected. Please contact the POCT Coordinator for advice, if required.
- Any abnormal results should be referred to the patient's physician or senior ward/clinic staff for action and advice.
- All results should be recorded in, transcribed or affixed to the patient's notes, along with the operator ID , and the time and date of analysis.

Audit trails

All POCT results must be of the same standard as laboratory results. This statement lies at the heart of successful POCT. One of the requirements of laboratory testing is the successful passing of audits. All POCT results must be able to pass an audit. This means that the results must be able to be traced to:

- the patient
- the operator
- the machine or process used
- relevant maintenance and QC logs
- the date and time
- current competency records held by the operator

External Quality Assessment and Proficiency Testing

All POCT must be quality controlled (QC) regularly. While QC is generally regarded as a day-to-day procedure for each analyser or procedure, External QA is performed at less regular intervals, typically monthly. Proficiency Testing or sample correlation testing is another approach to QC. Proficiency testing can involve more than a single laboratory or POCT site.

Quality Assurance in Routine Immuno-hematology

Every laboratory that performs immuno-hematological examinations must regularly conduct internal quality controls and participate in external quality control trials.

- Clinical validation of the results is not feasible
- Immuno-hematological methods are not quantitative
- Biological reagents with great variability and storage instability are used
- Reagents are subject to only limited controls by official overseeing institutions or government regulatory agencies
- Reagents and methods need to be compatible with each other
- Numerous sources for potential methodical errors as well as many patient-specific interfering factors exist
- Confirmation of the adequate sensitivity and specificity of the methods used by external quality control, e.g. inter laboratory surveys
- Regular internal control of the quality of the reagents used
- Regular internal control of the equipment used
- Constant monitoring of the accuracy of test performance, test sensitivity, and test specificity by internal quality controls
- Detection and differentiation of patient-specific interfering factors by regular internal quality control

Internal quality control includes controls of the tests, the reagents, and the equipment.

Test controls as performed in the individual test methods. They include positive and negative controls as well as auto-controls. These controls are indispensable even in emergency cases; they should not be postponed until after the other tests have become positive.

All reagents used on a daily basis should be examined under identical conditions (quality control location) at least once a week according to a standard protocol, if possible in the test methods used; the results of these examinations should be compared with a target-value protocol. If abnormalities are found, the test needs to be repeated. If the abnormal result is confirmed, the test is repeated again using a new sample from the same batch.

Reagents	Examination (frequency)
Test cells	Hemolysis (t), reactivity (w)
Test sera	Contamination (t), reactivity (w)
Albumin/LISS	Contamination (t), reactivity (w), pH (b), albumin contents (b), molarity (b)
Enzymes	Contamination (t), reactivity (w)
Wash solution	Contamination (t), pH (daily), electrolytes (b)
AB serum	Contamination (t), pH (t'), reactivity (w)

In Table given above controls are listed that should be performed ither each time the reagents are used (t), weekly as part of the reagent control (w) or only in the case of a new batch (b).

The presence of hemolysis or contamination is checked by visual inspection before the reagents are stirred and pipetted.

Control of the reactivity of reagents

The weekly reagent controls serve to check sensitivity and specificity

Target value protocol in conjunction with reagent controls							
Sera/media	Test media				Coomb's	O D	O D
	A1	A2	B	O	controls	weak	neg
AB serum	-	-	-	-	-	-	-
Anti-A	~4+	~2+	-	-	-	-	-
Anti-B	-	-	~ 4+	-	-	-	-
Anti-D	-	-	-	~4+	-	-	-
Anti-D	-	-	-	-	-	*~2+	-
Anti human globulin serum	-	-	-	-	~2+	-	-
Anti Le	-	-	-	~2+	-	-	-

*In the Indirect Antiglobulin Test

Equipment controls

The demands placed on equipment controls, as far as immuno hematology is concerned, do not differ from other, customarily performed equipment-specific controls in laboratories; some of these controls are also performed according to the manufacturers' specifications.

Therefore, no further details are presented here.

External quality controls

External quality control is primarily based on regular participation in inter laboratory surveys. In addition, all unresolved immuno-hematological problems should be mailed to reference laboratories for further examination.As part of inter laboratory surveys, all parameters should always be checked which are investigated in that particular laboratory. In the immuno-hematological laboratory, this applies especially to the ABO and Rh blood groups including the subgroups and weak variants, antibody screening, identification and quantification as well as the characterization of auto antibodies. If different methods are used for antibody screening and cross-matches, these tests must be checked independently from each other.

Analyses for inter laboratory surveys must be performed with coded material under routine conditions.

Quality Assurance for Routine Hemostasis Laboratories

Routine investigations for hemostasis are quite easy to perform and appear deceptively easy. A number of pretest variables effect accuracy and precision of coagulation results. The variables may arise out of collection techniques, sample processing, selection and preparation of reagents etc. In order to reduce variability and errors one must clearly understand the impact and ultimately elimination of the variations, thereby improving accuracy and reproducibility.

Patient preparation

Although no special patient preparation is necessary, however, samples should not be taken from patients who have had heavy meals or have heavily exercised. Heavy exercise alters coagulation factors and heavy meal alters plasma clarity by making it opaque (lipemic). Photo-optic instruments mis-read clot appearance timings. For the same reason turbid, hemolysed and icteric samples should ideally be avoided.

Sample collection techniques

Withdraw blood without undue venous stasis into a plastic syringe with a short 19 to 20 SWG needle. Venipuncture should be a clean one. On experiencing difficulty change both, the syringe and the needle AND the vein. Do not use tourniquet forextended periods of time, also do not pat the venipuncture site. A clean catch is essential to prevent formation of micro clots at the venipuncture site; this in turn consumes clotting factors, which will lead to artificially prolonged results. Using short, big bore needles allow free flow of blood and reduce contact with the metal surface, otherwise, extended metal contact initiates clotting or partial consumption of factors which leads to erroneous results. Frothing while dispensing blood into the tubes also induces micro clot formation.

Sample preparation

The anticoagulant of choice is buffered sodium citrate (3.2% or 0.109M). Factors V and VII remain stable in it. These factors are more labile in oxalate and heparin neutralizes thrombins action on fibrinogen. Buffered citrate neutralizes CO_2 that is absorbed while sample processing.

The buffered 3.2% Sodium Citrate negates the effects of absorbed CO_2 which happens on centrifugation of the sample for separating the plasma. Using 3.8% citrate would produce unduly prolonged timings. The optimum citrate to blood ratio is 1:9. With apt molarity of citrate, all available calcium is bound and clotting is prevented. A shift in the ratio leads to erroneous results as is explained: **More blood less citrate:** Calcium chelation will be inadequate leading to micro clot formation and consumption of factors which at the end would lead to unduly prolonged timings. **More citrate less blood:** Excess citrate would consume calcium from the reagents and would again give prolonged timings eventually. For APTT the optimum concentration of $CaCl_2$ is 0.02 M. This replaces the calcium necessary to activate the intrinsic coagulation cascade. This ultimately generates thrombin from the prothrombin via the coagulation cascade. Appropriate volumes of $CaCl_2$ should be aspirated for the days work. Prewarmed should always be discarded at the end of the day. The anticoagulant to blood ratio of 1:9 is for a normal hematocrit (one that falls within normal range for the age and sex of the patient).

The formula for the anemic and polycythemic patients is

$$C = 1.85 \times 10^{-3} (100-H) V$$

C = Volume of sodium citrate in ml.
V = Volume of whole blood-sodium citrate in ml.
H = Hematocrit in percentage.

When the PCV is higher than 55% the patient blood contains so little plasma that excess unutilized anticoagulant remains and is available to bind reagent calcium leading to prolongation of the results.

On the other hand if the PCV is less than 20% the patient blood contains excess of plasma but less of anticoagulant and the chelating activity of citrate will not be sufficient to bind the calcium present in sample. This will lead to formation of clots invitro, consumption of factors and prolongation of results.

Sample Processing and Storage

Ideally containers for collection and processing should be made of plastic or siliconised glass. They should be scrupulously clean and dry. Scratched glass surfaces can activate the clotting mechanism. Leavening agents used by the plastic industry have an inhibitory effect. All containers should be free from detergents, acids and alkalies. These chemicals have an effect on the pH. Change in pH effects factor stability. Detergents inhibit reactive characteristics of the sample/reagent mixture. Nothing is better than using clean disposable labware.

Time: Ideally the samples must be processed immediately. At room temperature (22°-24°C) the tests must be conducted within 2 hours while if held at 2°- 4°C the time available is 3 hours. Plasma samples should not be held at 4°- 8°C for prolonged periods as they can undergo cold activation. Samples obtained for factor and fibrinolysis assays should be stored in crushed ice if delay is anticipated. Citrated blood for platelet aggregation studies should remain in capped tubes at room temperature (20°-25°C) before testing.

Centrifugation: All samples collected must be tightly capped to prevent absorption of atmospheric CO_2, which can shift the pH, and hence the eventual results. Centrifugation speeds are also important. The PT uses platelet poor plasma (PPP) while the APTT uses platelet free plasma (PFP). Excessive centrifugation on account of heat generation can destroy the clotting factors. Under centrifugation would invariably leave platelets in the plasma leading to activation of the clotting mechanism in vitro again leading to erroneous results. Normally centrifugation for 15 minutes approximately 1500 G yields PPP and at 2000 G produces PFP. The "G" is the function of length of rotor head and RPM. Each laboratory should calculate its own ideal centrifugation time and speed.

Calibration of instruments / equipments

Water baths or heating blocks should be calibrated and preset at 37°C ± 0.5°C. The pH, ionic strength and the reaction temperature are very important. At manufacturer's level, all calibrations are conducted at optimal conditions and similar environment must be used at the end user labs. Sample and reagent dispensing volumes must be accurate and precise.

Storage of Reagents

The pipettes / tips used for sucking reagent should be absolutely clean and dry. Otherwise they can spoil the reagents quickly. Repeated intrusions into the reagent vial exponentially increase the chances of reagent contamination and destruction. After use, the reagent vials must be stored back at the recommended storage temperatures. Thermal and cryogenic stresses to the reagents should be avoided at all costs. Freezing destroys the colloidal nature of the reagents and these when used give erroneous results. Bringing the reagents /samples to room temperature should be a two-stage process. First, let the reagent attain room temperature and then the required dispensed volume can be taken to 37°C (the testing temperature).

End Point Reading

Used manually, the end point definition can vary. Ideally the end point should be read as "as soon as the first fibrin strand is visible and the gel clot formation begins". The background against which the reading is taken should be well lit. As user variations are important it is better not to change the testing personnel.

As all automated systems detect clots differently, they have their own ideal sets of circumstances. Preferably low turbidity reagents should be employed.

Drugs/Clinical Conditions influencing patient results

PT tests are influenced on administration of the following drugs

PT may be shortened	PT may be prolonged by
Drugs: antihistamines butabarbital phenobarbital caffeine oral contraceptives vitamin K	Drugs: corticosteroids EDTA asparaginase clofibrate erythromycin ethanol tetracycline, aspirin, anticoagulants (warfarin, heparin)

APTT tests are influenced by administration of the following drugs

APTT may be shortened	APTT may be prolonged by
Drugs: oral contraceptives conjugated estrogen therapy	Drugs: diphenylhydantoin heparin warfarin naloxone Radiographic reagent

Thrombin test time is prolonged in the following circumstances

Normal neonate, SLE, Macroglobulinemia, Presence of exogenous/ endogenous circulating anticoagulants, Hepatic diseases, Toxemia of pregnancy, Multiple myeloma.

MNPT and INR

MNPT is a critical requirement in the derivation of INR. Ideally each laboratory must derive its own MNPT from 20 or more normal subjects for a given PT reagent and Lot under consideration. This corrects intra laboratory test variables that influence the PT results. By definition INR is

$$INR = \left(\frac{\text{Patient PT in seconds}}{\text{Mean Normal Prothrombin Time}} \right)^{ISI}$$

It is advisable to use Prothrombin time reagent having ISI nearer to the value of one.

Quality Control Aspects

If using reconstituting lyophilized reagents then the quality of water used should be impeccable.

Additionally the quality assurance for coagulation-based reagents must be performed preferably on a daily basis. Normal and abnormal controls should be run everyday.

AT ALL COSTS ADHERE TO THE MANUFACTURER'S INSTRUCTIONS.

Prothrombin Time / INR

Oral anticoagulants are used for:

1. Prevention of primary and secondary venous thromboembolism
2. Prevention of systemic arterial embolism in patients with mechanical prosthetic valves or with atrial fibrillation.
3. Prevention of acute myocardial infarction in patients with peripheral arterial disease.
4. Prevention of stroke and recurrent infarction.

Warfarin sodium (a vitamin K antagonist) is the most commonly used oral anticoagulant in use today. Prothrombin time test is used to detect coagulation disorders related to the extrinsic pathway namely II, V, VII, X and fibrinogen and for monitoring oral anticoagulant therapy. Rate of depression of the individual clotting factors is determined by their biological half-life. Factor VII falls most rapidly followed by factors IX, X, and II. As the active clotting factor levels begin to fall, the PT results start to prolong. Factor V and Fibrinogen are not affected by anticoagulants. On the other hand upon withdrawal of the oral anticoagulant therapy these factors return to normal levels in the reverse order and so do the PT results.

Since the individual thromboplastin reagent preparations differ significantly in their sensitivities to the deficiencies of the vitamin K dependent coagulation factors, the use of 'seconds' or PTR (ratio) or PTI (index) as a reporting format has led to confusion among clinicians. In order to correctly dose deserving patients with oral anticoagulants so as to achieve the dual goal of adequate anti-coagulation and reduce the risk of bleeding simultaneously, the WHO introduced a method for monitoring patients stabilized on oral anticoagulant therapy using International Normalized Ratio (INR). The INR normalizes the PT ratio to an International Reference Preparation (IRP) of thromboplastin.

This has ensured that a uniform intensity of oral anticoagulant therapy is used world wide.

Reporting PT in seconds, or as PTR, or as PTI, or as % activity by using different reagents can give remarkably differing values. By mid 1970s, WHO and other agencies started work towards standardization of PT for monitoring oral anticoagulant therapy. The first step was preparation of IRP's (International Reference Preparations) of thromboplastins. This was done to have an accurate reference reagent which could serve as a standard for calibrating responsiveness / sensitivity of commercial thromboplastin reagents. The IRPs prepared were assigned ISI values of 1.0. The ISI (International Sensitivity Index) indicates the sensitivity of test thromboplastin in comparison to the IRP. When the calibration line has a slope=1.0, the test thromboplastin equates to the IRP in sensitivity and responsiveness. In other words, the ISI is a measure of the responsiveness (prolongation of PT values) of a given thromboplastin to the reduction of the vitamin K dependent coagulation factors. The lower the ISI (more close it is to 1.0) the more sensitive is the thromboplastin. Manufacturers assign ISI value for each lot of thromboplastin by the WHO recommended methods to assist laboratarians in calculation of the INR.

Results are reported by using the formula

$$INR = \frac{(\text{Patient PT in seconds})^{ISI}}{(\text{Mean Normal PT})}$$

Many automated and semi-automated coagulometers can do the calculation themselves.

Other Factors Influencing the INR

The variability of the responsiveness of the PT reagents, is corrected through the"ISI" calibration, however three additional technical factors influence the INR.

Derivation of MNPT: Ideally each laboratory must derive its own MNPT from 20 or more normal patients for a given PT reagent and Lot under use. This corrects within laboratory test variables that influence PT results. If "normal control plasmas" are used in place of patient plasma for arriving at the MNPT it can effect the evaluation of the patients level of anticoagulation. If the control time is greater than the MNPT, the PT ratio for any patient PT will be smaller, potentially leading to over coagulation. If the control time is lesser than MNPT

Magnitude of difference in the ISI value of test thromboplastin and IRP (ISI=1.0) and Method of clot detection employed during PT test: INR loses some precision when comparisons are made with thromboplastins with markedly different ISI values as against the IRP (ISI=1.0) and different methods of clot detection e.g., manual, mechanical, optical etc. Therefore manufacturers must provide ISI values adapted to the method used for clot detection. Also the reagent used for reporting results should ideally be as close to 1.0 as possible.

Since all manufacturers (for QC and ISI evaluation) and diagnostic community worldwide use 3.2% TriSodium Citrate as the preferred anticoagulant of choice for coagulometry studies, it is imperative that all laboratories also employ the same strength of TriSodium Citrate for their evaluations. This is mandatory to avoid giving falsely high INR results that may be obtained by using 3.8% TriSodium Citrate.

Practical Considerations for Warfarin therapy

Oral administration of Warfarin results in a rapid absorption of the drug, however an observable anticoagulant effect is delayed. This delay is due to the time required for des-carboxylated (dysfunctional) vitamin K dependent factor to replace the normal clotting factors. Depending upon the dose the delay may range from 1 to 7 days. The early anticoagulant effect is mainly caused by the loss of fully carboxylated procoagulant factor VII which has a half life of approximately 5 hours.

However these oral anticoagulant reagents also cause suppression in the synthesis of natural anticoagulant protein C and protein S. Due to this, in the early phase of initiation of oral anticoagulant therapy there is a potential for initial prothrombotic effect. This event underlines the syndrome of coumadin induced skin necrosis, especially in patients with hereditary deficiencies of protein C and protein S.

Therapy can begin with an anticipated maintenance dose (e.g.,: 5 mg/day). A small loading dose of about twice the average maintenance dose may also be used initially. This dosage achieves a steady state anticoagulant effect in 5-7 days. The use of large loading dose (e.g., 20-40 mg) has little benefit. Such dosing not only produces a marked factor VII deficiency (which alone may not protect against thrombosis) but also an acquired protein C deficiency, which could produce a prothrombotic state.

If the need for antithrombotic effect is more urgent, heparin should be given as indicated. Heparin is then discontinued when INR is in the therapeutic range.

Considerations for frequency of Laboratory tests for monitoring oral anticoagulant therapy

PT monitoring of patients initially should be performed daily for the first 5 days and 2 to 3 times a week for the first 1 to 2 weeks. Depending on the stability of the PT results from the third week onwards frequency of monitoring may be further reduced to every 4-8 weeks. While some patients on long term Warfarin therapy have unexpected fluctuations in dose response, some have unexplained requirement for increase in dosage.

The unexpected fluctuations in dose response could be due to: change in diet, undisclosed concomitant drug use, poor patient compliance, surreptitious self-medication, alcohol consumption, intermittent illness or unsuspected changes in the responsiveness of the PT reagent used to perform the PT test.

Advantages of the INR system

Major advantage of the INR system is that it helps alleviate confusion in the interpretation of PT results. Usually laboratory changes like change in thromboplastin and/ or equipments could go unnoticed by the attending physicians. The INR remains constant even with such changes.

INR system affords comparison of PT results between laboratories. INR system provides a more accurate and convenient means of monitoring patients who travel extensively.

INR therapeutic ranges for different clinical conditions are based on international collaborative studies. Usage of standardized dosage reduces the risk of thrombotic episodes or secondary bleeding.

Disadvantages of the INR system

The prothrombin time test is always a part of the preoperative screening panels. It is also frequently used to evaluate other hemostatic disorders such as liver disease, DIC, LA, hereditary factor deficiencies and acquired vitamin K deficiency. Since these disorders have been excluded from the derivation of the ISI, INR has a diagnostic and therapeutic value mainly applicable for patients stabilized on oral anticoagulants. Therefore laboratories may prefer to report both the INR and patient's time in seconds depending upon the clinical application.

- The INR systems effectiveness would still depend on the calibration of the coagulation instruments as well as thromboplastin reagents used.
- Derivation of the correct MNPT and use of the mean normal range in each laboratory.
- Usage of thromboplastin reagents with ISI of preferably 1.0 or as close to 1.0 as possible.
- The correct use of the formula to compute the INR.
- Uniform understanding of the INR system by clinicians as well as laboratarians.

Principles of Quality Assurance and Standards for Clinical Chemistry

I. Preanalytical Factors Important in Clinical Chemistry
II. Analytical Factors Important in Clinical Chemistry
III. Postanalytical Factors Important in Clinical Chemistry

I. Preanalytical Factors Important in Clinical Chemistry

A. Specimen Collection, Handling, and Transport to the Laboratory

Samples should be appropriately collected, handled and transported to the laboratory in a timely manner, dependent on the type of specimen and its stability. For any assay performed in the laboratory, information concerning sample requirements, proper collection, handling, and delivery or shipping procedures should be available to clients in a laboratory services manual, special information sheets, journal or newsletter articles, other written materials, or by personal or telephone conversation.

B. Specimen Identification

Specimens should be identified with pertinent information as determined by the laboratory, name of clinic or doctor, address, telephone and fax numbers, e-mail address, location from which the specimen was collected, etc. on the submission container and submission form.

C. Test Identification

The requested test(s) should be clearly stated on the submission form.

D. Specimen Accessioning

The specimen should be correctly entered into the laboratory system. Test request entry, delivery of the specimen to the correct location, and specimen aliquoting (if necessary) or sharing between laboratories or departments (i.e.,pharmacology, endocrinology, and clinical chemistry) should be coordinated.

E. Client Communication and Education

Communication between laboratory personnel and clients should be timely and courteous regarding pre-analytical factors influencing laboratory test results (e.g, incomplete submission forms, inappropriate sample or sample handling or poor sample quality). Clients should be informed of the expected time for receipt of preliminary and final reports.

F. Personnel Safety

Personal protective equipment should be appropriate for handling specimens and equipment used for clinical chemistry. Safety procedures and disposal of all samples and supplies should be appropriate for the type of specimen. Personnel should receive safety and biohazard training and information about exposure to potentially hazardous chemicals or infectious agents. All training should be documented.

G. Laboratory Environment

The laboratory space should be clean, well lit, and organized to ensure proper achievement of the above goals.

H. Personnel Requirements

Laboratory personnel should have training in specimen handling and sample preparation. Documentation of training, continuing education and periodic proficiency assessment should be at the discretion of the laboratory director.

II. Analytical Factors Important in Clinical Chemistry

A. Monitoring
B. Method Validation
C. Instrumentation
D. Quality Control
E. Procedures Manual
F. Comparison of Test Results

A. Monitoring

1. Internal monitoring should include the following

(a) Quality of water (as specified by instrumentation and assays).
(b) Stability of electrical power (as specified by instrumentation).
(c) Temperatures of water bath, refrigerator, and freeze(recommended at least monthly).
(d) Regular calibration of analytical balances and pipettes (recommended annually).
(e) Maintenance of up-to-date procedure manuals with clearly stated dates when procedures are first implemented and when any changes are made and implemented.
(e) Maintenance of adequate inventory, with proper storage and handling.
(g) Maintenance of a log of changes in any procedures, problems or other factors affecting methods, as well as actions that resolved the problem. All entries should be clearly dated and signed by laboratory personnel.

2. External monitoring should include participation in an external proficiency program

(a) All participating laboratories should analyze the same materials.
(b) Results should be tabulated regularly (monthly, quarterly) and distributed to participants with statistical summaries and comparison of participating laboratories with mean indices expressing the closeness of individual laboratory results to the group mean.
(c) Means should be calculated and analyzed based on identification of the method (same methods compared).
(d) Each laboratory should carefully assess the validity of their reported performance and consider any changes indicated by the proficiency program.

B. Method Validation

Method validation should be performed before a test procedure is placed into routine use. Validation may be accomplished by thoroughly testing reference materials or by comparison of results of tests performed by an alternative method. For each method, the laboratory should verify the manufacturer's claims and any adjustments before initiating patient testing.

Method validation should provide evidence of the following:

1. Accuracy : Perform either (a) or (b)

(a) Run known value substance and compare results to expected value.
(b) Perform split sample patient comparison between existing method of known accuracy and new method.

2. Precision : Perform either (a) or (b)

(a) Run 10 replicates of 2 levels of quality control (QC) samples.
(b) Gather 21 results; 7 results in each of 3 separate runs (better estimate of day -to-day precision, as well as within-run precision).

With results from (a) or (b) determine mean, standard deviation (SD) and coefficient of variation (CV). Determine whether within-run SD is acceptable.

3. Sensitivity : Perform (a), (b) or (c)

(a) Assess manufacturer's claims.
(b) Use concentration of low calibrator or another sample or fluid low levels of analyte.
(c) Run a series of dilutions and assess acceptability of performance

4. Specificity : Perform (a) or (b)

(a) Use published list of interfering substances, check with manufacturer.
(b) Assess known or suspected interfering substances by spiking specimens or use patient material with known conditions

5. Linear reportable range

(a) Establish upper and lower limits for reporting patient values based on calibration materials.
(b) For the lower limit, there should be confirmation of the discriminatory ability of the test.
(c) The highest calibration point is the maximum upper limit and the lowest calibration point or zero should be the minimum lower limit for reporting patient results.

6. Linearity : Perform either (a) or (b)

(a) Determine by analyzing multiple dilutions of either a high calibrator, control or patient samples with increased levels of analyte.
(b) Analyze calibrators of variable, known concentrations.
(c) Linearity should be established at the time of validation and whenever new or altered reagents are used.

7. Reference intervals

(a) The laboratory should establish or validate existing reference intervals for each method before reporting results.
(b) Parallel tests should be run to confirm reference intervals for controls when changing reagents or QC lot number.

C. Instrumentation

1. Instrument performance

The equipment and instrument used must be capable of providing test results within the laboratory's stated performance characteristics. These include: detection limits, precision, accuracy, specificity, sensitivity, freedom from interferences and related test variables (refer to previous section on method validation). Additional points to consider: Instruments with adjustable setting for different substances and/or species should be carefully checked for compliance performance characteristics as defined, Compare and make adjustments for performance characteristics as defined by the laboratory and the manufacturer, Make sure certain species differences are accommodated.

2. Functional checks

(a) Appropriate function checks should be made on all instruments. These are critical operating characteristics of an instrument, i.e., stray light, zeroing, electrical levels ,optical alignment, background checks, etc.
(b) Laboratory personnel should recheck and /or calibrate each instrument daily or once per shift, prior to patient testing, to ensure that it is functioning correctly and is properly calibrated. This includes QC.

3. Calibration

(a) Instruments should be calibrated every 6 months or more frequently if indicated by: manufacturer's recommendation, after major service, QC outside limits or troubleshooting indicates need, laboratory determination that volume, equipment performance or reagent stability indicate a need for more frequent calibration.
(b) After calibration, controls should be run.

4. Laboratory personnel knowledge of equipment and its use, including, but not limited to:

(a) Linearity differences from possible manufacturer's range (human) to animal.
(b) Effects of hemolysis, lipemia, icterus, caretenoid pigments (especially large animals), and different anticoagulants on each assay.
(c) Reportable ranges.
(d) Species-specific ranges and reference intervals.
(e) Expected abnormal ranges..
(f) Common problems encountered with veterinary samples.
(g) Regular instrument maintenance schedule.
(h) Replacement of inadequate or faulty equipment.
(i) Problem-solving procedures, troubleshooting.

D. Quality Control

1. For each run, at least 2 controls should be assayed. Use of 'high' and 'low' abnormal controls is recommended.
2. Maximum length of a run is 24 hours. If the instrument manufacturer requires more frequent controls, observe the recommended frequency (i.e, some blood gas instruments).
3. Verify that the instrument is stable over the "run time". During a validation check, controls are assayed more frequently to establish run time.
4. Establish QC frequency; consider the following:

(a) Test volume (number performed each run or day) and frequency.
(b) Technique dependence of the method.
(c) Analyte or reagent stability.
(d) Frequency of QC failures.
(e) Training and experience of personnel.
(f) Cost of QC (increasing frequency adds to cost-per-test).

5. Quality control parameters

(a) Mean, SD and CV should be calculated (minimum number = 20).
(b) Controls should be assayed in the same manner as patient specimens.
(c) A mechanism should be in place to determine whether testing personnel follow policies and procedures correctly.
(d) Use of Westgard multirule procedures or other rules based on QC validation is recommended.
(e) Policies and procedures should be written and available in a laboratory. Standard Operating Procedures (SOP) manual to ensure accurate and reliable test results .
(f) An SOP manual should have clearly marked and dated entries of current procedures (manufacturer package inserts are sufficient as long as verified) and when any changes are made and implemented.
(g) QC records should be reviewed frequently to ensure that when QC values fail to meet the criteria for acceptability, suitable action is taken.
(h) Control products should be purchased commercially, if possible. If using calibrators as controls, use a different lot as QC material. If patient pooled samples are used, establish the mean value of all analytes (minimum n = 10 to establish a mean).
(i) Monitor results of clinical specimens for various sources of error by use of parameters such as anion gap, comparison of test results with previous submissions from same patient (delta checks), and investigation of markedly abnormal results (limit checks).

E. Procedures Manual

All procedures currently in use should be included. Protocols may be organized in manuals and/or stored in computers, and be in written form. They should contain such information as: patient preparation, specimen

collection, processing and handling, criteria for rejection of specimens, limitations and things that interfere with the method in use, step-by-step procedures, reagent preparation, manufacturer reference interval, reportable range, literature references, reagent labelling: content, storage requirements, expiration and laboratory-specific information, such as identification of instrument used, result reporting method, actions to take when system is down, criteria for specimen referrals to outside laboratories ("send outs"), quality control procedures, documentation of critical values, clearly stated and dated entries of procedure implementation or change. If the laboratory performs the same test by more than one method or a more than one test site, or the test is sometimes also sent to a referral laboratory, comparisons should be run at least twice annually to define the relationships between methods and sites. Comparison of different test methods for the same analyte within the laboratory or between laboratories (if samples are tested in-house and at a referral laboratory) is recommended. This should be done every 6 months or at a frequency determined by the laboratory manager. The following steps should be included:

1. Perform a 20-sample or greater comparison using specimens covering the analytical range: (a) group data in an x-y comparison plot, (b) calculate slope and intercept by a least squares method
2. Laboratory director or qualified personnel should define acceptable performance limits
3. If individual test results performed on the same patient or material do not correlate with each other (i.e., BUN/creatinine, electrolyte balance), the cause should be investigated and corrective action taken.

Postanalytical Factors Important in Clinical Chemistry

A. Computer Entry of Data
B. Report Generation
C. Report Delivery
D. Specimen Disposal
E. Personnel Safety
F. Laboratory Environment
G. Personnel Training Requirements.

Quality Assurance in Antibiotic Susceptibility Testing

ANTIBIOTIC SUSCEPTIBILITY testing has become a very essential step for properly treating infectious diseases and monitoring antimicrobial resistance in various pathogens. The choice of antibiotic needs to be made taking into consideration the susceptibility profile of the pathogen, pharmacology of the antibiotic, the need for antibiotic therapy, and its cost effectiveness.

Indications for routine susceptibility testing: A susceptibility test may be performed in the clinical laboratory for two main purposes: To guide the clinician in selecting the best antimicrobial agent for an individual patient. To accumulate epidemiological information on the resistance of microorganisms of public health importance within the community.

Susceptibility test as a guide for treatment: Susceptibility tests should never be performed on contaminants or commensals belonging to the normal flora, or on other organisms that have no causal relationship to the infectious process. These should be carried out only on pure cultures of organisms considered to be causing the infectious process. The organisms should also be identified since not every microorganism isolated from a patient with an infection requires an antibiogram. Routine susceptibility tests are not indicated when the causative organism belongs to a species with predictable susceptibility to specific drugs. This is the case for *Streptococcus pyogenes* and *Neisseria meningitidis*, which are still generally susceptible to penicillin. If resistance of these microorganisms is suspected on clinical grounds, repres-entative strains should be submitted to a competent reference laboratory.

Susceptibility test as an epidemiological tool: Routine susceptibility tests on major pathogens (e.g. *S.typhi*, shigellae) are useful as part of a comprehensive programme of surveillance of enteric infections. These are essential for informing the physician of the emergence of resistant strains (chloramphenicol resistant *S.typhi,* co-trimoxazole resistant and ampicillin resistant shigellae) and indicate a need to modify standard treatment schemes. Continued surveillance of the results of routine suscepti-bility tests is an excellent source of information on the preva-lence of resistant staphylococci and Gram-negative bacilli that may be responsible for cross-infections in the hospital. Periodic reporting of the susceptibility pattern of the prevalent strains is an invaluable aid to forming a sound policy on antibiotic usage in the hospital by restriction and/or rotation of life-saving drugs, such as the aminoglycosides and cephalosporins.

Choice of drugs: The choice of drugs used in a routine antibiogram is governed by considerations of the antibacterial spectrum of the drugs, their pharmacokinetic properties, toxicity, efficacy, and availability, as well as their cost to both the patient and the community. Among the many antibacterial agents that could be used to treat a patient infected with a given organism, only a limited number of carefully selected drugs should be included in the susceptibility test. Table 1 indicates the drugs to be tested in various situations. These agents are divided into two sets. Set 1 includes the drugs that are available in most hospitals and for which routine testing should be carried out for every strain. Tests for drugs in Set 2 are to be performed only at the special request of the physician, or when the causative organism is resistant to the first-choice drugs, or when other reasons (allergy to a drug, or its unavailability) make further testing justified.

Direct versus indirect susceptibility tests: In the standardized method, the inoculum is prepared from colonies on a primary culture plate or from a pure culture. This is called an "indirect sensitivity test". In certain cases, where a rapid answer is important, the standardized inoculum may be replaced by the pathological specimen itself, e.g. urine, a positive blood culture, or a swab of pus. For urine specimens, a microscopic examination of the sediment should first be made in order to see if there is evidence of infection, i.e. the presence of pus cells and/or organisms. The urine may then be used as the inoculum in the standard test. Likewise, susceptibility tests may be performed on incubated blood cultures showing evidence of bacterial growth, or a swab of pus may be used as a direct inoculum, when a Gram stained smear shows the presence of large numbers of a single type of organism. This is called a "direct susceptibility test"; its advantage over the indirect test is that a result is obtained 24 hours earlier. The disadvantage is that the inoculum cannot be properly controlled. When the susceptibility plate shows too light or too heavy growth, or a mixed culture, the results should be interpreted with caution or the test repeated on pure cultures.

Table 1: Basic sets of drugs for routine susceptibility tests

	Set 1	Set 2
Staphylococcus	Benzylpenicillin Oxacillin Erythromycin Tetracycline Chloramphenicol	Gentamicin Amikacin Co-trimoxazole Clindamycin -
Intestinal	Ampicillin Chloramphenicol Co-trimoxazole Nalidixic acid Tetracycline	Norfloxacin
Enterobacteriaceae Urinary	Sulfonamide Trimethoprim Co-trimoxazole Ampicillin Nitrofurantoin Nalidixic acid Tetracycline	Norfloxacin Chloramphenicol Gentamicin
Blood and tissues	Ampicillin Chloramphenicol Co-trimoxazole Tetracycline Cefalotin Gentamicin	Cefuroxime Ceftriaxone Ciprofloxacin Piperacillin Amikacin
Pseudomonas aeruginosa	Piperacillin Gentamicin Tobramycin	Amikacin

GENERAL PRINCIPLES OF ANTIMICROBIAL SUSCEPTIBILITY TESTING: Antimicrobial susceptibility tests measure the ability of an antibiotic or other antimicrobial agent to inhibit bacterial growth *in vitro*. This ability may be estimated by either the dilution method or the diffusion method. **The dilution method:** For quantitative estimates of antibiotic activity, dilutions of the antibiotic may be incorporated into broth or agar medium, which is then inoculated with the test organism. The lowest concentration that prevents growth after overnight incubation

is known as the minimum inhibitory concentration (MIC) of the agent. The MIC value is then compared with known concentrations of the drug obtainable in the serum and in other body fluids to assess the likely clinical response. **The diffusion method:** Paper discs impregnated with a defined quantity of antimicrobial agent are placed on agar medium uniformly seeded with the test organism. A concentration gradient of the antibiotic forms by diffusion from the disc and the growth of the test organism is inhibited at a distance from the disc that is related among other factors to the susceptibility of the organism. The recommended method for intermediate and peripheral laboratories is the modified Kirby-Bauer method, the methodology of which is given below: This method has been recommended by National Committee on Clinical Laboratory Services (NCCLS-USA) Subcommittee on Antimicrobial Susceptibility Testing. This is the most thoroughly described disc diffusion method for which interpretive standards have been developed and which is supported by laboratory and clinical data.

The Modified Kirby-Bauer Method: Reagents: Mueller-Hinton agar: 1. Mueller-Hinton agar should be prepared from a dehydrated base according to the manufacturer's recommendations. The medium should be such that control zone sizes within the standard limits are produced. It is important not to overheat the medium. 2. Cool the medium to 45-50°C and pour into the plates. Allow to set on a level surface, to a depth of approximately 4 mm. A 9 cm diameter plate requires approximately 25 mL of the medium. 3. When the agar has solidified, dry the plates for immediate use for 10-30 minutes at 36°C by placing them in an upright position in the incubator with the lids tilted. 4. Any unused plates may be stored in a plastic bag, which should be sealed and placed in the refrigerator. Plates stored in this way can be kept for 2 weeks. To ensure that the zone diameters are sufficiently reliable for testing susceptibility to sulfonamides and co-trimoxazole, Mueller-Hinton agar must have low concentrations of the inhibitors thymidine and thymine. Each new lot of Mueller-Hinton agar should therefore be tested with a control strain of *Enterococcus faecalis* (ATCC 29212 or 33186) and a disc of co-trimoxazole. A satisfactory lot of medium will give a distinct inhibition zone of 20 mm or more that is essentially free of hazy growth or fine colonies. **Antibiotic Discs:** Any commercially available discs with the proper diameter and potency can be used. Stocks of antibiotic discs should preferably be kept at -20°C; the freezer compartment of a home refrigerator is convenient. A small working supply of discs can be kept in the refrigerator for upto 1 month. On removal from the refrigerator, the containers should be left at room temperature for about 1 hour to allow the temperature to equilibrate. This procedure reduces the amount of condensation that occurs when warm air reaches the cold container. **Turbidity Standard:** Prepare the turbidity standard by pouring 0.6 mL of a 1% (10 g/L) solution of barium chloride dihydrate into a 100-mL graduated cylinder, and filling to 100 mL with 1% (10 mL/L) sulfuric acid. The turbidity standard solution should be placed in a tube identical to the one used for the broth sample. It can be stored in the dark at room temperature for 6 months, provided it is sealed to prevent evaporation. **Swabs:** A supply of cotton wool swabs on wooden applicator sticks should be prepared. These can be sterilized in tins, culture tubes, or on paper, either in the autoclave or by dry heat.

Procedure: To prepare the inoculum from a primary culture plate, touch with a loop the tops of each of 3-5 colonies of similar appearance of the organism to be tested. When the inoculum has to be made from a pure culture, a loopful of the confluent growth is similarly suspended in saline. Compare the tube with the turbidity standard and adjust the density of the test suspension to that of the standard by adding more bacteria or more sterile saline. Proper adjustment to the turbidity of the inoculum is essential to ensure that the resulting lawn of growth is confluent or almost confluent. Inoculate the plates by dipping a sterile swab into the inoculum. Remove excess inoculum by pressing and rotating the swabs firmly against the side of the tube above the level of the liquid. Streak the swab all over the surface of the medium three times, rotating the plate through an angle of 60° after each application. Finally, pass the swab round the edge of the agar surface. Leave the inoculum to dry for a few minutes at room temperature with the lid closed. The antibiotic discs may be placed on the inoculated plates using a pair of sterile forceps. A sterile needle tip may also be used to place the antibiotic discs on the plate. Alternatively, an antibiotic disc dispenser can be used to apply the discs to the inoculated plate. A maximum of seven discs can be placed on a 9-10 cm diameter plate. Six discs may be spaced evenly, approximately 15 mm from the edge of the plate, and 1 disc placed in the centre of the plate. Each disc should be gently pressed down to ensure even contact with the medium. The plates should be placed in an incubator at 35°C within 30 minutes of preparation. Temperatures above 35°C invalidate results for oxacillin/methicillin. Do not incubate in an atmosphere of carbon dioxide. After overnight incubation, the diameter of each zone (including the diameter of the disc) should be measured and recorded in mm. The results should then be interpreted according to the critical diameters by comparing them with standard tables. The measurements can be made with a ruler on the under surface of the plate without opening the lid. The endpoint of inhibition is judged by the naked eye at the edge where the growth starts, but there are three exceptions. With sulfonamides and co-trimoxazole, slight growth occurs within the inhibition zone; such growth should be ignored. When β-lactamase producing staphylococci are tested against penicillin, zones of inhibition are produced with a heaped-up, clearly defined edge; these are readily recognizable when compared with the sensitive control, and regardless of the size of the zone of inhibition, they should be reported as resistant. Certain *Proteus* species may swarm into the area of inhibition around some antibiotics, but the zone of inhibition is usually clearly outlined and the thin layer of swarming growth should be ignored.

Clinical definitions of terms resistant and susceptible: the three-category system: The result of the susceptibility test, as reported to the clinician, is the classification of the microorganism in one of two or more categories of susceptibility. The simplest system comprises only two categories, susceptible and resistant. This classification, although offering many advantages for statistical and epidemiological purposes, is too inflexible for the clinician to use. Therefore, a three-category classification is often adopted. The Kirby-Bauer method recognizes three categories of susceptibility and it is important that both the clinician and the laboratory worker understand the exact definitions and the clinical significance of these categories. **Susceptible:** An organism is called "susceptible" to a drug when the infection caused by it is likely to respond to treatment with this drug at the recommended dosage.

Intermediate susceptibility: This term covers two situations. It is applicable to strains that are "moderately susceptible" to an antibiotic that can be used for treatment at a higher dosage because of its low toxicity or because the antibiotic is concentrated in the focus of infection (e.g. urine). The term also applies to those strains that are susceptible to a more toxic antibiotic that cannot be used at a higher dosage. In this situation this category serves as a buffer zone between susceptible and resistant. **Resistant:** This term implies that the organism is expected not to respond to a given drug, irrespective of the dosage and of the location of the infection. For testing the response of staphylococci to benzylpenicillin, only the categories 'susceptible' and 'resistant' (corresponding to the production of b-lactamase) are recognized. Factors influencing zone size and common problems encountered in performing susceptibility test are shown in Tables 2 and 3.

Table 2: Factors influencing zone size in antibiotic susceptibility testing

Factor	Influence
Inoculum density	Larger zones with light inoculum and vice versa
Timing of disc application	If after application of disc, the plate is kept for longer time at room temperature, small zones may form
Temperature of incubation	Larger zones are seen with temperatures <35°C
Incubation time	Ideal 16-18 hours; less time does not give reliable results
Size of the plate	Smaller plates accommodate less number of discs
Depth of the agar medium	Thin media yield excessively large inhibition zones and vice versa
Proper spacing of the discs	Avoids overlapping of zones
Potency of antibiotic discs	Deterioration in contents leads to reduced size
Composition of medium	Affects rate of growth, diffusion of antibiotics and activity of antibiotics
Acidic pH of medium	Tetracycline, novobiocin, methicillin zones are larger
Alkaline pH of medium	Aminoglycosides, erythromycin zones are larger
Incubation in the presence of CO_2	Increases zone size of tetracycline and methicillin
Addition of thymidine to medium	Decreases activity of trimethoprim
Addition of defibrinated blood	Decreases activity of sulfonamides
On chocolate agar, decreased activity of	Sulfonamides, trimethoprim, aminoglycosides
Reading of zones	Subjective errors in determining the clear edge
Chelating agents such as calcium, magnesium and iron	Decreases diffusion of tetracycline and gentamicin

Table 3: Troubleshooting guide for disc diffusion test in antibiotic susceptibility testing

Aberrant results	Probable cause
Tetracycline zone too small	pH of medium too low
Aminoglycoside zone too small	pH of medium too high
Aminoglycoside zone too large	Ca^{2+} and/or Mg^{2+} level too high in medium Ca^{2+} and/or Mg^{2+} level too low in medium
Too large zone on control plates	Inoculum too light Nutritionally poor medium Slow growing organisms (not seen with controls) Improper medium depth (too thin)
Zone universally too small on control plates	Inoculum too heavy
Methicillin zone indeterminant in disc test	Methicillin degraded by strong β-lactamase producing staphylococci
Carbenicillin zone disappears with *Pseudomonas* control	Resistant mutant has been selected for testing
Single disc result above or below control limits	Error in reading, fuzzy zone edge, transcription error, bad disc Disc may not be pressed firmly onto agar surface
Colonies within zone of inhibition	Mixed culture Resistant mutants within zone
Zones overlap	Discs too close together
Zones indistinct	Poorly streaked plates
Zone within zone phenomenon	Swarming *Proteus* species Feather edge of zones around penicillin or ampicillin discs usually with b lactamase negative strains of *Staph. aureus*
Enterococcus appears sensitive to aminoglycoside discs	Assessment of aminoglycosides inaccurate in disc test

Need for quality control in susceptibility test: The final result of a disc diffusion test is influenced by a large number of variables. Some of the factors, such as the inoculum density and the incubation temperature, are easy to control, but a laboratory rarely knows the exact composition of a commercial medium or the batch-to-batch variations in its quality, and it cannot take for granted the antimicrobial content of the discs. The results of the test must, therefore, be monitored constantly by a quality control programme which should be considered part of the procedure itself. The precision and accuracy of the test are controlled by the parallel use of a set of control strains, with known susceptibility to the antimicrobial agents. These quality control strains are tested using exactly the same procedure as for the test organisms. The zone sizes shown by the control organisms should fall within the range of diameters given. When results regularly fall outside this range, they should be regarded as evidence that a technical error has been introduced into the test, or that the reagents are at fault. Each reagent and each step in the test should then be investigated until the cause of the error has been found and eliminated. **Standard procedure for quality control:** The quality control programme should use standard reference strains of bacteria that are tested in parallel with the clinical culture. They should preferably be run every week, or with every fifth batch of tests, and in addition, every time that a new batch of Mueller Hinton agar or a new batch of discs is used. **Standard Strains:** These are: *Staphylococcus aureus* (ATCC 25923)– *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) Culture for day-to-day use should be grown on slants of nutrient agar (tryptic soya agar is convenient) and stored in the refrigerator. These should be subcultured onto fresh slants every 2 weeks. **Frequency of quality control testing:** Salient features of quality control in antibiotic susceptibility testing are: Use antibiotic discs of 6 mm diameter, Use correct content of antimicrobial agent per disc, Store supply of antimicrobial discs at -20°C, Use Mueller-Hinton medium for antibiotic sensitivity determination, Use appropriate control cultures, Use standard methodology for the test, Use coded strains from time to time for internal quality control, Keep the antibiotic discs at room temperature for one hour before use, Incubate the sensitivity plates for 16-18 hours before reporting, Incubate the sensitivity plates at 35°C, Space the antibiotic discs properly to avoid overlapping of inhibition zone, Use inoculum size that produces 'near confluent' growth, Ensure even contact of the antibiotic disc with the inoculated medium, Measure zone sizes precisely, Interpret zone sizes by referring to standard charts. The zone size produced by an antimicrobial agent indicates its activity against the organism. However, zone sizes of two agents to which the organism is sensitive are not comparable and should not give an erroneous impression that the test organism is more sensitive to the drug which has yielded a bigger zone size.

Quality Assurance in Urine Testing

QUALITY ASSURANCE (Q.A.):

It is a program of checks and balances to assure the quality of laboratory services. It is a mechanism to detect problems and improve services that includes plans, policies, and procedures. Q. A. encompasses every ancillary service of the institution. The following components are part of the Q. A. process in the laboratory; (1) how tests are ordered, (2) the quality of the request forms, (3) identification of patients, (4) how specimens are procured, (5) how specimens are labeled, (6) how specimens are transported to the lab, (7) how specimens are processed, (8) how tests are performed and if there is an established procedure for each test, (9) laboratory procedure manuals for every department, (10) laboratory instruments, (11) quality of reagents, (12) turnaround time, (13) accuracy of results that are reported, (14) how is quality control in the laboratory conducted, (15) procedures for going about to solve problems, (16) how are patient's records kept, (17) are critical values established, and (18) what proficiency test procedures are in place to assure accuracy of results. Q. A. is a process that included the interaction between the patient, the laboratory, other departments, and the physicians. Included in this process is included evaluation, monitoring, documentation, and communication to remove obstacles to appropriate patient care. Its intent is to give self confidence to the laboratory staff performing the tests.

QUALITY CONTROL (Q. C.) IN THE LABORATORY:

Q. C. is a system that ensures reliable test results in the clinical laboratory. This system monitors the facilities, test methods, test equipment, reagents, supplies, procedures, equipment maintenance, calibration strategies, control procedures, remedial actions, and maintains a record book.

RELIABILITY, ACCURACY, PRECISION, AND VARIANCE ARE PART OF THE QUALITY CONTROL PROGRAM:

Reliability is dependent upon the accuracy and precision of test results. An accurately performed test describes how close the test results are to the actual value of the test. A test that can be repeated over and over, still obtaining the same results describes the obtained that are statistically close to the actual value and when repeated and the same results are obtained, then the test is reliable. Accuracy and precision of a test depends upon the laboratory using standardized procedures, control standards, reputable reagents and supplies, and precise equipment. Variance is a statistical term that describes how much fluctuation occurs when a specimen is tested repeatedly. The smaller the fluctuation the smaller the variance and the more reliable the test.

CONTROL SPECIMENS AND THE Q. C. PROGRAM:

A control specimen is an sample specimen that resembles the patient's test specimen, but the constituents are known. Generally control specimens consists of low abnormal, normal, and high abnormal values. The control specimens are entered into the testing procedure and treated as a patient specimen. In this way, by knowing the expected outcome of a control specimen, any variable that would affect the patient specimen will also affect the control. Through the control specimen; the parameters of reliability, precision, accuracy, and variance are monitored and the results of the testing procedure assured.

SEVEN FACTORS THAT MAY CAUSE A TEST RESULT TO BE UNACCEPTABLE:

(1) The reagents or controls have deteriorated. (2) Defect in equipment/ instrument. (3) Glassware improperly cleaned or is contaminated. (4) Failure in correctly timing the incubation temperatures. (5) Failure to use a method appropriate for the equipment / instruments. (6) Carelessness/ indifference by the laboratory staff performing the test. (7) Murphy's law. If something can go wrong it will and you won't know why. Statistically for some unknown reason a test result will be in error.

Q. C. AS PART OF SPECIMEN EVALUATION FOR ACCEPTABILITY OF A URINE SPECIMEN FOR TESTING:

Specimens must be received into the laboratory that are suitable for testing. The laboratorian must be able to recognize whether or not a specimen will be able to provide the kind of results that a physician can use in the care of a patient. The following seven points address this process. (1) Incorrect preservative used in the urine. If a urine is preserved with formalin and a leukocyte esterase test is requested, the results will be a false positive. (2) An insufficient volume of urine for the requested test. If procedure calls for 1.0 mL of urine and the lab receives 0.5 mL, another specimen should be collected. (3) The specimen is not labeled. It is impossible for the laboratory to guarantee that the results are for a specific patient. (4) The specimen or requisition form is improperly labeled. Same reason as #3. (5) The specimen is inappropriate for the test requested. If a random specimen is brought in for a culture and sensitivity in an unclean container, the results would be invalid and unnecessary costs to the Patient. (6) Visibly contaminated urine. If the urine contains fecal material, it is invalid for tests. (7) Specimen not collected properly for transportation to a reference lab. The preservative may not be correct or the amount in container is wrong. The lab cannot guaranteed reliable results.

TEN ERRORS THAT CAN OCCUR IN URINE TESTING IF A LABORATORY FAILS TO HOLD TO Q. A. PRINCIPLES:

(1) Inadequate care of reagents (failure to replace bottle cap or improper temperature storage). (2) Testing urine that is not fresh (usually more than 1-2 hours old). (3) Failure to mix urine before testing (this is the most common error in the lab). (4) Failure to follow directions, tends to use short cuts, poor technique. (5) Not able to recognize false positive or false negative results. The laboratorian does not understand what substances can interfere with a test. (6) Using chemically unclean containers. There may be residual cleaning materials present. (7) Failure to recognize critical values or when values are inconsistent with each other. (8) Failure to recognize when a test must be repeated. The tech has a disregard for test results. (9) Recording wrong results. 2.22 mg/dL is different from 22.2 mg/dL. (10) Lack of understanding of the importance of incorporating test data from several tests to make a clinical diagnosis. The laboratorian can use such data to verify his/her results.

LIST OF EQUIPMENT ITEMS AND THE FREQUENCY AT WHICH PERFORMANCE EVALUATION MUST BE DONE:

(1) Thermometers: Test when received into the department and test once annually using a National Bureau of Standards certified thermometer. (2) Temperature devices (refrigerators, dry-block baths, etc.): Test daily by recording the temperature and recording in a log. (3) Reagent strip readers: Test daily by using standard reagent strips and control urine. (4) Microscopes: Perform daily. Perform maintenance as prescribed in the procedure manual. (5) Centrifuges: Clean daily with disinfectant. Check r.p.m. with a tachometer and timer with a stop watch on an annual basis. (6) Automatic pipettes: Test when received into the laboratory and then annually per the laboratory procedure manual.

HOW TO CORRECTLY HANDLE MISLABELED OR NON-LABELED URINE SPECIMENS:

(1) If unlabeled or mis-labeled, set the specimen and its requisition aside in a container. (2) Obtain an "incident sheet" and record responses and actions. (3) Notify the collection source (nursing station, clinic, etc.). State the problem and request that a new specimen be collected. Record the name of the person talked to, date, time, and any other data. (4) Do not discard the specimen. Retain until the matter is resolved and determined to be completed. (5) If the specimen cannot be re-collected, then one of the following must occur: (a) The physician must contact the laboratory and request testing on the questionable sample. He must also signature the incident document. (b) The person responsible for collecting the specimen must come to the laboratory and identify the specimen, properly label the specimen and/or requisition form. They must also sign off on the incident form. (6) Perform the test and report results after signatures are secured. (7) Dependent upon the circumstances, the report may need to contain the following comment, "This specimen was improperly labeled (or whatever the problem), but was approved for testing. The laboratory cannot otherwise guarantee that the reported values are from this patient. (8) Forward a copy of the incident report to the Q. A. committee and the unit which sent the specimen.

Quality Control in Hematology

Quality control in medical laboratories encompasses a set of procedures which ensure that reliable and timely test results are received by the users of laboratory service. Reliability implies both precision and accuracy.

There are four components of quality assurance program:

A. Internal quality control (IQC)
B. External quality control (EQA)
C. Standardization.
D. Proficiency surveillance.

A. Internal Quality Control

Since now most of the tabs are dependent on automated machine, it has become extremely important to maintain good internal quality control, which is done by:

1. Testing Control Sample

The best known method is by testing a control sample along side the routine specimen in each batch of test. Control material is either obtained commercially or prepared individually, but its stability and homogeneity should be ensured.

2. Control Chart (Levy-Jenninqs or L-J chart)

In this process when a batch of samples is dispensed (after being run along a control sample), the mean and standard deviation of each parameter is obtained and linear graphs are ruled, showing the +2 standard deviation (SD) limits. Statistically, not more than 1 in 20 samples should fall outside these limits if the system is in control.

3. Cusum Analysis

Cumulative Sum (Cusum) Charting was introduced in 1960s. Deviation from the largest is plotted in a cumulative manner so that each point represents the sum of all the deviations to date from the mean or target value. This method of plotting exaggerates trends in the data. And makes shifts of the mean much more obvious than by other plots. The rules for using the cusum system for quality control are less well defined than for the L-J system.

4. Duplicate Tests

A well known method for checking precision in clinical analysis is duplicate testing. In this process a few of the specimens that were measured in an earlier batch, are rechecked with the next batch control.

5. Inbuilt Quality Control

This includes:

Use of cumulative reports of a single patient

Clinical correlation: if a physician can not interpret a report on clinical grounds, a repeat test with a fresh specimen is indicated.

Red cell indices: if reports are giving erroneous rise or fall in the red cells indices, this usually points to an error in analysis.

Blood film examination ultimately helps in double checking the analysis done by the instrument.

B. External Quality Assessment

The college of American Pathologist first introduced "proficiency testing" survey program in 1960. In the late 60s, The British Committee for Standards in Hematology, finally developed the National External Quality Assessment Scheme (NEQAS) for Hematology. Such methods are used by various laboratories all over the world to keep up with international standards.

C. Standardization

Modern diagnostic systems depend on a calibration procedure for accurate performance. Calibrators or testing standards are commercially prepared products, made by a direct comparison with a primary international standard. They are used for accuracy and inter laboratory harmonization of test results. The calibrator has an assigned value as close to the true value as can be established.

The WHO (World Health Organization) provides a wide range of biologically important international reference standard material.

Some examples of these which are available for use in hematology are:

a) Hemoglobin preparation.
b) Hemoglobin A2 and F.
c) Thromboplastin.
d) Blood typing sera.
e) Various coagulation factors.

D. Proficiency Surveillance

This is concerned with the pre-analytical and post-analytical parts of the process that require control, if tests are to be reliable and effective. This involves following a standard guideline at various steps of a laboratory analysis.

The steps are:

1) Standard of blood collection tube
2) Phlebotomy technique
3) Identification of sample with special reference to hazardous specimens
4) Maintenance of transportation standards
5) Data processing of results
6) Establishing normal reference values, assessment of the significance of results and taking decisions for further tests.

TECHNICAL PROFICIENCY HAS ALWAYS BEEN THE CORNER STONE OF THE LABORATORY, BUT IN RECENT YEARS WITH THE ADVENT OF SOPHISTICATED INSTRUMENTS AND AUTOMATION, QUALITY CONTROL HAS ASSUMED AN EVEN MORE IMPORTANT ROLE IN GOOD LABORATORY PRACTICE.

IT IS THE DUTY OF THE LABORATORY STAFF TO ENSURE THAT THE TESTS WHICH ARE CARRIED OUT ARE APPROPRIATE AND TO PROVIDE RELIABLE ANALYTICAL RESULTS.

Quality Control in the New Environment: Automated Hematology

The hematology laboratory has undergone a technological revolution over the last 20 years-- from tedious manual methods to relatively simple instrumentation to complex multiparameter instruments. Any discussion of cost savings in quality control must begin with the question of whether QC methods have kept pace with the technological changes. In fact, many hematology quality control procedures are really holdovers from that earlier era when instruments were fundamentally less reliable than they are today. Modern microprocessor-driven instruments are rather precise and stable and almost certainly do not need the degree of precision control common 15 to 20 years ago when procedures were semiautomated or manual. Therefore, many hematology laboratories could save money on QC while continuing to maintain quality standards. The way to do that, however, is not through a bottom-line approach that trims frequency of controls or cuts back on programs subscribed to in an attempt to reach some predetermined budget figure. Instead, we need to take a systems approach to the problem. This means analyzing every procedure in terms of the instruments and methods currently in use. The analysis should include not only the obvious costs of QC (reagents, computer time and programs, and labor) but also the hidden costs of too many repetitive runs. We need to discover how much extra cost is generated by quality control protocols that are too restrictive or rigid. Within the systems analysis framework, there are three specific programs we can institute that are highly cost-effective in hematology: the use of retained patient specimens, weighted moving averages of red cell indices, and clinical quality control. We will discuss these in some detail a little later in the article, along with quality control for some of the newer analyte additions to automated hematology. The systems approach requires us first to address the issues of selection, maintenance, and calibration of analytic systems.

Selecting and maintaining analytic systems

Selection of an analytic system is usually based on a number of factors, including costs of acquisition and operation (labor and consumables); throughput; ease of operation; training requirements; reliability in terms of downtime; accuracy, linearity, sensitivity, and precision data; and manufacturer support and reputation. Additional factors, harder to define, relate broadly to how the proposed instrumentation fits with the existing mix of space, personnel, and equipment. We cannot ignore these intangibles. Indeed, an analytic system cannot be viewed simply as an instrument. It is an integral unit composed of instrument, reagents, personnel, and performance documentation. For this unit to function effectively, appropriate training and continuing education of system operators are essential, as are readily available and user-friendly operating instructions, maintenance schedules, troubleshooting protocols, and convenient documentation. To take this reasoning a step further, selecting instrumentation on the basis of ease of process control and the ability to use available quality control material, techniques, and programs can itself be a prudent and cost-effective strategy.

Calibration. The International Committee for Standardization in Hematology has defined a calibrator as "a substance... used to calibrate, graduate, or adjust a measurement' that is "traceable to a national or international reference preparation or reference material.' Once an instrument is calibrated, it does not ordinarily have to be recalibrated until there is a major change in an instrument component or a significant drift in control measurements. Calibration becomes an issue in hematology because of the absence of stable standard substances and the relative instability of most materials that are available. Although there are exceptions, QC materials do not in general meet the ICSH guidelines and thus should not be used for calibration. Indeed, hematology calibration is mostly a patchwork affair. The cyanmethemoglobin method as described by the ICSH is the accepted reference standard for hemoglobin determination. Microhematocrit procedures, on the other hand, depend for precision on operational standardization with "selected' and "reference' methods. Calibration of hematocytometers can be done in two ways, neither very satisfactory. The choice is between tedious, poorly reproducible manual counting methods that identify cells and only cells and semiautomated counting instruments that may produce erroneous counts due to particles, bubbles, or other optical/electrical interference. Most labs opt for the latter, with appropriate precautions and corrections. Automated instruments present their own set of calibration problems. Some authorities still recommend repetitive analysis of fresh whole blood specimens, but the disadvantages of this approach are legion: the amount of blood needed; the time it takes to run 10 to 20 determinations on multiple specimens; and the cost of space, personnel, and instrument maintenance resources devoted solely to calibration. Many laboratories find whole blood calibration impractical and have turned to commercial stabilized calibrators. The problem is that stabilized cells differ substantially from whole blood in the way they are processed and viewed by aperture impedance and optical light-scattering instruments and in their transferability from one instrument to another.

Process control with commercial reagents

A number of control materials and alternative strategies should be considered in establishing cost-effective and reliable quality control protocols. Manufactured quality control material offers convenience and stability, but it is expensive, and efforts to extend stability have often compromised the degree to which the material resembles fresh whole blood. Nevertheless, most laboratories buy stabilized commercial QC material. It has been common practice to document control determinations on Levey-Jennings charts and to look for shifts and drifts. Today, many laboratories are implementing alternative statistical options, including cusum analysis and multirule analyses of the Shewhart type.

Interlab comparison and proficiency testing

A valuable quality control tool -- and one we recommend -- is interlaboratory comparison. These programs, whereby laboratories share a common pool of extended-stability control material and a common database, may be sponsored by a vendor, a local pathology society, the CAP's QAS committee, or a multiregional group of labs. Whatever the source, the purpose of the program is to establish comparisons based on method/ instrument/ reagent peer groups, as well as to provide intralaboratory summary statistics. Limitations of interlaboratory comparisons include their cost, the limited stability of control materials, and the difficulty in establishing meaningful databases because of small participant groups further splintered by numerous biases in methods, instruments, and reagents. In addition, problems are created by the characteristics of manufactured control products and their interaction with specific instruments. The limitations aside, participants can derive great benefit from interlab comparisons. The programs provide control materials at reduced cost as well as access to statistical resources and group data. Moreover, the spread of microcomputers and telecommunication increases the potential for on-line data acquisition and comparison. Proficiency testing, on the other hand, has limited value in real time. Its advantage lies in identifying trends and defining method bias, educating staff, and satisfying a need for peer group comparison and external validation of results.

Retained patient specimens

No other area of the laboratory is as adaptable as hematology is to the use of retained patient specimens. With the exception of the white cell subpopulations, properly aliquoted, refrigerated blood specimens show no significant change in major hematologic parameters for 24 hours. This makes them ideal for run-to-run or shift-to-shift calibration control (at absolutely no cost). The biggest advantage of retained patient specimens is their transferability from major instruments to backup or satellite instruments, in contrast to commercial control materials, which are subject to significant instrument/ method biases.

Weighted moving averages

The technique that offers a significant opportunity for cost savings in hematology also involves a conceptual leap from what has been standard in the past. Weighted moving averages is a patient-result-based system for process control of red-cell-related parameters. It is inexpensive and admirably suited to the control requirements of modern multiparameter automated hematology analyzers. The technique is based on the empirical observation that averaged red cell indices from patient populations in acute care general hospitals are approximately Gaussian, consistently stable, and similar in all institutions studied. These properties reflect the physiologic consistency of red cell size and hemoglobin content in health, disease, and even many hematologic disorders. The dimensions of the properties are expressed by the Wintrobe indices, ratios independent of certain procedural errors (dilution, inadequate mixing) that may seriously compromise hemoglobin and hematocrit measurements and red cell counts. Weighted moving averages anchors the validity of the indices by referencing one primary measurement, hemoglobin, to a defined calibration event. It then uses a complex, statistical algorithm to evaluate successive batches of patient sample indices and incorporate them into a continually updated mean. Means are trimmed (outliers eliminated) and smoothed (data from previous batches incorporated into the new mean), thereby diluting the effect of random error and abnormal results. Deviations of the means from specific limits indicate loss of calibration, a shift in the characteristics of the population under study, or specific types of instrument malfunction.

Several caveats

The method should not be used by laboratories performing fewer than 100 CBCs daily. In addition, because of small sample sizes, random entry of raw data is mandatory, and each group of patients should be representative of the patient population as a whole. No more than one-third of a run should be made up of patients with mean corpuscular volume deviations in the same direction (chemotherapy, pediatric, iron-deficient). The system takes a little getting used to because it is so statistically intensive and because it completely abandons commercial controls. Many labs may opt for periodic use of manufactured controls or retained patient specimens as a kind of security blanket. Controversy still colors the subject of weighted moving averages. Some studies, concluding that stabilized whole blood controls are better at separating calibration change from patient variation, recommend that they be used in tandem with weighted moving averages. In addition, the system cannot be used for process control of leukocyte and platelet counts because of the very high physiologic variability of these analytes. Nevertheless, the hematology resource committee of the CAP has approved, with some reservations, the use of the weighted moving averages for longitudinal process control of seven- and eight-parameter analyzers. Recognition has also been accorded by the CAP's Commission on Laboratory Accreditation. Questions pertaining to the system are included in the current laboratory accreditation checklist.

Clinical quality control

Costs can also be cut in hematology if we eliminate or identify as far as possible the sources of results variation that occur before the specimen is analyzed. This really has more to do with quality assurance than quality control but should not be overlooked in our efforts to improve quality assurance and control costs. The causes of preanalytic variation range from patient-specific changes, resulting from physiologic, environmental, or pathologic conditions, to a multitude of variables during the process of obtaining and transporting the specimen to the analytic site. Specimen collection variables include choice of needles, collection systems, containers, anticoagulants, and transport conditions. Systematic variation is associated with specimen source--earstick versus fingerstick, capillary versus venous--as well as specimen preparation. QC for new analyte additions: At first glance, new automated hematology instruments that offer additional analytes would seem to increase rather than save costs. After all, if you have to perform quality control on a parameter that did not exist before, it will cost you money. While that is true, new instruments do make cost savings possible in a number of ways--the much smaller amounts of reagents, for example, and the elimination of labor-intensive manual differentials. Let's look briefly at some of the latest analyte additions. Automated platelet counting: Automation of the platelet count and its incorporation into the routine hemogram are increasingly common on newer multiparameter instruments. These instruments are increasingly precise, although evaluation of specimens with moderate to severe thrombocytopenia is still an area of concern. Quality control for automated platelet counting has been simplified by stabilized whole blood control material. However, instruments using different technologies may perceive the stabilized or fixed platelets differently from fresh patient samples. Among the trilevel controls that many manufacturers offer, those with platelet counts within the normal and low ranges are most important for quality control monitoring. Smaller amounts of reagents and less labor account for the major cost savings from automated platelet counting. Red cell, platelet histograms, and related parameters: Another recent addition to hematology instrumentation is the graphic display of red cells and platelet volume histograms with related indices such as the red cell distribution width, the red cell morphology index, and the mean platelet volume. Used in conjunction with the MCV, these new parameters hold promise in the differential diagnosis of anemia and in identifying specimens that require careful review. In particular, the RDW is of use in the differential diagnosis of iron deficiency and beta thalassemia minor. An elevated RDW has been noted in association with hemoglobinopathies. Platelet histograms and the MPV offer information beyond a mere count of platelets in the blood specimen. For example, certain disease states such as chronic myelogenous leukemia are associated with platelets of abnormal size. Quality control is a problem in this group of parameters: Interpretation of the MPV is complicated by the fact that it varies inversely with the platelet count in normal individuals. Furthermore, Threatte et al have demonstrated significant changes in MPV resulting from use of different anticoagulants and the temperature at which the analysis is performed. Matrix problems with stabilized control materials also appear to be significant. These various studies highlighting MPV difficulties underscore the need for each laboratory to confirm its reference range with regard to instrument, reagent system, and type of anticoagulant used. It is possible that stabilized control material may be limited to providing comparison data only for a similar instrument-reagent combination. MPV may be best controlled by monitoring groups of patient specimens with similar platelet counts analogous to Bull's algorithm for the red cell indices. The same may be true of the red cell histogram and related parameters. It is unlikely that different levels of

manufactured control material will show significant differences in the RDW and red cell histogram distributions since that might be costly to produce. Automated white cell differential: The manual 100-cell differential is a rather imprecise test, a fact that poses serious limitations to the clinical usefulness of the test. This conclusion is supported by recent clinical studies that question the inherent value of routine manual differential counts, both in the inpatient and outpatient settings. These studies and others, prompted at least in part by medical cost containment, lend support and impetus not only for reducing the number of manual differential counts performed but also for using the alternatives provided in the various automated methodologies. In general, automated differential instrumentation can be classified as either image analysis or flow analysis systems. Image analysis systems try to replicate the classic manual differential, although cell identification criteria are standardized and test precision is increased. Quality control procedures for these systems are relatively simple: The control material (stained slides) is not consumed by the analysis and can be used over and over to check the system. Image analysis systems are expensive and limited, however. They are also dedicated instruments and do not provide data on such other parameters as hemoglobin, hematocrit, platelet count, and total white cell count. For these reasons, they are not really suited to small or even medium size hospitals. Flow analysis systems, on the other hand, do provide additional hematologic data, and the required hardware and software can sometimes be added to present laboratory instrumentation via upgrades. These systems therefore offer many institutions a practical alternative to the labor-intensive manual differential. Various studies have estimated that 60 to 80 per cent of manual differentials could be replaced by such automated differentials without compromising patient care. Quality control for flow analytic systems is still in the formative stage. One possibility is to compare automated results with values obtained from the same patient specimens done manually. This is both expensive and tedious, however, since 400- to 500-cell differentials would be required on several samples to achieve meaningful precision for comparison purposes. The limited stability of white cell populations in the fresh state also raises an obstacle to periodic replicate analysis of patient specimens as control material for differentials. Stabilized quality control material for flow analytic systems is a relatively recent development and is still not perfected. An ideal control material compatible with all instruments may be extremely difficult if not impossible to produce. If this is the case, then interlaboratory comparison would be limited to similar instrument-reagent combinations. It is our opinion that the optimal quality assurance system for this area will involve the use of stabilized control materials; limited comparison analysis of patient samples using extended (400-cell) manual differentials coupled with clinical criteria to eliminate unnecessary manual differentials; and laboratory criteria, including instrument flage, review of histograms, and action limits to determine which patient specimen requires careful morphologic review of the blood smear. By eliminating unnecessary manual differentials and concentrating on those specimens that are abnormal or potentially abnormal, it is possible not only to achieve cost savings but also to increase the medical usefulness of this laboratory test.

Issues Faced with Automated Cell Counters

Measurement of complete blood cell count is one of the essential laboratory tests. Electronic blood cell counters simplify and speed up the performance of blood counts and the calculation of red cell indexes. Because of their high precision, physicians tend to accept their results as accurate. However, these counters can be "fooled" by changes in cell size with platelet clumping, agglutination of erythrocytes, or precipitation of abnormal proteins. Failure of the physician to recognize these errors may lead to patients being subjected to unnecessary procedures and therapy. In this issue of Crux, we present four patients in whom the results of red blood cell indexes measured by an automated counter were incompatible and unreasonable. These patients had cold agglutinins: two of them had a respiratory infection, one had cytomegalovirus mononucleosis, and the fourth had a B cell lymphoproliferative disease. One of the patients also had a clinically overt autoimmune hemolytic anemia. Cold agglutinins are polyclonal or monoclonal autoantibodies, usually of immunoglobulin M subtype, directed against I or i antigens and preferentially binding erythrocytes at cold temperatures. These autoantibodies may be associated with malignant or benign disorders (e.g., B cell neoplasm, post-infection, collagen vascular disease) and can be manifested by transient laboratory abnormalities up to severe autoimmune hemolytic anemia. With automated analyzers, cold agglutinin laboratory abnormalities typically present as a discrepancy between the red blood cell indexes. The agglutinated erythrocytes may be recognized as single cells or may be too large to be counted as RBC; therefore, measured mean corpuscular volume is falsely elevated and the red blood cell count is disproportionately low. While the measured hemoglobin is correct, the calculated indexes are incorrect: the hematocrit (red cell count x MCV) is low, while the mean corpuscular hemoglobin (hemoglobin/red cell count) and the mean corpuscular hemoglobin concentration (hemoglobin/ hematocrit) are elevated. By rewarming the blood sample to 37 degrees C, the erythrocyte agglutination is abolished and correct values will be read. In the blood sample, hemagglutination may be visible to the unaided eye and examination of the peripheral blood smear may reveal erythrocyte clumping. Another problem might appear with the presence of cryoglobulins. Cryoglobulins are immunoglobulins that precipitate at temperatures below 37 degrees C, producing high molecular weight aggregates. The first clue to a diagnosis of cryoglobulinemia could be laboratory artifacts detected in the automated blood cell counts. The precipitated cryoglobulin particles of various sizes may falsely be recognized as leukocytes or platelets causing pseudoleukocytosis and pseudothrombocytosis. At the same time, the RBC indexes are generally unaffected. Reliable automated counts can be obtained by warming the blood to 37 degrees C or by keeping the blood at 37 degrees C from the time of venipuncture to analysis. May-Grunwald-Giemsa-stained blood films are usually normal, extracellular material is occasionally seen, and leukocyte cytoplasmic inclusion is rarely found. Another important laboratory artifact seen with the automated analyzers is pseudothrombocytopenia. This condition is caused by diverse mechanisms, including: anticoagulant-induced pseudothrombocytopenia, platelet satellitism, giant platelets, and cold agglutinin-induced platelet agglutination. The anticoagulant-induced pseudothrombocytopenia is an in vitro platelet agglutination generally seen in specimens collected into EDTA. It has been reported both in healthy subjects and in patients with various diseases (e.g., collagen vascular disease, neoplasm, and in severely ill patients) and has an overall incidence of approximately 0.1%. Although the agglutination is most pronounced with EDTA, it may occur with other anticoagulants as well, such as heparin, citrate or oxalate. Because the generated platelet aggregates are large, the automated counters do not recognize them as platelets, leading to lower platelet counts. In some cases the aggregates are large enough to be counted as leukocytes, causing a concomitant pseudoleukocytosis. The aggregation in pseudothrombocytopenia is time-dependent and usually temperature-sensitive, with maximal activity at room temperature. The EDTA-induced pseudothrombocytopenia is mediated by autoantibodies of IgG, IgM and IgA subclasses directed at an epitope on glycoprotein IIb. This epitope is normally hidden in the membrane GP IIb/IIIa. Ionized calcium has an important role in maintaining the heterodimeric structure of the GP IIIb/IIa complex. The EDTA, through its chelating effect, dissociates the GP IIIb/IIa complex with epitope exposure. In Glanzmann's thrombasthenia, a disorder characterized by the quantitative and/or qualitative abnormality of glycoprotein IIb/IIIa, pseudothrombocytopenia does not occur. Interestingly, in recent years, Abciximab-a GP IIb/IIIa antagonist has been associated with pseudothrombocytopenia. If anticoagulant-induced pseudothrombocytopenia is suspected a peripheral blood smear should be examined for platelet clumping. Platelet satellitism is similar to anticoagulant pseudothrombocytopenia. In the presence of EDTA, platelets bind to leukocytes and form rosettes. The binding is usually to neutrophils but binding to other white blood cells has been reported. The automated analyzers do not correctly recognize platelet-neutrophil clumping, resulting in pseudothrombocytopenia. Platelet satellitism is mediated by autoantibodies of IgG type. These autoantibodies are directed at GP IIIb/IIa on the platelet membrane and to an Fc gamma receptor III on the neutrophil membrane. Pseudothrombocytopenia occurs with giant platelets. Because of their size, the giant platelets are excluded from electronic platelet counting. Platelet cold agglutinin-induced pseudothrombocytopenia is a rare condition. The platelet agglutination is anticoagulant-independent, occurs at maximal activity at 48 degrees C, and is mediated by IgM autoantibody directed against GP IIb/IIIa. Because this autoantibody has little activity at temperatures above 30 degrees C, no clinical complication occurs. Other technical problems and less known situations may cause abnormal cell counts and indexes with automated analyzers. These include clots or overfilling of tubes, hypertriglyceridemia, hyperbilirubinemia, and extreme high white blood count ± any of which may interfere with cell counting and cell indexes. Severe microcytosis, microorganisms, and cytoplasmic fragments of leukocytes may cause spurious elevation of the platelet counts. These conditions are characterized by small particles that are wrongly counted as platelets. Larger particles may be recognized as leukocytes, e.g., circulating normoblasts, giant platelets, and erythrocytes with more resistance to lysis. The latter occurs in automated analyzers when leukocyte counting is based on prior erythrocyte lysis. Erythrocyte resistance to lysis, causing interference with leukocyte counting, was reported in hemoglobinopathies (e.g., hemoglobin C trait, CC, SC, and SS) and fetal (cord) red cells. EDTA-dependent leukoagglutination (similar to platelet satellitism) and cold-induced leukoagglutination uncommonly cause pseudoleukopenia. Rarely does severe hyperglycemia cause spurious macrocytosis. The hyperosmolar glucose-"loaded" erythrocytes become swollen when they are diluted into a relatively hypotonic counting medium, but after hyperglycemia is corrected the MCV returns to normal. To conclude, in our modern era, automated analyzers are able to increasingly recognize pathologic conditions and artifacts. First, the results are presented in numbers, histograms, and scatter plots with or without flags for internal laboratory review. The results are then transferred to the clinician, usually as numbers only. Still, undetected artifacts occur and go unnoticed. The clinician should be alert to those artifacts, thus avoiding unnecessary investigations and therapies.

ICSH (International Council of Standardization of Hematology) Recommendations for Measurement of ESR

The increased mobility of patients and the benefits to laboratories of sharing their experience has led to the need for measurements between laboratories to be comparable. This can be achieved by using a reference method. ICSH has defined this as exactly described technique which, in the opinion of and Expert Panel, provides sufficiently accurate and precise measurement for it to be used to assess the validity of other such laboratory methods.

The original ICSH reference method for measuring ESR was based on the methodology of Fahreaeus and Westergren using diluted blood (4 vols blood plus 1 vol of citrate) in an open ended glass tubing of 300 mm in length, mounted vertically in a rack or stand. Modifications of these specifications in particular to use of undiluted blood, are now recommended as a basis of a new ICSH reference method.

Recent developments, including biohazard awareness and difficulty in obtaining equipment to perform the reference method, have prompted ICSH to introduce a standardized method as an alternative to, and potential replacement for, the reference method.

For working (routine) methods, ICSH now recommends specifications for selected methods. These are procedures whose reliability has been verified against the reference or standardized method and which minimize the biohazard risk of the test procedure.

ICSH has prepared new recommendations for measurement of erythrocyte sedimentation rate (ESR) under the following categories:

1. ICSH reference method:
   ICSH now recognizes, as its reference method for the ESR, the sedimentation of EDTA-anticoagulated but undiluted blood in traditional Westergren pipettes that meet ICSH specifications.

2. ICSH standardised method:
   ICSH recommends specifications for a new standardised method for the ESR based on the sedimentation of EDTA-anticoagulated, but undiluted blood in pipettes with a 200 mm scale and which are designed to avoid spillage of blood or aerosol generation. This standardised method may be use for verification or quality control of other ESR methods and, in future, may replace the reference method.

3. ICSH selected methods
   ICSH recommends specifications for working methods, using diluted or undiluted blood, which may be considered as ICSH selected methods for routine use. A protocol is outlined for evaluation of such working methods against ICSH reference method or the new ICSH standardised method.

TEMPERATURE CORRECTION OF WESTERGREN ESR (SED RATE) RESULTS

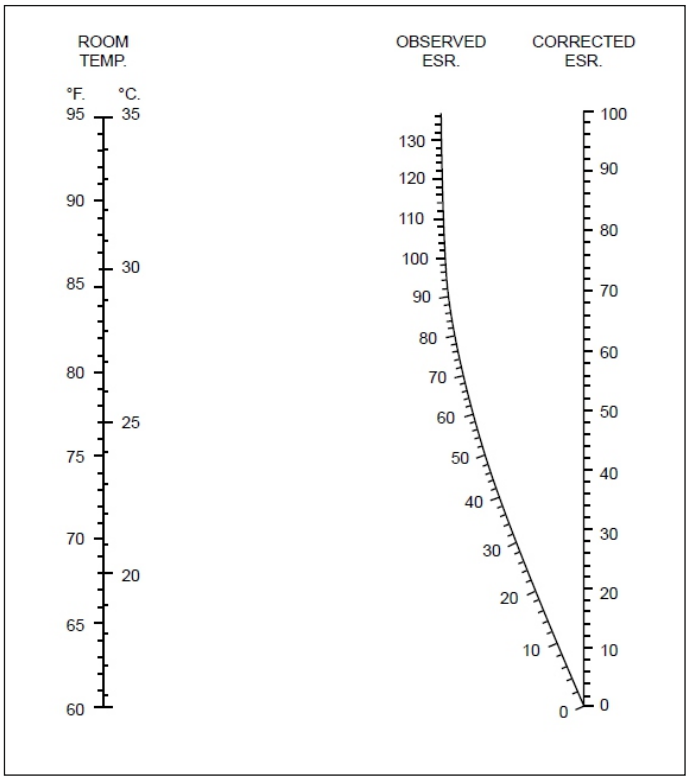
The International Council for Standardisation in Haematology and the Clinical Standards Institute recommend that the Erythrocyte Sedimentation Rate (Sed Rate) test be performed within the temperature range 18 to 25 ^{0}C (64 to 77 ^{0}C). However it is understood that these temperatures may be exceeded in some parts of the world where temperature control of the environment is not available or may have broken down.

Personnel performing the test must always keep a record of the temperature at the start of the timed hour and at the end when reading the result- thus being able to calculate and record an average temperature over the testing period. If the average temperature thus obtained is at or above 25 ^{0}C (77 ^{0}C) the observed result should be corrected for temperature using the chart or nomogram devised and published in the Journal of Clinical Pathology in 1957; Volume 10, pages 354-356.

Note: This nomogram applies only to the Westergren Method of ESR estimation, the Wintrobe method requires a different chart and most automated systems have a correction system built into the software.

A sample of the correction chart and nomogram is presented below

Room Temperature ^{0}C	Observed ESR	Corrected ESR
34	100	68
33	24	14
25	40	32
25	80	68



Quality Control in Bacteriology

QUALITY CONTROL OF MEDIA AND STAINS

Culture Media are used in the laboratory for a variety of purposes. These are used to support the growth of microorganisms showing typical colonial and morphological appearance.

Media are also used to demonstrate many other properties of organisms, e.g. production of acid and gas in carbohydrate fermentation media or haemolysis on blood agar. Variations in the composition of the medium may alter these characters.

Quality Control of Media

Sources of Error

A few years back media used to be prepared from basic chemical ingredients, but laboratories are no longer required to do this now.

Dehydrated Media

These are commercially available and require only the addition of water to be reconstituted for use. The responsibility for quality control lies with the manufacturer.

However, it has to be tested for its quality, after preparation, because of changes that can be brought about by the process of reconstitution and sterilization.

Dehydration with Additive

For isolation of fastidious organisms, certain additives need to be used when media are prepared in the laboratory. The additives usually are unstable materials such as blood, serum or other growth factors. Hence, quality control needs to be maintained.

Commercially Prepared Media

Ready to use media are commercially available. In these media also the responsibility for quality control maintenance lies with the manufacturer but laboratories need to keep a watch on their behaviour.

Sources of Error

Inappropriate Medium

Since dehydrated media are usually arranged alphabetically on a shelf, one may select the wrong bottle inadvertently, or an improper additive might be selected, making the medium unsuitable for use.

It is always important to read the label, particularly when a new lot of medium has been received in the laboratory.

Water

Measure carefully the amount of water that is added when reconstituting media. Since impurities render tap water unsuitable for the preparation of most biological media, laboratories should use either distilled water, deionized water, or water that has been treated in both ways.

Weighing

Accurate balances should be used for weighing dry materials. Weighing errors significantly alter the composition of the final product.

Dispensing

Media should be dispensed accurately and aseptically in plates and tubes. Failure to measure the amount accurately may result, for example, in too shallow or too deep agar medium, either of which may make the medium unsuitable for use.

Proper Sterilization

A common error in media preparation is sterilizing media at too high a temperature or for too long a period, or both. This may result in deterioration or decomposition of some constituents of the media, which will render the media useless for the intended purpose.

Glassware

Care should be taken to use clean glassware, since residues on glass may be inhibitory to some fastidious microorganisms, particularly viruses grown in cell culture, or to the cells themselves.

Quality Control

Any quality programme for culture media must in the final analysis assure that a medium will support the growth of the organisms likely to be in the specimen. It must, if specified inhibit the growth of commensal organisms, exhibit a typical biochemical response, be stable and have a reasonable shelf life. Because laboratories usually have no control over the reparation, shipping or storage of these products it is very important that they document the information that is available for each.

Physical Appearance

If the medium is stored for an excessively long time under adverse conditions or has been improperly prepared, the following signs may develop and these should be documented.

- Presence of turbidity or a precipitate indicates that some constituent has come out of the solution.
- Colours darker than normal may indicate overcooking of sugar containing media, incorrect pH or incorrect mixture of ingredients.
- Colours lighter than normal may also indicate incorrect mixture of ingredients or a wrong pH.
- Prolonged storage of medium after pouring in plates causes its dehydration and makes it unfit for use. Dehydration of the medium can be reduced by preparing only required number of plates of media and storing them by sealing plates in plastic bags.

Sterility

A few media are used without terminal sterilization, but these are exceptions; most media must be sterile when they are inoculated. Each batch of medium, whether prepared in the laboratory or received from a commercial source, should be sampled for sterility.

This is best done by removing 1-5% of the batch and placing it in a bacteriologic incubator at 35°C for 48 hours. If contaminants appear in the medium as a result of inadequate sterilization, a new lot should be obtained.

Those containers that are used for sterility testing should be discarded at the completion of the test, since they are unsuitable for inoculation because of the dehydration that occurs after up to 48 hours in the incubator.

Growth

Determine the ability of the medium to support the growth of suspected organisms by inoculating the medium with a typical stock culture isolate. A frequent quality control error is the use of a heavy inoculum for this purpose. For most media, inoculating with a stock culture that is too heavy may result in misleading growth.

In a specimen, the organism may be much more fastidious or present in very small numbers; therefore, the medium may not support its growth.

When testing for the ability to support growth, it is good to prepare a dilute suspension to use as the inoculum. This suspension will give greater assurance that the medium is adequate for the growth of a small number of organisms in a patient's specimen.In selecting an organism for testing, one should select from among the more fastidious species of organisms that one may be looking for in specimens received from patients.

Biochemical Response

When inoculating media used to identify a specific reaction, such as fermentation or H_2S production, it is necessary to use only a species or strain of organism that will produce the desired reaction.

Selective media

Since selective media are designed not only to support the growth of organisms but to inhibit the growth of others, it is necessary to inoculate the medium with representatives of both groups of organisms.

To demonstrate the inhibitory effect, one can challenge the medium with a heavy inoculum, since, if the medium will prevent the growth of a large inoculum, it will inhibit the small number of organisms that may be present in the primary specimen. The medium must also support the growth of the selected organisms.

As a matter of general principle, each batch of culture medium should be checked before use with control strains to ensure that it supports the growth of bacteria and, in the case of selective media, inhibits the growth of undesirable organisms. However, if economics does not permit this approach, those media which are known from experience to be trouble free and reliable need not be subjected to such a regular quality control regimen. The laboratory has to identify such reliable media and accordingly establish quality control schedules. This concept must be periodically reviewed. However, whenever a new batch of medium, new supplier or a new product is to be used it is prudent to subject it to rigorous quality control measures until confidence in the quality of the product is established. A "batch" of the medium refers to all the tubes, plates or containers of medium prepared at the same time in the laboratory, or all the plates, tubes or containers having the same lot number that are received in a single shipment from an outside supplier.

Spectrum of Quality Control

The frequency of performing quality control procedures needs to be determined from the experience of the laboratory. To meet certification requirements, laboratories need to perform quality control procedures according to a prescribed pattern. Careful records of quality control procedures should be made and maintained which should be reviewed periodically to determine the stability of media so that corrective measures can be taken in time.

Quality control of culture media should not be a blind procedure, but should be approached in a rational and disciplined manner.

Performance of Plated Media

Samples of plates from each batch are selected for performance testing and are inoculated with the appropriate stock cultures. For each type of medium, at least two or three microorganisms having growth characteristics with 'positive' and 'negative' results for the medium should be used. The size of inoculum and method of inoculating the test plates must be standardized as closely as possible.

In general, control organisms should be selected from an actively growing broth culture and a standard loopful of culture seeded directly onto the test medium, which is then streaked so as to obtain isolated colonies. After appropriate incubation, the results of the performance test are recorded.

The medium is released for use in the clinical laboratory only if the results indicate satisfactory performance. In initiating a quality control programme, one must establish some priorities, such as beginning by testing those media that are most likely to demonstrate deficiencies.

Top priority should be given to blood agar, chocolate agar and Thayer Martin agar media. Secondary priority should be accorded to selective enteric media such as MacConkey agar, EMB, XLD and bile salt agars.

A quantitative approach may be more useful for testing of performance of selective or inhibitory media such as Thayer Martin agar. *N.gonorrhoeae* and *N.meningitidis* usually grow on Thayer Martin agar when the inoculum is heavy, but when a fairly light inoculum is used, the pathogens might be inhibited.

Consequently, a somewhat quantitative performance test could detect deficiencies that would be overlooked if one simply inoculated test plates with undiluted stock cultures.

Quality Control of Stains

Test all stains at appropriate intervals for their ability to distinguish positive and negative organisms and document the results.

Quality control of stains need to be performed on weekly basis and also as and when a new lot of reagents for staining are procured.

Various Biochemical tests are performed in the laboratory on the isolates obtained from the clinical specimen. These tests help in identification of the organism.

Quality Control of Bacteriological Techniques

Quality control procedures are essential for these tests to avoid generation of wrong results which may lead to erroneous diagnoses. Organisms known to give positive or negative reactions with various biochemical tests have been identified. These must be used frequently in the laboratory to assess the authenticity of results of biochemical reactions.

It is also essential to undertake quality control procedures at regular intervals.

These should be performed:

- With each new batch of reagents
- With each new vial of reagent
- Daily for catalyse, oxidase, and coagulase
- Weekly for bacitracin, optochin and ONPG

A test procedure not giving anticipated results with the control organisms should not be used till such time that remedial steps have been taken to correct the problem.

TESTING METHODS

TroubleShooting Guidelines

A Compilation from

The Crux
BIMONTHLY FORUM FOR THE LABORATARIANS

Selection and Troubleshooting of Immunoassays

The most common assays used in workplace drug testing regimes include homogenous assays which are used with different automatic analyzers. Therefore, one single instrument would never meet the requirements of all the laboratories and that is why, dealers offer variety of shapes and sizes in the instrument kits. A laboratory should therefore, identify its goals and objectives so that it can accomplish those using appropriate testing systems. This template can then be utilized to evaluate different options while considering instruments and reagents. Even though, the importance of different factors varies according to different laboratories, some important points to consider are as follows:

- The environment of the laboratory including the labor, space and the compatibility of different systems and the LIS – laboratory information system interface
- The present and predicted sample volumes, thereby allowing enough capacity for growth
- The speed of analyzer – i.e. the reportable test quantity per hour along with sample throughput
- The capacity of test analyzer – i.e. the quantity of reagent slows available
- Validated instruments available for selected assay reagents
- The costs, such as capital equipment, service agreements, time, requirements for reagent volume and labor
- Referral to other laboratories for further consultation
- The advantages and disadvantages of utilizing single or many dealers for supplying instruments and reagents

All analytical systems require regular monitoring of assays and their performance so that accurate results are ensured. For assays, monitoring is done by open Quality control specimens, below and above the cutoff ranges. When systems don't meet the acceptable criteria, trouble shooting is done to find out the basic cause of failure. Therefore, at times, it is not appropriate to separate the reagent from the analyzer in order to find out where the fault is, and it can be a combination of both the reagent and the instrument. So, routine preventive and daily maintenances are done along with operational checks so that the mechanical as well as the performance operations of the instrument can be verified. Enthusiastic performance as well as the documentation of these tasks minimize the time and provide real time instrument conditions to be used in the troubleshooting procedures. Recording of lot numbers for reagents, quality control material and calibrators with their daily calibration can ensure quality control results.

Some considerations while troubleshooting assay's performance are given below:

Quality Control

The most frequent problem associated is the lack of quality control material to fulfill the acceptability criteria. This can occur as an individual occurrence or can be due to a bias developed over some time. Random failures can likely result due to deterioration or insufficient volume of quality material in the sample, which is resolved by replacing the material. An ongoing bias or shift indicates systematic issue. Though, minor change in the quality control can be due to improper preparation, storage or shipping of the reagent. The manufacturer can provide information related to in-house reagent testing and reports from other alternate users for comparison. The development of quality control bias over a lot of time can be due to the deterioration of quality control reagents or materials or may indicate problems with the storage parts that require temperature control or refrigeration. Mechanisms such as reagent dispensing and pipetting of instruments along with technological errors like improper placement of quality control material should be given consideration.

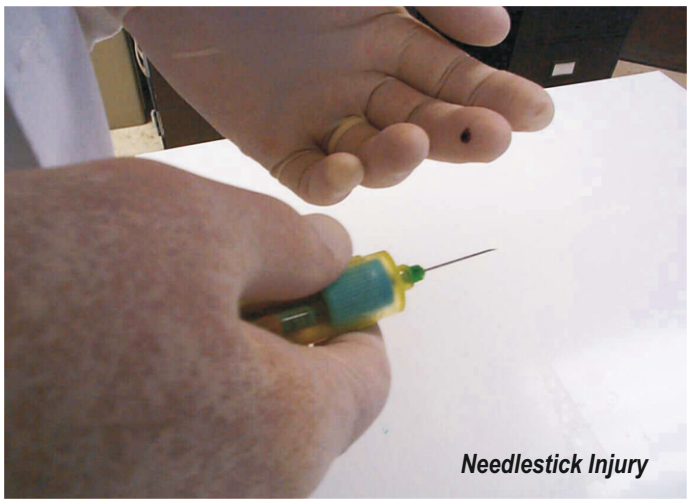
Other Considerations for Reagents

Monoclonal antibodies used for drug abuse testing have enhanced the consistency of reagents. However, even changes occur in these systems over a period of time and the antibodies shift their affinity and reagents alter their formulations which can gravely impact the performance of immunoassays. The monitoring of calibration, quality control and reaction curves can provide insight about the shifts in reagents. Even though, minute changes don't impact the results' accuracy, the change in the affinity of the antibody and its specificity over time can cause poor recovery and assay performance.

Treating HIV Exposure

DECREASING HIV RISK WITH POST-EXPOSURE PROPHYLAXIS

Treating HIV exposure has been shown to decrease the risk of HIV infection. Workers in health care settings are constantly exposed to occupational hazards; wet floors that lead to slip and falls, toxic chemicals that cause burns to the hands or face. But there is one hazard that people in the health care field fear most, the needle stick. Occupational exposure to blood borne infections, including HIV infection, via the needle stick occurs all too often. Some sources report that nearly 1 million healthcare workers suffer needle stick injuries each year. As a result, hundreds of workers are infected with diseases such as Hepatitis B, Hepatitis C and HIV. But treating HIV exposure with HIV medications has been shown to decrease the incidence of sero-conversion and HIV infection. Because of the increasing problem of HIV infection from needle sticks, Internationally institutions now recommend treating HIV exposure with what they call as post-exposure prophylaxis (PEP) for those workers thought to be exposed to HIV in the workplace.



Needlestick Injury

What is Post Exposure Prophylaxis (PEP)?

PEP is just what the name suggests; prophylaxis (preventative) medications given after an HIV or suspected HIV exposure in hopes of decreasing the likelihood of HIV infection from the exposure. The PEP medication combinations used depends on the degree of exposure and the HIV status of the source of the exposure. But before any medications are prescribed, it has to be determined if PEP is indicated and appropriate.

WHEN IS PEP INDICATED?

The following scenarios warrant PEP.

Two drug PEP recommended when

- exposure to asymptomatic HIV+ person by solid needle stick or superficial injury that break the skin
- a mucous membrane exposure to a large volume of HIV infected blood that's source is asymptomatic (consider for a lesser volume, a few drops)
- a mucous membrane exposure to a small volume of HIV infected blood that's source is symptomatic.

Three drug PEP recommended

- exposure to asymptomatic HIV+ person via deep puncture from a large bore hollow needle
- a puncture from a needle with visible blood on the needle
- a puncture from a needle used in a patient's vein or artery.

Three or More Drug PEP Recommended

- any needle stick exposure from any type needle used on a symptomatic HIV+ person
- a mucous membrane exposure to a large volume of HIV infected blood whose source is symptomatic.

Possibility of Two Drug PEP under Certain Circumstances

- needle stick with any type needle and any degree of exposure if the source has an unknown HIV status but has HIV risk factors
- needle stick with any type needle and any degree of exposure if the source has an unknown HIV status and unknown risk factors but a setting in which exposure to HIV+ persons is likely
- a mucous membrane exposure to any volume of blood whose source has an unknown HIV status but has HIV risk factors
- a mucous membrane exposure to any volume of blood whose source has an unknown HIV status but is in a setting where where HIV exposure is likely.

No PEP Warranted

- any needle stick injury involving a known HIV negative source
- a mucous membrane exposure to any volume of HIV negative blood.

WHAT MEDICATION COMBINATION IS USED?

PEP regimens are chosen depending on the type of exposure. Typically regimens are prescribed for a four week period. PEP should be started within hours of the potential exposure not days. The sooner PEP is began the better.

Preferred Two-Drug Regimen

Option 1 - (Zidovudine, AZT)+ (Lamivudine) twice daily. (Retrovir + Epivir) twice daily is typically substituted for ease of administration. This twice a day regimen is a bit harder to take but is recommended in pregnancy.

Option 2 - (Tenofovir + Emtricitabine) taken once daily. This one drug regimen is easier to take but does have the risk of liver toxicity.

Preferred Three-Drug Expanded Regimen

Basic two drug regimen option 1 or 2 above with the addition of (Lopinavir + Ritonavir) twice daily.

CONCERNS ASSOCIATED WITH PEP

While the benefits of PEP have been documented, there are some concerns as well. It's these concerns that cause practitioners to consider the need for PEP thoroughly before prescribing it. PEP is not without risk and should only be given in those people that absolutely need it. That being said concerns associated with PEP include: **Adherence Issues and the Problem of Resistance** - It's no secret that HIV medications have some unpleasant side effects. Because of these side effects the people who have been exposed find it difficult to take their PEP regimen as prescribed and/or complete the four week course. Both of these barriers result in poor adherence. And as in the case of HIV+ people on medication, poor adherence leads to viral resistance and poor control of HIV. That could make the difference between the PEP being successful or not.

THE LAST WORD ON PEP

PEP is a viable option for occupational exposures to HIV. While it is not without it's downfalls, it is effective in reducing the risk of HIV infection from a needle stick. But, without addressing the problem of needle sticks, more people are going to become infected by this route, health care cost will continue to rise and the epidemic will continue to grow.

Rapid HIV Testing Issues and Usage

Objectives of this article

- To know the capabilities of rapid HIV testing.
- To understand the significance of reactive and nonreactive rapid HIV test results.
- To recognize the clinical indications for rapid HIV testing.

Recent breakthroughs in technology have produced tests for HIV antibody that are highly accurate and easy to use and can give a preliminary result in 20 minutes or less. These rapid HIV tests will be used increasingly in labor and delivery wards, emergency departments, urgent care centers, and the primary care office. They have unique applications for healthcare worker exposures, military operations, public health venues, and developing countries. In this article, the advantages and limitations of rapid HIV testing in various settings are presented. Though based on US (CDC) guidelines, the presentation that ensues may not be acceptable as it is in many different nations, as each nation has set its own diagnostic protocols. By and large, the matter presented can not be refuted by most learned authorities.

The conventional HIV testing algorithm starts with a sensitive enzyme immunoassay (EIA). The EIA can be performed with serum, plasma, urine, or oral fluid, and the result is typically available after 3 to 4 days. If the EIA is negative, the result is considered definitive, and no further testing is indicated. A limitation is that HIV antibodies can take up to 3 months to develop after infection occurs. During this window period, antibody tests may remain negative. If the EIA is repeatedly positive, more specific testing, using the Western blot technique, is done for confirmation. The testing process--from the time a specimen is submitted until a final result is available--can take a week or longer.

With rapid HIV antibody testing, a preliminary result is available in 10 to 20 minutes, depending on the brand of test used. The result is comparable in clinical significance to a sensitive EIA result. Patients who have nonreactive rapid tests can be counseled that they are negative for HIV, just as with a negative EIA (including the caveat about the window period). Those who have a reactive rapid HIV test must be advised that this is a preliminary result: it could indicate HIV infection or could be falsely reactive. Confirmation with a Western blot test must be performed before a diagnosis of HIV can be made. This confirmation can take 5 to 7 days. Importantly, the EIA lacks the specificity to be a confirmatory test. Good pretest counseling is critical with rapid testing, and the patient needs to understand that a false-positive test is a possibility.

Conventional testing has two advantages over rapid testing. First, by the time a patient learns the HIV test result, it is definitely positive or negative (except for the occasional indeterminate result). Second, in the case of a positive test, the physician usually has the result a day or two in advance and can be better prepared to discuss it with the patient. A disadvantage of conventional testing is that many patients, especially those in transient care and public health settings, fail to return for test results.

Rapid testing has the obvious advantage of a short turnaround time for obtaining negative or preliminary positive results. This time savings can be critical in clinical situations that require prompt initiation of antiretroviral therapy.

When is a rapid test indicated?

Rapid HIV tests may be indicated and used most often in obstetric wards, healthcare worker occupational exposures, urgent care clinics and emergency departments, military medicine, public health settings, developing countries, and the primary care office.

Obstetric wards:

In women with untreated HIV infection, mother-to-child transmission occurs in about 25% of pregnancies. Much of this transmission (60% to 70%) is thought to occur during the birthing process. Knowledge of maternal serostatus is the first step in all measures to decrease mother-to-child transmission.

In urban hospitals many women who present to the labor and delivery ward may Obstetric wards:have unknown HIV status. The patient has either received no prenatal care or received prenatal care but had no record of an HIV test. An added concern is that patients without prenatal care often have risk factors, such as illicit drug use or history of unsafe sex, that puts them at higher risk for HIV infection. These women can now be offered rapid HIV testing. In the event of a reactive test, the woman can be advised of the possibility of HIV infection and started on prophylaxis to prevent mother-to-child transmission. Fortunately, serious shortor medium-term adverse effects of antiretroviral therapy for the mother or neonate have not been detected. Studies of long-term adverse effects are pending. Pretest counseling of women having a rapid HIV test in labor must be thorough. The patient needs to understand that a false-positive test is a possibility and that HIV therapy will likely be initiated on the basis of a preliminary positive test. Post-test counseling for a reactive test is stressful for both patient and physician. The physician should be able to explain, again, the possibility of a falsely reactive test, the need for confirmatory testing, and the rationale for urgent antiretroviral therapy. In the obstetric setting, the physician should use the most specific test possible to minimize false positives.

Healthcare worker occupational exposure:

The number of healthcare workers having a documented HIV seroconversion from occupational exposure is rising steadily. Healthcare exposures can be stressful for everybody involved, including the treating physician. Often, an emergency department physician is called on in the middle of the night to make the decision to treat or not to treat. Confirmation of the source patient's HIV status can be crucial. With a rapid HIV test, the source patient's HIV status can be determined within 20 minutes. If the source patient does not have high-risk behavior and the rapid test is negative, the physician, along with the healthcare worker, might elect not to begin prophylactic therapy. With non-rapid EIA, results might not be available for 3 to 4 anxiety-filled days. An emergency department study has showed that rapid HIV testing is cost-effective in treatment of healthcare worker exposures.

Urgent care clinics and emergency departments:

Chart reviews have shown that most patients with newly diagnosed HIV or AIDS have had missed opportunities for earlier diagnosis. Often, these missed opportunities occurred in visits to an urgent care clinic or emergency department. The CDC has long recommended routine HIV testing in urgent care clinics and in emergency departments in areas where HIV prevalence is greater than 1%. Persons who access these episodic care settings are at increased risk for HIV infection because of underinsurance, lack of primary care, and acute medical concerns.

Military medicine:

In battle, occupational exposures can be extensive and unavoidable for soldiers and medics. The technical simplicity and portability of rapid HIV testing make it convenient for frontline use. A negative rapid test in the source patient could resolve anxiety and prevent unnecessary post-exposure prophylaxis; a reactive test would be an indication for prophylaxis in the exposed person and for confirmatory testing in the source patient. Rapid testing is not approved by the FDA for screening donated blood, but it could be used for screening emergency blood donations in combat.

Public health settings:
A huge problem with publicly funded HIV testing is that 30% to 40% of patients do not return for their test result. Groups least likely to return include adolescents and persons tested at a clinic for sexually transmitted disease. As a consequence, valuable HIV testing resources are squandered and opportunities for timely treatment and prevention counseling are lost.

Developing countries:
Rapid HIV testing greatly improves the percentage of patients who learn their test result. The average interval between doing the testing and learning the test result is usually about 30 minutes. With this short turnaround time, 99.7% of patients can learn their test result on the spot. Rapid HIV tests are already used widely in resource poor countries because the tests are technically simple, accurate, and cost effective. Also, they can accurately be done by non laboratarians and do not require refrigeration or expensive laboratory equipment. A definitive diagnosis of HIV infection can be achieved by using two or three different rapid HIV tests in combination. These protocols yield sensitivity and specificity equal to those of standard EIA and Western blot methodologies and are recommended by the World Health Organization.

Primary health centers:
There are clinical situations where rapid HIV testing makes sense in the primary care setting. A rapid HIV test is recommended for patients with a high probability of not returning (e.g., sex workers).

TORCH Syndrome

What is TORCH syndrome?

Many women think that TORCH syndrome is a single health disorder. However, according to health experts, TORCH syndrome is a group of different infectious diseases. They can cause serious problems and harm the health of a fetus.

TORCH diseases in pregnancy

Developing TORCH infection in pregnancy is dangerous for your unborn baby. It spreads rapidly through your blood to the baby. At this level, the immune system of your baby is not strong enough to fight the infection so he/she develops the infection as well. Moreover, if the infection or disease remains in your baby's blood, he or she might not develop vital organs properly. There are the risks of numerous health problems as well. For instance, jaundice or hearing problems. TORCH diseases in pregnancy increase the risk of stillbirth and miscarriage as well.

Among the diseases that are associated with TORCH syndrome are:

Toxoplasmosis

Caused by parasites, toxoplasmosis is a rare condition. It happens when parasite enters your body through the mouth. You can get this parasite from eating uncooked meat. The infection transfers into your unborn baby as well, which might result in brain damage, excess fluid in the brain, seizures, inflammation in certain parts of the eyes, and delays in the ability to use muscles.

Rubella

Rubella or German measles is caused by a virus and is a contagious disease. Pregnant women, who have rubella, develop a sore throat, rash, along with low-grade fever. Moreover, if you are pregnant and get rubella (especially in your first trimester), you need to visit your doctor as your unborn baby might get it as well.

Cytomegalovirus (CMV)

It is an infection in the herpes virus group and affects around 50% of adults by the time they are 30. Although there is no cure for it, it gets better on its own very quickly and it does not cause serious problems. However, the case is different for pregnant women; if they develop the condition, CMV may pass on to their baby. Cytomegalovirus is also one of the common viral infections that affect newborn babies.

If a baby is born with congenital CMV, it may get sick or encounter the following chronic issues:

- jaundice
- small birth size
- hearing loss
- vision loss
- mental disability
- muscle weakness
- Lung problems
- seizures

Herpes Simplex virus

Herpes is one of the very common chronic infections. There are two types of herpes, i.e. HSV-1, which causes blisters around your mouth. It can pass on to your genitals as well. HSV-2 is an STD that leads to genital herpes. It causes blisters and opens sores which are painful. This infection can also contribute to oral herpes. Herpes simplex can affect your baby when he or she is in the uterus, which is rare. Moreover, babies often get the infection during delivery. The risk of infection increases in babies when mothers get the first outbreak of herpes while they are pregnant. at the start of pregnancy. This is because pregnant women shed excess virus particles for a long time. However, the risks of herpes infection in your baby increase if you get herpes later in your pregnancy. So, you should consult with the doctor about this situation. With suitable precautions, you can reduce the risk of infection to some extent.

TORCH test before pregnancy

TORCH screen is done to detect whether you have an infectious disease (covered by screening) or had one in the past. This test is also necessary to find out if you are immune to infectious diseases such as rubella. Many health experts recommend TORCH tests before conception for the healthy development of a fetus and safe pregnancy. Furthermore, the results of a TORCH test are termed positive and negative. A negative test result is considered normal unless it is for a disease that you should be vaccinated against. This indicates that there are no antibodies in your body. And, there is no recent or past infection in your body. On the other hand, a positive test result indicates that IgG or IgM antibodies are found. These antibodies can be a sign of one or more infections that are covered in your TORCH test. These can also suggest that you have been vaccinated for a disease before. Your doctor will further elaborate if your test results are positive and recommend the best treatment.

TORCH infections treatment

The treatment or management of TORCH diseases differs and is based on the symptoms.

Treatment of TORCH infections during pregnancy

- To treat toxoplasmosis, your doctor may suggest sulfadiazine and pyrimethamine.
- The treatment of cytomegalovirus is done according to the patient's symptoms, such as fever or fatigue.
- In the case of Herpes Simplex virus, your doctor might suggest cesarean delivery if active lesions are found.
- Pregnant women who develop Rubella can treat it by resting and mild analgesics.
- If the mother has developed chicken pox in her life, then it is not a major concern. However, if a pregnant mother has never had chicken pox, its risk increases during pregnancy. You can protect your baby from congenital varicella syndrome by avoiding people who have chicken pox.
- Health experts do not recommend getting vaccinated against varicella infection while you are pregnant.

TORCH infection treatment before pregnancy

TORCH infection treatment is less stressful before pregnancy. However, you need to eliminate TORCH infection from your body to promote the healthy development of the fetus. Health experts recommend TORCH tests before you conceive. These tests help with disease or infection detection. In case of positive results, your doctor might suggest the treatment on the basis of your symptoms. Although negative results are not a significant concern, your doctor may suggest precautionary steps for a safe pregnancy. TORCH infections can lead to critical health conditions and affect the health of your unborn baby. It is highly recommended to discuss TORCH syndrome with your doctor before your pregnancy to avoid unwanted outcomes.

FUO/PUO (Fever/Pyrexia of Unknown Origin)

Fever of unknown origin (FUO) in adults is defined as a temperature higher than 38.3°C (100.9°F) that lasts for more than three weeks with no obvious source despite appropriate investigation. The four categories of potential etiology of FUO are classic, nosocomial, immune deficient, and human immunodeficiency virus related. The four subgroups of the differential diagnosis of FUO are infections, malignancies, autoimmune conditions, and miscellaneous. A thorough history, physical examination, and standard laboratory testing remain the basis of the initial evaluation of the patient with FUO.

Newer diagnostic modalities, including updated serology, viral cultures, computed tomography, and magnetic resonance imaging, have important roles in the assessment of these patients.

For appropriate diagnosis, the level or generation of tests that may suffice may vary with individual cases. For instance, in an attempt to diagnose tuberculosis, sometimes ppd with X-ray may be sufficient, while in other cases you may need PCR-NAT technologies. Likewise for diagnosing SLE, in a few cases anti-deoxy-ribonuclear protein antibodies may be enough while in other cases complete ANA profile may be essential.

Category of FUO	Definition	Common etiologies
Classic	Temperature >38.3°C (100.9°F) Duration of >3 weeks Evaluation of at least 3 outpatient visits or 3 days in hospital	Infection, malignancy, collagen vascular disease
Nosocomial	Temperature >38.3°C Patient hospitalized 24 hours but no fever or incubating on admission	Clostridium difficile enterocolitis, Drug- induced, pulmonary embolism, septic thrombophlebitis, sinusitis
Immune deficient	Temperature >38.3°C (100.9°F) Neutrophil count 500mm Evaluation of at least 3 days	Opportunistic infections, neutropenic) aspergillosis, candidiasis, herpes virus
HIV-associated	Temperature >38.3°C (100.9°F) Duration of > 4 weeks for Outpatients, >3 days for inpatient complex, HIV infection confirmed	Cytomegalovirus, Mycobacterium avium intracellular Pneumocystis carinii pneumonia, Drug-induced, Kaposi's sarcoma, lymphoma

Common Aetiologies of FUO

Infections

Tuberculosis(especially extrapulmonary) , abdominal abscesses, pelvic abscesses, dental abscesses, endocarditis, osteomyelitis, sinusitis, cytomegalovirus, Epstein-Barr virus, HIV, Lyme disease, prostatitis, sinusitis, malignancies, chronic leukemia, metastatic cancers, renal cell carcinoma, colon carcinoma, hepatoma, myelodysplastic syndromes, pancreatic carcinoma, sarcomas.

Autoimmune conditions

Adult Still's disease, polymyalgia rheumatica, temporal arteritis, rheumatoid arthritis, rheumatoid fever, inflammatory bowel disease, Reiter's syndrome, systemic lupus erythematosus, vasculitides.

Miscellaneous

Drug-induced fever, complications from cirrhosis, factitious fever, hepatitis (alcoholic, granulomatous or lupoid), Deep venous thrombosis, sarcodoisis.

Agents commonly associated with drug-induced fever

Allopurinol, Captopril, Clofibrate, Erythromycin, Heparin, Hydralazine, Hydrochlorothiazide, Isoniazid, Meperidine, Nifedipine, Nitrofuranatoin, Pencillin, Phenytoin, Procainamide, Quinidine.

Laboratory-acquired Brucellosis

Brucellosis is a serious disease seen worldwide and has been historically known as undulant fever, Bang's disease, Gibraltar fever, Mediterranean fever, and Malta fever. Brucellosis has a limited geographic distribution but remains a major problem in Mediterranean and Middle Eastern countries. In 2001, a total of 15,510 brucellosis cases were reported in Turkey. In our centre there are approximately 20 to 25 new cases each year. Brucella is most commonly transmitted by the consumption of contaminated raw or unpasteurised milk and cheese. Laboratory-acquired brucellosis has also been documented and is considered the most important laboratory-acquired bacterial infection. All *Brucella* spp. have been implicated in laboratory-associated infections, and they may account for up to 2% of all laboratory-associated infections.

In this paper, we report 3 laboratory-acquired brucellosis.

Case 1 was a 26-year-old female laboratory worker, who works as microbiology technologist. She presented with joint pain and fever of 1 week's duration. There was no history of trauma. The patient had given birth 2 weeks prior. Physical examination and haematological parameters were normal. However, the levels of aminotranferases were elevated [aspartate transferase (AST), 45 U/L; alanine transferase (ALT), 55 U/L]. The past history of the patient did not reveal any raw cheese consumption. She had been working on the determination of subgroups of *Brucella* positive cultures. She was tested for brucella using the Rose Bengal microagglutination test (Tulip Diagnostics Ltd. Goa, India) as well as serological titre of anti-*Brucella abortus* antibodies were evaluated by using a standard tube agglutination test (Seromed Laboratory Products, Turkey). The Rose Bengal microagglutination test was positive. The *Brucella* serum agglutination test was reactive (1/640). Two sets of blood samples were obtained for culture. The blood cultures showed bacterial growth (Bactec 9050, Becton Dickinson, USA) following 72 hours of incubation. Bacteria were isolated in 5% sheep blood agar. Grams' stain revealed small gram-negative coccobacilli. The organism was confirmed to be *B. melitensis* by standard biochemical reactions (production of urease, catalase - positive, oxidase-positive, H_2S and indole negative, the dyes basic fuchsin, thionine, thionine blue are positive). In addition, biochemical identification using an API 20 NE (BioMerieux, France) was done. Treatment was initiated with a combination of 2 g of ceftriaxone plus 600 mg of rifampin every day for 6 weeks. The patient had a full recovery without any coexisting problem.

Case 2 was a 28-year-old female laboratory worker who presented with non-specific symptoms of malaise, vomiting and fever. She had been working in the same microbiology laboratory as Case 1 and Case 3. She had worked with the same *Brucella* samples as the patient in Case 1 two weeks prior. She did not have any risk factors for brucellosis exposure such as ingestion of unpasteurised milk products. The results of her physical examination and haematological and biochemical studies were normal. Brucella serology was positive at 1/160. The blood culture revealed *B.melitensis* growth after 3 days. She received doxycycline at a dosage of 100 mg po twice daily and rifampin at a dosage of 600 mg po qd for 6 weeks. She recovered completely.

Case 3 was a 24-year-old female microbiology technologist who was suffering from fever and pain of the lower extremities. Her physical examination and routine laboratory tests were normal. Brucella serology was positive at 1/640. *B.melitensis* was isolated from the blood culture. She received the same treatment as the second case and recovered fully. Like the preceeding 2 patients, she denied other exposure to brucellosis. Brucellosis has been considered the most important laboratory-acquired bacterial infection. Aerosol transmission generated accidentally or during microbiologic techniques from contaminated materials are the proposed routes of transmission. Our present report includes 3 patients with an exposure history of working *Brucella* bacteria in a microbiology laboratory. No accident occurred in the laboratory during the time they were exposed. All 3 cases were found to be working on the specimen of the index case patient. The index case was a 45-year-old man who had a *Brucella* serology of 1/640 and blood culture that grew *B.melitensis*. Only 3 of the microbiology staff work on the specimen. The other personnel in the laboratory who did not work with the specimen were also examined with Rose-Bengal test, brucella antibody and brucella-related symptoms. The results were negative for brucellosis. Brucellosis is an endemic disease in our country. The 3 patients presented did not have any suspicious history of unpasteurised milk consumption or animal contact. The absence of this kind of contamination led us to believe that the transmission to our patients was through the laboratory route. The patients were thought to have been infected during subculturing for collecting bacteria. These laboratory workers were unaware of the hazards of aerosol transmission of *Brucella* spp. They handled the biosafety cabinet, and used gloves and masks. Sniffing culture plates is another risk factor, and is a common practice in bacteriology laboratories in Turkey, as in other countries. *Brucella* spp. are highly infectious because the infectious dose by an aerosol is only 10 to 100 organisms. Laboratory-acquired brucella infections are very important in developing countries and in countries with endemic disease, such as Turkey. In our country, biosafety cabinets do not exist in most hospital laboratories. Therefore, using gloves, masks and goggles and also continuous education on biosafety where brucellosis is endemic is very important. The transmission in our cases was probably due to aerosol contamination because of the current practice of sniffing culture plates. Additionally, catalase test used for bacteria identification might have produced aerosol. In 1986, the World Health Organization recommended the use of doxycycline in combination with rifampin for 6 weeks as the preferred treatment for adult acute brucellosis. The second and third cases received doxycycline and rifampin for 6 weeks. However, the first case received rifampin + ceftriaxone for 6 weeks. It is known that tetracyclines accumulate in fetal bones and teeth, and furthermore, pass into breast milk.1 For this reason, the combination of rifampin + ceftriaxone was preferred instead of the combination of rifampin + doxycycline for Case 1, who had given birth 2 weeks prior and was breastfeeding. Laboratories should be aware of laboratory-associated hazards and take adequate safety precautions even after the use of biosafety cabinet, gloves and masks. In addition, all laboratory workers should be educated periodically for occupational risks in the laboratory. In our hospital, all microbiology laboratory staff were educated concerning laboratory-acquired bacterial infections. The laboratory staff are discouraged from smelling the culture plaques. In order to prevent future infections, close collaboration between clinicians and laboratory staff was initiated. Biosafety level 3 has to be advocated and used whenworking with micro-organisms such as *Brucella* spp. Clinicians should alert laboratory personnel if they suspect Brucellosis in patients so they can take extra precautions.

Helicobacter pylori Tests

Helicobacter pylori tests are used to detect a *Helicobacter pylori* (*H. pylori*) infection in the stomach and upper part of the small intestine (duodenum). *H. pylori* can cause peptic ulcers; however, most people with *H. pylori* in their digestive systems do not develop ulcers. Four tests are used to detect *H. pylori*: Blood antibody test: A blood test to ascertain if the patient has antibodies to *H. pylori* bacteria. If found to be positive it implies that the patient is infected or has been infected in the past. Urea breath test: A urea breath test checks to find if the patient has *H. pylori* bacteria in his stomach. The breath test is not always available. Stool antigen test: A stool antigen test checks to see if substances that trigger the immune system to fight an *H. pylori* infection (*H. pylori* antigens) are present in the feces. Stool antigen testing may be done to help support a diagnosis of *H. pylori* infection or to determine whether treatment for an *H. pylori* infection has been successful. Stomach biopsy: A small sample (biopsy) is taken from the mucosa of the stomach and small intestine during an endoscopy. Several different tests may be done on the biopsy sample. Elisa/rapid immunochromatographic formats are also available.

Why it is done

A *Helicobacter pylori* (*H. pylori*) test is done to: Determine whether an infection with *H. pylori* bacteria may be causing an ulcer or gastritis. Determine whether treatment for an *H. pylori* infection has been successful.

How to prepare

Blood antibody test or stool antigen test: No special preparation is required.

Stomach biopsy or urea breath test: The patient should not eat or drink for at least 6 hours before a breath test or a stomach biopsy. Many medicines may change the results of this test. The clinician must be informed about all the prescription and nonprescription medicines one takes. He may recommend that the patient should stop taking some of your medicines for up to 1 week before having this test. The patient should not take antibiotics, proton pump inhibitors (such as omeprazole or pantoprazole), or medicines containing bismuth (such as Pepto-Bismol) for 1 to 2 weeks before the test. The patient should not take H_2 blockers, such as Pepcid AC, Zantac, Axid, or Tagamet for 24 hours before the test.

How it is done

Blood antibody test: By venipuncture withdraw adequate amount of blood.

Urea breath test: The breath sample is collected when the patient blows into a balloon or blow bubbles into a bottle of liquid. The health professional taking a sample of your breath will: Collect a sample of your breath before the test starts. Give the patient a capsule or some water to swallow that contains tagged or radioactive material. Collect samples of your breath at different times. The breath samples will be tested to see whether they contain material formed when *H. pylori* comes into contact with the radioactive material. The urea breath test usually takes about 1.5 hours.

Stool antigen test: The stool sample for this test may be collected at home. If the patient is in the hospital, a health professional will help collect the sample. To collect the sample, one needs to: Pass stool into a dry container. Either solid or liquid stools can be collected. Be careful not to get urine or toilet tissue in with the stool sample. Replace the container cap and label the container with the patients name, referring doctor's name, and the date the sample is collected. The clinical attendent/ laboratarian should wash his/ her hands well after collecting the sample to avoid spreading bacteria. Deliver the sealed container as soon as possible to the laboratory. Sometimes the clinician may also use a cotton swab inserted into the patients rectum to collect a stool sample during an exam.

Stomach biopsy: Endoscopy is used to collect samples of tissue from the stomach and duodenum. The gastroenterologist may collect up to ten tissue samples. The tissue samples are tested in the lab to see if they contain *H. pylori*. In rare cases, a biopsy sample may be placed in a container that promotes the growth of *H. pylori* bacteria. A culture and sensitivity test may be conducted if felt appropriate.

How it feels

Blood antibody test: Venipuncture usually doesn't cause any problem.

Urea breath test: A urea breath test does not normally cause discomfort.

Stool antigen test: Collecting a stool sample normally does not cause any discomfort. If the clinician collects the sample during a rectal exam, one may feel some pressure or discomfort as the cotton swab is inserted into the rectum.

Stomach biopsy: The local anesthetic sprayed into the throat usually tastes slightly bitter and will make the tongue and throat feel numb and swollen. Some people report that they feel as if they cannot breathe at times because of the tube in their throat, but this is a false sensation caused by the anesthetic. There is always plenty of breathing space around the tube in the mouth and throat. One should try to relax and take slow, deep breaths. One may experience some gagging, nausea, bloating, or mild abdominal cramping as the tube is moved. Even though the patient won't be able to talk during the procedure because he has a tube in his throat, he can still communicate. If the discomfort is severe, alert the doctor with an agreed-upon signal or a tap on the arm. The IV medications may make one feel sleepy. Other side effects-such as heavy eyelids, difficulty speaking, a dry mouth, or blurred vision-may last for several hours after the test. The medications may also cause one not to remember much of what happens during the test.

Risks

Blood antibody test: There is very little chance of a problem from having a blood sample taken from a vein. One may get a small bruise at the site. The patient can lower the chance of bruising by keeping pressure on the site for several minutes. In rare cases, the vein may become swollen after the blood sample is taken. This problem is called phlebitis. A warm compress can be used several times a day to treat this. Ongoing bleeding can be a problem for people with bleeding disorders. Aspirin, warfarin (Coumadin), and other medicines that thin blood can make bleeding more likely. If the patient has bleeding or clotting problems, or if on anti-coagulnts, the patient should inform the phlebotomist before the blood sample is taken.

Urea breath test: There are no known risks or complications with a urea breath test. If radioactive carbon is used, the amount of radioactivity

exposure is extremely small-less than one normally gets from being outside during the day.

Stool antigen test: There are no risks or complications with a stool sample. However, if you do not wash your hands well after collecting the sample, you may spread germs.

Stomach biopsy: There is a slight risk (1 in 10,000) of puncturing the wall of the esophagus, stomach, or duodenum during an endoscopy to collect stomach biopsy samples. The biopsy may also cause some bleeding at the site where the samples are collected. However, the bleeding usually stops without treatment.

After the test

After the test, one may belch and feel bloated for a while. He also may have a tickling, dry throat; slight hoarseness; or a mild sore throat. These symptoms may last several days. Appropriate lozenges and warm saltwater gargles can help relieve the throat symptoms. One should not drink alcohol after the test. After the test, the patient should contact his clinician immediately if he: Vomit blood or notice black or bloody stools. Has trouble swallowing or talking. Is short of breath or have a fast heartbeat. Has increasing chest or abdominal pain. Has neck or shoulder pain. Has a fever.

Results

Helicobacter pylori tests are used to detect a *Helicobacter pylori* (*H. pylori*) infection in the stomach and duoenum. Results from the urea breath test or a stool antigen test are generally available within a few hours. Results from a blood antibody test are usually available within 24 hours. Results from biopsy samples obtained by endoscopy are usually available within 48 hours. Results from a biopsy sample that is cultured can take up to 10 days.

Blood antibody test: Normal: The blood sample does not contain *H. pylori* antibodies. Abnormal: The blood sample contains *H. pylori* antibodies.

Urea breath test: Normal: The breath sample does not contain the tagged hydrocarbon. Abnormal: The breath sample contains the tagged hydrocarbon.

Stool antigen test: Normal: The stool sample does not contain *H. pylori* antigens. However, a negative stool antigen test does not always mean that the patient does not have an *H. pylori* infection. Abnormal: The stool sample contains *H. pylori* antigens.

Stomach biopsy:
Normal: The biopsy sample does not contain *H. pylori* bacteria. *H. pylori* bacteria does not grow in a culture of the tissue biopsy samples.
Abnormal: The biopsy sample contains *H. pylori* bacteria. *H. pylori* bacteria grows in a culture of the tissue biopsy samples.

What Affects the Test

Reasons one may not be able to have the test or why the results may not be helpful include the following: The radioactive urea breath test for *H. pylori* is not usually done during pregnancy or while the patient is breast-feeding, because the radiation could harm the child. Use of antibiotics may affect the results of the urea breath test, the stool antigen test, and stomach biopsy by reducing the number of *H. pylori* bacteria in the stomach and duodenum. The use of lansoprazole, rabeprazole, sucralfate, omeprazole, famotidine, ranitidine, nizatidine, cimetidine, or medicines containing bismuth (such as Pepto-Bismol) can also interfere with the results of the urea breath test and stomach biopsy. A stomach biopsy may not detect an *H. pylori* infection that is present if the biopsy samples are taken from areas that are not infected by the *H. pylori* bacteria. Rough handling, contamination, or inadequate refrigeration of the blood sample can cause inaccurate blood antibody test results. When a blood antibody test is done early in an *H. pylori* infection, the results may be falsely negative because the level of antibodies is too low to measure. The likelihood of infection with *H. pylori* increases with age; older adults are more likely to have detectable amounts of the bacteria in their body.

Important Considerations

The radioactive urea breath test is not recommended for children or for pregnant or breast-feeding women because of exposure to a small amount of radioactivity. The stool antigen test is the newest and least expensive of the four tests for *Helicobacter pylori*, but it may not be as accurate as the other tests. The stomach biopsy is very accurate, but it is the most expensive and most risky of the four tests. A negative stool antigen test does not always mean that an *H. pylori* infection is not present. Although many people are infected with *H. pylori* bacteria, only a few of them will develop peptic ulcer disease. For this reason, other factors (such as a person's symptoms) should be considered when interpreting the results of an *H. pylori* test. Blood tests for *H. pylori* may be positive for several years after the infection; therefore, the urea breath test or a biopsy may be used to determine if treatment has been effective. If one's symptoms persist, an endoscopy may be needed. Having an infection with *H. pylori* increases one's chances of having cancer of the stomach; but the risk is very low.

Understand ICT Tests

Introduction

Immunochromatography assay (ICA), namely lateral flow test, is a simple device intended to detect the presence or absence of the target analyte. The concept of immune-chromatography is a combination of chromatography (separation of components of a sample based on differences in their movement through a sorbent) and immunochemical reactions. The most widespread immunochromatographic system is the test strip (Figure 1).

Developing an ICA

Strips used for ICA contain four main components:

1. Sample Application Pad

It is made of cellulose and/or glass fiber and sample is applied on this pad to start the assay. Its function is to transport the sample to other components. Sample pad should be capable of transportation of the sample in a smooth, continuous and homogenous manner. This pretreatment may include separation of sample components, removal of interferences, adjustment of the pH, etc. analyte sample should be added to the sample application pad to start the test.

2. Conjugate Pad

It is the place where labeled biorecognition molecules (labeled antibodies, usually nano colloid gold particle) are dispensed. Material of conjugate pad should immediately release labeled conjugate upon contact with moving liquid sample. Labeled conjugate should stay stable over entire life span of the lateral flow strip. Any variations in dispensing, drying or release of conjugate can change the results of assay significantly. Poor preparation of labeled conjugate can adversely affect sensitivity of the assay. Glass fiber, cellulose, polyesters and some other materials are used to make conjugate pad.

3. Substrate (Nitrocellulose) Membrane

It is highly critical in determining sensitivity of ICA. Test and control lines are drawn over this piece of membrane. So an ideal membrane should provide support and good binding to capture probes (antibodies, etc.). Nonspecific adsorption over test and control lines may affect results of assay significantly, thus a good membrane will be characterized by lesser non-specific adsorption in the regions of test and control lines. Proper dispensing of bioreagents, drying and blocking play a role in improving sensitivity of the assay.

4. Adsorbent Pad

It works as sink at the end of the strip. It also helps in maintaining flow rate of the liquid over the membrane and stops back flow of the sample. Adsorbent capacity to hold liquid can play an important role in results of assay.

All these components are fixed or mounted over a backing card. Materials for backing card are highly flexible because they have nothing to do with ICA except providing a platform for proper assembling of all the components. Thus, backing card serves as a support and it makes easy to handle the strip.

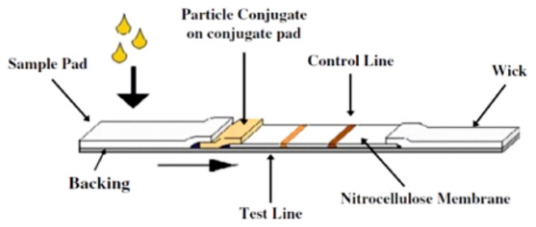


Figure 1. Typical layout of a lateral flow test strip.

Major steps in ICA are:

(i) Preparation of labeled antibody and capture antibody against target analyte;
(ii) Immobilizing the labeled antibody onto conjugate pad, and the capture antibody onto the strip membrane to form the Test/Control line.
(iii) Assembling of all components onto a backing card after dispensing of reagents at their proper pads.
(iv) Add samples and buffer onto sample pad.
(v) Wait the sample flow through the test and control line for 5-10min.
(vi) Read the result when the color reveal.

ICA Format

Lateral flow assay basically combines a number of variants such as formats, biorecognition molecules, labels, detection systems and applications. There are three types of ICAs based on detection format, which are:

1. Sandwich Assay

In this assay format, label (Enzymes or nanoparticles or fluorescence dyes) coated antibody is immobilized at conjugate pad. This is a temporary adsorption which can be flushed away by flow of any buffer solution. A capture antibody against target analyte is immobilized over test line. A secondary antibody against labeled antibody is immobilized at control zone (Figure 2).

To start a test, sample containing the analyte is applied to the sample application pad and it subsequently migrates to the other parts of strip. At conjugate pad, target analyte is captured by the immobilized labeled antibody and results in the formation of analyte-labeled antibody complex. This complex now reaches to nitrocellulose membrane and moves under capillary action. At test line, analyte-labeled antibody complex is captured by another antibody which is primary to the analyte. Analyte becomes sandwiched between labeled and primary antibodies forming labeled antibody-analyte-primary antibody complex. Excess labeled antibody will be captured at the control zone by secondary antibody. Buffer or excess solution goes to absorption pad. Intensity of color at test line corresponds to the amount of target analyte and is measured with an optical strip reader or visually inspected. Appearance of color at control line ensures that a strip is functioning properly.

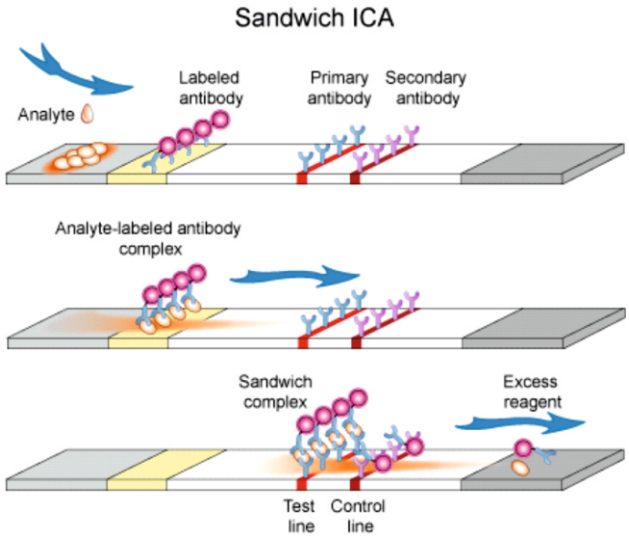


Figure 2. Schematic diagram of Sandwich ICA.

2. Competitive Assay

Competitive format has two layouts. In the first layout, solution containing target analyte is applied onto the sample application pad and prefixed labeled antibody gets hydrated and starts flowing with moving liquid. Test line contains pre-immobilized antigen (same analyte to be detected) which binds specifically to label conjugate. Control line contains pre-immobilized secondary antibody which has the ability to bind with labeled antibody. When liquid sample reaches at the test line, pre-immobilized antigen will bind to the labeled conjugate in case target analyte in sample solution is absent or present in such a low quantity that some sites of labeled antibody conjugate were vacant. Antigen in the sample solution and the one which is immobilized at test line of strip compete to bind with labeled conjugate (Figure 3.). In another layout, labeled analyte conjugate is dispensed at conjugate pad while a primary antibody to analyte is dispensed at test line. After application of analyte solution, a competition takes place between analyte and labeled analyte to bind with primary antibody at test line.

Such format suits best for low molecular weight compounds which cannot bind two antibodies simultaneously. Absence of color at test line is an indication for the presence of analyte while appearance of color both at test and control lines indicates a negative result.

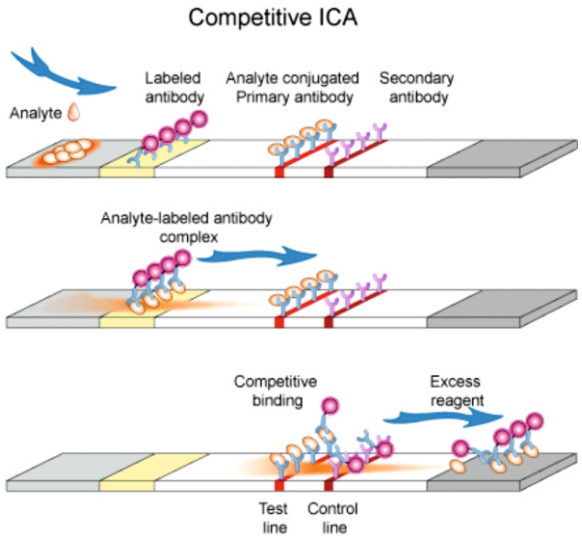


Figure 3. Schematic diagram of competitive ICA.

3. Multiplex Detection Assay

Multiplex detection format is used for detection of more than one target species and assay is performed over the strip containing test lines equal to number of target species to be analyzed. It is highly desirable to analyze multiple analytes simultaneously under the same set of conditions. Multiplex detection format is very useful in clinical diagnosis where multiple analytes which are inter-dependent in deciding about the stage of a disease are to be detected. Lateral flow strips for this purpose can be built in various ways, for example, by increasing length and test lines on conventional strip, making other structures like parallel threads, stars or T-shapes. Shape of strip for ICA will be dictated by number of target analytes.

Lateral flow immunoassays represent a well-established and very appropriate technology when applied to a wide variety of point-of-care (POC) or field use applications.

The advantages of the lateral flow immunoassay system (LFIA) are well known:

- Relative ease of manufacture - equipment and processes already developed and available. Easily scalable to high-volume production
- Stable - shelf-lives of 12-24 months often without refrigeration
- Ease of use: minimal operator-dependent steps and interpretation
- Can handle small volumes of multiple sample types
- Can be integrated with onboard electronics, reader systems, and information systems
- Relatively low cost and short timeline for development and approval
- Market presence and acceptance - minimal education required for users and regulators

Traditionally designed lateral flow immunoassays, however, have also suffered from performance limitations, most notably sensitivity and reproducibility. Some of these issues are listed below:

- Miniaturization of sample volume requirements below microliter level
- Multiplexing: simultaneous analysis of multiple markers difficult
- Integration with onboard electronics and built-in QC functions challenging
- Sensitivity issues in some systems
- Test-to-test reproducibility challenging - limits applications in quantitative systems

Benefit from its rapid test procedure and naked eyes visible characteristics, lateral flow immunoassays have achieved broad penetration in a variety of markets. Here we summarize some of the applications in figure 4.

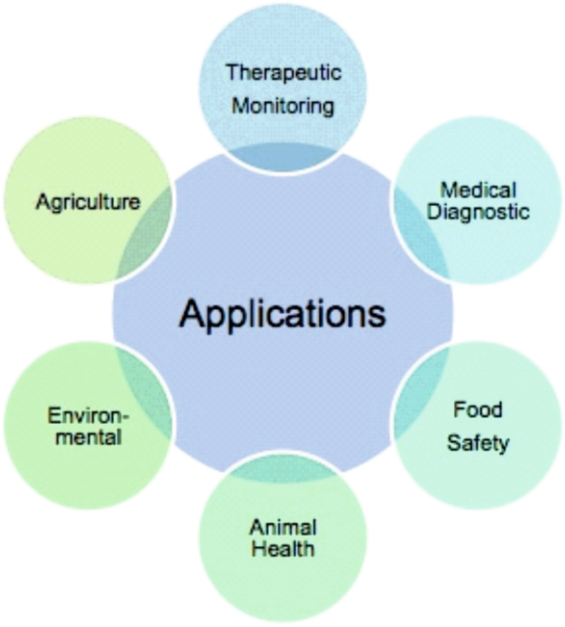


Figure 4. Applications of Immunochromatography assay.

Point-of-care Immunodiagnostic Tests for COVID-19

Scientific Brief

In response to the growing COVID-19 pandemic and shortages of laboratory-based molecular testing capacity and reagents, multiple diagnostic test manufacturers have developed and begun selling rapid and easy-to-use devices to facilitate testing outside of laboratory settings. These simple test kits are based either on detection of proteins from the COVID-19 virus in respiratory samples (e.g. sputum, throat swab) or detection, in blood or serum, of human antibodies generated in response to infection.

WHO applauds the efforts of test developers to innovate and respond to the needs of the population.

However, before these tests can be recommended, they must be validated in the appropriate populations and settings. Inadequate tests may miss patients with active infection or falsely categorize patients as having the disease when they do not, further hampering disease control efforts. **At present, based on current evidence, WHO recommends the use of these new point-of-care immunodiagnostic tests only in research settings. They should not be used in any other setting, including for clinical decision-making, until evidence supporting use for specific indications is available.**

WHO continues to evaluate available immunodiagnostics tests for COVID-19 and will update this scientific brief when necessary.

Rapid diagnostic tests based on antigen detection

One type of rapid diagnostic test (RDT) detects the presence of viral proteins (antigens) expressed by the COVID-19 virus in a sample from the respiratory tract of a person. If the target antigen is present in sufficient concentrations in the sample, it will bind to specific antibodies fixed to a paper strip enclosed in a plastic casing and generate a visually detectable signal, typically within 30 minutes. The antigen(s) detected are expressed only when the virus is actively replicating; therefore, such tests are best used to identify acute or early infection.

How well the tests work depends on several factors, including the time from onset of illness, the concentration of virus in the specimen, the quality of the specimen collected from a person and how it is processed, and the precise formulation of the reagents in the test kits. Based on experience with antigen-based RDTs for other respiratory diseases such as influenza, in which affected patients have comparable concentrations of influenza virus in respiratory samples as seen in COVID-19, the sensitivity of these tests might be expected to vary from 34% to 80%.

Based on this information, half or more of COVID-19 infected patients might be missed by such tests, depending on the group of patients tested. These assumptions urgently require further study to understand whether they are accurate. Additionally, false-positive results – that is, a test showing that a person is infected when they are not – could occur if the antibodies on the test strip also recognize antigens of viruses other than COVID-19, such as from human coronaviruses that cause the common cold. If any of the antigen detection tests that are under development or commercialized demonstrate adequate performance, they could potentially be used as triage tests to rapidly identify patients who are very likely to have COVID-19, reducing or eliminating the need for expensive molecular confirmatory testing.

With the limited data now available, **WHO does not currently recommend the use of antigen-detecting rapid diagnostic tests for patient care, although research into their performance and potential diagnostic utility is highly encouraged.**

Rapid diagnostic tests based on host antibody detection

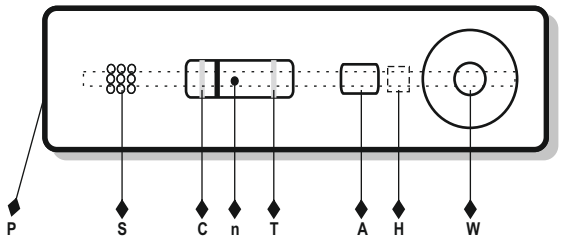
There is another, more common type of rapid diagnostic test marketed for COVID-19; a test that detects the presence of antibodies in the blood of people believed to have been infected with COVID-19. Antibodies are produced over days to weeks after infection with the virus. The strength of antibody response depends on several factors, including age, nutritional status, severity of disease, and certain medications or infections like HIV that suppress the immune system. In some people with COVID-19, disease confirmed by molecular testing (e.g. reverse transcription polymerase chain reaction: RT-PCR), weak, late or absent antibody responses have been reported. Studies suggest that the majority of patients develop antibody response only in the second week after onset of symptoms. This means that a diagnosis of COVID-19 infection based on antibody response will often only be possible in the recovery phase, when many of the opportunities for clinical intervention or interruption of disease transmission have already passed. Antibody detection tests targeting COVID-19 may also cross-react with other pathogens, including other human coronaviruses and give false-positive results. Lastly, there has been discussion about whether RDTs detecting antibodies could predict whether an individual was immune to reinfection with the COVID-19 virus. There is no evidence to date to support this.

Tests to detect antibody responses to COVID-19 in the population will be critical to support the development of vaccines, and to add to our understanding of the extent of infection among people who are not identified through active case finding and surveillance efforts, the attack rate in the population, and the infection fatality rate. For clinical diagnosis, however, such tests have limited utility because they cannot quickly diagnose acute infection to inform actions needed to determine the course of treatment. Some clinicians have used these tests for antibody responses to make a presumptive diagnosis of recent COVID-19 disease in cases where molecular testing was negative but where there was a strong epidemiological link to COVID-19 infection and paired blood samples (acute and convalescent) showing rising antibody levels.

Based on current data, **WHO does not recommend the use of antibody-detecting rapid diagnostic tests for patient care but encourages the continuation of work to establish their usefulness in disease surveillance and epidemiologic research.**

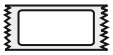
Malaria - RDTs

Background: Early rapid diagnosis of malaria is crucial to the healthcare programmes in endemic countries. Their increasing importance is in response to increasing drug costs and recognition of the importance of early and correct treatment to attain reduction in malaria morbidity and mortality. Malaria RDT's in the last decade have played a vital role in this regard in reaching objective treatment to affected populations even in resource poor settings, where the traditional microscopic diagnosis is either impractical or impossible. In the last decade there has been an increase in the operational use of RDT's as well as an increase in number of companies manufacturing or dealing in malaria RDT's. Wide range of field and laboratory trials have been conducted in the last decade to assess the accuracy and effectiveness of various products to establish their performance, quality and appropriateness for use in the endemic areas. These trials have given the users and decision makers a huge database to review. In addition, local testing in country of use works to further supplement and validate this performance data for decision making. Published field trials of Malaria RDT's have however shown high variability in performance due to the following reasons: Poor study methods, analysis & reporting. Inexperienced manpower. Incorrect handling. Poor preparation and interpretation. Attempts have been made to undertake larger centralized trials to evaluate the performance of Malaria RDT's. Before discussing the alternative approaches to the performance evaluation of Malaria RDTs, it would be useful to understand the architecture and functioning of Malaria RDTs.



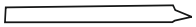
The Essential Components of A Malaria RDT: W = Wicking area for sample / buffer; H = HAMA Blocking Reagents embedded on sample pad; A = Conjugate pad containing the gold conjugate to target antigen specific antibody; T = Test line striped to target antigen specific antibody; n = Nitrocellulose membrane; C = Control line striped with relevant antibody to give a test run validation; S = Soak pad that absorbs the unreacted sample post test conclusion; P = Plastic housing.

Components To Be Used With RDTs:


Desiccant with indicator to ensure moisture free pack.


Buffer bottle for test run.


Sample dispensing loop.


Lancet for obtaining finger prick blood


Alcohol swab

Malaria RDT's: Immunological Considerations

RDT Combinations	Possible Detection Systems	Detects
Pf only	Monoclonal Anti Pf HRP-II specific	*P. falciparum* infection
Pf only	Monoclonal Anti Pf pLDH specific	*P. falciparum* infection
Pf and Pan	Monoclonal Anti Pf HRP-II specific + Monoclonal Anti Pan pLDH specific (co-specific to *P. falciparum, P. vivax, P. ovale* and *P. malariae)*	*P. falciparum* infection & Differentiate *P. falciparum* infection and non *P. falciparum* infection
Pf and Pan	Monoclonal Anti Pf HRP-II specific + Monoclonal Anti Pan Aldolase specific (co-specific to *P. falciparum, P. vivax, P. ovale* and *P. malariae)*	*P. falciparum* infection & Differentiate *P. falciparum* infection and non *P. falciparum* infection
Pf and Pan	Monoclonal Anti Pf pLDH specific + Monoclonal Anti Pan Aldolase specific (co-specific to *P. falciparum, P. vivax, P. ovale* and *P. malariae)*	*P. falciparum* infection & Differentiate *P. falciparum* infection and non *P. falciparum* infection
Pf and Pan	Monoclonal Anti Pf pLDH specific + Monoclonal Anti pan pLDH specific (co-specific to *P. falciparum, P. vivax, P. ovale* and *P. malariae)*	*P. falciparum* infection & Differentiate *P. falciparum* infection and non *P. falciparum* infection
Pf and Pv	Monoclonal Anti Pf HRP-II specific + Monoclonal Anti *P. vivax* pLDH specific	*Speciates P. falciparum* infection & *P. vivax* infection
Pan only	Monoclonal Anti pan pLDH specific (co-specific to *P. falciparum, P. vivax, P. ovale* and *P. malariae)*	All malaria parasite infection
Pf, Pv and Pan	Monoclonal Anti Pf HRP-II specific + Monoclonal Anti *P. vivax* pLDH specific + Monoclonal Anti pan pLDH specific (co-specific to *P. falciparum, P. vivax, P. ovale* and *P. malariae)*	*Speciates P. falciparum* infection, P. vivax infection, Detects non *P. falciparum* and *P. vivax* infections

General Comments: All HRP-II detection tests, have a better analytical sensitivity and a lower limit of detection for P. falciparum malaria as compared to pLDH and Aldolase based assays. Since HRP-II persists in the blood circulation 2-3 weeks even after successful treatment testing within the said "hang over"period will give "false positive results". Hangover period of pLDH assays is much shorter than for HRP-II based assays. HRP-II detection systems by and large do display better thermal stability over other detection systems based on pLDH and aldolase. However, product to product the stability may vary. pLDH based detection assays have lower analytical sensitivity as compared to HRP-II based assays for detection of P. falciparum infections. pLDH and Aldolase based assays also have lower thermal stability as compared to HRP-II assays.

Similarities and Differences in Malaria RDTs: Though the various RDT's appear to be similar, they vary considerably in their functioning due to the intrinsic character of the critical components employed. The performance of each RDT is based on the optimization of all these functional components, which vary from manufacturer to manufacturer in terms of their basic characteristics and processing methods employed during manufacture.

Component	Typical function	Impact on RDT
Sample Pad	Usually contains HAMA blocking agents.	Improves specificity by blocking RF and heterophilic antibodies.
Buffer	Brings about lysis of the red cells, especially of the sequestered parasite and release of target antigens.	The lysing efficiency of the buffer • dictates the revelation of the target antigen from the parasitized red cells. • Formulation effectiveness impacts sensitivity of test.

Component	Typical function	Impact on RDT
Gold Conjugate	Binds to the Target antigen as the lysed blood flows through the conjugate pad.	Primary binding agent to the target antigen, it influences sensitivity, specificity and binding affinity to target antigen.
Nitrocellulose Membrane	Serves as a migration template /platform for the reaction and directs reactants and reaction kinetics of each test.	**Large porosity membranes:** reactants move fast. Better background clearance, however, leads to loss of test sensitivity since accords less time for reagents to react and bind. **Small porosity membranes:** Reactants move slowly, slower background clearance, improves test sensitivity as reagents have more time to react and bind.
Test lines	Capture antibody directed towards the specific malaria antigen in the sample.	Secondary binding agent in tandem with gold conjugate; drives sensitivity, specificity and affinity of binding and signal intensity.
Control line	Reacts with excess / unreacted conjugate to validate test run.	Ideally should be the weakest visible intensity to serve as a practical comparator for test intensity.
Soak pad	Absorbs excess reactants at test culmination.	Absorbance capacity impacts back flow of reactants post test completion.

The final optimization of each product will then have an impact on its functioning and the resultant: Migration and Reaction Kinetics, Sensitivity, Specificity, Thermal stability, Ease of use.

Problems associated with the usage of Malaria RDT's: RDT's retrieved from cold storage (2-8°C must be allowed to come to room temperature (ambient temperature, in case of field conditions) before the pouches are opened and the test used. When specimen is added to a cold device, it attacks a 'moisture rush' thereby altering the migration properties of the membrane. If specimen is added to 'cold' devices the blood flow is usually slowed down affecting the background clearance and visualization of test results; especially of specimen containing low target antigen concentration. At lower temperatures the antigen-antibody binding is less than optimum, leading to loss in sensitivity and resultant signals. Use of specimen transfer devices such as loops and straws are extremely simple to use. However, to ensure accuracy and precision of sample delivery, user training has to be imparted to build usage competence with actual users. The end point of a Malaria RDT reaction is qualitative. Its interpretation has an element of personal subjectivity, that introduces variability. What appears as a weak positive to our reader may well be negative for another. Usually “tie break”method or “best of 3” is the best way to resolve interpretative subjectivity. User training, hands on experience in reading and interpreting RDT end points must be assessed for consistency as a prerequisite. This is especially relevant when samples used during evaluation are low analyte concentrations, nearing the detection limits of RDT's are being used that generate very low signal intensities.

Antigen Vs Antibody Tests in Malaria Diagnosis

Malaria has been recognized to be a major source of mortality and morbidity worldwide. With the emergence of drug resistant strains, the infection presents a diagnostic challenge to laboratories, globally.

Although microscopy still remains the "Gold Standard" for diagnosis, in the recent years, laboratory diagnosis of malaria has been enhanced by the introduction of easy to use, affordable, simple immunochromatography assays.

Given the limitations of conventional diagnostic methods, it is not surprising that pathologists and clinicians have looked to these rapid immunochromatography techniques or Rapid Diagnostic Tests (RDT's) as additional, and perhaps more definitive, means of diagnosing and differentiating malaria species.

Currently available RDT's can be classified according to the analyte they detect:

Antibody based RDT's

Antibodies to the asexual blood stages of the parasite appear a few days after malarial infection and increase in titre over a few weeks. After successful therapy, the antibodies titers may fall more rapidly and are undetectable within 3-6 months.

Reinfection or relapse induces a secondary immune response with a rapid increase in antibody titre. It is also observed that antibody titre (due to past exposure) may persist for upto 10 years in endemic areas such as India.

Delay in appearance of detectable levels of antibodies limit the used of Antibody based RDT's and are not suitable for early detection of malarial infection.

Presence of antibodies to the Plasmodium parasite though provide useful information with regards to exposure to malaria infection, it does not differentiate between current and successfully treated past infection.

Therefore Antibody based RDT's have limited use in routine malaria diagnosis and is more useful for sero-epidemiological studies on malaria.

Most antibody based RDT's detect the presence of anti-malarial antibodies by employing blood stage antigen prepared from primate blood infection or from *P. falciparum* cultures in the laboratory. Blood stage schizonts coated on the test band often tends to cross react with the antibodies directed against *P.ovale, P.malariae* and *P.vivax.*

Further some studies have also stated that cross reaction can also occur between Plasmodium and Babesia species.

To conclude, employing an antibody based test for routine diagnosis of malaria may lead to improper patient management due to delayed diagnosis, false classification and incorrect speciation.

Antigen based RDT's

Antigen based RDT's for malaria detect circulating antigens in the infected individual by the corresponding antibody. Malaria antigens currently targeted by RDT's are Histidine Rich Protein II (HRP-II) and parasite Lactate Dehydrogenase (pLDH).

Many field and laboratory studies have compared these antigen based RDT's with conventional microscopy, fluorescence microscopy and PCR and have concluded that antigen based RDT's are a better alternative for diagnosing malaria, in field and laboratory conditions alike.

Recently, RDT's for combined detection of Pf. HRP-II for *P.falciparum* detection, *P.vivax* specific pLDH for *P.vivax* detection and pan specific pLDH for all 4 Plasmodium species have been developed.

These combo RDT's offer benefits of accurate detection and true speciation of the 'Big Two'; *P. falciparum* and *P. vivax*, and can also be employed for monitoring success of anti-malarial therapy.

Further, these combo RDT's are simple, rapid, sensitive, specific and suitable for on the spot diagnosis of malaria, even in field settings.

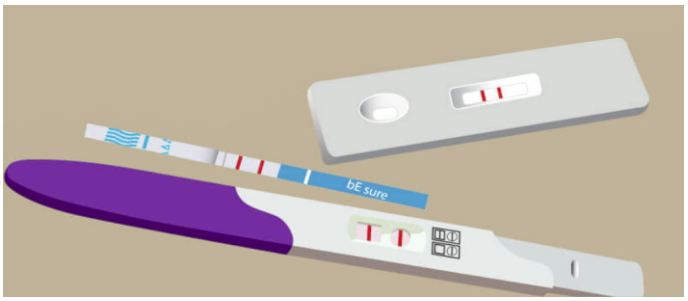
Home Pregnancy Tests

Home pregnancy tests are accurate as long as you follow the instructions correctly.

A positive test result is almost certainly correct. However, a negative test result is less reliable. The result may not be reliable if you:

- don't follow the instructions properly
- take the test too early

Some medications can also affect the results.



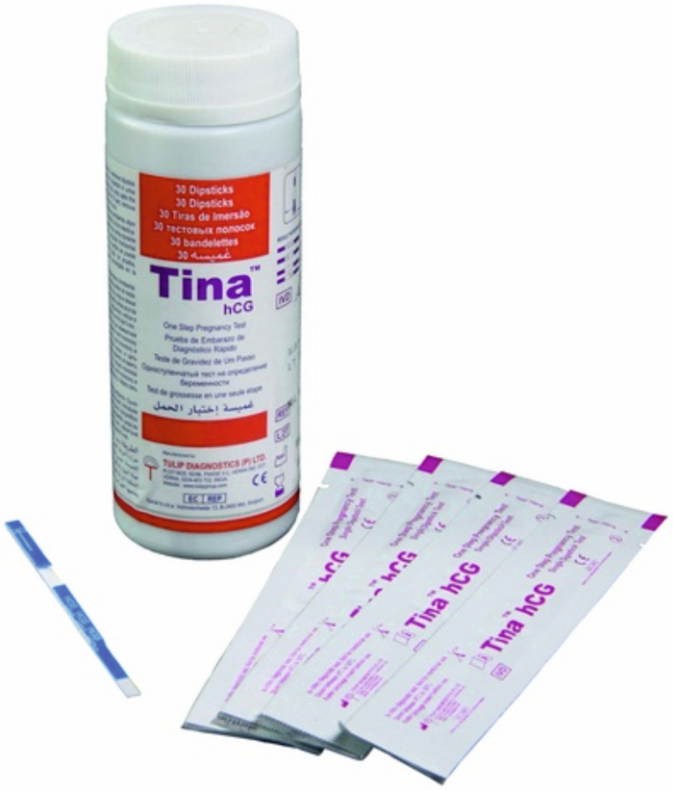
Carrying out a test

When you become pregnant, your body produces the pregnancy hormone, human chorionic gonadotrophin (HCG). Home pregnancy tests detect HCG in your urine.

You can take most pregnancy tests from the first day of your missed period. Tests carried out earlier than this are not always accurate. For more information, see How soon can I do a pregnancy test?

Check the instructions to make sure you can do the test at any time of day. It's usually best to take the test first thing in the morning as your urine will have the highest concentration of hormones at this time.

Avoid drinking too much fluid beforehand, as this can dilute the level of HCG in your urine.



Positive test results

If the test result is positive, you're almost certainly pregnant. Contact your GP surgery as soon as possible. Because home pregnancy tests are so accurate, your GP may not repeat the test.

If you want to continue with your pregnancy, it's a good idea to start your antenatal care as soon as possible. If you're not sure whether you want to continue with the pregnancy, you can find more information about your options here: Am I pregnant?

If you want to know when the baby is due, you can use our pregnancy due date calculator.

Negative test results

If the test result is negative, you may not be pregnant. However, negative results are less reliable. For example, if you do a pregnancy test too early, you could be pregnant, but there may not be enough HCG in your body to give a positive test result.

Pregnancy tests vary in their sensitivity (how soon they can detect HCG and what level of HCG needs to be present). You can find information on the packaging about how sensitive your test is.

If you still think you're pregnant after a negative result, wait a few days and try again. Speak to your GP if you get a negative result after a second test but your period hasn't arrived.

Medications

Some medications can affect test results, including:

- promethazine – used to treat conditions such as allergies
- medicines used to treat Parkinson's disease
- sleeping tablets (hypnotics)
- tranquillisers
- diuretics (medicines that increase the amount of urine produced) – used to treat conditions such as heart failure
- anticonvulsants (medicines that prevent seizures or fits) – used to treat conditions such as epilepsy
- medicines used for infertility

If you're taking any medication, the patient information leaflet that comes with it will tell you if it affects test results. You can also ask a pharmacist.

Early miscarriage

If your first pregnancy test result is positive, but a later one is negative or your period arrives, it's possible you've had an early miscarriage. Speak to your GP or midwife for advice.

Guidelines for Blood Grouping and Antibody Testing in Pregnancy

INTRODUCTION

Purpose of the Guideline

The purpose of the guideline is to define the red cell immunohaematology tests which should be applied in pregnancy. The aim of the testing programme is the prevention of haemolytic disease of the fetus and newborn.

Since the majority of publications use the term 'haemolytic disease of the newborn', HDN, to refer to both fetus and newborn, it is used here too.

The Significant Developments

The use of monoclonal reagents and automation has considerably improved the related diagnostic scenario. The Serious Hazards of Transfusion [SHOT] haemovigilance scheme has focused attention on blood grouping and red cell serology practice and revised guidelines for compatibility testing in blood transfusion laboratories have been brought to the fore.

The introduction of non-invasive techniques to monitor fetal anaemia has influenced the management of allo-immunised pregnancies and the concentration of care of these cases in fetal medicine units has resulted in improved outcomes of intra-uterine transfusion.

It is endorsed that routine antenatal anti-D prophylaxis (RAADP) should be offered to all D negative women who have no detectable immune anti-D.

Injections at 28 weeks and again at 34 weeks gestation are recommended.

As the relative incidence of immune anti-D has declined the incidence of positive antibody screens due to prophylactic anti- D has increased and the two types of antibody cannot be distinguished by laboratory tests.

The risks associated with the misinterpretation of passive and immune anti D are clear: if passive anti-D is misinterpreted as immune, anti-D prophylaxis may be omitted leaving the women unprotected from sensitization. If immune anti-D is misinterpreted as passive, appropriate follow-up of the antibody level during pregnancy may be curtailed putting the fetus at risk. The testing protocols recommended here are designed to provide clarity for practice in order to protect pregnant women including those who are D negative, and their infants.

Informed consent

Providing information about any blood test and obtaining consent is a clinical responsibility and ideally informed consent should be obtained and documented prior to samples being taken.

Purposes of laboratory tests

ABO and D typing to identify D negative women who require anti-D prophylaxis.

Screening and identification of red cell alloantibodies

To detect clinically significant antibodies which might affect the fetus and/or newborn to highlight possible transfusion problems.

Follow-up tests when clinically significant red cell antibodies are present

-To monitor the strength of antibodies to identify those pregnancies which are at risk of HDN and to predict fetuses/infants who are likely to require treatment for HDN.

-To identify additional maternal alloantibodies. Women who have developed one or more antibodies may go on to form further antibodies of different specificities.

Recommendations for samples and request forms

Identification of samples

It is essential that samples from pregnant women are correctly identified and that request forms are accurately completed. SHOT reports provide evidence that errors in patient identification and sample labelling may lead to ABO incompatible transfusions. The record of ABO/D type derived from an antenatal sample may be used as the basis for the provision of suitable blood for transfusion, and the sample could be used for a crossmatch.

Misidentification can also lead to failure in, or inappropriate, administration of prophylactic anti-D.

Therefore, the same minimum patient identification on antenatal samples and request forms is required as for pre-transfusion samples i.e.

I] Surname/family name [correctly spelt]

ii] First name[s] in full

iii] Date of birth [not age or year of birth]

iv] Unique identifier number e.g. Hospital number or IPD/ OPD number.

When these are not readily 'address' is a suitable alternative identifier if it is given on both the sample and the request form.

Recommendation 1 Samples for antenatal screening are identified to the same standard as pre-transfusion samples (Good Practice Point [GPP])

Recommendation 2 Samples should be dated, labelled and signed by the person taking them, in the presence of the patient who should be asked to confirm demographic details. Any labels pre-printed away from the phlebotomy procedure, should not be accepted on the specimen

It is essential that any previous administration of prophylactic anti-D in the current pregnancy, including date and dose, is recorded on the laboratory request form.

Clinical history, particularly of HDN and previous transfusions, is also essential information on the request form.

Recommendations for laboratory testing

All test procedures must be well established and validated in compliance with published guidelines. Ideally testing should be performed on automated equipment which ensures positive sample identification and with electronic transfer of results to the woman's computer record.

Recommendation 3 ABO and D grouping must be performed in accordance with the guidelines for compatibility procedures in blood transfusion laboratories.

TESTING

Antibody screening.

Approximately 1% of pregnant women are found to have clinically significant red cell antibodies. Of these, the commonest specificity is anti-D, although the universal introduction of RAADP is predicted to reduce this sensitisation rate. However, with the introduction of RAADP there is a significant rise in positive antibody screening results, due to the detection of prophylactic anti-D.

Screening methods.

The Indirect Antiglobulin Test [IAT] using reagent red cells suspended in LISS is the most suitable for detection of clinically significant red cell antibodies. Column agglutination methods, liquid-phase tube tests and solid-phase methods have been found to be suitable [Poole, 1996]. There is no additional value in using an enzyme technique in antibody screening. Testing for high levels of immune anti-A or anti-B in pregnant women is not recommended as their presence neither predicts ABO HDN nor does it cause problems *in utero*.

Reagent cells.

Cells used for antibody screening should comply with the

recommendations of the guidelines for compatibility procedures in blood transfusion laboratories. The following antigens should be expressed on screening cells: C,c,D,E,e,K,k,Fya, Fyb, Jka, Jkb,S, s, M, N, Lea. It is recommended that one of the screening cells should be R1R1 and another should be R2R2 and that the Fya, Fyb, Jka, Jkb, S and s antigens should be represented on reagent cells with homozygous expression. Screening cells must not be pooled. It is not necessary to include screening cells which express low frequency antigens such as Cw, Kpa or Lua.

***Recommendation 5:* The screening cells and methods used for red cell antibody screening should comply with the guidelines for compatibility procedures in blood transfusion laboratories; BCSH b 2004 (Level IV, Grade C)**

ANTENATAL TESTING PROTOCOLS

See algorithm for samples and testing requirements.

Routine Testing

All pregnant women should have samples taken early in pregnancy, ideally at 10-16 weeks gestation, for ABO and D typing and for screening for the presence of red cell alloantibodies. When an antibody screen is positive further tests should be carried out to determine the antibody specificity and significance.

All pregnant women, whether D positive or D negative, should have a further blood sample taken at 28 weeks gestation for re-checking the ABO and D group and further screening for red cell allo-antibodies [National Collaborating Centre for Women's and Children's Health, 2003]. D positive women are just as likely as D negative women to form antibodies, other than anti-D, late in pregnancy [Thompson et al, 2003]. No further routine blood grouping or antibody screening is necessary after 28 weeks.

There is evidence that antibodies detected only in the third trimester do not cause HDN [Rothenberg et al, 1999; Heddle et al, 1993]. Further, and significantly, the introduction of RAADP has resulted in the detection of anti-D in samples taken after 28 weeks from D negative women [Dalton 2003; Parker 2003]. Since it is not possible to differentiate between prophylactic and immune anti-D there is the potential for confusion between the two [New et al, 2001].

***Recommendation 6:* All pregnant women should be ABO and D typed and screened for the presence of red cell antibodies early in pregnancy and at 28 weeks gestation [National Collaborating Centre for Women's and Children's Health, 2003]. (Level III, Grade B)**

Local policies must ensure that D-negative women eligible for RAADP have the third trimester antibody screening sample taken before the first RAADP injection is administered at 28 weeks. Samples taken after the injection could result in passive anti-D being detected which may be mistaken for immune anti-D.

Sensitising Episodes during pregnancy

This section is a synopsis of recommendations of the Guideline for administration of anti-D prophylaxis [In addition to RAADP, pregnant women who are D negative should be considered for anti-D prophylaxis for the following potentially sensitising episodes:

- Amniocentesis
- Cordocentesis
- Other in-utero therapeutic intervention/surgery (e.g. intrauterine transfusion, shunting)
- Ante partum haemorrhage (APH)
- Chorionic villus sampling
- Ectopic pregnancy
- External cephalic version
- Fall / Abdominal trauma
- Intrauterine death
- Miscarriage
- Termination of pregnancy

Before 12 weeks gestation, confirmed by scan, in uncomplicated miscarriage or mild and painless vaginal bleeding, anti-D should not be administered because the risk of feto-maternal haemorrhage [FMH] is minimal.

Between 12 and 20 weeks gestation, for any potential sensitising event outlined above, a sample should be tested to confirm that the woman is D negative and that she has not become sensitised to the D antigen. At least 250 iu anti-D immunoglobulin should be administered as soon as possible and certainly within 72 hours of the event. There is no need to assess the volume of FMH under 20 weeks gestation.

After 20 weeks gestation there is a requirement to assess the volume of FMH.

If the acid elution technique is used and a FMH of more than 4mL is indicated, the test should be repeated using flow cytometry. At least 500 iu anti-D immunoglobulin should be administered intramuscularly as soon as possible, and certainly within 72 hours of the potentially sensitising event. If FMH of more than 4mL is confirmed by flow cytometry, more anti-D will be required [BCSH-c, 2005].

After the birth, a cord sample must be tested to obtain the ABO and D type of the baby. If this is not collected for any reason, a heel prick sample should be obtained as soon as possible.

Maternal samples for confirmatory ABO and D type and FMH testing should be collected after sufficient time has elapsed for any FMH to be dispersed in the maternal circulation. A period of 30-45 minutes is considered adequate.

Following birth of a D positive infant at least 500 iu anti-D must be administered to the woman if the FMH is 4 mL or less.

If the pregnancy is non-viable and no sample can be obtained from the baby, prophylactic anti-D must be administered to the woman.

Red cell antibodies detected in pregnancy:

When red cell antibodies are detected, further testing of maternal blood should be undertaken to determine the specificity, concentration, origin and level of antibody or antibodies, and the likelihood of HDN.

Anti-D, anti-c and anti-K are the antibodies most often implicated in causing haemolytic disease severe enough to warrant antenatal intervention.

Women with anti-D present Distinguishing between prophylactic and immune anti-D

In addition to the administration of prophylactic anti-D following sensitising events the use of RAADP is increasing as the NICE recommendations [NICE, 2002] are being adopted. It is therefore inevitable that more antenatal samples containing low levels of anti-D will present the problem of determining whether the anti-D is prophylactic or immune.

Following administration of an intramuscular injection of anti-D immunoglobulin, serologically detectable levels of anti-D are quickly reached and peak blood levels are reached within three to seven days. The half-life of prophylactic anti-D immunoglobulin is approximately 3 weeks [Eklund et al, 1982]. Prophylactic anti-D can be detected by serological tests for several weeks: by IAT for up to 8 weeks or more and by other more sensitive techniques for up to 12 weeks and in exceptional cases for several months.

Immune anti-D becomes detectable approximately 4 weeks after exposure to D positive cells, and reaches a peak level after 6-8 weeks [Mollison et al c, 1997].

Both prophylactic and immune anti-D are detectable by laboratory tests and cannot be distinguished. While prophylactic anti-D levels will fall with time, immune anti-D levels will usually remain stable or rise if there is re-stimulation of the antibody.
The level of anti-D in maternal samples post prophylaxis rarely exceeds 1 iu/mL unless a dose[s] of more than1250 iu has been administered.

Procedure if prophylactic anti-D is suspected

a. If there is a record of administration of anti-D within the past 8 weeks and the antibody reaction is weak, testing should be as for non-sensitised women i.e. no antibody testing after 28 weeks and Rh prophylaxis should continue.
b. If there is no record of anti-D administration or information regarding prophylaxis is not available the antibody should be monitored by both IAT and anti-D quantification as for immunised women i.e. at 4 weekly intervals to 28 weeks or at 2 weekly intervals if after 28 weeks. If the anti-D becomes undetectable by IAT and the quantified level is falling it is probably prophylactic. A rising or steady level indicates immune anti-D.
If there is significant doubt about the immune or passive nature of anti D, the sample should be referred for quantification.
In either case anti-D prophylaxis should continue unless it is established beyond doubt that the anti-D is immune.
The pregnant woman with anti-D should not be issued with an antibody card documenting the finding of anti-D until it is established that the anti-D is immune.
If the sample in which anti-D is detected is referred for routine antibody screening or is pre-transfusion, a panel of D negative cells, selected to provide all relevant red cell antigens, should be used to detect or exclude the presence of allo-antibodies of other specificities.

Recommendation 7:

Blood transfusion laboratories should keep a record of anti-D administration to provide a basis for distinguishing between immune and prophylactic anti-D (Level IV, Grade C).

Women with immune anti-D

Blood samples from women with immune anti-D should normally be tested at least monthly until 28 weeks gestation and every 2 weeks thereafter to monitor the level of anti-D and to identify any additional antibodies that may develop.
The antibody level should be quantified, in iu/mL, using the national anti-D standard [National Institute for Biological Standards and Control (NIBSC),2003].
Each sample should be tested in parallel with the previous sample and the results compared to identify significant changes in antibody level.
Where the level is more than 1.0 iu/mL an increase of anti-D level of 50% or greater over the previous level indicates a significant increase, irrespective of the period of gestation.
Anti-D is the most frequent antibody responsible for serious HDN. The following levels of anti-D have been used to guide the management of pregnancies since the publication of the previous guideline [BCSH a, 1996; Nicolaides & Rodeck,1992].
Anti-D Less than 4 iu/mL HDN unlikely
Anti-D 4-15 iu/mL Moderate risk of HDN
Anti-D More than 15 iu/mL High risk of hydrops fetalis
As a consequence of developments in the assessment of fetal anaemia and in the technique of IUT the significant anti-D level is that which triggers referral to a specialist feto-maternal unit. Non-invasive assessment can then be used to monitor fetal anaemia [Scheier et al, 2004]. A woman whose anti-D level is 4 iu/mL or greater and/or has a rising anti-D level and/or has a history of HDN affected offspring must be referred to such a unit. It should also be noted that HDN has been reported at levels less than 4 iu/mL [Bowell et al, 1982]. Once the referral to the feto-maternal unit has been made the value of subsequent samples for anti-D quantification is doubtful. A sample at 28 weeks should be tested for the presence of further red cell antibodies.
It is possible to determine the D type of the fetus from a maternal peripheral blood sample using polymerase chain reaction (PCR) see section 7. [Daniels et al a, 2004]

Women with apparent anti-C + D, possible anti-G

A proportion of antibodies with apparent anti-C+D specificity but with disproportionately high anti-C titres may be demonstrated, by advanced serological techniques, to be anti-G. [Maley et al, 2001]. Since women with anti-G, without anti-D, should be eligible for RAADP and post-delivery anti-D immunoglobulin it is important that a reference centre should confirm examples of apparent anti-C+D specificity.

Women with immune anti-c present

Women with anti-c should be re-tested with the same frequency as women with anti-D, i.e. at least monthly to 28 weeks gestation and every 2 weeks thereafter.
Samples from women with anti-c should be quantified with reference to the international anti-c standard [NIBSC 2003] with the previous sample tested in parallel, as for anti-D [above], and any additional antibodies should be identified.
Quantification of anti-c is useful in monitoring increases in the antibody concentration.
When account has been taken of previous history of HDN the following levels of anti-c are indicative of the need to refer to a specialist unit [Kozlowski et al, 1995].

Anti-c level:

Less than 7.5 iu/mL Continue to monitor.
7.5 to 20 iu/mL Risk of moderate HDN, refer to specialist unit.
More than 20 iu/mL Risk of severe HDN Refer to specialist unit.
It is important to note that anti-c may cause delayed anaemia in the neonate.

Women with immune anti-K, or other Kell system antibodies.

HDN due to anti-K is characterised by low haemoglobin, but amniotic and/or cord bilirubin levels are not generally reported. The fetal anaemia associated 11 with anti-K may be due to the inhibition of K positive erythroid early progenitor cells [Vaughan et al, 1998] or to promotion of their immune destruction [Daniels et al b, 2003].
While it has been stated that the severity of HDN due to anti-K is not correlated with titre of the antibody reports of affected pregnancies are associated with antibodies with a titre of at least 32. [McKenna et al, 1999; Ahaded et al, 2000]
However, samples from women with anti-K should be titrated by IAT when first identified in the pregnancy, as for any clinically significant antibody.
The majority of cases of anti-K in pregnant women are the consequence of previous K positive transfusions. The incidence of anti-K could be reduced by selecting K negative units for transfusion to females with potential for childbearing [Lee & de Silva, 2004]. Therefore selecting K-negative units for females under the age of 60 is considered good practice. However urgent transfusions should not be delayed if suitable K-negative units are not immediately available.
The transfusion history of women with anti-K should be established and a sample from the father of the fetus should be K typed. If the woman has not been transfused and the father is K positive, the patient should be referred to a specialist unit and titration of samples should be performed at monthly intervals to 28 weeks, and at fortnightly intervals thereafter. If

the father is K negative and a confidential enquiry establishes paternity, no further samples are required until 28 weeks when the antibody should be titrated and further antibodies excluded.

The fetus can be K typed from an amniocentesis sample, but this sampling involves physical intervention with associated risks to the fetus and of stimulating the antibody level. See section 7, 'Fetal genotyping', for reference to the development of K genotyping from peripheral maternal blood samples.

Recommendation 8: ***Cases of anti-D, anti-c and anti-K [unless the father is confirmed K negative] should be assessed at monthly intervals to 28 weeks gestation and at fortnightly intervals thereafter. Such cases must be referred to a specialist fetal medicine unit if the antibody reaches the critical level and/or the level is rising significantly. (Grade B)***

Women with other red cell antibodies

Only IgG antibodies are capable of entering the fetal circulation. Red cell antibodies with a significant IgG component are detectable by IAT. 'Cold reactive', IgM and low affinity antibodies to high frequency antigens [e.g. CR1 and CR4 related antibodies] have not been implicated in HDN.

In addition to anti-D, -c and K, the following specificities are most commonly associated with HDN: anti-C [-Ce], -E [-cE], -Fya, and -Jka [Moise, 2000; Moran 2000; Goodrick et al, 1997]. However, many other specificities have been reported as the cause of HDN, and a summary, by blood group system, is given by Daniels et al [Daniels et al c, 2002]. In all these cases re-testing at 28 weeks generally provides sufficient information to determine management of 12 the pregnancy. A medical decision should be made regarding the more frequent testing of women with a previous history of children with HDN.

Where an antibody has been detected, testing of both booking and 28-week samples should include titration and testing by IAT against reagent cells heterozygous for the corresponding antigen. Careful attention to technique is necessary to minimise the variables in the method and titrating the national anti-D standard [NIBSC,2003] in parallel, as an internal control, is recommended [BCSH d, 1999]. Given the wide spread implementation of RAADP programmes prophylactic anti-D may be present in addition to alloantibodies and selecting D negative reagent cells for titration should be considered.

In general, a titre of 32 or greater is likely to cause HDN, although a clear-cut association between titre and HDN has not been established.

The presence of any further antibodies should be established and any clinically significant antibodies should be titrated as above.

Recommendation 9:

Clinically significant antibodies, other than anti-D, -c or K, should be assessed, and other antibodies excluded, at 'first appointment' and at 28 weeks gestation. (level IIb Grade B)

Recommendation 10:

All women who have previously had an infant affected by HDN should be referred before 20 weeks to a specialist unit for advice and for assessment of fetal haemolysis, irrespective of antibody level. (Level IIa Grade B)

PATERNAL TESTING

Where a clinically significant antibody capable of causing HDN, particularly anti-D, anti-c or anti-K, is present in a maternal sample, determining the father's phenotype provides useful information to predict the likelihood of a fetus carrying the relevant red cell antigen. The complexities of paternal testing and the potential for misidentification of the father need to be acknowledged [National Collaborating Centre for Women's and Children's Health, 2003].

FETAL GENOTYPING

When a clinically significant antibody of high concentration is present, and/or the woman has a history of HDN and the father is heterozygous for the relevant antigen, it can be clinically relevant to determine the genotype of the fetus. Until recently fetal DNA for genotyping by PCR assay was obtained by amniocentesis or chorionic villus sampling. These invasive techniques carry a small risk of spontaneous miscarriage and may boost maternal antibody levels.

A technique is now available for the accurate determination of fetal D genotype from samples of maternal peripheral plasma.

The same testing service for fetal c and K type is under development and should be used as it becomes available.

REPORTS OF LABORATORY INVESTIGATIONS

In addition to blood group and specificity of any red cell alloantibodies present, reports must inform the clinician[s] responsible for the woman's antenatal care of the likely significance of the antibody/ies, with respect to both the development of HDN and transfusion problems [National Collaborating Centre for Women's and Children's Health 2003]. Reports should also, where relevant, alert the clinician to the need to refer the woman to a specialist unit.

Details of the timing of further samples required should also be given.

Recommendation 11:

Women with clinical significant red cell antibodies should be issued with a card giving details of the antibody. (GPP)

ACTION AT TIME OF BIRTH

D negative women with no immune anti-D

A maternal sample and a cord blood sample should be taken. The cord blood sample should be used to determine the infant's D group, thus identifying women who must receive post-delivery prophylactic anti-D immunoglobulin.

Since there is minimal evidence that DVI on fetal red cells can cause maternal sensitisation, and since detecting DVI on cord samples would require different reagents from those used on adult patients, with the potential for confusion and inappropriate testing of adult patients, testing cord samples for DVI is not recommended, i.e. anti-D reagents for typing cord samples should not react with DVI. Most examples of weak D antigen can be easily detected by selecting high affinity anti-D reagents. [BCSH b, 2004]

A test should be performed on the maternal blood sample to detect and estimate the volume of fetal cells present, so that additional anti-D immunoglobulin may be given if the feto-maternal haemorrhage [FMH] exceeds 4mL. Samples showing a FMH result of more than 4mL by acid elution technique, should be referred for a more accurate assessment of the volume of bleed by flow cytometry. The dose calculation for prophylactic anti-D is based on the volume of FMH. [BCSH c, 2006]

Direct Antiglobulin Test [DAT] on cord samples

Routine DAT on the cord samples of D positive infants born to D negative women

This is not recommended. It has been shown that following RAADP anti-D immunoglobulin can cross the placenta, enter the fetal circulation and bind to fetal D antigen sites. Consequently, up to 3-6% of D positive cord samples have been found to have a positive DAT [Dalton, 2003; Parker 2003] and this may result in unnecessary additional investigations being undertaken and in anxiety for the parents. There is evidence that prophylactic anti-D does not cause destruction of fetal/neonatal red cells [Maayan-Metzger et al, 2001].

DAT on infants of women who have IAT reactive red cell antibodies.

Whenever the maternal serum has been found to contain an immune, IAT reactive red cell antibody/ies a DAT should be done on the cord sample.

A positive DAT in itself is not diagnostic of HDN. However if it is positive, the infant's haemoglobin and bilirubin levels should be checked to diagnose/exclude HDN. Where the DAT is positive and the infant shows symptoms of HDN, a red cell eluate may be helpful to confirm the red cell antibody specificity. In cases of suspected HDN wherever possible the red cells from the cord should be tested for the corresponding antigen[s].

Infants who have been transfused *in utero* with units negative for the relevant antigen the DAT may be negative and the baby may type as antigen negative for several months after birth. [BCSH b, 2004]

Recommendation 12:

All infants born to women who have clinically significant antibodies should be closely observed for evidence of HDN. A DAT should be performed and if positive, haemoglobin and bilirubin levels should be measured. (Level IV, Grade C).

Pre and Post delivery testing of maternal samples

Routine antibody screening of immediate pre and/or post delivery samples is not required, as this information does not influence management of the pregnant woman or her infant. Blood grouping and antibody screening of maternal samples other than confirmatory D typing should be undertaken only if pre-transfusion compatibility tests are required.

FUTURE DEVELOPMENTS

The NICE guidance on the use of RAADP [NICE, 2002] endorses the importance of feasibility studies of mass testing for fetal blood group antenatally by analysis of circulating fetal DNA in maternal plasma. "If fetal Rh blood type could be determined before 28 weeks gestation, antenatal anti-D prophylaxis would be necessary only if the fetus was RhD positive, and knowledge of the father's blood group would no longer be required' It is anticipated that such large scale testing will become available in the near future [Daniels et al a, 2004].

Sperm Counts

Safety Precautions

Safety precautions should be observed when handling seminal fluid. The following guidelines should be followed:

- If non-disposable items are used, soak contaminated items (e.g. hemacytometers and coverslips) in 70% alcohol or Alconox.
- All disposable items should be placed in a biohazard bag for autoclaving.
- Gloves must be worn and hands thoroughly washed when the examination is completed.
- Seminal fluids that are to be discarded should be placed in biohazard bags for autoclaving.

Sperm counting methods

Sperm can be counted either manually or by automated methods. Although automated counting has some advantages for assessment of motility parameters, manual counting is still performed by most laboratories. There are several **manual counting methods** available for semen.

These include:

- Neubauer hemacytometer.
- Makler chamber.
- CellVu.
- MicroCell.

The Makler, CellVu, and MicroCell methods have the advantage of requiring no dilution of the semen. Since semen is viscous, accurate dilution can be problematic. These methods also allow counting of motile and non-motile sperm at the same time and thus avoid the need for separate assessment via wet mount. Each laboratory should determine the best most reproducible method for their own situation, equipment, and expertise.

Calculating sperm count on a hemacytometer:

The formula for calculating the sperm count when 5 small squares within the large center square are counted is:

- Number of sperm counted in 25 squares on each of 2 sides x dilution factor/volume x 1000 = sperm/ml.
- Example: 100 sperm are counted in the five small squares of one side of the hemacytometer, 110 sperm are counted in 5 small squares of the other. The dilution is 1:20.
- Number of sperm in 25 squares on 2 sides = 210 x 5 = 1050 Sperm/ml = 1050 x 20 (dilution factor) divided by 0.2 mm^3 x 1000 = 105 million sperm/ml.

Diluting a specimen for counting on a hemacytometer

Following liquefaction (20-30 minutes), mix the sample manually by swirling the container several times. Thorough mixing is essential for accurate counting. Calibrated automatic pipettes are used to prepare a dilution. Because of the viscosity of semen, the semen should be added to the diluent using a positive pressure pipettor.

The dilution often used for routine sperm counts is 1:20 but the actual dilution factor will vary depending on the total sperm count. For high concentration specimens a greater dilution will be necessary. For low concentrations an undiluted or minimally diluted specimen may be required. The appropriate dilution is determined by estimating the concentration needed to do a count of at least 100 cells per side of the loaded hemacytometer.

The diluent that may be used for sperm counts on a hemacytometer can be as follows: 5 gm of sodium bicarbonate in 100 ml of distilled water, plus 1ml of formalin (neutral).

Other counting chambers

Some professionals believe that sperm counts done by hemacytometer are not accuate because of the need to dilute the viscous semen prior to counting. There are several other counting methods available to assess sperm concentration.

The advantages of the following methods are:

- The specimen does not have to be diluted.
- Motile and non-motile sperm can both be counted avoiding the need for wet mount evaluation of motile cells.

Note that counting moving sperm can be difficult and takes significant practice to avoid error.

For each of these methods accurate counts are best obtained when at least 100 sperm per replicate are counted.

Makler: An undiluted sample is placed on the chamber and covered with the coverglass. Ten squares on the grid contain 0.000001ml.

CellVu: Two sides of a special slide are loaded with a drop of undiluted semen. Coverslips with special grids are placed on top of the sperm according to manufacturer's directions. Sperm on both sides are counted.

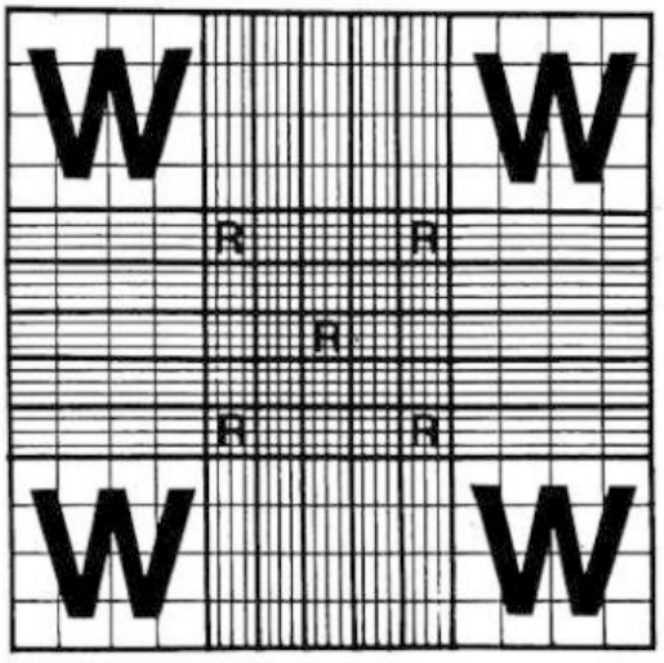
MicroCell: It has two chambers on a single, disposable slide. A special eyepiece with a grid is needed for counting.

Loading and counting using a hemacytometer

Fill both sides of the hemacytometer. Focus on the large center square with the 20X objective. The counting area consists of five small squares in the large center square. The squares usually counted are the four corner squares and the center square, all of which are marked R. A minimum of 100 sperm should be counted in the five small “R” squares. If the number of sperm is low then 10 squares or all squares may be counted to obtain the 100 per side. Count both sides of the hemacytometer and take the average of the two counts to calculate the actual count per ml.

Neubauer hemacytometer

The picture below shows the counting chamber of the Neubauer hemacytometer. This counting method is used to count many types of cells. To use this chamber for counting sperm the specimen will usually need to be diluted. Proper loading of the hemacytometer is also important for accurate sperm counts to be obtained.



Glycosylated Hemoglobin

Introduction:

Glycosylated (or glycated) hemoglobin (*hemoglobin A1c, Hb_{1c} , or Hb_{A1c}, A1C*) is a form of hemoglobin used primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic pathway by hemoglobin's normal exposure to high plasma levels of glucose. Glycosylation of hemoglobin has been implicated in nephropathy and retinopathy in diabetes mellitus. Monitoring the Hb_{A1c} in type-1 diabetic patients may improve treatment.

History:

Hemoglobin A1c was first separated from other forms of hemoglobin by Huisman and Meyering in 1958 using a chromatographic column. It was first characterized as a glycoprotein by Bookchin and Gallop in 1968. Its increase in diabetes was first described in 1969 by Samuel Rahbar and coworkersThe reactions leading to its formation were characterized by Bunn and his co-workers in 1975. The use of hemoglobin A1c for monitoring the degree of control of glucose metabolism in diabetic patients was proposed in 1976 by Koenig and coworkers.

Terminology:

Glycohemoglobin (GHb, glycated hemoglobin, glycosylated hemoglobin) is a generic term for hemoglobin bound irreversibly (ketoamine form) to glucose. Often, the term is used to mean total glycated hemoglobin, and sometimes to mean hemoglobin A1c. Total glycated hemoglobin (Total GHb) refers to all the glycated hemoglobins, including glycated hemoglobin variants. Total glycated hemoglobin is usually determined by affinity chromatography or immunoassays. Hemoglobin A1c (HbA1c) is the major subfraction of the glycated normal hemoglobin (HbA1). Determination of HbA1c is usually achieved by ion-exchange HPLC or gel electrophoresis.

Glycated Hemoglobins:

Glycated hemoglobins are hemoglobin components formed through a two-step non-enzymatic reaction between hemoglobin and blood glucose. The first step consists of the formation of a reversible aldimine form of hemoglobin to glucose linkage. In the second step, the labile aldimine form is converted slowly to the stable and irreversible ketoamine form through an Amadori rearrangement. The level of glycated hemoglobins in the blood is directly related to the average blood glucose levels over the life span of the hemoglobin in the circulation. Since the half life of red blood cells is about 120 days, a single determination of glycated hemoglobin reflects the average blood glucose level during the preceding 8 to 12 weeks. The test is therefore a very good monitor for long-term (2 to 3 months) blood glucose control in patients with diabetes mellitus.

Underlying principle:

In the normal 120-day life span of the red blood cell, glucose molecules join hemoglobin, forming glycated hemoglobin. In individuals with poorly controlled diabetes, increases in the quantities of these glycated hemoglobins are noted. Once a hemoglobin molecule is glycated, it remains that way. A buildup of glycated hemoglobin within the red cell reflects the average level of glucose to which the cell has been exposed during its life cycle. Measuring glycated hemoglobin assesses the effectiveness of therapy by monitoring long-term serum glucose regulation. The Hb_{A1c} level is proportional to average blood glucose concentration over the previous four weeks to three months. Some researchers state that the major proportion of its value is related to a rather short term period of two to four weeks.

Specimen Requirements: EDTA Whole Blood (Lavender top tube), 2.0 mL (minimum 1.0 mL). **Storage and Specimen Stability:** Stable at room temperature for up to 12 hours. Refrigerated below 4°C for up to 7 days. Frozen (below -20°C) for long-term storage. Glucose level over the past 60 days, and not the glucose value of the specimen obtained at the same time as the HbA1c.

Measuring A1c:

There are a number of techniques used to measure A1c. Laboratories use: high-performance liquid chromatography (HPLC) Immunoassay. Point of care (e.g. doctors surgery) devices use: Immunoassay. Boronate Affinity Chromatography. POC A1c tests are certified by the National Glycohemoglobin Standardization Program (NGSP) to standardise them against the results of the 1993 Diabetes Control and Complications Trial (DCCT).

Interpretation of results:

Laboratory results may differ depending on the analytical technique, the age of the subject, and biological variation among individuals. Two individuals with the same average blood sugar can have A1C values that differ by as much as 1 percentage point. In general, the reference range (that found in healthy persons), is about 4 - 5.9%. Higher levels of Hb_{A1c} are found in people with persistently elevated blood sugar, as in diabetes mellitus. While diabetic patient treatment goals vary, many include a target range of Hb_{A1c} values. A diabetic person with good glucose control has a Hb_{A1c} level that is close to or within the reference range. The International Diabetes Federation and American College of Endocrinology recommend Hb_{A1c} values below 6.5%, while American Diabetes Association recommends that the Hb_{A1c} be below 7.0% for most patients. A high Hb_{A1c} represents poor glucose control. Persistent elevations in blood sugar (and therefore Hb_{A1c}) increase the risk for the long-term vascular complications of diabetes such as coronary disease, heart attack, stroke, heart failure, kidney failure, blindness, erectile dysfunction, neuropathy (loss of sensation, especially in the feet), gangrene, and gastroparesis (slowed emptying of the stomach). Poor blood glucose control also increases the risk of short-term complications of surgery such as poor wound healing. Lower than expected levels of Hb_{A1c} can be seen in people with shortened red blood cell life span, such as with glucose-6-phosphate dehydrogenase deficiency, sickle-cell disease, or any other condition causing premature red blood cell death. Conversely, higher than expected levels can be seen in people with a longer red blood cell life span, such as with Vitamin B12 or folate deficiency. The approximate mapping between Hb_{A1c} values and average blood glucose measurements over the previous 4-12 weeks is shown in the table.

Correlation with Mean Blood Glucose Levels:

A single fasting blood glucose measurement only gives an indication of the patient's immediate past (last 1 to 2 hours) condition, and may not represent the true status of blood glucose regulation. In contrast, the level of glycated hemoglobin is directly related to the average glucose concentration over the life-span of the hemoglobin in the circulation. Various formulae have been proposed to demonstrate the correlation between the mean blood glucose (MBG) and Hemoglobin A1c (HbA1c). The following is one from Nathan, et al, N Engl J Med (1984). MBG = 33.3 * HbA1c - 86 To verify the correlation, the mean blood glucose level for each patient was obtained as the average of up to 4 daily determinations

over a period of 2 months (the average of over 200 glucose readings). Hemoglobin A1c was determined by ion-exchange HPLC at the end of the two month period. Note that the mean blood glucose value is the average.

HbA1c (%)	Avg. Blood Sugar	
	(mmol/L)	(mg/dL)
4	3.3	60
5	5.0	90
6	6.7	120
7	8.3	150
8	10.0	180
9	11.7	210
10	13.3	240
11	15.0	270
12	16.7	300
13	18.3	330
14	20.0	360

Reference Guidelines

Degree of glucose control	Total GHb	Hb A1c
Normal (non-diabetic)	< 7%	< 6%
Near normoglycemic	7 to 8%	6 to 7%
DCCT therapeutic goal		Less than 7%
In good control	8 to 9%	7 to 8%
Actions suggested	9 to 11%	8 to 9%
Not in control	> 11 %	> 9%

In patients with uncontrolled diabetes, the % of glycated hemoglobin is substantially higher than in diabetics in good control and in non-diabetics. The determination of a glycated hemoglobin level may therefore also assist in the initial diagnosis of diabetes, or it may be used to indicate the degree of long-term diabetic control in diabetic patients. The significance of a low glycated hemoglobin level has not been established.

Indications and use:

Glycosylated hemoglobin is recommended for both (a) checking blood sugar control in people who might be pre-diabetic and (b) monitoring blood sugar control in patients with more elevated levels, termed diabetes mellitus. For a single blood sample, it provides far more revealing information on glycemic behavior than a fasting blood sugar value. That being said, fasting blood sugar tests are crucial in making treatment decisions. The American Diabetes Association guidelines are similar to others in advising that the glycosylated hemoglobin test be performed at least two times a year in patients with diabetes who are meeting treatment goals (and who have stable glycemic control) and quarterly in patients with diabetes whose therapy has changed or who are not meeting glycemic goals. Glycosylated hemoglobin measurement is not appropriate where there has been a change in diet or treatment within 6 weeks. Likewise the test assumes a normal red blood cell aging process and mix of hemoglobin subtypes (predominantly HbA in normal adults). Hence people with recent blood loss or hemolytic anemia, or genetic differences in the hemoglobin molecule (hemoglobinopathy) such as sickle cell disease and other conditions are not suitable for this test. The alternative fructosamine test may be used in these circumstances and it similarly reflects an average of blood glucose levels over the preceding 2 to 3 weeks. There is variation among laboratories and a lack of consensus on a diagnostic threshold for glycosolated hemoglobin. For these and other reasons, no medical organization recommends the use of this test alone to diagnose diabetes. Instead, fasting plasma glucose or an oral glucose tolerance test are used.

HbA1c Measurement

The measurement of HbA1c/GHb in blood has become the gold standard for the long term control of the glycemic state of the diabetic patients. The optimal therapy of diabetic patients requires carefully validated/ method independent therapeutic target values for the HbA1c levels of diabetic patients in order to reduce the long-term risk of late complications such as retinopathy, nephropathy and neuropathy as well as short term risk of life threatening hypoglycemia. The % HbA1c values can also be converted into Mean Plasma Glucose values as described in American Diabetes Association (ADA) recommendations for assays traceable to the DCCT reference method as described below:

% HbA1c	Approximate mean blood glucose		Interpretation
	Mg/dl	Mmol/L	
4	65	3.5	Non- diabetic range
5	100	5.5	
6	135	7.5	
7	170	9.5	ADA Target
8	205	11.5	Action Suggested
9	240	13.5	
10	275	15.5	
11	310	17.5	
12	345	19.5	

GHb comprises several different hemoglobin-glucose adducts including HbA1a, HbA1b and HbA1c. The different GHb assay methods available to the routine clinical laboratory can be divided into two major categories: those based on charge differences between GHb and non-GHb (these include cation-exchange chromatography, electrophoresis, and isoelectric focusing) and those based on structural characteristics of glycogroups on hemoglobin (these include affinity chromatography and immunoassay). Most methods quantify HbA1c, defined as HbA with glucose attached to the NH2-terminal valine of one or both β-chains. Other methods (boronate affinity) quantify "total glycated hemoglobin," which includes both HbA1c and other GHb adducts (e.g., glucose-lysine adducts and glucose α-chain NH2-terminal valine adducts). These factors have led to considerable variation in reference intervals and results reported by different laboratories. Proper interpretation of GHb test results is not easy and requires that health care providers understand the relationship between the test results and average blood glucose, kinetics of GHb, specific assay limitations, and patient factors (other than blood glucose levels) that can affect the results. To bring semblance in monitoring glycemic control by HbA1c it is important to understand the important aspects of different methods and interpret the results accordingly. A review of preanalytical and analytical variables affecting GHb assays has been reviewed and described below: In general, any situation that shortens erythrocyte survival or decreases mean erythrocyte age falsely lowers GHb test results regardless of the assay method. Vitamins C and E are reported to falsely lower test results, possibly by inhibiting glycation of hemoglobin. Iron-deficiency anemia is reported to increase test results. Hypertriglyceridemia, hyperbilirubinemia, uremia, chronic alcoholism, and chronic ingestion of salicylates, opiate addiction, hemoglobinopathies, and chemically modified derivatives of hemoglobin may interfere with some assay methods. Interferences from hemoglobin variants should be considered before interpreting the GHb test results. Interferences from hemoglobin variants and adducts are summarized on the National Glycohemoglobin Standardization Program (NGSP) Web site at www.ngsp.org. Laboratories should use GHb assay methods with an interassay coefficient of variation (CV) of <4% (ideally <3%). Each laboratory should also determine its own reference interval following NCCLS guidelines (Document C28A). In renal failure, carbamylated compounds abound, which also interfere with GHb estimation.

Common Analytical variables: Most common analytical variable in HbA1c/GHb measurement is the preparation of specimen Lysate for testing recommended by several methods. For preparing Lysate different methods recommend a small volume of whole blood (anywhere between 5-10µl whole blood) that has to be diluted in 500-1000 µl of hemolysing solution. A small variation in delivery of whole blood sample can significantly affect the test results. Loss in calibration of pipette can significantly affect the GHb/HbA1c test results. Homogenizing of whole blood is essential to evenly distribute the erythrocytes before aspirating specimen from the specimen tube. The time suggested for lysis of erythrocytes during lysate preparation is also very important and is the determinant in eliminating the interference of Schiff's base in several assays. Most GHb methods need to measure both total hemoglobin and glycated hemoglobin. The percent HbA1c is calculated from the total and glycated hemoglobin. Error in calculation of total or glycated hemoglobin can contribute significantly in conversion to percent HbA1c.

Method specific variables: Cation exchange chromatography methods: Cation exchange chromatography can either be undertaken on mini columns or in a sophisticated, automated system. The pH and temperature conditions affect the results significantly; therefore require a sophisticated system where the conditions can be adequately controlled. In semi automated systems the room temperature varies at different times of the day and therefore can significantly influence the test results. HbA1c possesses less charge positivity and hence elutes faster from a cation exchange column. Pre-glycohemoglobin has similar mobility in this system and hence, needs to be removed before column chromatography. The kind of resin, lot to lot variation of resin , column size , buffer composition and elution time influences the test system. The presence of HbE affects the determination of GHb using cation-exchange based kits. Drugs that possess strong ionic charge like aspirin can alter GHb in ion-exchange chromatographic methods. Carbamyl-Hb has an isoelectric point similar to HbA1c and can thus interfere with charge-based methods of measuring gHb. In vitro carbamylation of Hb, to concentrations as high as 5.4% carbamyl-Hb, has been shown to produce significant spurious increases in HbA1c values in multiple cation-exchange methods. HbE1c frequently elutes as a shoulder to HbA1c in most HPLC or cation-exchange chromatographic methods.

Electrophoresis: HbA1c can be separated from HbAo by any electrophoretic method. The most commonly used method is agargel electrophoresis where HbA1 migrates to cathodic side of HbAo. Pre-GHb migrates with GHb in this system as well and hence has to be separated in advance. Comigration of Hb variants or derivatives with either HbA or HbA1c interferes with HbA1c determinations. Comigration of HbF or carbamylated Hb with HbA1c produces spuriously increased HbA1c values.

Boron affinity methods: Most semi-automated methods measure GHb and provide calculated HbA1c. This method is less influenced by pH, temperature and storage conditions. However, there is a batch-to-batch variation in gel characteristics, which makes application of this method difficult. A recent study demonstrated lot to lot variation in reagents of 6 of the eight HbA1c point of care instruments and proved that these do not meet the general accepted analytical performance criteria. Some of these points of care instruments used in the study were based on boron affinity method.

Immunological

methods: Immunoassays have been developed by using a HbA1c specific monoclonal antibody. These methods are more accurate and are suitable for both small and large laboratories. Most immunoassays are not affected by hemoglobin derivatives such as carbamylated or Acetylated Hb. They are also not affected by HbF, HbE, HbA2. These factors need to be considered when comparing results of immunoassays with other GHb methods. **Hb Variants:** A variety of patient-related factors and laboratory-related processes can lead to inaccurate determinations of gHb in the setting of variant Hbs. Samples should be evaluated for the presence of a Hb variant with any gHb reading >15%). In addition, any patient with a significant change in gHb coinciding with a change in laboratory gHb methods should be evaluated for the presence of variant or derivative Hb. Appropriate evaluation includes obtaining pertinent clinical history regarding hemoglobinopathies, alterations in red cell turnover, or conditions favoring the chemical modification of Hb. Manual review of cation-exchange chromatographs may identify the presence of aberrant peaks produced by variants. The gHb measurement should be repeated with a different assay method, and a Hb analysis by chromatography or electrophoresis should be performed to identify more common Hb variants. In some cases, variants may be identified only by ES-MS or by sequencing the expressed globin genes. Although most modern chromatographic and immunoassay methods are either unaffected by common heterozygous variants such as HbAS, HbAC, and HbAE or give warning flags concerning the likelihood of an underlying variant, less common variants may give no such warnings. Furthermore, all gHb methods are inadequate for the assessment of long-term glycemic control in patients homozygous for HbS, HbC, or with HbSC disease. Although technologies such as boronate affinity chromatography and ES-MS provide a means of accurately determining gHb in these individuals, results are unlikely to accurately reflect long-term glycemic control due to pathological conditions that affect the formation and turnover of gHb *in vivo*. In regions where populations have a high prevalence of variant Hbs, methods for the determination of gHb must be carefully selected to allow accurate determination of gHb in these individuals. When dealing with populations in which HbSS, HbCC, or HbSC disease are common and in which gHb determinations have limited utility, laboratories should offer alternative forms of testing, such as GSPs or GSA, to assist physicians with the determination of glycemic control in these individuals.

Hemoglobin A1C (HbA1c) Test

What is a hemoglobin A1c (HbA1c) test?

A hemoglobin A1c (HbA1c) test measures the amount of blood sugar (glucose) attached to hemoglobin. Hemoglobin is the part of your red blood cells that carries oxygen from your lungs to the rest of your body. An HbA1c test shows what the average amount of glucose attached to hemoglobin has been over the past three months. It's a three-month average because that's typically how long a red blood cell lives. If your HbA1c levels are high, it may be a sign of diabetes, a chronic condition that can cause serious health problems, including heart disease, kidney disease, and nerve damage. Other names: HbA1c, A1c, glycohemoglobin, glycated hemoglobin, glycosylated hemoglobin.

What is it used for?

An HbA1c test may be used to check for diabetes or prediabetes in adults. Prediabetes means your blood sugar levels show you are at risk for getting diabetes. If you already have diabetes, an HbA1c test can help monitor your condition and glucose levels.

Why do I need an HbA1c test?

The Centers for Disease Control (CDC) recommends that adults over the age of 45 get tested to screen for diabetes and prediabetes. If your results are normal, you should repeat the test every 3 years. If your results show you have prediabetes, you should get tested every 1-2 years. You should also talk to your health care provider about taking steps to reduce your risk of developing diabetes. If you are under 45, you may need this test if you have certain risk factors. These include:

- Being overweight or obese
- High blood pressure
- History of heart disease
- Physical inactivity

Testing should be done every 3 years, and more frequently if your results show you have prediabetes.

You may also need an HbA1c test if you have symptoms of diabetes. These include:

- Increased thirst
- Increased urination
- Blurred vision
- Fatigue

What happens during an HbA1c test?

A health care professional will take a blood sample from a vein in your arm, using a small needle. After the needle is inserted, a small amount of blood will be collected into a test tube or vial. You may feel a little sting when the needle goes in or out. This usually takes less than five minutes.

Will I need to do anything to prepare for the test?

You don't need any special preparations for an HbA1c test.

Are there any risks to the test?

There is very little risk to having a blood test. You may have slight pain or bruising at the spot where the needle was put in, but most symptoms go away quickly.

What do the results mean?

HbA1c results are given in percentages. Typical results are below.

- **Normal**: HbA1c below 5.7%
- **Prediabetes**: HbA1c between 5.7% and 6.4%
- **Diabetes**: HbA1c of 6.5% or higher

Your results may mean something different. If you have questions about your results, talk to your health care provider. If you have diabetes, the American Diabetes Association recommends keeping your HbA1c levels below 7%. Your health care provider may have other recommendations for you, depending on your overall health, age, weight, and other factors.

Is there anything else I need to know about an HbA1c test?

The HbA1c test is not used for gestational diabetes, a type of diabetes that only affects pregnant women, or for diagnosing diabetes in children. Also, if you have anemia or another type of blood disorder, an HbA1c test may be less accurate for diagnosing diabetes. If you have one of these disorders and are at risk for diabetes, your health care provider may recommend different tests.

DIAGNOSTIC TESTS

HbA1c Test for Diabetes- Other Considerations

HbA1c is an important blood test that gives a good indication of how well your diabetes is being controlled. Together with the fasting plasma glucose test, the HbA1c test is one of the main ways in which type 2 diabetes is diagnosed. HbA1c tests are not the primary The HbA1c test, also known as the haemoglobin A1c or glycated haemoglobin test, diagnostic test for type 1 diabetes but may sometimes be used together with other tests.

The **World Health Organisation (WHO**) suggests the following diagnostic guidelines for diabetes:

- **HbA1c below 42 mmol/mol (6.0%):** Non-diabetic
- **HbA1c between 42 and 47 mmol/mol (6.0–6.4%):** Impaired glucose regulation (IGR) or Prediabetes
- **HbA1c of 48 mmol/mol (6.5%) or over:** Type 2 diabetes

If your HbA1c test returns a reading of **6.0–6.4%,** that indicates prediabetes. Your doctor should work with you to suggest appropriate lifestyle changes that could reduce your risk of developing type 2 diabetes. HbA1c is **not** used to diagnose gestational diabetes in the UK. Instead, an oral glucose tolerance test is used. A random blood glucose test will usually be used to diagnose type 1 diabetes. However, in some cases, an HbA1c test may be used to support a diagnosis of type 1 diabetes.

Why is HbA1c important?

People with diabetes who **reduced their HbA1c by less than 1%** can **cut their risk of dying within 5 years by 50%** , according to Swedish research presented at the annual meeting of the European Association for the Study of Diabetes, Sept. 2012 (EASD).

How does the HBA1c test work?

HbA1c (glycated haemoglobi, haemoglobin A1C) occurs when haemoglobi, the oxygen-carrying protein in red blood cells, becomes

bonded with glucose in the bloodstream. The bonding with glucose is called glycation. The higher a person's blood glucose levels have been, the higher the number of red blood cells that will have become glycated, and therefore the higher HbA1c level they will have. Note that red blood cells exist in the body for around 3 months, therefore an HbA1c levels generally reflects a person's blood glucose levels over the previous 8-12 weeks.

Limitations of HbA1c tests

Whilst HbA1c tests are usually reliable, there are some limitations to the accuracy of the test. For example, people with forms of anaemia may not have sufficient haemoglobin for the test to be accurate and may need to have a fructosamine test instead. Being pregnant or having an uncommon form of haemoglobin (known as a haemoglobin variant) can also return an inaccurate HbA1c, while readings can also be affected by short term issues such as illness as they can cause a temporary rise in blood glucose. Because of the way the HbA1c test measures blood sugar, if you have higher blood sugar levels in the weeks leading up to your HbA1c test, this will have a greater impact on your test result than your glucose levels 2 to 3 months before the test.

Diabetes Mellitus Diagnosis

Symptoms of type 1 diabetes often appear suddenly and are often the reason for checking blood sugar levels. Because symptoms of other types of diabetes and prediabetes come on more gradually or may not be evident, the American Diabetes Association (ADA) has recommended screening guidelines. The ADA recommends that the following people be screened for diabetes:

- **Anyone with a body mass index higher than 25 (for whites) (23 for Asians), regardless of age,** who has additional risk factors, such as high blood pressure, abnormal cholesterol levels, a sedentary lifestyle, a history of polycystic ovary syndrome or heart disease, and who has a close relative with diabetes.
- **Anyone older than age 45** is advised to receive an initial blood sugar screening, and then, if the results are normal, to be screened every three years thereafter.
- **Women who have had gestational diabetes** are advised to be screened for diabetes every three years.
- **Anyone who has been diagnosed with prediabetes** is advised to be tested every year.

Tests for type 1 and type 2 diabetes and prediabetes

- **Glycated hemoglobin (A1C) test.** This blood test, which doesn't require fasting, indicates your average blood sugar level for the past two to three months. It measures the percentage of blood sugar attached to hemoglobin, the oxygen-carrying protein in red blood cells.

  The higher your blood sugar levels, the more hemoglobin you'll have with sugar attached. An A1C level of 6.5% or higher on two separate tests indicates that you have diabetes. An A1C between 5.7 and 6.4 % indicates prediabetes. Below 5.7 is considered normal.

  If the A1C test results aren't consistent, the test isn't available, or you have certain conditions that can make the A1C test inaccurate — such as if you are pregnant or have an uncommon form of hemoglobin (known as a hemoglobin variant) — your doctor may use the following tests to diagnose diabetes:
- **Random blood sugar test.** A blood sample will be taken at a random time. Regardless of when you last ate, a blood sugar level of 200 milligrams per deciliter (mg/dL) — 11.1 millimoles per liter (mmol/L) — or higher suggests diabetes.
- **Fasting blood sugar test.** A blood sample will be taken after an overnight fast. A fasting blood sugar level less than 100 mg/dL (5.6 mmol/L) is normal. A fasting blood sugar level from 100 to 125 mg/dL (5.6 to 6.9 mmol/L) is considered prediabetes. If it's 126 mg/dL (7 mmol/L) or higher on two separate tests, you have diabetes.
- **Oral glucose tolerance test.** For this test, you fast overnight, and the fasting blood sugar level is measured. Then you drink a sugary liquid, and blood sugar levels are tested periodically for the next two hours.

  A blood sugar level less than 140 mg/dL (7.8 mmol/L) is normal. A reading of more than 200 mg/dL (11.1 mmol/L) after two hours indicates diabetes. A reading between 140 and 199 mg/dL (7.8 mmol/L and 11.0 mmol/L) indicates prediabetes.

If type 1 diabetes is suspected, your urine will be tested to look for the presence of a byproduct produced when muscle and fat tissue are used for energy because the body doesn't have enough insulin to use the available glucose (ketones). Your doctor will also likely run a test to see if you have the destructive immune system cells associated with type 1 diabetes called autoantibodies.

Tests for gestational diabetes

Your doctor will likely evaluate your risk factors for gestational diabetes early in your pregnancy:

- **If you're at high risk of gestational diabetes** — for example, if you were obese at the start of your pregnancy; you had gestational diabetes during a previous pregnancy; or you have a mother, father, sibling or child with diabetes — your doctor may test for diabetes at your first prenatal visit.
- **If you're at average risk of gestational diabetes**, you'll likely have a screening test for gestational diabetes sometime during your second trimester — typically between 24 and 28 weeks of pregnancy.

Your doctor may use the following screening tests:

- **Initial glucose challenge test.** You'll begin the glucose challenge test by drinking a syrupy glucose solution. One hour later, you'll have a blood test to measure your blood sugar level. A blood sugar level below 140 mg/dL (7.8 mmol/L) is usually considered normal on a glucose challenge test, although this may vary at specific clinics or labs.

  If your blood sugar level is higher than normal, it only means you have a higher risk of gestational diabetes. Your doctor will order a follow-up test to determine if you have gestational diabetes.
- **Follow-up glucose tolerance testing.** For the follow-up test, you'll be asked to fast overnight and then have your fasting blood sugar level measured. Then you'll drink another sweet solution — this one containing a higher concentration of glucose — and your blood sugar level will be checked every hour for a period of three hours.

  If at least two of the blood sugar readings are higher than the normal values established for each of the three hours of the test, you'll be diagnosed with gestational diabetes.

Blood Calcium

Reference Range

Calcium concentration, both total and free, is characterized by a high physiological variation, depending on age, sex, physiological state (eg, pregnancy), and even season (owing to the seasonal variation of vitamin D, which is directly involved in the regulation of calcium concentration). Therefore, separate reference intervals have been established according to the age and sex of the individual being tested.

Total calcium reference ranges in males are as follows:

- Younger than 12 months: Not established
- Age 1-14 years: 9.6-10.6 mg/dL
- Age 15-16 years: 9.5-10.5 mg/dL
- Age 17-18 years: 9.5-10.4 mg/dL
- Age 19-21 years: 9.3-10.3 mg/dL
- Age 22 years and older: 8.9-10.1 mg/dL

Total calcium reference ranges in females are as follows:

- Younger than 12 months: Not established
- Age 1-11 years: 9.6-10.6 mg/dL
- Age 12-14 years: 9.5-10.4 mg/dL
- Age 15-18 years: 9.1-10.3 mg/dL
- Age 19 years and older: 8.9-10.1 mg/dL

Free (ionized) calciumreference ranges in males are as follows:

- Younger than 12 months: Not established
- 1-19 years: 5.1-5.9 mg/dL
- Age 20 years and older: 4.8-5.7 mg/dL

Free (ionized) calciumreference ranges in females are as follows:

- Younger than 12 months: Not established
- 1-17 years: 5.1-5.9 mg/dL
- Age 18 years and older: 4.8-5.7 mg/dL

Calcium (urine) reference ranges are as follows*:

- Males: 25-300 mg/24-hour urine collection
- Females: 20-275 mg/24-hour urine collection
- Hypercalciuria: >350 mg/specimen
- *Values are for persons with average calcium intake (ie, 600-800 mg/day)

Turbidimetry

Turbidity has long been one of the key indicators in determining water quality. However, it is well recognised within the industry that because of the effect of instrument design on readings, different instruments can give different results on the same sample. There are a number of reasons for this, some to do with the instruments incident light and scattered light detection angle, and some to do with how the sample is presented to the instrument. Turbidity can be a complex measurement to do, especially at the very low levels required in UK drinking water production. Measurement can be affected by the size and shape of the material scattering the light, the sample colour and then the distribution of the material in the sample. To overcome these issues, a new turbidity instrument has been developed, created by an expert team led by world-renowned turbidity expert Mike Sadar. The online instrument they designed sought to address the common issues with the presentation of the sample to the instrument.

The challenges of turbidity measurement:

Cause	Effect
Bubbles	High results
Sample cell variations	High or Low results. The impact of this issues is most severe when measuring low level turbidity.
Stray light	High results
Contamination	High results. Results from the build-up of particles/scale within the instrument, or from microbiological fouling.
Instrument optical aspects	High or low results. Degradation of the optical components within the instrument and the effect of calibration.
Absorbing coloured particles	Low results
Sample colour	Low results.(If using a wavelength in the visible region)
Particle Size	High or low results (wavelength dependent) Large particles scatter longer wavelengths of light more readily than small particles. Small particles scatter shorter wavelengths more efficiently than longer wavelengths
Particle settling	High or low results. Tends to be an issue with portable instrumentation rather than online.
Particle Density	Low results

A new way of measuring turbidity has been developed by Tulip Diagnostics.

Bubbles

Bubbles are the bane of turbidity measurement and a number of methods of removing them from the sample prior to measurement have been attempted by manufacturers over the years. To eliminate bubbles from entering the measurement chamber, the new instrument design incorporates an integral patented bubble trap mechanism, using both horizontal and vertical flow to trap and remove even microbubbles from the instrument prior to measurement.

Sample cell

Any surface that comes between the incident light and the detector is not ideal, bench-top turbidity measurement is inherently prone to defects on the cell caused by cleaning and use, these cause false positive turbidity results, so why use them in online instrumentation if you don't need to. Glass cells are prone to scratching and fouling. Added to this is the issue of condensation, a particular problem when using glass cells but also effects online instruments that don't when condensation builds up around the light source. To avoid these issues, the experts simply didn't use glass cells and incorporated a temperature controlled light source to prevent condensation on the optics.

Stray light

Stray light is another problem which can be reduced by a well thought out instrument design. Essentially, it is the light that is detected by the instrument that isn't associated with the scattering by the particles in the sample at that moment. Again, very low-level turbidity measurement is prone to error due to even the tiniest amounts of light bouncing around the sample chamber. The new instrument has been designed to channel stray light downwards, away from the detector and towards a 'stray light dump'. This specially developed component captures stray light and prevents it from bouncing around the measurement chamber and causing false positive results.

Contamination

Specially designed wetted parts are used and the measurement chamber design is completely smooth to minimise any places where sediment can build up. Additionally, the measurement chamber is designed to simple to access as making cleaning easy for operators is the key to avoiding contamination issues.

Instrument optical aspects

By using a LED light source, the instrument offers long and stable performance over time. In order to verify the optical system, a solid standard has been developed to enable operators to check their system, but the expert team wanted to go one step further and enable calibration on the primary calibration solution for turbidity instrumentation, formazin. Turbidity calibration is all based around the response of the instrument to formazin. Formazin was specially developed for the calibration of turbidity instruments and is a polymer which has relatively consistent light scattering properties. It and is the ONLY real primary calibration standard available, every other standard you can get for a turbidity instrument is a secondary standard which relates back to formazin and should only really be used for verification. Unfortunately, formazin has two major drawbacks in that it it's not very nice to handle and secondly, it isn't terribly stable in dilute solution. Given that drinking water works are working below 1NTU then the solutions you would use to calibrate would have to be made up fresh before use.

Sample colour/coloured absorbing particles

There is little that can be done about the absorption of light by colour, however as it tends to affect instruments using a wavelength below 800nm. As most turbidity instruments in use in the UK are designed to comply with the ISO 7027 standard which specifies at near infra red wavelength should be used, it is less of an issue.

Particle Size / density

Again, not a lot can be done about the instruments handling of the different scattering properties of particles of different size and density. The user must decide whether they want to be able to detect smaller particles by using a shorter wavelength (and hence run the risk of colour interference) or use the longer wavelength ISO compliant version of the instrument. The new instrument comes in both forms. With respect to particle settling, again, not a lot can be done in the instrument design but it is worth noting when online and portable instrumentation results are compared.

Conclusion

In conclusion, a new way of measuring turbidity has been developed, from Tulip Diagnostics which incorporates the expertise of a team with over 2 decades of experience in turbidity measurement. Whilst overcoming many of the technical issues associated with turbidity measurement, the team also designed it with the water treatment works in mind. This meant ensuring only small volumes of sample were required to reduce water consumption and introducing an App based interface for operation on a smart device where this is desirable. A new standard for turbidity measurement has been developed.

ELISA Troubleshooting Tips

ELISA troubleshooting tips guide is designed to help improve and troubleshoot the common problems that Labs have with their ELISA kits when performing assays. Optimising the ELISA and removing common mistakes that are made can dramatically improve the results and the sensitivity of the ELISA assays. This ELISA troubleshooting guide details the common areas where Labs encounter problems with their ELISA.

ELISA Troubleshooting areas

High Signal:

High Signal can occur for numbers reasons including insufficient plate washing, not stopping the reaction and adding too much Conjugate. If you have a high signal this can results in a lot of false positives and incorrect data.

Out of Range:

Sometimes this can happen based on your samples, insufficient washing or incorrect dilutions prepared. This can result in a loss of data due to negative or no results.

High Variation:

High variation can be due to sample preparation mistakes, pipette errors and inconsistencies, insufficient plate agitation among other problems. Data with high variation can skew the real results and cause inconsistencies in your data.

Background is high

High background may result from inadequate washing steps, cross reactivity of samples or contamination. Again high background may result in false positive/negative data and affect your results.

No Signal

No signal in your ELISA assay may result from numerous sample and assay problems including wash buffer, target below detection of assay or avidin-HRP was not added. No signal may mean no results from precious samples, have a read through the reasons below to avoid these problems.

Poor Standard Curve

A poor standard curve will prove unpublishable results if not prepared correctly. Reasons may included reagents are poorly mixed, the standard has degraded or pipetting errors.

ELISA troubleshooting for High Signal		
1.	TMB Substrate Solution was contaminated	*Use fresh TMB substrate solution which should be clear and colorless prior to addition to wells. Use a clean V bottom container prior to pipetting substrate solution into wells. Use a clean V bottom container prior to pipetting substrate solution into wells.*
2.	Reaction not stopped	*Colour will keep developing if the substrate reaction is not stopped.*
3.	Plate left too long before reading on the plate reader	*Colour will keep developing (though at a slower rate if stop solution has been added).*
4.	Contaminants from laboratory glassware	*Ensure reagents are fresh and prepared in clean glassware.*
5.	Substrate incubation carried out in the light	*Substrate incubation should be carried out in the dark. Ensure substrate is not exposed to light—store in a dark place. Limit exposure to light while running assay.*
6.	Wells are insufficiently washed	*Wash wells as per protocol recommendations.*
7.	Too much Conjugate added	*Ensure the reagent has been diluted and mixed properly.*
8.	Precipitate formed in wells upon substrate addition	*Increase dilution factor of sample or decrease concentration of substrate.*
9.	Dirty plate	*Clean the bottom of the plate.*
10.	Incorrect standard curve dilutions prepared	*Check your pipetting technique. Calibration of pipettes might be required.*
11.	Longer incubation times than recommended	*Make sure your incubation times are correct and adhere to the protocol provided with the technical manual.*
12.	Plate sealers or reagent reservoirs reused, resulting in presence of residual HRP. This will turn the TMB blue non-specifically	*Reuse of plate sealers may lead to the presence of residual HRP, leading to non- specific colour change of TMB. To avoid this use fresh plate sealer and reagent reservoir for each step.*
13.	Contamination of buffers	*Always make fresh buffers.*

ELISA Troubleshooting for out of Range		
1.	Samples contain no or below detectable levels of analyte	*If samples are below detectable levels, it may be possible to use high sample volume. Check with technical support for appropriate protocol modifications.*
2.	Samples contain analyte concentrations higher than the highest standard point	*Samples may require further dilution.*
3.	Insufficient washing	*Use appropriate washing procedure—see below. At the end of each washing step, invert plate on absorbent tissue and allow to completely drain, tapping forcefully if necessary to remove any residual fluid.*
4.	Plate sealers not used or reused	*During incubations, cover assay plates with plate sealers. Use a fresh sealer each time the plate is opened. This will prevent wells from contaminating each other.*
5.	Incorrect dilutions prepared	*Check pipetting technique—see below—and double-check calculations.*
6.	Longer incubation times than recommended	*Manufactured kits have optimized protocols. Make sure to follow recommended incubation times.*
7.	Substrate solution mixed too early and turned blue	*Substrate solution should be mixed and used immediately.*
8.	Too much Conjugate	*Check dilution, titrate if necessary.*
9.	Plate sealers or reagent reservoirs reused, resulting in presence of residual HRP. This will turn the TMB blue non-specifically	*Use fresh plate sealer and reagent reservoir for each step.*
10.	Buffers contaminated	*Make fresh buffers.*
ELISA Troubleshooting for High Variation		
1.	Multichannel pipette errors	*Calibrate the pipettes.*
2.	Plate washing was not adequate or uniform	*Make sure pipette tips are tightly secured. Confirm all reagents are removed complete in all wash steps.*
3.	Samples may have high particular matter	*Remove the particulate matter by centrifugation.*
4.	Insufficient plate agitation	*Wherever recommended; plate should be agitated during all incubation steps using an ELISA plate shaker at a speed where solutions in wells are within constant motion without splashing.*
5.	Cross well contamination	*When reusing plate sealers check that no reagent has touched the sealer. Care should be taken when using the same pipette tips used for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.*
6.	Plates stacked during the incubations	*Stacking of plates does not allow even distribution of temperature across the wells of the plates. Avoid stacking.*
7.	Pipette inconsistent	*Ensure pipettes are working correctly and are calibrated. Ensure pipette tips are pushed on far enough to create a good seal. Take particular care when diluting down the plate and watch to make sure the pipette tips are all picking up and releasing the correct amount of liquid.*
8.	Antibody dilutions/reagents are not well mixed	*To ensure a consistent concentration across all wells, ensure all reagents are mixed before pipetting onto the plates.*
9.	Well allowed to dry out	*Ensure lids are left on the plates at all times when incubating. Place a humidifying water tray (bottled clean/sterile water) in the bottom of the incubator.*

10.	Bottom of the plate is dirty	*Clean the bottom of the plate carefully before re-reading the plates.*
11.	Bubbles in wells	*Ensure no bubbles are present prior to reading the plate.*
12.	Edge effects	*Ensure the plate and all reagents are at room temperature.*
13.	Storage	*Ensure reagents and samples are stored at correct temperature.*
14.	Variations in protocols	*Adhere to the protocol that comes with your assay.*
15.	Improper calculations of standard curve	*Check calculations, make new standard curve & use internal controls.*
16.	Buffers contaminated	*Use fresh buffers.*
17.	Well bottom scrapped	*Avoid contact with the bottom of the well during pipetting. Aim the pipette tip to the side of the well to avoid disrupting the bottom.*
ELISA Troubleshooting for Background is high		
1.	Background wells were contaminated	*Avoid cross-well contamination by using the sealer appropriately. Use multichannel pipettes without touching the reagents on the plate.*
2.	Insufficient washes	*Refer to wash protocol for number of washes and soaking time between washes prior to addition of substrate solution.*
3.	Concentration of conjugate is too high	*Perform dilutions as per protocol.*
4.	Incorrect assay temperature	*Check that the incubation temperature did not exceed 37°C.*
5.	Inadequate washing	*Ensure all wells are filling with wash buffer and are being aspirated completely. Use an automated plate washer if available.*
6.	Contaminating enzymes present in sample	*Test sample with substrate alone to check for contaminating enzyme activity.*
7.	Wells are insufficiently washed	*Wash wells are per protocol recommendations.*
8.	Contaminated wash buffer	*Prepare fresh buffers.*
9.	Too much detection reagent	*Ensure the reagent has been diluted properly.*
10.	Waiting too long to read plate after addition of stop solution	*Read plate immediately after adding stop solution.*
11.	Substrate incubation is carried out in light	*Substrate incubations should be carried out in the dark or as recommended by manufacturer.*
12.	Precipitate formed in wells upon substrate addition	*Increase dilution factor of sample.*
13.	Dirty plate	*Clean the bottom of the plate with a wipe.*
ELISA Troubleshooting for No Signal		
1.	Incorrect concentration or no conjugate was added	*Add appropriate detection antibody and continue.*
2.	Substrate solution was not added	*Add substrate solution and continue.*
3.	Incubation time too short	*Follow the protocol for incubation time and temperature.*
4.	Analyte present below detection limits of assay	· Check sample dilution step · Check calibration · May be sample specific.

5.	Incompatible sample type	*Detection may be reduced or absent in untested samples types. Use recommended sample type.*
6.	Not enough conjugate reagent	*Prepare correct concentration of conjugate following manufacturer guidelines.*
7.	Sample prepared incorrectly	*Ensure proper sample preparation/dilution. Sample type used may be incompatible with microtiter plate assay format.*
8.	Incubation temperature is too low	*Ensure the incubations are carried out at the correct temperature. All reagents including plate should be at room temperature or as recommended by the manufacturer before proceeding.*
9.	Incorrect wavelength	*Verify the wavelength and read the plate again.*
10.	Plate washing is too vigorous	*Check the correct pressure in the automatic plate washer. Pipette wash buffer gently if washes are done manually.*
11.	Wells dried out	*Do not allow wells to become dry once the assay has started. Cover the plate using sealing film or tape for all incubations.*
12.	Slow colour development of enzymatic reactions	*Prepare substrate solution immediately before use.*
13.	Uneven Colour	*Ensure all wells are washed correctly, use a ELISA plate washer where possible.*
14.	Reagents not at room temperature	*All reagents should at room temperature from the start of the assay. Room temperature should be reached following 15–20 minutes on the bench.*
15.	Expired Reagents	*Ensure all reagents used are within date.*
ELISA Troubleshooting for Poor standard curve		
1.	Standard was incompletely reconstituted or was incorrectly stored	*Reconstitute standard according to the protocol provide and follow storage instructions.*
2.	Reagents were added to the wells at incorrect concentrations	*Check for pipetting errors and correct the reagent volume.*
3.	Incubations done at incorrect temperature	*Follow protocol for storage, incubation and agitation.*
4.	Wells not completely aspirated during washing	*Completely aspirate wash buffer between steps, use plate washer where possible.*
5.	Plates stacked during incubation	*Keep plates separated.*
6.	Poor dilution series	*Check dilution steps according to protocol.*
7.	Reagents poorly mixed	*Make sure to mix reagents thoroughly.*
8.	Standard degraded	*Check that standard was stored correctly.*
9.	Pipetting error	*Check pipettes and calibrate.*
10.	Not enough conjugate	*Check dilution.*
11.	Incorrect calculation of standard curve dilution	*Check your calculations and make a new curve.*
12.	Mixing or Substituting reagents from different kits	*Avoid this as it can affect the quality of your assay.*

Genetic Testing

An international body - Clinical Laboratory Improvement Advisory Committee (CLIAC) has reviewed quality concerns related to molecular genetic testing. The newly released report reflects CLIAC recommendations of "good laboratory practices for ensuring the quality of molecular genetic testing for heritable diseases and conditions."

History of Genetic Testing Recommendations:

This report cites previous studies of clinical laboratory tests in general, showing that more errors originate during preanalytic and postanalytic phases of testing than during the analytic process itself. Inappropriate test selection underlies many preanalytic errors. For example, a study of testing for the adenomatous polyposis coli gene found that in 17% of the cases, testing was unwarranted. "When you talk about errors in laboratory testing, you look at that in the broad sense, where errors can occur anywhere from what goes into the medical decision that the doctor uses in test selection. And that's the practice of medicine, which the guideline does not address," specialists say. "Good laboratory practice, and also part of the general regulatory requirement, is for the laboratory to make available to users information about the tests that they provide," "So the laboratory may be able to influence correct decision-making if physicians work with the laboratory to understand what tests are provided and the characteristics of that test" in terms of answering their questions. "The guideline strives to emphasize that role for laboratory practice but does not cross over that line of medical practice." The CLIAC Genetic Testing Good Laboratory Practices Workgroup evaluated factors in genetic testing likely to affect quality and identified areas in need of quality assurance guidelines to comply with current CLIA requirements. The recommended practices relate specifically to testing for heritable diseases. "We know that genetic testing is growing and is expanding, and so we can't really say for sure how many labs will pick up on it and where the testing will be done," noted a researcher "But the recommendations were set up the way that they were so that, regardless of where this testing is done in the future, if labs are following these recommendations, we could be assured of the quality of the testing, that it was producing accurate results."

Preanalytic Testing Phase Guidelines:

In the preanalytic testing phase, the report lists laboratory guidelines for providing information about molecular genetic tests to those who use their services. The information should include: Selection of appropriate tests; Information on proper methods for collecting, handling, transporting, and submitting specimens; Patient information needed for proper testing and reporting of results; Indication of potential implications of the results for family members; and Availability of laboratory consultations regarding the issues mentioned earlier. "What is included in this guidance are elements that users of laboratory services should look for when deciding to engage a laboratory, in terms of good laboratory practice," commented a scientist "[This] would be the laboratory making available to the user of its services information about the test that is offered.... When [physicians] are trying to evaluate laboratory services,...use those criteria to determine whether the test that you are thinking of ordering is actually valid for the purposes that you are ordering it for," emphasized the same scientist. Additional concerns addressed by the work group in the preanalytic phase were informed consent, test requests, specimen handling, and establishing policies to assess and correct problems in the preanalytic phase.

Analytic Testing Phase Guidelines:

The analytic phase of molecular genetic testing was already regulated by CLIA requirements to "establish or verify the analytic performance of all non-waived tests and test systems before introducing them for patient testing." Beyond adherence to the more general CLIA requirements, recommendations for molecular and genetic testing focus on the assurance of validity and reliability in the tests and proper interpretation of test results. "Many labs have pretty rigorous assurance quality management systems in place. What's less controlled is the lab's capacity to control or influence what comes to them from clinical settings," "So there are communication issues there sometimes, and [issues] in terms of interpreting results.... While errors are made, the data do suggest that [in] the vast majority of testing, there's not a significant problem that has really been documented." Recommendations regarding establishing and verifying performance specifications for molecular genetic tests list 5 guidelines to be followed for each test: "Conduct a review of available scientific studies and pertinent references, Define appropriate patient populations for which the test should be performed, Select the appropriate test methodology for the disease or condition being evaluated, Establish analytic performance specifications and determine quality control procedures using the appropriate number, type, and variety of samples, Ensure that test results and their implications can be interpreted for an individual patient or family and that the limitations of the test are defined and reported." Studies of the analytic phase of DNA-based genetic testing have reported errors in specimen handling and analysis in only 0.06% to 0.12% of nearly 100,000 tests studied. Recommendations for the analytic phase devote significant attention to control procedures and emphasize the importance of proficiency testing in evaluating laboratory competence (as well as in providing education for lab personnel).

Postanalytic Testing Phase Guidelines:

Postanalytic errors commonly reflect problems in preparing reports and interpreting results. Studies have shown that a major contributor to these problems is poor understanding among healthcare providers of the limitations of molecular genetic tests and their proper interpretation. For postanalytic testing, the recommendations focus on content, completeness, and interpretation of test reports. In addition, CLIA requires that test reports, records, and even the tested specimens be retained for specified periods ranging from "as long as possible" for tested specimens to 2 years for test reports and 10 years for pathology test reports. These recommendations recognize the potential importance of these materials for family members and for future diagnostic use, as medical technology and knowledge progress. Finally, the report speaks to issues of confidentiality, laboratory personnel, and the "quality management system" approach- a system widely adopted internationally. Development of a quality management system is likely to play an important role in the ability of labs to receive test referrals from international sources, as well as improving their quality management.

Highlights:

Laboratories should publicize which genetic tests they offer to all potential users, including clinicians and patients. This may include print and electronic media. Laboratories should also include information to guide decision making about the test itself. Such data should include the following: The recommended patient populations for testing, Test method used, The reliability of the test, Whether the test is performed with approved test system, Limitations of the test, A statement that the result may likely have implications for the family. Information regarding the cost of testing should also be included, if possible. Informed consent should remain the responsibility of the person ordering the genetic test.

However, laboratories may aid in the process of shared decision making by being available to answer questions during the consent process and using test order forms that acknowledge that patient consent was obtained before the test was ordered. Obtaining information on indications for testing, relevant clinical or laboratory information, patient racial/ethnic background, family history, and pedigree is critical for selecting appropriate test methods, determining the mutations or variants to be tested, interpreting test results, and reporting test results in a timely manner. Laboratories are required to establish analytic performance specifications and then establish quality-control procedures to ensure that these specifications are met consistently. This can be a particular challenge with molecular genetic testing in patients with diverse backgrounds. Performance assessment for each type of molecular genetic test offered should be performed at least twice annually. Formal proficiency testing is available for a limited number of genetic tests and is preferred. Reports from molecular genetic testing should be kept on file at the laboratory for at least 25 years after reporting. Maintenance of patient confidentiality is critically important with regard to results from genetic testing. Laboratories may release patient test information only to the authorized person ordering the test, healthcare providers authorized by the ordering person to receive the test, and the laboratory that initially requested the test. If a healthcare provider who provides care for a family member of the patient is authorized to request patient test information, the laboratory should request the patient's authorization before releasing the patient's genetic test results.

PCR

Requisites for Superior PCR Results

Adjustments to any of the components of a PCR reaction can alter the quality of the outcome, either improving or diminishing the product yield and quality, or reaction specificity and sensitivity. Parameters that influence the end result can be either physical (i.e. temperature, cycle times) or chemical (i.e. template concentration, type of enzyme used). The following are several key factors that influence the success of PCR.

1. Clean Laboratory Conditions

Since **PCR** is capable of detecting a single molecule of DNA, it is important to maintain pristine conditions in the laboratory. Always wear fresh gloves, use sterile glassware, tubes and pipette tips, and sterile solutions and clean the work area before beginning work. Always include control reactions, without template DNA and without enzyme, to ensure the results are truely due to amplification of the right sample. Most people make sure to have their own set of solutions that are not shared, in order to make trouble-shooting easier, and designated pipettes are often set aside for PCR only. DNase and RNase-free PCR tubes, aerosol-resistant pipette tips and working in a fumehood with UV light are also ways to minimize problems.

2. Chemical Components

Also of critical importance is the purity and integrity of template (purified DNA) and primer design. There are several software programs available online for designing primers. The best primers have 18-24 bases, no secondary structure (i.e. hairpin loops), balanced distribution of G/C and A/T pairs, are not complimentary to each other at the 3' ends and have melting temperatures (Tm) about 5-10 degrees celcius below the annealing temperature, which is usually between 55 and 65 degrees celcius. The Tm for both primers should be similar for best results.

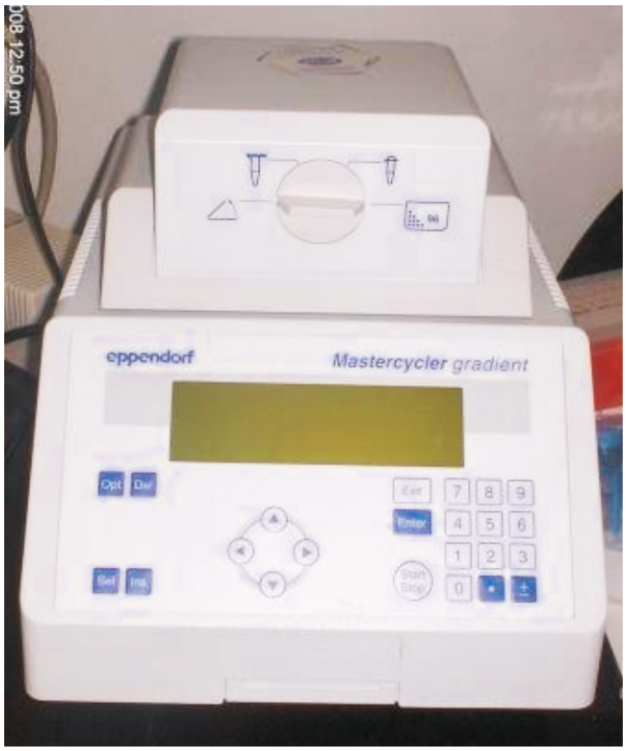
3. Reaction Mix: Template and Primers

The proportions of the reaction mixture have a huge influence of the quality of PCR results. There is a general formula for concentrations of template, enzyme, primers and nucleotides to use, but this can be tweaked a little bit. Optimal primer concentrations are between 0.1 and 0.6 micromoles/L. The amount of template varies depending on the type of DNA (human, bacterial, plasmid). With very low amounts of template there are other strategies to improve results, like increased cycle numbers or use of "hot start". Always test new primers with a positive control reaction, to be sure they work under your specific experimental conditions.

4. Reaction Mix: Enzyme Activity

The choice of **DNA polymerase** affects the fidelity of the reaction and quality of product. The traditional Taq polymerase has been replaced in many labs by higher fidelity enzymes (those that make less errors). The concentration of $MgCl_2$ in the reaction mix can influence the PCR outcome and could play an important role in more fastidious reactions. Magnesium cation forms soluble complexes with dNTPs to produce the actual substrate that the polymerase enzyme recognizes. Equal amounts of all four dNTPs also help reduce the polymerase error rate. Certain additives such as betaine, BSA, detergents, DMSO, glycerol and pyrophosphatase can also affect enzyme specificity or reaction efficiency.

5. Type of Thermocycler



When **shopping for laboratory equipment**, bear in mind that some thermocyclers are less precise than others at maintaining exact, desired temperatures. This is one area where going cheap might not pay off in the long run, if you are faced with a finicky reaction or using primers that require a narrow range of annealing temperature. Thin-walled reaction tubes designed to fit the precise brand of thermocycler you are using, also help optimize reaction temperatures.

6. Cycle Settings

Reaction cycle lengths, temperatures, and number of cycles, all have a critial role in determining how well a PCR will work. The initial heating step must be long enough to completely denature the template and cycles must be long enough to prevent melted DNA from reannealing to itself. Increased yield can be achieved by increasing the extension time about every 20 cycles, to compensate for less enzyme to amplify more template. Usually less than 40 cycles is enough to amplify less than 10 template molecules to a concentration large enough to view on an ethidium bromide-stained **agarose gel**.

PCR Troubleshooting Guide

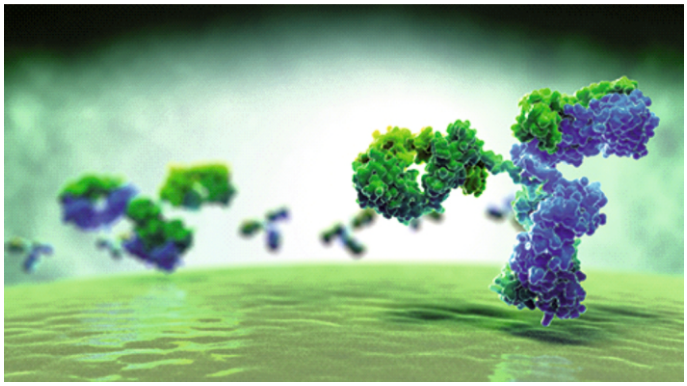
The following guide can be used to troubleshoot PCR reactions.

Observation	Possible Cause	Solution
SEQUENCE ERRORS	Low fidelity polymerase	• Choose a higher fidelity polymerase DNA Polymerases
	Suboptimal reaction conditions	• Reduce number of cycles • Decrease extension time • Decrease Mg^{++} concentration in the reaction
	Unbalanced nucleotide concentrations	• Prepare fresh deoxynucleotide mixes
	Template DNA has been damaged	• Start with a fresh template • Try repairing DNA template • Limit UV exposure time when analyzing or excising PCR product from the gel
	Desired sequence may be toxic to host	• Clone into a non-expression vector • Use a low-copy number cloning vector
	Incorrect annealing temperature	• Recalculate primer
INCORRECT PRODUCT SIZE	Mispriming	• Verify that primers have no additional complementary regions within the template DNA
	Improper Mg^{++} concentration	• Adjust Mg^{++} concentration in 0.2–1 mM increments
	Nuclease contamination	• Repeat reactions using fresh solutions
	Incorrect annealing temperature	• Recalculate primer • Test an annealing temperature gradient, starting at 5°C below the lower Tm of the primer pair
NO PRODUCT	Poor primer design	• Check specific product literature for recommended primer design • Verify that primers are non-complementary, both internally and to each other • Increase length of primer
	Poor primer specificity	• Verify that oligos are complementary to proper target sequence
	Insufficient primer concentration	• Primer concentration can range from 0.05–1µM in the reaction. Please see specific product literature for ideal conditions
	Missing reaction component	• Repeat reaction setup
	Suboptimal reaction conditions	• Optimize Mg^{++} concentration by testing 0.2–1 mM increments • Thoroughly mix Mg^{++} solution and buffer prior to adding to the reaction • Optimize annealing temperature by testing an annealing temperature gradient, starting at 5°C below the lower Tm of the primer pair

Observation	Possible Cause	Solution
NO PRODUCT	Poor template quality	• Analyze DNA via gel electrophoresis before and after incubation with Mg^{++} • Check 260/280 ratio of DNA template
	Presence of inhibitor in reaction	• Further purify starting template by alcohol precipitation, drop dialysis or commercial clean up kit • Decrease sample volume
	Insufficient number of cycles	• Rerun the reaction with more cycles
	Incorrect thermocycler programming	• Check program, verify times and temperatures
	Inconsistent block temperature	• Test calibration of heating block
	Contamination of reaction tubes or solutions	• Autoclave empty reaction tubes prior to use to eliminate biological inhibitors • Prepare fresh solutions or use new reagents and new tubes
	Complex template	• Use Q5 High-Fidelity • For GC-rich templates
	Premature replication	• Use a hot start polymerase • Set up reactions on ice using chilled components and add samples to thermocycler preheated to the denaturation temperature
MULTIPLE OR NON-SPECIFIC PRODUCTS	Primer annealing temperature too low	• Increase annealing temperature
	Incorrect Mg^{++} concentration	• Adjust Mg^{++} in 0.2–1 mM increments
	Poor primer design	• Check specific product literature for recommended primer design • Verify that primers are non-complementary, both internally and to each other • Increase length of primer • Avoid GC-rich 3´ ends
	Excess primer	• Primer concentration can range from 0.05–1µM in the reaction. Please see specific product literature for ideal conditions.
	Contamination with exogenous DNA	• Use positive displacement pipettes or non-aerosol tips • Set-up dedicated work area and pipettor for reaction setup • Wear gloves during reaction setup
	Incorrect template concentration	• For low complexity templates (i.e. plasmid, lambda, BAC DNA), use 1 pg–10 ng of DNA per 50 µl reaction • For higher complexity templates (i.e. genomic DNA), use 1 ng–1 µg of DNA per 50 µl reaction

Antinuclear Antibodies

Laboratories must consider several key factors before deciding which method is best for their patients and staff



Imagine your lab has decided to take the plunge and implement antinuclear antibody (ANA) testing in house, taking it off the send-out menu. You might first ask, What is the best method for ANA testing? Or, what if your lab already performs ANA testing, but the expert technologist who has been reading ANA indirect immunofluorescence (IIF) slides for 30 years has just announced that she is going to retire. This might prompt you to ask, Is it time for us to move from IIF ANA testing to a newer methodology? These are important and relevant questions, but without easy answers. This review aims to provide practical information on ANA testing methodologies, including their diagnostic utility and performance characteristics.

ANA TESTING HISTORY AND CONTEXT

ANAs refer to a collection of autoantibodies that target a variety of nuclear and cytoplasmic antigens. First described more than 50 years ago, ANAs remain the most sensitive serologic marker for evaluating patients with suspected connective tissue diseases (CTDs), also referred to as ANA-associated rheumatic diseases (AARDs) (1). The diagnostic potential of ANAs originated with the discovery of LE cells, described as mature polymorphonuclear leukocytes containing phagocytosed nuclear material. LE cells were so-named because they were found only in patients with systemic lupus erythematosus (SLE). LE cells could be produced in vitro by taking patient plasma and mixing it with peripheral blood from healthy controls that had been "damaged" by vortexing with glass beads. Ultimately, research demonstrated that immunoglobulin from patient plasma was binding to nuclei from the "damaged" peripheral blood, which neutrophils in turn phagocytosed. IIF was used to further characterize this immunoglobulin, demonstrating its specific binding to cellular nuclear material. This immunoglobulin is what we now know as the ANA. ANA testing generally involves two parts (2). First, for patients with a suspected AARD, a screening ANA is ordered to detect the ANA regardless of the antigen specificity. Second, for patients with positive screening assay results, additional tests characterize the antigen specificity of their ANA. Identifying the antigen specificity has important diagnostic and prognostic implications for patients. Although dozens of antigens have been associated with ANAs, only a small number are available for routine clinical testing. Depending on a patient's clinical scenario, a positive ANA may require testing for anti-double standard DNA antibodies, antibodies against one or more of the extractable nuclear antigens (SS-A, SS-B, Sm, Scl-70, Jo-1, and RNP), anti-ribosomal P antibodies, or anti-centromere antibodies.

METHODOLOGIES FOR ANA TESTING

Three primary methods are available to clinical laboratories as screening ANA tests: IIF, enzyme immunoassay (EIA), and multiplex immunoassay (MIA). IIF detects antibodies that bind to a tissue substrate which, for ANAs, is usually fixed HEp-2 cells. IIF accomplishes this detection with a fluorescently labeled anti-human immunoglobulin. With EIA, an antigen mixture adhered to a solid surface (usually a 96-well plate) takes the place of the HEp-2 cells, and detection occurs through an enzyme-labeled anti-human immunoglobulin. MIAs are based on polystyrene bead sets distinguished from one another based on their fluorescent signature. Each bead set is conjugated to a known ANA antigen, and the different sets are then combined into a bead cocktail. A patient sample is added to the bead cocktail, and binding of a patient antibody to any of the beads is accomplished with a fluorescently labeled anti-human immunoglobulin.

Reporting of ANA Test Results

From a physician's perspective, one of the most obvious differences between ANA screening methods is how results are reported. In most cases, MIAs are reported qualitatively as "ANA positive" or "ANA negative," with screen results being based on the collective assessment of all the individual antigen specificities included in an assay. If all the included antigen specificities are negative, then the ANA screen is interpreted as negative. Conversely, if one or more of the beads show fluorescence exceeding a certain threshold, a sample would be identified as positive. Importantly, for ANA positive samples, the identities of the antigen specificities are not revealed to the laboratory and thus are not reported to patients' medical records. If a clinician wants to determine the antigen specificity of a patient's ANA, he or she would need to order the clinically relevant tests. In contrast, most EIAs are reported as a numeric value with an arbitrary unit of measurement. There is no traceable standard for these assays, so each manufacturer establishes the units and analytical measuring range for its tests. EIAs' quantitation is based on light absorbance. The enzyme linked to the detection antibody converts a colorless substrate to a colored product, the absorbance of which is compared to a standard curve. Manufacturers will provide a recommended cutoff, which is the unit value above which a sample would be considered "ANA positive". As with MIAs, a positive EIA result does not reveal the antigen specificity of the ANA, and further testing would be necessary if a clinician wants to know those details. ANA by IIF is generally reported with both a titer and a pattern. Labs screen all samples initially at a single dilution, usually 1:40 or 1:80. Any sample identified as positive at the screening dilution is titered out either to endpoint or to a pre-defined dilution, depending on the laboratory's preference. The titer is determined by serial dilution, with the reported titer being the last dilution for which the IIF would be identified as positive. The pattern interpretation is based upon recognition of specific cellular features to which a patient's antibody has bound. Because IIF pattern interpretation is based on visual interpretation, standardization in reporting has been a challenge. The International Consensus on ANA Patterns (ICAP), a subcommittee of the Autoantibody Standardization Committee, promotes discussion and generates consensus regarding the morphologic features associated with specific ANA patterns (4). ICAP has also made recommendations regarding how laboratories should report ANA patterns. The group has defined six nuclear patterns as "Competent-Level": homogeneous; speckled; dense fine speckled (DFS); centromere; discrete nuclear dots; and nucleolar. ICAP recommends that any laboratory performing ANA by IIF should be able to accurately and reproducibly identify these patterns.

The remaining nuclear patterns are designated as "Expert-Level" and might be recognizable only by individuals with particular expertise in IIF analysis.

ANA CLINICAL SENSITIVITY AND SPECIFICITY

When considering which ANA test to implement, understanding each method's clinical sensitivity and specificity is critical. Many studies have compared the clinical sensitivity and specificity of the different methods. Because IIFs, EIAs, and MIAs report results so differently, these studies have focused primarily on qualitative agreement. Although seemingly very straight-forward, these types of comparisons are more difficult than they appear, largely because estimated sensitivities and specificities and the agreement between methods is heavily dependent on the cutoffs used to differentiate between positive and negative. Historically, IIF has been considered the most sensitive method for identifying patients with AARDs. In a 2009 position statement on ANA testing methods, the American College of Rheumatology identified IIF as the "gold standard for ANA testing" primarily based on its high sensitivity (>95%) for the diagnosis of SLE (5). However, the statement also acknowledges that the specificity of ANA by IIF is a limitation. In a cohort of patients for whom ANA testing was ordered as part of routine clinical care, we demonstrated that IIF at a titer cutoff of 1:40 had a sensitivity of 94% for the general diagnosis of AARDs (6). This was higher than the sensitivity of either EIA or MIA, at 74% and 67%, respectively. However, the IIF's higher sensitivity was at the expense of specificity, which, at the 1:40 cutoff, was only 43%. In comparison, the corresponding EIA and MIA specificities were 80% and 87%, respectively. When we increased the cutoff for IIF to 1:80, the specificity improved to 62% but the sensitivity decreased to 84%. Some data suggest that the titer of the ANA may help in distinguishing between patients with and without AARDs. In a study from 2011, Mariz et al. demonstrated that 45.8% of positive ANAs in healthy controls had a titer of 1:80, while 88.5% of ANA-positive AARD patients had an ANA titer ≥1:320 (7). Many laboratories that perform ANA by IIF are moving away from screening at the 1:40 dilution, opting for improved specificity even with some loss in sensitivity. When labs use higher screening dilutions, the sensitivities of IIFs are on par with those of EIAs and MIAs. Although IIFs have the capability of maximizing sensitivity, from a practical perspective, EIAs and MIAs provide a good balance of sensitivity and specificity. IIF's sensitivity is attributed to its broad antigen specificity. This method detects antibodies against any of the hundreds of nuclear and cytoplasmic antigens present in a cell. However, not all antigen specificities are relevant for the diagnosis of AARDs. For example, the DFS pattern appears almost exclusively in patients with no evidence of an AARD (7). It has been suggested that the presence of the DFS pattern could be used to rule out an AARD in an individual with a positive ANA. The antigen specificity associated with this pattern has been identified as lens epithelial-derived growth factor, also referred to as DFS70 (8). Further studies have confirmed that monospecificity for DFS70 in the context of a DFS pattern is not consistent with an AARD. This pattern, and perhaps others like it that have yet to be characterized, may help to address some of the specificity challenges associated with ANA testing by IIF.

PERFORMANCE CONSIDERATIONS FOR ANA METHODOLOGIES

When labs are considering which ANA method to implement, availability of a qualified technologist to perform the testing is likely a significant concern. Other key considerations include throughput, workflow, and automation of a method. Although automation of immunological testing has not reached the level of chemistry platforms, significant strides have been made over the last decade, particularly with EIAs and MIAs. EIAs can be performed manually, although more often than not, labs perform this testing on semi-automated or automated platforms. The semi-automated platforms may dilute patient samples and add reagents to the plate, but a technologist's intervention might be required to wash and move the plate to an absorbance reader. A fully automated system processes an EIA in its entirety, only requiring technologists to load samples and reagents. Most MIA systems are also fully automated. In addition, MIAs have the advantage of being random access, which facilitates improved workflows. In contrast, EIAs are batched, which, for labs with lower volumes of ANA orders, could have a negative impact on workflow and on turnaround times. Another advantage of MIA systems is they offer labs the opportunity to expand their test menus. Most MIA systems are not limited to ANA testing, and have reagents available for other autoimmune conditions (celiac disease, antiphospholipid syndrome, and vasculitis) and for infectious diseases (Epstein-Barr virus, HIV, and herpes simplex virus). Being able to perform additional testing and maximize an instrument's utilization could make an MIA system an attractive option. Historically, IIF has been the ANA method requiring the most clinical technologist resources and expertise, with automation limited to dilution of patient samples and perhaps addition of sample and reagents to slides. In addition, slide reading was a manual process that relied on experienced technologists to interpret numerous complex patterns. Now, however, systems are available that automate almost the entire process, from slide processing to reading. Processing the slides includes not only sample and reagent pipetting but also slide incubation and washing. After processing, the slides can be moved to an enclosed microscope with a high-resolution digital camera, which obviates the need for a darkroom. This means such systems can be used on a bench in an open laboratory. Cameras in these newer IIF systems capture several digital images from different areas of slides. The fluorescence intensity of the stain is measured, and values above a certain cutoff are considered positive. For samples identified as positive, the computer algorithm reads the pattern of and interprets the fluorescence intensities in the context of known ANA patterns. Although this step automates the previously manual process of slide reading, final qualitative and pattern interpretation still requires a technologist's expertise. For each sample, a technologist must confirm the computer-generated result. If he or she disagrees, the result can be changed. Most automated readers recognize the common ANA patterns, and some identify certain mixed patterns. More complex patterns unidentifiable by the computer still require a technologist's interpretation. Some automated readers not only automate pattern interpretation at least partially but also estimate titers. These instruments use the fluorescence intensity of an image to estimate a sample's titer rather than relying on serial dilutions. This can be accomplished either from a single patient dilution or a limited number of dilutions. As with pattern interpretation, an estimated titer can be replaced with a titer from serial dilutions, depending on the pattern and the technologist's judgment. Overall, although not completely automated by chemistry standards, the availability of automation for IIF, EIA, and MIA gives labs several options for complex ANA testing in a time of shrinking resources.

FOBT

Description

Fecal occult blood (FOB) refers to blood in the feces that is not visibly apparent. A **fecal occult blood test** (FOBT) checks for hidden (occult) blood in the stool (feces). Newer tests look for globin, DNA, or other blood factors including transferrin, while conventional stool guaiac tests look for heme.

Purpose

Fecal occult blood testing (FOBT), as its name implies, aims to detect subtle blood loss in the gastrointestinal tract, anywhere from the mouth to the colon. Positive tests ("positive stool") may result from either upper gastrointestinal bleeding or lower gastrointestinal bleeding and warrant further investigation for peptic ulcers or a malignancy (such as colorectal cancer or gastric cancer). The test does not directly detect colon cancer but is often used in clinical screening for that disease, but it can also be used to look for active occult blood loss in anemia or when there are gastrointestinal symptoms.

Nomenclature

In 2007 the nomenclature of overt, obscure and occult bleeding was clarified. The different methods of testing for "fecal occult blood" as broadly considered actually test for particular components of blood or for aberrantly expressed cellular markers from the intestinal mucosa.

Methodology

There are four methods in clinical use for testing for occult blood in feces. These look at different properties, such as antibodies, heme, globin, or porphyrins in blood, or at DNA from cellular material such as from lesions of the intestinal mucosa. **Fecal Immunochemical Testing (FIT),** and **immunochemical fecal occult blood test** (**iFOBT**). FIT products utilize specific antibodies to detect globin. FIT screening is more effective in terms of health outcomes and cost compared with guaiac FOBT. According to the guidelines of the American College of Gastroenterology, "Annual fecal immunochemical testing is the preferred colorectal cancer detection test." The FIT tests are clearly superior to traditional low sensitivity gFOBT for colorectal cancer screening. Although FIT has replaced most gFOBT tests in colon cancer screening, the high sensitivity gFOBT, such as Hemoccult SENSA, remains an accepted option although the FIT test is clearly preferred in recent guidelines. The number of fecal samples submitted for FIT may affect the clinical sensitivity and specificity of the methodology. This methodology can be adapted for automated test reading and to report quantitative results, which are potential factors in design of a widescale screening strategy. High-sensitivity FOBT may retain a role in monitoring gastrointestinal conditions such as ulcerative colitis. **Stool guaiac test for fecal occult blood (gFOBT):** The stool guaiac test involves smearing some feces on to some absorbent paper that has been treated with a chemical. Hydrogen peroxide is then dropped on to the paper; if trace amounts of blood are present, the paper will change color in one or two seconds. This method works as the heme component in hemoglobin has a peroxidase-like effect, rapidly breaking down hydrogen peroxide. In some settings such as gastric or proximal upper intestinal bleeding the guaiac method may be more sensitive than tests detecting globin because globin is broken down in the upper intestine to a greater extent than is heme. There are various commercially available gFOBT tests which have been categorized as being of low or high sensitivity, and only high sensitivity tests are now recommended in colon cancer screening. Optimal clinical performance of the stool guaiac test depends on preparatory dietary adjustment. **Fecal porphyrin quantification: HemoQuant,** unlike gFOBT and FIT, permits precise quantification of hemoglobin, and is analytically validated with gastric juice and urine, as well as stool samples. The heme moiety of intact hemoglobin is chemically converted by oxalic acid and ferrous oxalate or ferrous sulfate to protoporphyrin, and the porphyrin content of both the original sample and of the sample after hemoglobin conversion to porphyrin is quantified by comparative fluorescence against a reference standard; the specificity for hemoglobin is increased by subtracting the fluorescence of a sample blank prepared with citric acid to correct for the potential confounding effect of existing non-specific substances. Precise quantification measurement has been very useful in many clinical research applications. **Fecal DNA test:** The test extracts human DNA from the stool sample and tests it for alterations that have been associated with cancer. The test looks at 23 individual DNA alterations, including 21 specific point alterations in the APC, KRAS and p53 genes, as well as testing BAT26, a gene involved in microsatellite instability (MSI). and a proprietary DNA Integrity Assay (DIA). Additional methods of looking for occult blood are being explored, including transferrin dipstick and stool cytology.

Test performance

Reference standards: The estimates for test performance characteristics are based on comparison with a variety of reference methods including 51-chromium studies, analytical recovery studies in spiked stool samples, analytical recovery after ingestion of autologous blood, rarer studies of carefully quantified blood instilled at bowel surgery as well as other research approaches. Additionally, clinical studies look at variety of additional factors.

Gastrointestinal blood loss in health

In healthy people about 0.5 to 1.5 ml of blood escapes blood vessels into the stool each day. Significant amounts of blood can be lost without producing visible blood in the stool, estimated as 200 ml in the stomach, 100 ml in the duodenum, and lesser amounts in the lower intestine. Tests for occult blood identify lesser blood loss.

Clinical sensitivity and specificity

Stool guaiac test for fecal occult blood (**gFOBT**) sensitivity varies depending on the site of bleeding. Moderately sensitive gFOBT can pick up a daily blood loss of about 10 ml (about two teaspoonfuls), and higher sensitivity gFOBT can pick up lesser amounts, sometimes becoming positive at about 2 ml. The sensitivity of a single stool guaiac test to pick up bleeding has been quoted at 10 to 30%, but if a standard three tests are done as recommended the sensitivity rises to 92%. Further discussion of sensitivity and specificity issues that relate particularly to the guaiac method is found in the stool guaiac test article. **Fecal**

Immunochemical Testing (FIT) picks up as little as 0.3 ml but because it does not detect occult blood from the stomach and upper small intestine the test threshold doesn't cause undue false positives from normal upper intestinal blood leakage and it is much more specific for bleeding from the colon or lower gastrointestinal tract. The detection rate of the test decreases if the time from sample collection to laboratory processing is delayed. **Fecal porphyrin quantification** by **HemoQuant** can be false positive due to exogenous blood and various porphyrins. HemoQuant is the most sensitive test for upper gastrointestinal bleeding and therefore may be most appropriate fecal occult blood test to use in the evaluation of iron deficiency Advised to stop red meat and aspirin for 3 days prior to specimen collection. False positives can occur with myoglobin, catalase, or protohemes and in certain types of porphyria. The DNA based **PreGen-Plus** was four times more sensitive than fecal blood testing, including detection of early stage disease, when treatment is most effective. Sensitivity increased to 51.6% compared to 12.9%. Additional clinical trials of the PreGen-Plus method are underway to more fully characterize its clinical performance. Expanding the range of DNA testing by looking at additional known genetic markers, such as CTNNB1, or by analyzing epigenetically methylated genes such as MLH1 which is very common in serrated polyps with microsatellite instability (MSI) and in proximal colon tumours that have poorer differentiation, does not appear to appreciably increase the sensitivity of the method because CTNNB1 mutations are infrequent in sporadic colorectal cancer, and because BAT26 alterations and lack of MLH1 expression show a high degree of overlap.

Clinical application

The stool guaiac test for hidden (occult) blood in the stool can be done at home or in the doctor's office, or can be performed on samples submitted to a clinical laboratory. Testing kits are available at pharmacies in some countries without a prescription, or a health professional may order a testing kit for use at home. If a home fecal occult blood test detects blood in the stool it is recommended to see a health professional to arrange further testing.

Sources of gastrointestinal bleeding

Gastrointestinal bleeding has many potential sources, and positive results usually result in further testing for the bleeding site, usually looking for lower gastrointestinal bleeding before upper gastrointestinal bleeding causes unless there are other clinical clues. Colonoscopy is usually preferred to computerized tomographic colonography. An estimated 1–5% of large tested populations have a positive fecal occult blood test. Of those, about 2–10% have cancer, while 20–30% have adenomas. A positive test can result from upper gastrointestinal bleeding or lower gastrointestinal bleeding. The common causes are: 2–10%: cancer (colorectal cancer, gastric cancer), 20–30% adenoma or polyps, Bleeding peptic ulcer, Angiodysplasia of the colon, Sickle cell anemia. In the event of a positive fecal occult blood test, the next step in the workup is a form of visualization of the gastrointestinal tract by one of several means: Sigmoidoscopy, an examination of the rectum and lower colon with a lighted instrument to look for abnormalities, such as polyps. Colonoscopy, a more thorough examination of the rectum and entire colon. Virtual colonoscopy. Endoscopy refers to upper gastrointestinal endoscopy. It is sometimes performed with chromoendoscopy, a method that assists the endoscopist by enhancing the visual difference between cancerous and normal tissue, either by marking the abnormally increased DNA content (toluidine blue) or failing to stain the tumor, possibly due to decreased surface glycogen on tumor cells(Lugol). Infrared fluorescent endoscopy and ultrasonic endoscopy can interrogate vascular abnormalities such as esophageal varices. Double contrast barium enema: a series of x-rays of the colon and rectum.

Stool color

Although red or black stools can be an indication of bleeding, a dark or black color can be due to black licorice, blueberries, iron supplements, lead, Pepto-bismol, and a red color can come from natural or artificial coloring such as red gelatin, popsicles, Kool-Aid, and large amounts of beets.

Colorectal cancer screening

Screening methods for colon cancer depend on detecting either precancerous changes such as certain kinds of polyps or on finding early and thus more treatable cancer. The extent to which screening procedures reduce the incidence of gastrointestinal cancer or mortality depends on the rate of precancerous and cancerous disease in that population. gFOBT and flexible sigmoidoscopy screening have each shown benefit in randomized clinical trials. Evidence for other colon cancer screening tools such as iFOBT or colonoscopy is substantial and guidelines have been issued by several advisory groups but does not include randomized studies. Guaiac FOB testing of average risk populations may reduce the mortality associated with colon cancer by about 25%. It is not always cost effective to screen a large population. If colon cancer is suspected in an individual (such as in someone with an unexplained anemia) fecal occult blood tests may not be clinically helpful. If a doctor suspects colon cancer, more rigorous investigation is necessary, whether or not the test is positive. The 2009 recommendations of the American College of Gastroenterology (ACG) suggest that colon cancer screening modalities that are also directly preventive by removing precursor lesions should be given precedence, and prefer a colonoscopy every 10 years in average-risk individuals, beginning at age 50. The ACG suggests that cancer detection tests such as any type of FOB are an alternative that is less preferred and which should be offered to patients who decline colonoscopy or another cancer prevention test. However, two other recent guidelines, from the US Multisociety Task Force (MSTF) and the US Preventive Services Task Force (USPSTF) while permitting immediate colonoscopy as an option, did not categorize it as preferred. The ACG and MSTF also included CT colonography every 5 years, and fecal DNA testing as considerations. All three recommendation panels recommended replacing any older low-sensitivity, guaiac-based fecal occult blood testing (gFOBT) with either newer high-sensitivity guaiac-based fecal occult blood testing (gFOBT) or fecal immunochemical testing (FIT). MSTF looked at 6 studies that compared high sensitivity gFOBT (Hemoccult SENSA) to FIT, and concluded that there were no clear difference in overall performance between these methods. In colon cancer screening, using only one sample of feces collected by a doctor performing a digital rectal examination is strongly discouraged.

Iron deficiency anemia

An extensive literature has examined the clinical value of FOBT in iron deficiency anemia.

Gastrointestinal disease and medications

Conditions such as ulcerative colitis or certain types of relapsing infectious diarrhea can vary in severity over time, and FOBT may assist in assessing the severity of the disease. Medications associated with gastrointestinal bleeding such as Bortezomib are sometimes monitored by FOBT.

Alcoholism

Several aspects of FOBT in alcoholism warrant further discussion.

Outpatient clinics

Several studies have reported clinical benefit from gFOBT testing including urology and gynecology clinics.

Inpatient guaiac testing

Several studies have questioned the traditional Admission Screening Guaiac (ASG). The utility of following stool guaiac in ICU settings is also questioned.

Testing of upper gastrointestinal or aerodigestive tract secretions for occult blood

The use of tests for occult blood in disorders of the mouth, nasal passages, esophagus, lungs and stomach, while analogous to fecal testing, is often discouraged, due to technical considerations including poorly characterized test performance characteristics such as sensitivity, specificity, and analytical interference. However, chemical confirmation that coloration is due to blood rather than coffee, beets, medications, or food additives can be of significant clinical assistance.

A related concept to colon cancer screening by FOBT, based on most neoplasms affecting the surface epithelium and losing small amounts of blood but no visible blood loss, is screening in populations at high risk for esophageal or gastric cancers by testing for blood by swallowing a small capsule that is recovered after 3 to 5 minutes by gentle retrieval by means of an attached nylon thread.

Fecal occult blood in marathon runners

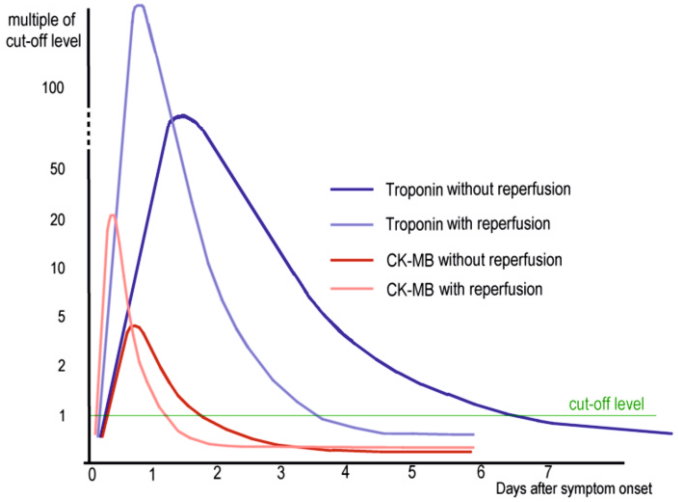
Gastrointestinal (GI) complaints and low intensity GI bleeding frequently occur in marathon runners. Strenuous exercise, particularly in elite athlete runners and less frequently in other exercise activities, can cause acute incapacitating gastrointestinal symptoms including heartburn, nausea, vomiting, abdominal pain, diarrhea and gastrointestinal bleeding. Approximately one third of endurance runners experience transient but exercise limiting symptoms, and repetitive gastrointestinal bleeding occasionally causes iron deficiency and anaemia. Runners can sometimes experience significant symptoms including hematemesis. Exercise is associated with extensive changes in gastrointestinal (GI) tract physiology, including diversion of blood flow from the GI tract to muscle and lungs, decreased GI absorption and small intestinal motility, increased colonic transit, neuroimmunoendocrine changes in hormones and peptides such as vasoactive intestinal peptide, secretin and peptide-histidine-methionine. Substantial changes occur in stress hormones including cortisol, in circulating concentrations and metabolic behavior of various leucocytes, and in immunoglobulin levels and major histocompatibility complex expression. Symptoms can be exacerbated by dehydration or by pre-exercise ingestion of certain foods and hypertonic liquids, and lessened by adequate training.

Cardiac Markers

Cardiac markers are biomarkers measured to evaluate heart function. They can be useful in the early prediction or diagnosis of disease. Although they are often discussed in the context of myocardial infarction, other conditions can lead to an elevation in cardiac marker level. Most of the early markers identified were enzymes, and as a result, the term "cardiac enzymes" is sometimes used. However, not all of the markers currently used are enzymes. For example, in formal usage, troponin would not be listed as a cardiac enzyme.

Applications of measurement

Measuring cardiac biomarkers can be a step toward making a diagnosis for a condition. Whereas cardiac imaging often confirms a diagnosis, simpler and less expensive cardiac biomarker measurements can advise a physician whether more complicated or invasive procedures are warranted. In many cases medical societies advise doctors to make biomarker measurements an initial testing strategy especially for patients at low risk of cardiac death. Many acute cardiac marker IVD products are targeted at nontraditional markets, e.g., the hospital ER instead of traditional hospital or clinical laboratory environments. Competition in the development of cardiac marker diagnostic products and their expansion into new markets is intense. Recently, the intentional destruction of myocardium by alcohol septal ablation has led to the identification of additional potential markers.



Kinetics of cardiac markers Troponin and CK-MB in myocardial infarction with or without reperfusion treatment.

Types

Types of cardiac markers include the following:

Test	Sensitivity and specificity	Approximate peak	Description
Troponin test	The most sensitive and specific test for myocardial damage. Because it has increased specificity compared with CK-MB, troponin is composed of 3 proteins- Troponin C, Cardic troponin I, and Cardiac troponin T. Troponin I especially has a high affinity for myocardial injury.	12 hours	Troponin is released during MI from the cytosolic pool of the myocytes. Its subsequent release is prolonged with degradation of actin and myosin filaments. Isoforms of the protein, T and I, are specific to myocardium. Differential diagnosis of troponin elevation includes acute infarction, severe pulmonary embolism causing acute right heart overload, heart failure, myocarditis. Troponins can also calculate infarct size but the peak must be measured in the 3rd day. After myocyte injury, troponin is released in 2–4 hours and persists for up to 7 days. Normal value are - Troponin I <0.3 ng/ml and Troponin T <0.2 ng/ml.
Creatine Kinase (CK-MB) test	It is relatively specific when skeletal muscle damage is not present.	10–24 hours	The CK-MB isoform of creatine kinase is expressed in heart muscle. It resides in the cytosol and facilitates movement of high energy phosphates into and out of mitochondria. Since it has a short duration, it cannot be used for late diagnosis of acute MI but can be used to suggest infarct extension if levels rise again. This is usually back to normal within 2–3 days. Normal range - 2-6 ng/ml.

Test	Sensitivity and specificity	Approximate peak	Description
Lactate dehydrogenase (LDH)	LDH is not as specific as troponin.	72 hours	Lactate dehydrogenase catalyses the conversion of pyruvate to lactate. LDH-1 isozyme is normally found in the heart muscle and LDH-2 is found predominantly in blood serum. A high LDH-1 level to LDH-2 suggest MI. LDH levels are also high in tissue breakdown or hemolysis. It can mean cancer, meningitis, encephalitis, or HIV. This is usually back to normal 10–14 days.
Aspartate transaminase (AST)			This was the first used. It is not specific for heart damage, and it is also one of the liver transaminases.
Myoglobin (Mb)	Low specificity for myocardial infarction.	2 hours	Myoglobin is used less than the other markers. Myoglobin is the primary oxygen-carrying pigment of muscle tissue. It is high when muscle tissue is damaged but it lacks specificity. It has the advantage of responding very rapidly, rising and falling earlier than CK-MB or troponin. It also has been used in assessing reperfusion after thrombolysis.
Ischemia-modified albumin (IMA)	Low specificity.		IMA can be detected via the albumin cobalt binding (ACB) test, a limited available FDA approved assay. Myocardial ischemia alters the N-terminus of albumin reducing the ability of cobalt to bind to albumin. IMA measures ischemia in the blood vessels and thus returns results in minutes rather than traditional markers of necrosis that take hours. ACB test has low specificity therefore generating high number of false positives and must be used in conjunction with typical acute approaches such as ECG and physical exam. Additional studies are required.
Pro-brain natriuretic peptide			This is increased in patients with heart failure. It has been approved as a marker for acute congestive heart failure. Pt with < 80 have a much higher rate of symptom-free survival within a year. Generally, Pt with CHF will have > 100.
Glycogen phosphorylase isoenzyme BB	0.854 and 0.767.	7 hours	Glycogen phosphorylase isoenzyme BB (abbreviation: GPBB) is one of the three isoforms of glycogen phosphorylase. This isoform of the enzyme exists in cardiac (heart) and brain tissue. Because of the blood–brain barrier, GP-BB can be seen as being specific to heart muscle. GP-BB is one of the "new cardiac markers" which are considered to improve early diagnosis in acute coronary syndrome. During the process of ischemia, GP-BB is converted into a soluble form and is released into the blood. A rapid rise in blood levels can be seen in myocardial infarction and unstable angina. GP-BB is elevated 1–3 hours after process of ischemia.

Limitations

Depending on the marker, it can take between 2 and 24 hours for the level to increase in the blood. Additionally, determining the levels of cardiac markers in the laboratory - like many other lab measurements - takes substantial time. Cardiac markers are therefore not useful in diagnosing a myocardial infarction in the acute phase. The clinical presentation and results from an ECG are more appropriate in the acute situation. However, in 2010, research at the Baylor College of Medicine revealed that, using diagnostic nanochips and a swab of the cheek, cardiac biomarker readings from saliva can, with the ECG readings, determine within minutes whether someone is likely to have had a heart attack.

Leucocyte Alkaline Phosphatase

How the Test is Performed

Blood is typically drawn from a vein, usually from the inside of the elbow or the back of the hand. The site is cleaned with germ-killing medicine (antiseptic). The health care provider wraps an elastic band around the upper arm to apply pressure to the area and make the vein swell with blood. Next, the health care provider gently inserts a needle into the vein. The blood collects into an airtight vial or tube attached to the needle. The elastic band is removed from your arm. Once the blood has been collected, the needle is removed, and the puncture site is covered to stop any bleeding. In infants or young children, a sharp tool called a lancet may be used to puncture the skin and make it bleed. The blood collects into a small glass tube called a pipette, or onto a slide or test strip. A bandage may be placed over the area if there is any bleeding. A laboratory specialist separates the white blood cells from the rest of the blood sample and watches to see if any substances stick to specific colored dyes. Substances that contain phosphate, such as ALP, attach to certain colored dyes.

How to Prepare for the Test

One should not eat or drink for 6 hours before the test. Certain medicines may affect the test results. Your health care provider may tell you to stop taking such medications. These medications include: Allopurinol, Androgens, Anti-inflammatory medicines, Birth control pills, Certain antibiotics, Certain arthritis drugs, Certain diabetes drugs (taken by mouth), Chlorpromazine, Cortisone, Methyldopa, Narcotics, Propranolol, Tranquilizers, Tricyclic antidepressants. No medicine should be stopped without medical advice.

Why the Test is Performed

ALP is found in different forms throughout the body. This test is done to confirm a number of different medical conditions, including certain types of anemia and leukemia. It may also be done if a person has an increase in platelet levels in the blood.

Normal Results

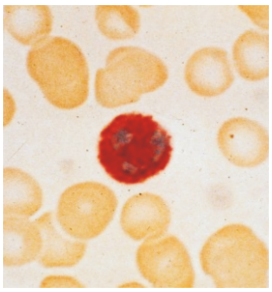
A staining score of 20 - 100 (out of a maximum of 400) is considered normal. Note: Normal value ranges may vary slightly among different laboratories. Talk to your doctor about the meaning of your specific test results. The examples above show the common measurements for results for these tests. Some laboratories use different measurements or may test different specimens.

What Abnormal Results Mean

Higher-than-normal results may be due to: Essential thrombocytosis, Leukemoid reaction, Myelofibrosis, Polycythemia vera.

Lower-than-normal results may be due to: Aplastic anemia, Chronic myeloid leukemia, Pernicious anemia.

Leukocyte alkaline phosphatase



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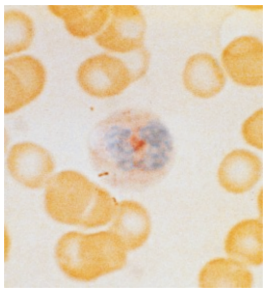


LAP NEGATIVE NEUTROPHIL

What if one suspects CML but doesn't have access to a cytogenetic or molecular lab (to look for the Philadelphia chromosome or the bcr-abl translocation)? Well, first you'd look for all the morphologic clues you could. CML usually presents with a marked leukocytosis (the WBC is often over 100,000), with a left shift all the way back to myeloblasts (though there are relatively few myeloblasts around). A benign left shift usually presents with a mild to moderate leukocytosis (the neutrophil count is often just above normal; it's generally nowhere near the magnitude often seen in CML), and the neutrophils are shifted back to the metamyelocyte or myelocyte stages (you'll very rarely see promyelocytes, and you'll virtually never see myeloblasts). Also, CML tends to have a "bulge" at the metamyelocyte stage, whereas a benign left shift does not (the cells are more or less present in decreasing amounts by stage of maturation, i.e., there are more segmented than band neutrophils, more bands than metamyelocytes, more metamyelocytes than myelocytes, more myelocytes than promyelocytes and blasts are basically nonexistent). Finally, CML has a basophilia, whereas a benign left shift does not. But if you wanted more proof that your case was CML, you could do a leukocyte (or neutrophil) alkaline phosphatase (LAP). This test is not done as much as it used to be, because now everyone goes right to cytogenetics or molecular testing in order to find the Philadelphia chromosome or the bcr-abl translocation. But it's still a good test, and it would be a good thing to do if you couldn't look for the Philadelphia chromosome. Here's the principle behind the test. LAP is an enzyme present in normal neutrophils, but absent (or present at very low concentrations) in malignant neutrophils (i.e., the ones in CML). So if you have a whole bunch of neutrophils around, and the LAP is strongly positive in those cells, as in the top image, you can be quite sure that it is a benign bunch of neutrophils. However, if the LAP is negative, or only weakly positive, as in the bottom image, that probably means that those neutrophils are malignant and that you're dealing with a case of CML. You'd still want to send off a blood or bone marrow specimen to a cytogenetics and/or molecular diagnostics lab, but in the meantime, the LAP can help you quickly assess and triage your patient.

Classification of acute leukemias by morphologic and cytochemical criteria (modified from Löffler)

Stem cell leukemia		
peroxidase	0	undifferentiated type
periodic acid Schiff	0	
alpha-napthylacetate esterase	0	
Myeloblastic leukemia (AML)		
peroxidase	(-)	Peroxidase type 1 and 2
periodic acid Schiff	0-(+)	
alpha-napthylacetate esterase	(+)	
Promyelocytic leukemia		
peroxidase	+	Peroxidase type 3
periodic acid Schiff	(+)	
alpha-napthylacetate esterase	(+)	
Myelomonocytic leukemia		
peroxidase	+	Peroxidase esterase type
periodic acid Schiff	(+)	
alpha-napthylacetate esterase	+	

Monocytic leukemia		
peroxidase	(+)	Esterase type
periodic acid Schiff	0-(+)	
alpha-napthylacetate esterase	+	
Lymphoblastic leukemia (ALL)		
preoxidase	0	periodic acid Schiff type
periodic acid Schiff	+	
alpha-naphthylacetate esterase	0	
0 = negative; (+) = weakly positive < 25%; + = strongly positive > 50		

Acute eosinophilic leukemia is diagnosed by examination of the bone marrow, since eosinophils usually are not increased in the peripheral blood. The predominant marrow cells are abnormal eosinophils (immature, pleomorphic forms, some with coarse, dark-blue granules, cytoplasmic vacuoles, distinct nucleoli). Diagnosis relies on the cytochemical detection of naphthol-AS-D-chloracetate esterase in the granules. Auer rods are unusual. Acute basophilic leukemia is evidenced by an extreme increase in the basophilic granulated cells of granulocytopoiesis. The granules are very atypical (large, coarse, hyperchromic), and Auer rods may be present The diagnosis is confirmed by the metachromatic reaction to toluidine blue in the cells. Four forms are recognized: (1) basophilic blast crises in CML, (2) promyelocytic basophilic type, (3) histiobasophilic type, (4) basophilic-eosinophilic type. It is very difficult to establish a diagnosis of acute megakaryoblastic leukemia, designated M7 in the FAB classification of acute leukemias. The blast cells in the peripheral blood and bone marrow present a variety of morphologies. They may appear as small cells with a narrow cytoplasmic border and dense chromatin, resembling lymphoblasts (L1), or they may resemble L2 cells with or without granules. The nuclei are round, finely reticular, and have one to three prominent nucleoli. The cells vary greatly in size and may be two to three times larger than normal lymphocytes. Sometimes one finds cytoplasmic vesicles or differentiated megakaryocytes with adjacent platelets or bare nuclei nested in clusters of platelets. Occasional megakaryocytic nuclei are found in the peripheral blood. It is often difficult to obtain a bone marrow specimen by aspiration, and marrow biopsy is usually indicated. Cytochemical methods may contribute to the diagnosis. The Sudan black and peroxidase reaction are negative. The monocytes can be a source of confusion. Often they are positive for alpha-napthylesterase and naphthyl-ASD-acetate esterase, in which case these enzymes canbe inhibited by fluoride. While the monocytes usually show a diffuse positivity for these esterase enzymes, the reaction in megakaryoblasts tends to be localized. PAS and acid phosphatase positivity are also localized. A platelet peroxidase occuring on a nuclear membrane and in the endoplasmic reticulum of megakaryoblasts can distinquish these cells from myeloblasts on electron microscope examination. This can also be accomplished by the use of monoclonal or polyclonal platelet-specific antibodies.

Vitamin D Assay

Insight into Vitamin D Assays In Clinical Laboratory

The status of vitamin D is usually assessed by measuring the serum concentration of 25-hydroxyvitamin D (25-OH-D).

Over the recent years there has been a dramatic increase in 25-OH-D requests, prompting many laboratories to consider the use of automated immunoassays. In this article, the two major techniques that are used for measuring vitamin D will be discussed and compared (binding assay and chemical assay techniques).

Dramatic Increase in Vitamin D Testing

Vitamin D was first recognized as a very important component of the diet back in the late 1800s when rickets was initially described. Presently, rickets has been eradicated from most developed countries. However, it is still a very common problem in areas of the world where food is scarce.

The recent dramatic increase in vitamin D testing is primarily due to two causes. First, there has been a marked rise in vitamin D deficiency throughout the world. The second reason is that vitamin D has increasingly being used as general health marker and several diseases were linked to vitamin D deficiency.

Metabolism

What we commonly refer to as vitamin D actually comes in two different forms: vitamin D2 and vitamin D3. Vitamin D2 is also known as ergocalciferol, calciferol, or just vitamin D. Vitamin D3 is also known as cholecalciferol (it derives from cholesterol). There are two main ways via which vitamin D gets into the body: through the skin and through diet. In the intestine, either previtamin D or vitamin D is absorbed and trapped in the chylomicron molecules. In the skin, under the effect of UV rays of sunlight, 7-dehydrocholesterol is converted to cholecalciferol (Vitamin D3). Vitamin D from the two sources is subjected to hydroxylation in the liver to form 25-OH-D. The hydroxylated vitamin is then alpha hydroxylated in the kidney to form 1,25$(OH)_2$vitamin D, the active form of vitamin D. 1,25 $(OH)_2$ vitamin D increases the absorption of calcium and phosphorous in the intestine. It also interacts with the parathyroid gland as feedback in the production of parathyroid hormone, therefore acting as a regulator of new bone formation. Vitamin D is also being recognized as a very important player in the signal transduction mechanisms in several organs like the brain, prostate, breast and colon tissue, as well as the immune cells. The cells in these organs have vitamin D receptors and respond to 1, 25 $(OH)_2$ vitamin D.

In the circulation, vitamin D is transported by the vitamin D - binding protein, which belongs to the albumin and alpha-fetoprotein gene family. The concentration of vitamin D - binding protein in the plasma greatly exceeds that of 25-OH-D (9 nM versus 50 nM), with less than 5% of available binding sites being occupied.

Measurement of 25-OH-D

The analytical measurement of vitamin D is performed for two major reasons: to determine the nutritional status of vitamin D, and to monitor its therapeutic level. As mentioned before, there are two different types of vitamin D. To adequately monitor therapy, we need to be aware of which vitamin D entity is the one measured in the different assays. Specifically, if an immunoassay or protein-binding assay is to be used, is the antibody reacting equally with both types of vitamin D? The answer to this question is that if the intention of measuring vitamin D is to monitor vitamin D2 therapy, then the assay must measure vitamin D2.

The assays currently available in the market (US and EU) can be classified as binding assays and chemical assays. Chemiluminescence immunoassays (CLIA), ELISA, radioimmunoassay (RIA), and binding protein assay belong to the binding assays group, while chemical assays include high-performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The specificity and accuracy of these methods are very variable. Both RIA and CLIA are immunoassays in which the accuracy of the method will depend on the specificity of the antibody used (how well the antibody recognizes D2 and D3). The binding assays are affected by the matrix effects due to the tight binding of the vitamin D - binding protein to vitamin D.

The first automated vitamin D assay was based on Competitive-Protein Binding Assays (CPBA) for the Nicholis Advantage analyzer. It has the advantages of being inexpensive, can be performed on small sample size, and is co-specific for 25-OH-D2 and 25-OH-D3. This assay underestimated 25-OH-D at low levels and overestimated it at high levels. Immunoassay methods were first reported in the 1980s with a radioimmunoassay (RIA). This assay formed the basis for a subsequent chemiluminescent detection – based system. The Radioimmunoassay (RIA) requires a small sample size and the incorporation of Iodine – 125 as a tracer. It is not subjected to nonspecific interference, and in addition to being rapid it is inexpensive and accurate. Nevertheless, it still requires the use of radionuclides, and some RIA assays discriminate between 25-OH-D2 and 25-OH-D3.

Chemical assays have been originally more technically involved but are also now able to accommodate a large number of tests per day. Chemical methods (HPLC and LC-MS/MS) can report vitamin D2 and D3 independently. Ultraviolet quantitation following HPLC is a very stable, repeatable assay, and provides separate quantitation of 25-OH-D2 and 25-OH-D3. Nevertheless, it requires a larger sample size, needs a preparation step before chromatography and sometimes is subject to interferences with other compounds measured in the ultraviolet spectrum. Also, this assay requires a high level of technical expertise.

LC-MS/MS has been referred to as a "Gold Standard" technique for 25-OH-D3, although results can also be erroneous. This technique requires the skills of an experienced analyst. Another caveat with LC-MS/MS is the presence of the 25-OH-D2 and C3 epimers of vitamin D3 in pediatric specimens. If the assay is not optimised, vitamin D2/D3 result may be

higher than expected in the pediatric population due to this epimer. Publications have shown that the C3 epimer may be present in adults as well.

Standardization and External Quality Control Assessment:

With the availability of many vitamin D assays, differences in the reported 25-OH-D values for the same samples were observed among different assays.

These differences could impact the classification of patients' vitamin D status and therefore affect the clinical management of some patients. The question is if it is appropriate to have a clinical decision limit without assay standardization. To address this issue, the Vitamin D Standardization Program (VDSP), an initiative of the National Institutes of Health Office of Dietary Supplements (NIH ODS), was launched in 2010 in collaboration with the National Institutes of Health, the Center for Disease Control and Prevention (CDC), the National Institute for Standards and Technology (NIST), and Ghent University in Belgium.

Following, the CDC has introduced a Vitamin D Standardization-Certification Program to ensure reliable clinical measurement of vitamin D. It was recommended that all assay manufacturers should participate in the CDC's Standardization-Certification Program. This is especially important for the in-house reference method of the manufacturers and for the assay measurement systems as they are being developed. The primary steps to standardization are as follows:

(1) develop a reference system,

(2) establish metrological traceability and

(3) verify "end-user" test performance.

When participants pass four consecutive surveys, they are awarded a certification for one year, which can be renewed annually.

Controversies Regarding Vitamin D Testing

Past: Over the past decade, a big number of studies linking low vitamin D levels to cancer, heart disease, diabetes, and other diseases haveled many doctors to routinely test vitamin D levels for their healthy patients. Consequently, laboratory professionals are confronted with the challenge of helping clinicians navigate the complexities of vitamin D assays.

The current evidence suggest that the main beneficial effects of vitamin D supplementation relate to musculoskeletal, rather than extraskeletal. Moreover, the exponential increase in vitamin D testing and supplements used in the past few years, have raised justifiable concerns if many vitamin D measurements are being undertaken without evidence-supported indications and if many individuals are being supplemented with little evidence of the benefit.

Present: In response to these concerns in 2013, the Royal College of Pathologists of Australasia (RCPA) published a position statement to clarify the role of vitamin D testing in the context of vitamin D deficiency, with guidelines about who should be tested and when repeat testing should be performed. Also, the U.S. Preventive Services Task Force (USPSTF) published a new recommendation in November 2014, which stated that there is no practical reason for most people to get a vitamin D test. Testing for vitamin D might be indicated in patients with osteoporosis or other bone-related problems, conditions that affect absorption of fat, including celiac disease or weight-loss surgery or who are taking medications that interfere with vitamin D activity, including anticonvulsants and glucocorticoids.

Troubleshooting Thyroid Hormone Results

What are Normal Thyroid Hormone Levels?

What is thyroid hormone?

Thyroid hormone is made by the thyroid gland, a butterfly-shaped endocrine gland normally located in the lower front of the neck. Thyroid hormone is released into the blood where it is carried to all the tissues in the body. It helps the body use energy, stay warm and keeps the brain, heart, muscles, and other organs working as they should. Thyroid hormone exists in two main forms: thyroxine (T4) and triiodothyronine (T3). T4 is the primary form of thyroid hormone circulating in the blood (about 95%). To exert its effects, T4 is converted to T3 by the removal of an iodine atom; this occurs mainly in the liver and in certain tissues where T3 acts, such as in the brain. T3 normally accounts for about 5% of thyroid hormone circulating in the blood. Most thyroid hormone in the blood is bound by protein, while only a small fraction is "free" to enter tissues and have a biologic effect. Thyroid tests may measure total (protein bound and free) or free hormone levels. Production of thyroid hormone by the thyroid gland is controlled by the pituitary, another endocrine gland in the brain. The pituitary releases Thyroid Stimulating Hormone (abbreviated TSH) into the blood to stimulate the thyroid to make more thyroid hormone. The amount of TSH that the pituitary sends into the bloodstream depends on the amount of thyroid hormone in the body. Like a thermostat, if the pituitary sense low thyroid hormone, then it produces more TSH to tell the thyroid gland to produce more. Once the T4 in the bloodstream goes above a certain level, the pituitary's production of TSH is shut off. In this way, the pituitary senses and controls thyroid gland production of thyroid hormone. Endocrinologists use a combination of thyroid hormone and TSH testing to understand thyroid hormone levels in the body.

What is a TSH test?

Thyroid tests

Blood tests to measure thyroid hormones are readily available and widely used. Not all thyroid tests are useful in all situations.

TSH Test

The best way to initially test thyroid function is to measure the TSH (Thyroid Stimulating Hormone) level in a blood sample. Changes in TSH can serve as an "early warning system" – often occurring before the actual level of thyroid hormones in the body becomes too high or too low. A high TSH level indicates that the thyroid gland is not making enough thyroid hormone (primary hypothyroidism). On the other hand, a low TSH level usually indicates that the thyroid is producing too much thyroid hormone (hyperthyroidism). Occasionally, a low TSH may result from an abnormality in the pituitary gland, which prevents it from making enough TSH to stimulate the thyroid (central hypothyroidism). In most healthy individuals, a normal TSH value means that the thyroid is functioning properly.

What is a T4 test?

T4 Tests

A **Total T4** test measures the bound and free thyroxine (T4) hormone in the blood. A **Free T4** measures what is not bound and able to freely enter and affect the body tissues.

What does it mean if T4 levels are abnormal?

Importantly, Total T4 levels are affected by medications and medical conditions that change thyroid hormone binding proteins. Estrogen, oral contraceptive pills, pregnancy, liver disease, and hepatitis C virus infection are common causes of *increased* thyroid hormone binding proteins and will result in a high Total T4. Testosterone or androgens and anabolic steroids are common causes of *decreased* thyroid hormone binding proteins and will result in a low Total T4. In some circumstances, like pregnancy, a person may have normal thyroid function but Total T4 levels outside of the normal reference range. Tests measuring free T4 – either a free T4 (FT4) or free T4 index (FTI) – may more accurately reflect how the thyroid gland is functioning in these circumstances. An endocrinologist can determine when thyroid disease is present in the context of abnormal thyroid binding proteins.

What is a T3 test?

T3 Tests

T3 tests measure triiodothyronine (T3) levels in the blood. A Total T3 test measures the bound and free fractions of triiodothyronine. Hyperthyroid patients typically have an elevated Total T3 level. T3 tests can be used to support a diagnosis of hyperthyroidism and can determine the severity hyperthyroidism. In some thyroid diseases, the proportions of T3 and T4 in the blood change and can provide diagnostic information. A pattern of increased T3 *vs* T4 is characteristic of Graves' disease. On the other hand, medications like steroids and amiodarone, and severe illness can decrease the amount of thyroid hormone the body converts from T4 to T3 (active form) resulting in a lower proportion of T3. T3 levels fall late in the course of hypothyroidism and therefore are not routinely used to evaluate patients with underactive or surgically absent thyroid glands. Measurement of Free T3 is possible, but is often not reliable and therefore may not be helpful.

What is reverse T3?

REVERSE T3

Reverse T3 is a biologically inactive protein that is structurally very similar to T3, but the iodine atoms are placed in different locations, which makes it inactive. Some reverse T3 is produced normally in the body, but is then rapidly degraded. In healthy, non-hospitalized people, measurement of reverse T3 does not help determine whether hypothyroidism exists or not, and is not clinically useful.

What is a normal thyroid (hormone) level?

Tests often used to assess thyroid hormone status include TSH and FT4 tests. The normal value for a laboratory test is determined by measuring the hormone in a large population of healthy individuals and finding the normal reference range. Normal ranges for thyroid tests may vary slightly among different laboratories, and typical ranges for common tests are given below. TSH normal values are 0.5 to 5.0 mIU/L. Pregnancy, a history of thyroid cancer, history of pituitary gland disease, and older age are some situations when TSH is optimally maintained in different range as guided by an endocrinologist. FT4 normal values are 0.7 to 1.9ng/dL. Individuals taking medications that modify thyroid hormone metabolism and those with a history of thyroid cancer or pituitary disease may be optimally managed with a different normal FT4 range. Total T4 and Total T3 levels measure bound and free thyroid hormone in the blood. These levels are influenced by many factors that affect protein levels in the body, including medications, sex hormones, and liver disease.

A normal Total T4 level in adults ranges from 5.0 to 12.0µg/dL.

A normal Total T3 level in adults ranges from 80-220 ng/dL.

Free T3 assays are often unreliable and not routinely used to assess thyroid function.

What does it mean if my thyroid levels are abnormal?

Lab results	Consider...
High TSH, low thyroid hormone level	**Primary hypothyroidism**
High TSH, normal thyroid hormone level	**Subclinical hypothyroidism**
Low TSH, high thyroid hormone level	**Primary hyperthyroidism**
Low TSH, normal thyroid hormone level	**Early or mild hyperthyroidism**
Low TSH, high thyroid hormone level Followed by… High TSH, low thyroid hormone level	**Thyroiditis (Thyroid Inflammation)**
Low TSH, low thyroid hormone level	**Pituitary disease**

Patterns of thyroid tests associated with thyroid disease

Primary Hypothyroidism

A high TSH and low thyroid hormone level (*e.g.* low FT4) can indicate primary hypothyroidism. Primary hypothyroidism occurs when the thyroid gland makes too little thyroid hormone. Symptoms of hyperthyroidism can include feeling cold, constipation, weight gain, slowed thinking, and decreased energy.

Causes of primary **hypo**thyroidism include:

- Autoimmune thyroid disease, including Hashimoto's thyroiditis
- Thyroid gland dysfunction due to a medication (e.g. amiodarone, tyrosine kinase inhibitors, or cancer immunotherapy)
- Removal of all or part of the thyroid gland
- Radiation injury to the thyroid (e.g. external beam radiation, radioactive iodine ablation treatment)
- Excess treatment with anti-thyroid medications (e.g. methimazole, propylthiouracil)

Early or mild hypothyroidism may present as a persistently elevated TSH and a normal FT4 hormone level. This pattern is called subclinical hypothyroidism and your doctor may recommend treatment. Over time, untreated subclinical hypothyroidism can contribute to heart disease. It is important to remember that normal TSH levels in older individuals (ages 70 and above) are higher than the normal ranges for younger individuals.

Primary Hyperthyroidism

A low TSH and a high thyroid hormone level (e.g. high FT4) can indicate primary hyperthyroidism. Primary **hyper**thyroidism occurs when the thyroid gland makes or releases too much thyroid hormone. Symptoms of hyperthyroidism can include tremors, palpitations, restlessness, feeling too warm, frequent bowel movements, disrupted sleep, and unintentional weight loss.

Causes of primary **hyper**thyroidism include:

- Graves' disease
- Toxic or autonomously functioning thyroid nodule
- Multinodular goiter
- Thyroid inflammation (called thyroiditis) early in the course of disease
- Thyroid gland dysfunction due to a medication (e.g. amiodarone or cancer immunotherapy)
- Excess thyroid hormone therapy

Early or mild hyperthyroidism may present as a persistently low TSH and a normal FT4 hormone level. This pattern is called **subclinical hyperthyroidism** and your doctor may recommend treatment. Over time, untreated subclinical hyperthyroidism can worsen osteoporosis and contribute to abnormal heart rhythms.

Thyroiditis

Thyroid inflammation, also called thyroiditis, causes injury to the thyroid gland and release of thyroid hormone. Individuals with thyroiditis usually have a brief period of hyperthyroidism (low TSH and high FT4 or Total T4) followed by development of hypothyroidism (high TSH and low FT4 or Total T4) or resolution. Some forms of thyroiditis are transient, like post-partum thyroiditis or thyroiditis following an infection, and often resolve on their own without need for medication. Other forms of thyroiditis, like thyroiditis resulting from cancer immunotherapy, interferon alpha, or tyrosine kinase inhibitors, usually result in permanent hypothyroidism and require long term treatment with thyroid hormone replacement. Your endocrinologist will monitor your thyroid tests during thyroiditis and can help determine if you need short and long term medications to balance your thyroid function and control any symptoms.

Central Hypothyroidism

A low TSH and a low FT4 may indicate pituitary disease. Detection of central hypothyroidism should prompt your doctor to check for problems in other pituitary hormones, an underlying cause, and you may need imaging tests to look at the pituitary gland. Central hypothyroidism is treated with thyroid hormone replacement. Importantly, adequacy of thyroid replacement in central hyperthyroidism is assessed with FT4 and Total T4 tests not TSH as in primary hyperthyroidism, and deficiency in stress hormone cortisol should be assessed before starting thyroid treatment to prevent an adrenal crisis. Causes of central hypothyroidism include pituitary gland disease, such as a pituitary mass or tumor, history of pituitary surgery or radiation, pituitary inflammation (called hypophysitis) resulting from autoimmune disease or cancer immunotherapy, and infiltrative diseases.

Rare causes of abnormal thyroid function

- Thyroid hormone resistance
- Iodine induced hyperthyroidism
- TSH-secreting tumor (TSH-oma)
- Germ cell tumors
- Trophoblastic disease
- Infiltrative diseases, such as systemic scleroderma, hemochromatosis, or amyloidosis

What to do if thyroid tests are abnormal, but patient feels fine.
Or…
When thyroid tests are abnormal, but clinician says there is no thyroid problem.

What does it mean?

While blood tests to measure thyroid hormones and thyroid stimulating hormone (TSH) are widely available, it is important to remember that no all tests are useful in all circumstances and many factors including medications, supplements, and non-thyroid medical conditions can affect thyroid test results. An endocrinologist can help you make sense of thyroid test results when there is a discrepancy between your results and how you feel. A good first step is often to repeat the test and ensure there are no medications that might interfere with the test results. Below are some common reasons for mismatch between thyroid tests and thyroid disease.

When abnormal thyroid function tests are not due to thyroid disease

Non-thyroidal illness

Significant illness, such as an infection, cancer, heart failure, or kidney disease, or recent recovery from an illness can cause changes transient changes in the TSH. Fasting or starvation can also cause a low TSH. An endocrinologist can help to interpret changes in thyroid function tests in

these circumstances to distinguish non-thyroid illness from true thyroid dysfunction.

Test interference

Biotin, a common supplement for hair and nail growth, interferes with many thyroid function tests and can lead to inaccurate results. Endocrinologists recommend stopping biotin supplements for 3 days before having a blood test for thyroid function. Individuals who have exposure to mice, like laboratory researchers and veterinarians, may develop antibodies against mouse proteins in their blood. These antibodies cross react with reagents in multiple thyroid function tests and cause unpredictable results. A specialized assay can accurately measure thyroid hormone levels and TSH in this circumstance.

Hypothyroidism Treatment

What is thyroid medication?

Thyroid Hormone Treatment

Levothyroxine is the standard of care in thyroid hormone replacement therapy and treatment of hypothyroidism. Levothyroxine (also called LT4) is equivalent to the T4 form of naturally occurring thyroid hormone and is available in generic and brand name forms.

How to take levothyroxine?

To optimize absorption of your thyroid medication, it should be taken with water at a regular time each day. Multiple medications and supplements decrease absorption of thyroid hormone and should be taken 3-4 hours apart, including calcium and iron supplements, proton pump inhibitors, soy, and multivitamins with minerals. Because of the way levothyroxine is metabolized by the body, your doctor may ask you to take an extra pill or skip a pill on some days of the week. This helps us to fine tune your medication dose for your body and should be guided by an endocrinologist. For patients with celiac disease (autoimmune disease against gluten) or gluten sensitivity, a gluten free formulation of levothyroxine is available. Some individuals may have genetic variant that affects how the body converts T4 to T3 and these individuals may benefit from the addition of a small dose of triiodothyronine.

Liothyronine is replacement T3 (triiodothyronine) thyroid hormone. This medication has a short half-life and is taken twice per day or in combination with levothyroxine. Liothyronine alone is not used for treatment of hypothyroidism long term.

Other formulations of thyroid hormone replacement include **natural or desiccated thyroid hormone extracts** from animal sources. Natural or desiccated thyroid extract preparations have greater variability in the dose of thyroid hormone between batches and imbalanced ratios if T4 *vs* T3. Natural or animal sources of thyroid hormone typically contain 75% T4 and 25% T3, compared to the normal human balance of 95% T4 and 5% T3. Treatment with a correct balance of T4 and T3 is important to replicate normal thyroid function and prevent adverse effects of excess T3, including osteoporosis, heart problems, and mood and sleep disturbance. An endocrinologist can evaluate symptoms and thyroid tests to help balance thyroid hormone medications.

How to verify if the thyroid dose is correct?

Monitoring thyroid levels on medication

Correct dosing of thyroid hormone is usually assessed using the same tests for diagnosis of thyroid disease, including TSH and FT4. Thyroid tests are typically checked every 4-6 weeks initially and then every 6 to 12 months once stable. In special circumstances, such as pregnancy, a history of thyroid cancer, central hypothyroidism, amiodarone therapy, or use of combination T4 and T3 thyroid hormone replacement, your endocrinologist may check different thyroid tests. Additionally, your endocrinologist will evaluate for symptoms of hyperthyroidism and hypothyroidism and peform a physicial exam. Women who are **pregnant** and women who **may become pregnant** should only be treated with levothyroxine (T4). Only T4 efficiently crosses the placenta to provide thyroid hormone to the developing fetus. Thyroid hormone is critical in early pregnancy for brain development. Normal ranges for thyroid tests in pregnancy are different and change by trimester. Women with thyroid disease in pregnancy or who are considering pregnancy should be under the care of an endocrinologist to guide therapy. Individuals with a history of **thyroid cancer,** even if only a portion of the thyroid was removed, also have different target ranges for TSH and FT4 tests. Thyroid hormone replacement in these individuals is closely tied to ongoing thyroid cancer surveillance, monitoring of thyroid cancer tumor markers, and dynamic assessment of recurrence risk. These patients are optimally managed by a multidisciplinary team including an endocrinologist and endocrine surgeon.

Atypical Thyroid Patterns

The five thyroid patterns

1. Hypothyroidism caused by pituitary dysfunction

This pattern is caused by elevated cortisol, which is in turn caused by active infection, blood sugar imbalances, chronic stress, pregnancy, hypoglycemia or insulin resistance. These stressors fatigue the pituitary gland at the base of the brain so that it can no longer signal the thyroid to release enough thyroid hormone. There may be nothing wrong with the thyroid gland itself. The pituitary isn't sending it the right messages. With this pattern, you'll have hypothyroid symptoms and a TSH **below** the functional range (1.8 – 3.0) but within the standard range (0.5 – 5.0). The T4 will be low in the functional range (and possibly the lab range too).

2. Under-conversion of T4 to T3

T4 is the inactive form of thyroid hormone. It must be converted to T3 before the body can use it. More than 90% of thyroid hormone produced is T4. This common pattern is caused by inflammation and elevated cortisol levels. T4 to T3 conversion happens in cell membranes. Inflammatory cytokines damage cell membranes and impair the body's ability to convert T4 to T3. High cortisol also suppresses the conversion of T4 to T3. With this pattern you'll have hypothyroid symptoms, but your TSH and T4 will be normal. If you have your T3 tested, which it rarely is in conventional settings, it will be **low**.

3. Hypothyroidism caused by elevated TBG

Thyroid binding globulin (TBG) is the protein that transports thyroid hormone through the blood. When thyroid hormone is bound to TBG, it is inactive and unavailable to the tissues. When TBG levels are high, levels of unbound (free) thyroid hormone will be low, leading to hypothyroid symptoms. With this pattern, TSH and T4 will be normal. If tested, T3 will be **low**, and T3 uptake and TBG will be **high**. Elevated TBG is caused by high estrogen levels, which are often often associated with birth control pills or estrogen replacement (i.e. Premarin or estrogen creams). To treat this pattern, excess estrogen must be cleared from the body.

4. Hypothyroidism caused by decreased TBG

This is the mirror image of the pattern above. When TBG levels are low, levels of free thyroid hormone will be high. You might think this would cause hyperthyroid symptoms. But too much free thyroid hormone in the bloodstream causes the cells to develop resistance to it. So, even though there's more than enough thyroid hormone, the cells can't use it and you'll have hypothyroid – not hyperthyroid – symptoms. With this pattern, TSH and T4 will be normal. If tested, T3 will be **high**, and T3 uptake and TBG will be **low**. Decreased TBG is caused by high testosterone levels. In women, it is commonly associated with PCOS and insulin resistance. Reversing insulin resistance and restoring blood sugar balance is the key to treating this pattern.

5. Thyroid resistance

In this pattern, both the thyroid and pituitary glands are functioning normally, but the hormones aren't getting into the cells where they're needed. This causes hypothyroid symptoms. Note that **all lab test markers will be normal** in this pattern, because we don't have a way to test the function of cellular receptors directly. Thyroid resistance is usually caused by chronic stress and high cortisol levels. It can also be caused by high homocysteine and genetic factors.

Conclusion

The five patterns above are only a partial list. Several others also cause hypothyroid symptoms and don't show up on standard lab tests. If you have hypothyroid symptoms, but your lab tests are normal, it's likely you have one of them.

Not only do these patterns fail to show up on standard lab work, they **don't respond well** to conventional thyroid hormone replacement. If your body can't convert T4 to T3, or you have too much thyroid binding protein, or your cells are resistant, it doesn't matter how much T4 you take; you won't be able to use it. Unfortunately, if you have one of these patterns and tell your doctor your medication isn't working, all too often the doctor's response is to simply increase the dose. When that doesn't work, the doctor increases it yet again. As I said at the beginning of this article, the key to a successful treatment is an accurate diagnosis. The reason the conventional approach fails is that it skips this step and gives the same treatment to everyone, regardless of the cause of their problem. The good news is that, once the correct diagnosis is made, patients respond very well to treatment.

Problems in the Interpretation of Thyroid Function Tests

It is difficult to guarantee reliable thyroid function results in patients with nonthyroidal illness. Abnormal results may occur in patients with infections, malignancy, myocardial infarction, following surgery, etc., who do not have thyroid disease. Typically, during the acute phase of an illness, free T3 (FT3) concentration and less often, free T4 (FT4) concentration is decreased. The TSH is usually normal but may be undetectable in the severely ill. During recovery, TSH may rise transiently into the hypothyroid range as free hormone concentrations return to normal. In chronic illness, for example, chronic renal failure, free hormone concentrations are decreased (to an extent that may reflect the severity of the underlying disease); TSH is usually normal, but it is occasionally decreased. The occurrence of abnormalities of thyroid function tests in patients with nonthyroidal illness has been termed the 'Sick Euthyroid Syndrome'. Causes include decreased peripheral conversion of T4 to T3; changes in the concentration of binding; increased plasma concentrations of free fatty acids, which displace thyroid hormones from their binding sites, and nonthyroidal influences on the hypothalamicpituitary- thyroid axis, for example, by cortisol, which can inhibit TSH secretion. Furthermore, many drugs can influence the results of tests of thyroid function. Many times, the levels of FT3, FT4 and TSH do not correlate.

Common Causes of TSH/FT4/FT3 Discrepancies

● Over replacement of thyroid hormone (TSH low, free T4 normal) ● Recent dose adjustment (TSH high, free T4 normal) ● Patient taking T3 (TSH low, free T4 normal) ● Patient noncompliant with hormone replacement (TSH high, free T4 normal) ● Nonthyroidal illness ● Drugs affecting thyroid hormones: Glucocorticoids, dopamine ● Thyroid hormone resistance (TSH high, freeT4 high, patient euthyroid) ● TSH-secreting tumour (TSH high, free T4 high, patient hyperthyroid) ● During antithyroid drug therapy, there can be patients who have persistent serum T3 excess, despite normal or low serum T4 values.

Effects of Drugs on Thyroid Function

● Altered hypothalamic or pituitary function ● Altered biosynthesis or release of thyroid hormones ● Displacement of T4 and T3 from binding proteins ● Reduced peripheral conversions of T4 to T3 ● Inhibition of peripheral hormone activity.

Drugs that Affect Results of Thyroid Function Tests

Drugs	*Effect*	*Cause*
Salicylates, Phenylbutzone, Dphenylhydantoin	Total T4 and T3 reduced, Free T4 normal	Inhibition of serum protein binding
Propylthiouracil, Methimazole, release Lithium, Iodides	Total T4 and T3 reduced	Inhibition of TSH production or
Propylthiouracil	Total T3 reduced both T4 and	Inhibition of conversion of T4 to T3
Propranolol	Total T3 reduced, T4 normal, TSH normal	Inhibition of conversion of T4 to T3
Glucocorticoids	Total T3 reduced, T4 and TSH low or normal	Inhibition of conversion of T4 to T3
Oral radiographic dyes	Total T3 reduced, both T4 and TSH high	Inhibition of conversion of T4 to T3
Dopamine, L-dopa, Glucocorticoids	Basal TSH and response to TRH reduced	Direct effect to inhibit TSH Production in pituitary gland
Amiodarone, Bensamide (transient), Metopramide, Sulpiride	Basal TSH increased TRH,	Increased TSH production

Relationship Between Serum Total T4 and Total T3 Concentrations in Various Disorders

Serum T3 Concentration	Serum T4 Concentration: LOW	NORMAL	HIGH
High	Iodide deficiency, T3 treatment, Antithyroid drug therapy,	T3 – thyrotoxicosis, T3 – binding auto-antibodies,	Thyrotoxicosis of any cause, Excess T4 ingestion Thyroid hormone resistance, TBG excess,
Normal	Iodine deficiency, T3 treatment, Hypothy-roidism		T4 treatment, Euthyroid hyperthy-roxinemia, Thyrotoxicosis with acute or moderate nonthyroidal illness, T4 binding autoantibodies
Low	Severe hypothy-roidism, TBG deficiency, Drugs, Severe nonthyroidal illness,	Acute and chronic non-thyroidal illness, Drugs, Fetal life, Restricted nutrition	Thyrotoxicosis with severe nonthyroidal illness,

Relationship Between Serum FT4, FT3 and TSH Concentrations in Various Disorders

Serum TSH Concentration	Serum T4 Concentration		
	HIGH	NORMAL	LOW
High	TSH-secreting tumor (rare) (FT3 = ↑)	Borderline/ compen-sated hypothyroi-dism,	Hypothyroid (primary), Recovery from sick euthyroid state,
Normal	Euthyroid with T4 autoanti-bodies (uncommon)	Euthyroid	Sick euthyroid (FT3 = ↓), Hypopituitarism (other pituitary hormones = ↓),
Low	Hyperthy-roidism (FT3 = ↑)	T3 thyrotoxi-cosis (FT3 = ↑), Sub-clinical hyperthyroi-dism (FT3 = N/↓),	Hypopituitarism (other pituitary hormones = ↓), Sick euthyroid (severe) (FT3 = ↓),

Free Thyroxine Measurements in Common Conditions Affecting Thyroid-Binding Proteins

Clinical conditions	Free T4 Levels
Near Normal Concentration of Serum-Binding Proteins	
Hypothyroidism	Low
Hyperthyroidism	High
Hyperoestrogenism	Low
Abnormal Concentration of Serum-Binding Proteins	
TBG excess	Normal
TBG deficiency	High
Dysalbuminaemia	Normal
Hypoalbuminaemia	Normal
T4 autoantibody	Normal
Low total T4 non-thyroidal illness	Normal or High
High total T4 Non-thyroidal illness	Normal or High

Free T4 and Free T3 in Various Disease Conditions

1. Hyperthyroidism

Hyperthyroidism produces a primary increase in Free T4, whereas oestrogens and idiopathic or genetic conditions may produce a primary increase in TBP. In both cases [T4 and TBP] increase, but in the former, the patient is ill and requires treatment; in the latter, the patient is euthyroid. Likewise, a low serum [T4 and TBP] may be due to a primary decrease in [FT4] or to a primary decrease in [TBP]. It is, therefore, clinically important to differentiate between changes in [T4 and TBP] that are due to primary changes in [FT4] (e.g. hyper-or hypothyroidism) and those that are due to primary changes in [TBP]. Serum TSH level is low in all forms of hyperthyroidism except in rare cases in which hyperthyroidism is mediated by TSH itself. When TSH level is low, free T4 concentration should be measured and will be elevated in most cases of hyperthyroidism. Finding a low TSH level and an elevated free T4 level is usually sufficient to establish the diagnosis of hyperthyroidism. If TSH level is low but free T4 level is normal, a T3 measurement should be performed, since serum T3 concentration is often elevated earlier in the course of hyperthyroidism and to a greater degree than is T4 concentration. Because only the free fraction of T3 is active, the estimation of free T3 is helpful in adjusting the total T3 for variations in binding proteins. It should be remembered that numerous medications as well as both acute and chronic illness may cause a transient lowering of T3 concentration as well as a reduction in TSH level. In Grave's disease or toxic adenomas, serum total T3 and free T3 levels are typically elevated to a greater degree than total T4 and free T4. T3 toxicosis—encountered in about 5 per cent of hyperthyroid population—total T3 and free T3 values increase. Serum total T4 and free T4 are disproportionately elevated to a greater degree than total T3 and Free T3 values in most patients with toxic multinodular goitre. Monitoring total T3 and free T3 values may also be of importance in evaluating both the severity and the response therapy in patients being treated for thyroid storm or crisis in that the antithyroid drug therapies are focused on reducing both thyroid gland T3 secretion and peripheral tissue T3 production from T4.

2. Nonthyroidal Illness

In nonthyroidal illness (NTI) and altered states of nutrition there are two categories: *Low T3 state:* Decrease in total T3 and free T3 while maintaining normal total T4 and free T4. Observed in mild or moderate NTIs or states of caloric deprivation (< 400 cal) *Low T3-T4 state:* Total T4 also decreased, a case of severe NTI. Free T4 levels remain within or near the normal range of values as serum total T4 levels decline. Decreased total T3 or normal free T4 or increased free T4 results from acquired defect in serum T4-binding proteins which accompany NTI. Also common are increases in the levels of the free fraction of T4 and T3 which are caused by decrease in serum concentrations of thyroid hormone-binding proteins, changes in binding properties induced by circulating inhibitors and drugs, or both. Low levels of total T4 may be seen in nonthyroidal illnesses, but total T4 concentrations in these patients are usually normal or above normal as determined using reference methods. Thus in nonthyroidal illnesses, abnormal thyroid test results are not necessarily indicative of thyroid disease but may demonstrate adaptations to the catabolic state, many of these changes revert to normal when the patient recovers. Several test abnormalities may be seen in nonthyroidal illnesses in euthyroid patients (the 'euthyroid sick syndrome'). The most common abnormalities are a reduction in the serum total T3 concentration and an elevation in the serum level of fT3. Also common are increases in the levels of the free fraction of T4 and T3 which are caused by decrease in serum

concentrations of thyroid hormone binding proteins, changes in binding properties induced by circulating inhibitors and drugs, or both. Low levels of total T4 may be seen in nonthyroidal illnesses, but total T4 concentrations in these patients are usually normal or above normal as determined using reference methods.

3. Hypothyroidism

If total T4 (or free T4) level is normal, hypothyroidism is most unlikely: however, a low T4 concentration is often seen in the euthyroid sick.

Assay Choice Application

For definitive diagnosis, assessment of both serum TSH and free T4 is required, but a more limited approach can be used for initial case finding and follow-up. In the interests of cost effectiveness, evaluation of thyroid status may often begin with an assay for either serum TSH or free T4, followed by further algorithm-based assessment if the initial result is abnormal. As an initial test, serum total T4 measurements give an unacceptable rate of abnormal results, due to the frequency of abnormalities in serum thyroid hormone-binding proteins. Four distinct clinical situations in which evaluation of thyroid function is done can be considered: testing of unselected populations for case finding or screening, testing of untreated patients who have clinical features that suggest thyroid disease, assessment of the response to treatment for thyroid dysfunction, and evaluation of patients in whom associated illness or drug therapy are likely to complicate clinical and laboratory assessment or whose initial results are atypical or unclear.

Screening and Case Finding

About 2 per cent to 7 per cent of women over age 40 years may have slightly elevated serum TSH concentrations. The case for routine assessment of thyroid status is strongest in elderly women who have any symptoms that could be consistent with hypothyroidism. Among hospitalised patients, the large majority of abnormal results are due to nonthyroidal illness or medications. Most persons found to have either high or low serum TSH values in screening or casefinding studies have subclinical disease. That is, they have no clinical manifestations of thyroid dysfunction and normal serum free T4 and T3 concentrations. Regardless of which initial test is used, assessment of thyoid status has a high priority in patients at increased risk of having thyroid dysfunction, as for example in those with goitre, those treated previously for thyrotoxicosis or receiving lithium or amiodarone, and patients with associated autoimmune disease or connective tissue diseases or a history of neck or whole body irradiation.

Untreated Patients

In untreated ambulatory patients, a normal serum TSH concentration has high negative predictive value in ruling out thyroid disease. If serum TSH is abnormal, serum free T4 is done. Diagnostic strategies have been evaluated in which serum T4 measurements are done routinely only if the serum TSH is abnormal, unless pituitary disease is suspected. Long-term assessment of this approach will need to balance cost savings against potentially serious adverse outcomes; for example, if thyrotoxicosis is missed because of normal serum TSH values, or central hypothyroidism is missed on the basis of normal serum TSH values. The following groups of patients will be incompletely or incorrectly assessed if either serum TSH or free TSH or free T4 alone is measured. Patients with subclinical hypothyroidism (high serum TSH, normal free T4) in whom replacement therapy may be beneficial. Those with subclinical thyrotoxicosis (low serum TSH, normal free T4) in whom treatment with an antithyroid drug or thyroid ablation may be beneficial. Those being treated for thyrotoxicosis, in whom suppression of TSH secretion may persist for weeks or months after normalization of serum T4 and T3 on drug. Those with central (secondary or hypothyrotropic) hypothyroidism (low serum free T4 low or normal TSH), who should be evaluated for adrenal insufficiency before T4 therapy is initiated. Those with binding abnormalities such as familial dysalbuminaemic hyperthyroxinaemia (FDH) or T4 or T4 binding autoantibodies in whom some serum free T4 estimates are invalid. Those with thyroid hormone resistance with high serum T4 and T3 concentrations and normal or high serum TSH concentrations, who are often not recognised until after inappropriate treatment has been given. Those with thyrotoxicosis caused by excess TSH secretion caused by a pituitary tumour or selective pituitary resistance to thyroid hormone. Not withstanding the widespread acceptance of serum TSH as a single initial test, some still advocate an estimate of free T4 as the best initial test for suspected thyrotoxicosis.

Assessment of the Response to Treatment

In the testing of ambulatory patients with known thyroid disease, the use of serum TSH alone can also be considered. In a study of ambulatory patients attending a thyroid clinic, hyperthyroid patients taking T4 for either replacement or suppression, seldom needed a serum free T4 measurement of the serum TSH was greater than 0.05 mU/L; although at lower values, the magnitude of hyperthyroxinaemia did influence management. In contrast, in patients with newly diagnosed thyrotoxicosis, measurements of serum free T4 or free T3, or both, were necessary in addition to serum TSH not only to establish the degree of hormone excess but also to evaluate the response to treatment. This study included a few new cases of hypothyroidism, in whom serum T4 measurement also would be required to establish the degree of hormone deficiency. In patients with thyroiditis and pituitary-hypothalamic disease, combined assessment was required. In evaluating patients receiving T4 therapy, some have suggested that hormone measurements add little to a clinical assessment made by experts, but there is justification for periodic serum TSH assessment to avoid subtle tissue effects of thyroid hormone excess of deficiency. A serum TSH value in the low-normal range is, probably, the best single indicator of appropriate dosage and is certainly of more use than a serum free T4 value alone, which may be increased slightly depending on the time interval between dose and sampling. In some situations (e.g. patients with ischaemic heart disease and hypothyroidism), the appropriate dose of T4 should be based on clinical judgement rather than laboratory findings.

Difficult Diagnostic Situations

The prevalence of abnormal serum T4 or TSH values in patients with acute medical or psychiatric illness is high, but there is controversy as to the value of thyroid function testing in these situations, because most of the abnormalities do not indicate the presence of thyroid disease in acutely ill patients because of the potential importance of intercurrent thyroid disease and the difficulty in assessing clinical features of thyroid dysfunction, others suggest that testing should not be done without some clinical indication. In patients hospitalised for acute illness one or more of the assumptions outlined above may not be justified; for example, when there are wide fluctuations from the steady state. Serum TSH values frequently are subnormal in the absence of thyrotoxicosis and serum free T4 estimates are subject to multiple interfering influences, depending often on the particular method. Dual assessment clearly is necessary to identify the serum free T4 TSH combinations that indicate true thyroid dysfunction. When a patient has both thyroid dysfunction and a severe nonthyroidal illness, assessment becomes especially difficult because the effects of the illness, medications, or changes in nutrition can alter the expected changes in serum free T4 or TSH. Only clinical re-evaluation and repeated sampling may resolve the dilemma.

Thyroid Diagnosis and Treatment

There are three general principles upon which the physician should focus when evaluating thyroid function in a patient. These principles are: The thyroid gland is the principal site of thyroid dysfunction. Autoimmune thyroid disease is the most common aetiology producing the dysfunction. Thyroid status is best determined by a combined measurement employing a serum free thyroxine (FT4) estimate and thyrotrophin (TSH). For both hypothyroidism and hyperthyroidism, TSH and an estimate of free T4 (FT4) are recommended. T3 or free T3 may be needed to confirm hyperthyroidism if free T4 is within limits. Anti-thyroid antibodies, preferably antithyroid peroxidase (anti-TPO), may establish an autoimmune mechanism.

Recommendations for Thyroid Testing

Hyperthyroid	
Symptomatic	Free T4, TSH
Post-therapy	Free T4 (Free T3)
Hypothyroid	
Symptomatic	TSH, Free T4 (anti-TPO)
Subclinical	TSH-first (T4, anti-TPO)
Monitor replacement	TSH
Hypopituitary	TSH and Free T4
Acutely ill	None without suspicion
Pregnant	
Diagnosis	TSH, Free T4
Hypo, treated	TSH
Elderly	
Healthy	None
ill	None
Women > 60 years	TSH
High risk*	TSH
Healthy adults	None without suspicion

*Ambigious symptoms, concurrent illness associated with thyroid disease, drugs associated with thyroid dysfunction.

Diagnostic Approach to Anomalous Serum T3, T4, and TSH Values

Clinical re-evaluation, with particular attention to long-term features suggestive of thyroid disease and to the medication history. Measurement of serum TSH by a third generation method to identify conclusively the degree of TSH suppression. Measurement of the serum T3 concentration with appropriate binding correction (free T3). An authentic estimate of serum free T4 (particularly in euthyroid hyperthyroxinaemia). Follow-up to establish whether the abnormality is transient or persistent. Search for evidence of unusual binding abnormalities or hormone resistance in the propositus and family members.

Typical Reference Ranges for Serum Thyroid Hormones and TSH in Humans*

Hormone	*Reference ranges*	*Variations unrelated to thyroid disease*
Total T3	69-202 ng/dl	Binding protein changes, binding competitors, age-related changes, nutrition, illness, surgery, drugs
Free T3	1.4-4.2 pg/ ml	Methodologic factors and influences on total T3
Total T4	4.4-11.6 µg/dl	Binding protein changes, binding competitors
Free T4	0.8-2.0 ng/dl	Methodologic factors, pregnancy
TSH	0.28-6.82 µlU/ ml	Diurnal variation, pulse secretion, age-related changes, drugs

*These ranges should be determined for the particular methods used in each laboratory. The neonatal period is excluded. Higher values in childhood.

CULTURES & STAINS

TroubleShooting Guidelines

A Compilation from

The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

Blood Cultures

HOW TO OBTAIN, PROCESS, REPORT, AND INTERPRET

The detection and identification of microorganisms circulating in the bloodstream of patients is arguably one of the most important functions of the clinical microbiology laboratory. Effective implementation of this function requires careful consideration of specimen collection and processing, culture techniques, result reporting, and, perhaps most importantly, result interpretation by the physician. The purpose of this review is to provide a synopsis of the current state of the art for each of these areas, with the intention of providing adequate information to enable clinical laboratory personnel and physicians to critically evaluate and, if required, improve their current blood culture practices.

Introduction

Bloodstream infections (BSIs) represent an important cause of human morbidity and mortality. The evaluation of patients suspected of having a BSI routinely includes blood cultures, which optimally yield an aetiological diagnosis and provide the opportunity to perform antimicrobial susceptibility testing to guide therapeutic intervention when necessary. The clinical significance of positive blood cultures has been extensively evaluated over the past several decades. These studies have served to define the most frequent aetiological agents responsible for BSIs and the range of agents, and have improved our understanding of the risks and outcomes associated with such infections. As the baseline characteristics of patients have changed with advances in medicine (e.g. more immunocompromised hosts, more indwelling catheters and other intravascular devices, and changes in therapy for human immunodeficiency virus), the epidemiology of BSIs has also evolved, with more infections occurring in patients with intravascular devices and in outpatient settings. Additionally, there appears to be a trend towards improved outcomes in patients with BSIs, perhaps as a consequence of earlier therapeutic intervention, whereas the number of BSIs appears to be increasing, especially those occurring in populations that were previously less affected (outpatients). Because BSIs remain an important cause of morbidity and mortality, and prompt targeted therapeutic intervention may improve patient outcomes, there has been significant interest in improving the speed and accuracy of blood culture methods in the clinical microbiology laboratory. Despite these efforts, little has changed since the introduction of continuous-monitoring blood culture systems in the 1990s, but incremental advances in more rapid identification and susceptibility prediction have occurred, especially for some particularly troublesome pathogens. Moreover, greater advances appear to be on the horizon.

Blood Culture Collection

The utility of blood culture for detecting BSI is directly influenced by the collection of optimal specimens only from patients with clinical findings compatible with BSI; routine 'surveillance' blood cultures are costly and of little clinical value. Clearly, venipuncture is the preferred method for blood culture collection. Arterial blood samples do not increase diagnostic yield, and blood specimens obtained from intravascular lines have demonstrated increased rates of contamination in some studies. The American College of Physicians guidelines recommend that collecting blood for culture from intravascular devices be avoided, and the CLSI recommends that, if one must collect a blood culture from an intravenous line, it should be paired with a culture that is obtained via venipuncture to assist in the interpretation of positive results. The timing of blood culture collection does not appear to significantly affect the recovery of clinically relevant microorganisms, and most authorities therefore recommend collecting multiple sets simultaneously or over a short period of time, except when documentation of continuous bacteraemia is required for patients with endovascular infection. Whenever possible, two to four sets of blood specimens should be collected from independent venipuncture sites, and, for adult patients, each set should consist of 20–40 mL of blood. The volume of blood drawn from infants and children is less well prescribed, but should be based on the child's age and not exceed 1% of the patient's total blood volume. It is clear that the total volume of blood cultured from adult patients is directly proportional to the yield of microorganisms recovered. This is a consequence of the fact that most adult patients with BSIs have very low circulating concentrations of viable microorganisms. Inadequate blood volume or the collection of a single blood culture set significantly reduces the sensitivity of the test, and also makes the interpretation of results far more difficult. Collection of multiple sets of blood cultures from a single venipuncture or intravascular line should also be avoided. For optimal recovery of diverse BSI aetiological agents, each set of blood cultures should include paired aerobic and anaerobic blood culture bottles, and the aerobic bottle should be filled first. Proper skin antisepsis prior to collection of blood cultures via peripheral venipuncture is paramount, to reduce blood culture contamination rates and facilitate result interpretation for the clinician. A variety of skin disinfectants have been clinically evaluated, and reports comparing their relative efficacy have been published. On the basis of these data, current guidance documents conclude that tincture of iodine, chlorine peroxide and chlorhexidine gluconate are superior to povidineiodine preparations, and that tincture of iodine and chlorhexidine gluconate are probably equivalent for skin antisepsis prior to blood culture collection . Although chlorhexidine gluconate is an adequate disinfectant for older infants, children, and adults, it should not be used on infants <2 months of age, and an alternative is therefore required in centres where this disinfectant is otherwise routinely employed. Once optimal blood culture specimens are collected according to the principles outlined above, they should be sent to the laboratory as promptly as possible. These specimens should never be refrigerated or frozen, and should be held at room temperature for no more than a few hours if necessary. Although an extended delay between blood culture collection and incubation in a continuous-monitoring blood culture instrument is not recommended, a significant diminution in pathogen recovery has only been experimentally observed when blood culture bottles have been held for >24 h at 4°C or room temperature and for >12 h at 37°C. Lengthy incubation of blood culture bottles prior to entering them into a continuous-monitoring blood culture instrument may delay or impede the detection of growth by the instrument, and is discouraged.

Laboratory Techniques for Blood Culture

In the vast majority of institutions, most blood culture specimens delivered to the laboratory are entered into an incubation protocol on a continuously monitored blood culture device. There are several manufacturers of such devices, and their performance characteristics are similar. These devices incubate the blood culture bottles for a prescribed period of time (determined by the user) and signal audibly and/or visually if growth is detected. Each automated blood culture system has its own associated medium formulations that must be selected by the user. The blood culture bottles typically contain proprietary mixtures of culture medium, an anticoagulant, and, in many cases, resins or charcoal mixtures to reduce the effects of antimicrobials

and other toxic compounds. Generally, combinations of medium formulations that are complementary to each other are chosen to enhance the recovery of the most diverse range of microorganisms. Medium combinations typically include aerobic and anaerobic formulations and, in select circumstances, a formulation containing reagents that are ideal for recovering mycobacteria and/or yeasts may be inoculated as well. Controlled studies comparing the performance of media with and without the addition of antimicrobial binding or absorbing agents (resins and/or charcoal compounds) have repeatedly demonstrated that the latter formulations are clearly superior for the recovery of microorganisms, especially staphylococci and yeasts. Blood cultures entered into automated, continuous-monitoring protocols should routinely be incubated for 5 days. Multiple studies have shown that this incubation time is adequate for the detection of the majority of pathogens, including fastidious bacteria that belong to the Haemophilus, Actinobacillus, Cardiobacterium, Eikinella and Kingella (HACEK) group, and that incubation beyond 5 days increases the number of contaminants recovered. Longer incubation times may be required when dimorphic fungaemia or bacteraemia caused by Legionella, Brucella, Bartonella or Nocardia spp. is suspected. Blood cultures for Mycobacterium spp. should be incubated for 4 weeks. The detection of some microorganisms is enhanced by employing blood culture techniques in addition to or in place of standard instrumented blood culture systems. The most common example of this principle is the utilization of the Isolator blood culture system (Wampole Laboratories, Cranbury, NJ, USA) for the enhanced detection of dimorphic fungi and Bartonella spp.. This system is unique in that it is non-broth-based. Instead, blood samples obtained by venipuncture are collected into a tube that contains a lysing solution. The tubes are then transported to the laboratory, where they are centrifuged. The supernatant is discarded, and the pellet is inoculated onto solid medium, the composition of which may be tailored to recover the organisms (bacteria, fungi, and/or mycobacteria) that are most likely or highly suspected on the basis of clinical findings. Although there are clear advantages to using this approach in select circumstances (suspicion of BSI caused by dimorphic fungi or Bartonella spp.), it is not routinely employed, as it is quite labour-intensive, it poses a greater risk of laboratory-acquired infection to technologists, and it is inferior to standard blood culture methods for the detection of anaerobes, Haemophilus spp., and pneumococci .

Special Considerations for Select Microorganisms

Fungal blood cultures

Fungi represent an emerging group of organisms that are responsible for BSI with increasing frequency. The growth requirements for fungi often differ from those for bacteria, most notably with regard to optimal growth temperature and media. For example, most yeasts grow best at 37°C, whereas filamentous fungi often grow best at lower (27–30°C) temperatures. Most routine manual and automated blood culture systems are able to support the growth of yeasts such as Candida spp. However, if suspicion is high for a BSI being caused by yeast, and routine blood cultures are negative, then it may be reasonable to consider a request for alternative test methods that are optimally designed to support the growth of most yeasts. Moulds, and especially dimorphic fungi, often grow poorly in typical instrumented blood culture systems. Furthermore, the recovery of moulds such as Aspergillus spp. is often of unclear clinical significance when they are isolated with these methods. In cases where fungaemia caused by a mould is suspected, alternative blood culture methods should be employed, such as the lysis centrifugation method, in which the lysed and pelleted blood specimen can be plated on medium that specifically supports the growth of moulds and dimorphic fungi. Some fungi require highly specialized medium supplements, the most noteworthy example being the requirement for lipid supplementation for Malasezzia furfur, which is often achieved by overlaying fungal medium with olive oil.

Mycobacterial blood cultures

Mycobacterial BSIs occur in immunocompromised patients (either as a consequence of iatrogenic immunosuppression, or associated with an immunosuppressive condition) and patients with long-term vascular access devices. Investigation tailored to the recovery of mycobacterial blood isolates should thus generally be limited to patients with such characteristics. As mycobacteria are commonly located intracellularly, approaches to growing them in vitro often include lysis of leukocytes prior to incubation in a rich medium that contains fatty acids. Mycobacteria may be optimally recovered, with extended incubation of 4 weeks, with manual methods such as lysis centrifugation or the use of commercial 'lytic' media in manual or instrumented systems. These blood culture formulations typically contain a proprietary mixture of fatty acids that support mycobacterial growth, along with antimicrobial agents. Limited comparisons between formulations suggest some variability in the performance of lytic culture media, but comprehensive comparative studies of all formulations have not been performed.

Fastidious microorganisms

Fastidious microorganisms are rarely implicated in BSI in clinical practice, but when they are isolated from blood cultures they often represent serious infection. In some cases, the observation of signal-positive Gram stain-negative blood culture results provides a clue that a fastidious microorganism might be implicated as the BSI aetiological agent. In those cases, collaboration between the laboratory and clinician is essential to ensure that appropriate steps are taken to increase the odds of isolation of such organisms. Some organisms may be small or of unusual morphology, and not readily recognized by the technologist. In other cases, the organism may not stain well with standard Gram stain protocols (e.g. Mycoplasma and Campylobacter). In those cases, alternative staining techniques may be employed, including the use of acridine orange (to stain bacterial nucleic acids) or the use of carbol fuschin as an alternative to safranin as a counterstain in the Gram stain protocol to enhance the staining of Campylobacter, Helicobacter, and Brucella. Perhaps the most frequently encountered fastidious bacteria are the members of the HACEK group as the aetiological agents for subacute bacterial endocarditis. As noted above, in the vast majority of cases, these organisms are isolated with standard blood culture techniques without the need for special protocols or procedures. This is also generally true for Brucella spp., Campylobacter spp., and Francisella spp., but is not true for all fastidious bacteria. Abiotrophia and Granulicatella are usually detected with automated blood culture instruments, but do not grow well on standard, unsupplemented solid media, as they require pyridoxal or cysteine for growth. This can be accomplished by co-cultivation with staphylococci, by the use of pyridoxalimpregnated disks placed on the surface of standard blood agar plates, or by the use of specially supplemented or enriched media. The yield of standard blood culture media for the cultivation of Bartonella spp. is typically low. Special techniques, including lysis centrifugation methods and/or serological investigations, are thus indicated for the diagnosis of BSI caused by Bartonella spp. Legionella spp. require buffered charcoal yeast extract (BCYE) for optimal growth. Recovery of Legionella can be achieved by subculturing standard blood culture medium that has been incubated according to the standard protocol for 5 days into BCYE, or by utilizing BCYE in conjunction with lysis centrifugation methods. A detailed description of all of the special

techniques required for the culture of other rarely encountered fastidious organisms (e.g. Helicobacter and Leptospira) is beyond the scope of this review, and has been provided elsewhere.

ISOLATION, IDENTIFICATION, AND SUSCEPTIBILITY TESTING

Once blood cultures become positive for growth, either by manual subculture techniques or signalling from automated systems, a Gram stain is performed. A positive Gram stain result should be regarded as a critical value, and immediately phoned to the ordering clinician or another responsible member of the healthcare team providing care to the patient. Subcultures are performed at this point, and these allow identification and, if indicated, susceptibility testing to be performed, typically over the next 24–48 h. Laboratories should have a comprehensive protocol in place to guide the appropriate work-up of organisms isolated from blood cultures. To optimally utilize resources, complete organism identification and organism-specific susceptibility testing should only be performed on clinically important isolates, and not on organisms that probably represent contaminants. Isolates that are probably associated with true BSI (as per the laboratory protocol) should be saved in the laboratory (by serial subculture) for several days to allow additional testing if required, and may be retained for longer periods of time (in a frozen archive) to allow investigation of recurrent BSIs in appropriate patients.

Interpretation of Positive Blood Cultures

The interpretation of positive blood culture results is often straightforward, but sometimes presents a significant dilemma for physicians and clinical microbiologists. For the latter circumstance, a variety of laboratory data must be evaluated in the context of the clinical picture to arrive at an accurate interpretation. The pattern of positivity of blood cultures is often useful; when the majority of or all blood culture sets obtained by independent venipuncture are positive for the same microorganism, the likelihood that this represents true BSI is exceedingly high, regardless of the organism's identity. Likewise, the identities of organisms isolated from positive blood cultures also have value. Staphylococcus aureus, Streptococcus pneumoniae, Enterobacteriaceae, Pseudomonas aeruginosa and Candida albicans are almost always predictive of true BSI. Conversely, Corynebacterium spp. and Propionibacterium spp. almost always represent contamination. The recovery of viridans group streptococci, coagulase-negative staphylococci (CoNS) and enterococci is more difficult to interpret, as some studies have demonstrated that they represent true BSI in 38%, 15% and 78% of cases, respectively. Notably, CoNS represent one of the most commonly encountered blood culture contaminants, but also constitute an important cause of BSI in the ever-expanding population of patients with implanted devices and indwelling catheters. Interpretation for these cases may be aided by identifying CoNS to the species level when more than one set of blood cultures becomes positive. If the same species of CoNS is isolated from multiple blood culture sets, the odds that it represents true bacteraemia as opposed to contamination increase. Without this additional information, using the number of positive blood culture sets that are generically positive for CoNS is a less reliable predictor. Finally, some have suggested that the number of blood culture bottles (as opposed to the number of sets) has predictive value, in that the more that are positive for CoNS, the more likely it is that the patient has bacteraemia caused by CoNS. However, systematic evaluations of this approach have proven it to be unreliable.

Rapid Methods for Identification and Susceptibility Testing of Isolates from Positive Blood Cultures

Prompt detection, identification and susceptibility testing of the aetiological agents responsible for BSIs is critical, as it allows clinicians to make the most informed decisions about possible therapeutic interventions. Blood cultures incubated in modern instrumented systems that are ultimately positive for most bacterial pathogens typically signal positive in a median time of 12–36 h, whereas the time to positivity from collection to detection is longer for some fastidious bacteria, anaerobes, and fungi. Following detection, Gram stain rapidly provides some information to the clinician that may be useful for determining the significance of the positive result and/or determining initial antimicrobial therapy. Standard microbiological protocols that rely on biochemical identification of microorganisms plus phenotypic antimicrobial susceptibility testing follow, and may take an additional 48–72 h, assuming that the results obtained are easily interpreted. It may take days longer to generate final results for organisms that are difficult to identify biochemically or grow slowly in vitro. Given the usual delay of 3–5 days from the collection of blood cultures to the time at which final identification and susceptibility results are obtained, there has been keen interest in reducing this interval by employing a variety of rapid methods. In some cases, the time to delivery of results may be reduced by employing traditional microbiological protocols earlier in the work-up. For example, the coagulase test, which is traditionally used to distinguish CoNS isolates from coagulase-positive isolates, may be performed directly on signal-positive blood culture broths that show Gram-positive cocci in clusters on Gram staining. This approach allows rapid distinction between CoNS and coagulase-positive staphylococci (which are mostly S. aureus), and may influence the ability of clinicians to interpret the clinical significance of a positive blood culture result and their ability to begin appropriate antimicrobial therapy. Obviously, this is not a complete solution, as it does not definitively identify the organism and nor does it provide susceptibility information. To augment such an approach, some laboratories may couple direct coagulase testing with the use of chromogenic agar medium, which allows identification of methicillin-resistant S. aureus isolates within 18–24 h. Clearly, this solution represents an improvement over traditional methods, but applies only to one specific organism and gives susceptibility results for only one drug. More robust approaches to improving the turn-around time for the laboratory diagnosis of BSIs have focused largely on newer or novel technology. Molecular methods, including nucleic acid amplification assays (NAATs), DNA sequencing approaches, DNA microarrays, and probe hybridization, have emerged as useful tools for microorganism identification, and, in some cases, the prediction of antimicrobial susceptibility for select antibiotics. Novel phenotypic approaches have also been shown to reduce turn-around time for the identification and limited susceptibility testing of select organisms. Finally, matrix-assisted laser desorption ionization time-of-flight mass spectrometry, which has already demonstrated widespread utility in the routine identification of microorganisms in the clinical microbiology laboratory, appears to be a very promising approach to the rapid identification of organisms directly from signal-positive blood culture broths. The major drawback of this approach, like most of the others, is the lack of rapid susceptibility information to accompany the organism identity. It is not yet clear when or if this capability will be possible with matrix-assisted laser desorption ionization time-of-flight mass spectrometry or other rapid methods, and the actual clinical utility of microorganism identification in the absence of susceptibility information is narrow and unproven.

Rapid Methods for Detection of Microorganisms Directly in Blood Specimens

Although technological improvements have led to reductions in the time required for identification and (in limited cases) susceptibility testing of isolates from signal-positive blood cultures, a further improvement would obviously be the ability to rapidly and directly detect and identify microorganisms in blood samples from patients with a suspected BSI. Currently available solutions involve the use of NAATs that are designed to detect specific microorganisms in blood samples. Controlled trials evaluating the performance of such solutions as compared with standard blood cultures have demonstrated reasonably good performance, with the obvious limitation that NAATs will only detect a subset of possible BSI pathogens, and provide no susceptibility information. As conventional phenotypic susceptibility testing requires isolated organisms, if the direct NAAT is positive and the corresponding culture is negative, susceptibility information may never be available. Thus, at the present time, such an approach may serve only as an adjunct to standard of care protocols. As is the case for rapid methods for blood culture isolate identification, the actual clinical impact of rapid methods for direct pathogen detection in blood specimens has not been extensively studied.

Conclusions

Technological advances have resulted in the ability to more rapidly identify and, in some cases, predict the susceptibility of the aetiological agents of BSIs to a limited extent. Although these methods hold great promise for the future, conventional blood culture methods remain the dominant approach to diagnosing most patients with BSIs. Therefore, the traditional principles of patient selection, adequate and careful specimen collection, appropriate cultivation and accurate result interpretation remain critical to the delivery of the most effective care for our patients with suspected BSIs.

Anaerobic Bacteria Culture

Definition

An anaerobic bacteria culture is a method used to grow anaerobes from a clinical specimen. Obligate anaerobes are bacteria that can live only in the absence of oxygen. Obligate anaerobes are destroyed when exposed to the atmosphere for as briefly as 10 minutes. Some anaerobes are tolerant to small amounts of oxygen. Facultative anaerobes are those organisms that will grow with or without oxygen. The methods of obtaining specimens for anaerobic culture and the culturing procedure are performed to ensure that the organisms are protected from oxygen.

Purpose

Anaerobic bacterial cultures are performed to identify bacteria that grow only in the absence of oxygen and which may cause human infection. If overlooked or killed by exposure to oxygen, anaerobic infections result in such serious consequences as amputation, organ failure, sepsis, meningitis, and death. Culture is required to correctly identify anaerobic pathogens and institute effective antibiotic treatment.

Precautions

It is crucial that the health care provider obtain the sample for culture via aseptic technique. Anaerobes are commonly found on mucous membranes and other sites such as the vagina and oral cavity. Therefore, specimens likely to be contaminated with these organisms should not be submitted for culture (e.g., a throat or vaginal swab). Some types of specimens should always be cultured for anaerobes if an infection is suspected. These include abscesses, bites, blood, cerebrospinal fluid and exudative body fluids, deep wounds, and dead tissues. The specimen must be protected from oxygen during collection and transport and must be transported to the laboratory immediately.

Description

Anaerobes are normally found within certain areas of the body but result in serious infection when they have access to a normally sterile body fluid or deep tissue that is poorly oxygenated. Some anaerobes normally live in the crevices of the skin, in the nose, mouth, throat, intestine, and vagina. Injury to these tissues (i.e., cuts, puncture wounds, or trauma) especially at or adjacent to the mucous membranes allows anaerobes entry into otherwise sterile areas of the body and is the primary cause of anaerobic infection. A second source of anaerobic infection occurs from the introduction of spores into a normally sterile site. Spore-producing anaerobes live in the soil and water, and spores may be introduced via wounds, especially punctures. Anaerobic infections are most likely to be found in persons who are immunosuppressed, those treated recently with broad-spectrum antibiotics, and persons who have a decaying tissue injury on or near a mucous membrane, especially if the site is foul-smelling.

Some specimens from which anaerobes are likely to be isolated are:

- blood
- bile
- bone marrow
- cerebrospinal fluid
- direct lung aspirate
- tissue biopsy from a normally sterile site
- fluid from a normally sterile site (like a joint)
- dental abscess
- abdominal or pelvic abscess
- knife, gunshot, or surgical wound
- severe burn.

Some of the specimens that are not suitable for anaerobic cultures include:

- coughed throat discharge (sputum)
- rectal swab
- nasal or throat swab
- urethral swab
- Voided urine

Specimen collection

The keys to effective anaerobic bacteria cultures include collecting a contamination-free specimen and protecting it from oxygen exposure. Anaerobic bacteria cultures should be obtained from an appropriate site without the health care professional contaminating the sample with bacteria from the adjacent skin, mucus membrane, or tissue. Swabs should be avoided when collecting specimens for anaerobic culture because cotton fibers may be detrimental to anaerobes. Abscesses or fluids can be aspirated using a sterile syringe that is then tightly capped to prevent entry of air. Tissue samples should be placed into a degassed bag and sealed, or into a gassed out screw top vial that may contain oxygen-free prereduced culture medium and tightly capped. The specimens should be plated as rapidly as possible onto culture media that has been prepared.

Culture

Cultures should be placed in an environment that is free of oxygen, at 95°F (35°C) for at least 48 hours before the plates are examined for growth. Gram staining is performed on the specimen at the time of culture. While infections can be caused by aerobic or anaerobic bacteria or a mixture of both, some infections have a high probability of being caused by anaerobic bacteria. These infections include brain abscesses, lung abscesses, aspiration pneumonia, and dental infections. Anaerobic organisms can often be suspected because many anaerobes have characteristic microscopic morphology (appearance). For example, Bacteroides spp. are gram-negative rods that are pleomorphic (variable in size and shape) and exhibit irregular bipolar staining. Fusobacterium spp. are often pale gram-negative spindle-shaped rods having pointed ends. Clostridium spp. are large gram-positive rods that form spores. The location of the spore (central, subterminal, terminal, or absent) is a useful differential characteristic. The presence of growth, oxygen tolerance, and Gram stain results are sufficient to establish a diagnosis of an anaerobic infection and begin antibiotic treatment with a drug appropriate for most anaerobes such as clindamycin, metronidazole, or vancomycin.

Gram-negative anaerobes and some of the infections they produce include the following genera:

- Bacteroides (the most commonly found anaerobes in cultures; intra-abdominal infections, rectal abscesses, soft tissue infections, liver infection)
- Fusobacterium (abscesses, wound infections, pulmonary and intracranial infections)

- Porphyromonas (aspiration pneumonia, periodontitis)
- Prevotella (intra-abdominal infections, soft tissue infections)

Gram-positive anaerobes include the following:

- Actinomyces (head, neck, pelvic infections; aspiration pneumonia)
- Bifidobacterium (ear infections, abdominal infections)
- Clostridium (gas, gangrene, food poisoning, tetanus, pseudomembranous colitis)
- Peptostreptococcus (oral, respiratory, and intra-abdominal infections)
- Propionibacterium (shunt infections)

The identification of anaerobes is highly complex, and laboratories may use different identification systems. Partial identification is often the goal. For example, there are six species of the Bacteroides genus that may be identified as the Bacteroides fragilis group rather than identified individually. Organisms are identified by their colonial and microscopic morphology, growth on selective media, oxygen tolerance, and biochemical characteristics. These include sugar fermentation, bile solubility, esculin, starch, and gelatin hydrolysis, casein and gelatin digestion, catalase, lipase, lecithinase, and indole production, nitrate reduction, volatile fatty acids as determined by gas chromatography, and susceptibility to antibiotics. The antibiotic susceptibility profile is determined by the microtube broth dilution method. Many species of anaerobes are resistant to penicillin, and some are resistant to clindamycin and other commonly used antibiotics.

Diagnosis/Preparation

The health care provider should take special care to collect a contamination-free specimen. All procedures must be performed aseptically. The health care professional who collects the specimen should be prepared to take two samples, one for anaerobic culture and one for aerobic culture, since it is unknown whether the pathogen can grow with or without oxygen. In addition, health care professionals should document any antibiotics that the patient is currently taking and any medical conditions that could influence growth of bacteria.

Aftercare

In the case of vein puncture for anaerobic blood cultures, direct pressure should be applied to the vein puncture site for several minutes or until the bleeding has stopped. An adhesive bandage may be applied, if appropriate. If swelling or bruising occurs, ice can be applied to the site. For collection of specimens other than blood, the patient and the collection site should be monitored for any complications after the procedure.

Risks

Special care must be taken by the health care team obtaining, transporting, and preparing the specimen for anaerobic culture. Poor methodology may delay the identification of the bacterium, may allow the patient's condition to deteriorate, and may require the patient to provide more samples than would otherwise be required. Patients may experience bruising, discomfort, or swelling at the collection site when tissue, blood, or other fluids are obtained.

Results

Negative results will show no pathogenic growth in the sample. Positive results will show growth, the identification of each specific bacterium, and its antibiotic susceptibility profile.

Patient education

A health care team member should explain the specimen collection procedure to the patient. If the patient is seriously ill, the team member should explain the procedure to the patient's family members. The patient and his or her family should understand that because bacteria need time to grow in the laboratory, several days may be required for bacterium identification.

Urine Culture

Test Overview

A urine culture is a test to detects micro-organisms - bacteria in the urine that can cause an infection. Urine in the bladder is normally sterile. This means it does not contain any bacteria or other organisms (such as fungi). But bacteria can enter the urethra and cause a urinary tract infection (UTI). A sample of urine is added to a substance that promotes the growth of germs. If no germs grow, the culture is negative. If germs grow, the culture is positive. The type of germ may be identified using a microscope or chemical tests. Sometimes other tests are done to find the right medicine for treating the infection. This is called sensitivity testing. UTIs are more common in women and girls than in men. This may be partly because the female urethra is shorter and closer to the anus. This allows bacteria from the intestines to come into contact more easily with the urethra. Men also have an antibacterial substance in their prostate gland that lowers their risk.

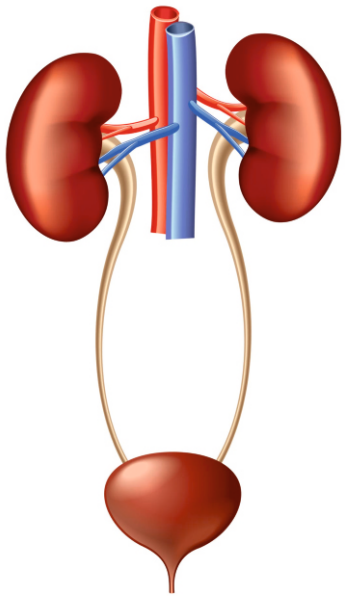
Why it is done

A urine culture may be done to:

- Find the cause of a urinary tract infection (UTI).
- Make decisions about the best treatment for a UTI. This is called sensitivity testing.
- Find out if treatment for a UTI worked.

How to prepare

You do not need to do anything before you have this test. If you are taking or have recently taken antibiotics, tell your doctor.



You will need to collect a urine sample. Avoid urinating just before having this test. Talk to your doctor if you have any concerns about the need for the test, its risks, how it will be done, or what the results will mean.

How it is done

You will be asked to collect a clean-catch midstream urine sample for testing. The first urine of the day is best because bacterial levels will be higher.

Clean-catch midstream urine collection

This method helps protect the urine sample from germs that are normally found on the penis or vagina.

- Wash your hands before you collect the urine.
- If the collection container has a lid, remove it carefully. Set it down with the inner surface up. Do not touch the inside of the cup with your fingers.
- Clean the area around your genitals.
  - For men: Pull back the foreskin, if you have one. Clean the head of the penis thoroughly. Use medicated towelettes or swabs.
  - For women: Spread open the folds of skin around the vagina with one hand. Then use the other hand to clean the area around the vagina and urethra thoroughly. Use medicated towelettes or swabs. Wipe the area from front to back to avoid spreading bacteria from the anus to the urethra.
- Start to urinate into the toilet or urinal. Women should keep holding apart the folds of skin around the vagina while they urinate.
- After the urine has flowed for several seconds, place the collection cup into the stream. Collect about 60 mL (2 fl oz) of this "midstream" urine without stopping the flow.
- Do not touch the rim of the container to your genital area. And don't get toilet paper, pubic hair, stool (feces), menstrual blood, or other foreign matter in the urine sample.
- Finish urinating into the toilet or urinal.
- Carefully replace the lid on the cup. Wash your hands. Return the cup to the lab. If you are collecting the urine at home and can't get it to the lab in an hour, refrigerate it.

How it feels

Collecting a urine sample is not painful.

Risks

Collecting a urine sample does not cause problems.

Results

A urine culture is a test to find germs (such as bacteria) in the urine that can cause an infection. Urine culture results are usually ready in 1 to 3 days. But some germs take longer to grow in the culture. So results may not be available for several days.

Urine culture

Normal:

No bacteria or other germs (such as fungi) grow in the culture. The culture result is **negative**.

Abnormal:

Organisms (usually bacteria) grow in the culture. The culture result is **positive**. A count of 100,000 or more bacteria per milliliter (mL) of urine may be caused by an infection. A count ranging from 100 to 100,000 could be caused either by infection or by contamination of the sample. You may need to repeat the urine culture. If the count is 100 or less, infection is unlikely. But you may have a count of 100 or less if you are already taking antibiotics. If test results are positive, sensitivity testing may be done to help make decisions about treatment.

What Affects the Test

You may not be able to have the test, or the results may not be helpful, if:

- You take antibiotics or have just finished taking them.
- You take water pills (diuretics) or drink a large amount of liquid. This may dilute your urine and reduce the number of bacteria in the sample.
- You take a lot of vitamin C.

What to Think About

- A urine culture done in the early stage of a urinary tract infection (UTI) may be less accurate than one that is done after the infection becomes established.

- A urine culture may be done when an abnormal result from a urinalysis (such as an increased number of white blood cells) shows signs of an infection. To learn more, see the topic Urine Test.
- A urine culture may be repeated after the UTI has been treated. This is to make sure the infection is cured.
- A health professional may collect a urine sample by placing a urinary catheter into the bladder. This method is sometimes used to collect urine from a person in the hospital who is very ill or who can't provide a clean-catch sample. Using a catheter to collect a urine sample reduces the chance of getting bacteria from the skin or genital area in the urine sample. But catheter use sometimes causes a UTI.
- People who have a urinary catheter in place for a long time are at high risk for getting a UTI.
- Collecting a urine sample from a small child or baby may be done by using a special plastic bag with tape around its opening (a U bag). The bag is attached around the child's genitals until he or she urinates (usually within an hour). Then the bag is carefully removed. To collect a urine sample from a very sick baby, a doctor may insert a needle through the baby's belly directly into the bladder. (This is called a suprapubic tap.)
- To diagnose tuberculosis that has spread to the urinary tract, a special test will be done using all of the first morning urine on 3 separate days.
- Sensitivity testing helps your doctor choose the best medicine to treat specific types of bacteria or fungus that may be causing a UTI.
- Some types of bacteria or fungi may take several weeks to grow in the culture.

Urinalysis

Urine Specimens – an overview of collection methods, collection devices, specimen handling and transportation

After blood, urine is the most commonly used specimen for diagnostic testing, monitoring of disease status and detection of drugs. Urine testing, using both automated and traditional manual methods, is growing rapidly. As with all clinical laboratory specimens, preanalytical error in urine specimens is often difficult to detect. Because of this, it is important for laboratories to have processes in place to ensure compliance with best practice in specimen collection, handling and transport – including the use of preservatives where appropriate.

Types of Urine Collection Methods

Urine specimens may be collected in a variety of ways according to the type of specimen required, the collection site and patient type. Randomly Collected Specimens are not regarded as specimens of choice because of the potential for dilution of the specimen when collection occurs soon after the patient has consumed fluids. First Morning Specimen is the specimen of choice for urinalysis and microscopic analysis, since the urine is generally more concentrated. Midstream Clean Catch Specimens are strongly recommended for microbiological culture and antibiotic susceptibility testing because of the reduced incidence of cellular and microbial contamination. Timed Collection Specimens may be required for quantitative measurement of certain analytes, including those subject to diurnal variation. Analytes commonly tested using timed collection include creatinine, urea, potassium, sodium, uric acid, cortisol, calcium, citrate, amino acids, catecholamines, metanephrines, vanillylmandelic acid (VMA), 5-hydroxyindoleacetic acid, protein, oxalate, copper, 17-ketosteroids, and 17-hydroxysteroids. Collection from Catheters (e.g. Foley catheter) using a syringe, followed by transfer to a specimen tube or cup. Alternatively, urine can be drawn directly from the catheter to an evacuated tube using an appropriate adaptor. Supra-pubic Aspiration may be necessary when a non-ambulatory patient cannot be catheterized or where there are concerns about obtaining a sterile specimen by conventional means. Pediatric Specimens present many challenges. For infants and small children, a special urine collection bag can be adhered to the skin surrounding the urethral area.

Urine Collection Devices

An extensive array of urine collection products is available on the market. Information on features, intended use and instructions for use should be obtained from the device manufacturer and reviewed before being incorporated into a specimen collection protocol.

Urine Collection Containers (cups for collection and transport): Urine collection container cups are available in a variety of shapes and sizes with lids that are either 'snap-on' or 'screw-on'. Leakage is a common problem with low quality products. To protect healthcare workers from exposure to the specimen and protect the specimen from exposure to contaminants, leak-proof cups should be utilized. Some urine specimen containers have closures with special access ports that allow closed-system transfer of urine directly from the collection device to the tube (further information).

Urine Collection Containers for 24-hour Collection: Urine collection containers for 24-hour specimens commonly have a 3 liter capacity. As for the urine collection cups above, closure types vary with some containers featuring anintegrated port for transfer of an aliquot of the specimen to an evacuated urine collection tube (further information). This provides the option for the laboratory to receive only the aliquot tube and specimen weight (with the large 24-hour container and contents discarded at the point of collection). Additional precautions need to be taken when a preservative is required (further information).

Urine Specimen Tubes: Urine specimens may be poured directly into tubes with 'screw-on' or 'snap-on' caps. Additionally, evacuated tubes, similar to those used in blood collection, are available (further information).

Urine Specimen Collection and Transportation Guidelines

As for any type of clinical laboratory specimen, certain criteria for collection and transportation (further information) of urine specimens must be met to ensure high quality specimens free of preanalytical artifact are obtained consistently. Without this, accurate test results cannot be guaranteed.

Urine Specimen Preservation

For urinalysis and culture and sensitivity testing, CLSI Guidelines recommend testing within two hours of collection. Different time limits may apply to specimens required for molecular testing of infectious agents (e.g. testing for Neisseria gonorrhoeae, Chlamydia trachomatis). For this type of testing, laboratories should ensure they are able to comply with specimen transportation conditions prescribed by the assay manufacturers. Where compliance with these and/or CLSI recommendations is not possible, consideration should be given to the use of a preservative (further information). Specimen collection tubes with preservatives for chemical urinalysis (further information) and culture and antibiotic susceptibility are available (further information).

Urine Specimen Reception in the Laboratory

In addition to routine checks and precautions taken for all specimens received in the clinical laboratory, the following additional 'check items' apply to urine specimens. Labels, Volume, Collection Date and Time, Collection Method, Specimen Preservation, Light Protection.

Randomly Collected Specimens: Randomly collected specimens are suitable for urinalysis in the clinical chemistry laboratory and for microscopic analysis. However, they are not regarded as specimens of choice because of the potential for dilution of the specimen when collection occurs soon after the patient has consumed fluids. In this situation, analyte values may be artificially low. Of necessity, pediatric urine specimens for urinalysis and microscopy are often of this type.

First Morning Specimen is the Specimen: First morning specimens are the specimen of choice for urinalysis and microscopic analysis since the urine is generally more concentrated (due to the length of time the urine is allowed to remain in the bladder) and, therefore, contains relatively higher levels of cellular elements and analytes. Abnormal constituents are also likely to be present in higher concentration and, thus, more likely to be detected.

Midstream Clean Catch Specimens: Midstream specimens are strongly recommended for microbiological culture and antibiotic susceptibility testing because of the reduced incidence of cellular and microbial contamination. Following instruction from a healthcare professional, patients are required to follow a prescribed procedure commencing with cleansing the urethral area. The patient should then void the first portion of the urine stream into the toilet. These first steps significantly reduce the opportunities for contaminants to enter the urine stream during collection of the clinical specimen. The urine midstream is then collected into a clean container after which the remaining urine is voided into the toilet. This method of collection can be conducted at any time of day or night.

Timed Collection Specimens: Timed specimens may be required for quantitative measurement of certain analytes, including those subject to diurnal variation. Analytes commonly tested using timed collection include creatinine, urea, potassium, sodium, uric acid, cortisol, calcium,

citrate, amino acids, catecholamines, metanephrines, vanillylmandelic acid (VMA), 5-hydroxyindoleacetic acid, protein, oxalate, copper, 17-ketosteroids, and 17-hydroxysteroids. A timed collection allows measurement of the excretion of these substances in urine over a specified length of time, usually, but not always, 8 or 24 hours. In this collection method, the bladder is emptied prior to beginning the timed collection. Then, for the duration of the designated time period, all urine is collected and pooled into a collection container, with the final collection taking place at the very end of that period. Half an hour before the end of the collection period, it is helpful to ask the patient to drink a glass of water, so that the last urine specimen can be obtained. If no specimen is produced, then the total volume and time of collection cannot be determined. It is also important to caution the patient not to lose urine specimens to the toilet during defecation. When a 24-hour urine specimen is required for the assay of catecholamines, metanephrines and/or VMA, for the diagnosis of pheochromocytoma, which causes persistent or episodic hypertension, it is advisable to monitor the blood pressure of the patient and collect the urine specimen when the blood pressure is high, in order to improve the chance of a positive finding. Timed specimens should be refrigerated during the collection period, unless otherwise directed by the laboratory. Accurate timing is very important as this information forms a critical part of the calculations performed to determine urine clearance values (e.g. creatinine clearance). Interpretations based on faulty calculations can result in improper diagnoses or medical treatment.

Collection from Catheters: Urine specimens can be collected from catheters (e.g. Foley catheter) using a syringe, followed by transfer to a specimen tube or cup. Alternatively, urine can be drawn directly from the catheter to an evacuated tube using an appropriate adaptor.

Direct draw adaptor for urine specimen collection from Foley catheter

Supra-pubic Aspiration: Supra-pubic aspiration may be necessary when a non-ambulatory patient cannot be catheterized or where there are concerns about obtaining a sterile specimen by conventional means. This procedure involves collection of the specimen by needle aspiration through the abdominal wall into the bladder.

Pediatric Specimens: Urine collection from pediatric patients presents many challenges. For infants and small children, a special urine collection bag can be adhered to the skin surrounding the urethral area. Once the collection is completed, the urine is poured into a collection cup or transferred directly into an evacuated tube with a transfer straw. Urine collected from a diaper is not recommended for laboratory testing since contamination from the diaper material may affect test results.

Urine Collection Containers

Urine collection containers with integrated port for transfer of specimen to evacuated urine collection tube.

24 Hour Urine Collection Container: 24 hour urine collection container with integrated port for transfer of specimen to evacuated urine collection tube. This provides the option for the laboratory to receive only the aliquot tube and specimen weight (with the large 24-hour container and contents discarded at the point of collection).

Preservatives for 24 Hour Specimens: When a preservative is required, it should be added to the collection container before the urine collection begins. Commonly used preservatives for 24 hour specimens are hydrochloric acid, boric acid, acetic acid, thymol and toluene. If more than one acceptable preservative is available for the analyte(s) being tested, the least hazardous one should, of course, be selected. Appropriate warning labels should be placed on the container to alert patients to possible harm arising from contact with the preservatives. This should be reinforced by appropriate instruction from the attending healthcare worker. A corresponding Material Safety Data Sheet (MSDS) should also be provided for the patient.

Urine Specimen Tubes: Evacuated tubes, similar to those used in blood collection, are available for urine collection. These can be filled using a straw device, from urine specimen containers with integrated transfer devices, or from direct sampling devices that are used to access catheter sampling ports.

Urine transfer 'straw' with adaptor for transfer of specimen to evacuated urine collection tube

Urine collection containers with integrated port for transfer of specimen to evacuated urine collection tube

Urinalysis tubes are available in a variety of shapes: conical bottom, round bottom, or flat bottom. Conical bottom tubes offer advantages for microscopic examination of urine sediment. The laboratory's tube selection process must include consideration of centrifugation conditions and compatibility with automated instrument systems. Tube fill volumes are typically within the range of 4 to 10mL with dimensions of 13 x 75mm and 16 x 100mm.

Evacuated urine specimen collection tubes

Collection and Transport Guidelines

All urine collection and/or transport containers should be clean and free of particles or interfering substances. The collection and/or transport container should have a secure lid and be leak-proof. Leak-proof containers reduce specimen loss and risk of healthcare worker exposure to the specimen while also protecting the specimen from contaminants. The use of containers that are made from break-resistant plastic is strongly recommended. The container material should not leach interfering substances into the specimen. Specimen containers must not be re-used. Specimen tubes should be compatible with automated systems and instruments used by the laboratory. Collection containers and/or specimen tubes should be compatible with pneumatic tube systems where these are used for urine specimen transport. Use of leak-proof containers is essential in this situation.

The CLSI Guidelines makes the following recommendations for urine collection: Primary (routine) specimen containers to have a wide base and a capacity of at least 50 mL. 24 hour specimen containers to have a capacity of at least 3 litres. Sterile collection containers for all microbiology specimens. Specimen containers to have secure closures to prevent specimen loss and to protect the specimen from contaminants. Amber colored containers for specimens required for assay of light sensitive analytes such as urobilinogen and porphyrins.

Urine Tubes Preservatives

For chemical urinalysis and conventional (culture based) microbiological testing, unpreserved specimens exceeding the two hour limit that have not been refrigerated should not be accepted for analysis due to potential bacterial overgrowth leading to disintegration of cells and casts*, invalidation of bacterial colony counts and errors in chemical urinalysis. When specimens for such testing are directly transferred from a collection cup to a tube containing a suitable preservative, a stable environment is provided for the specimen until testing can be conducted. Preservatives are also available for some molecular tests (e.g. BD UPT urine specimen tube for use with BD ProbeTec™ ET assay system). When a decision to use a preservative is made – for any type of testing, potential interference with assay methods should be considered. Laboratories should validate all test procedures intended to be used for preserved specimens. Specimens may need to be split if various tests requiring different preservatives are requested.

* Bacterial growth increases the pH of the urine leading to lysis of red blood cells and white blood cells. Increased pH (alkalinity) can also cause casts to dissolve.

Chemical Urinalysis Preservatives: A variety of urine preservatives is available that allow urine to be maintained at room temperature while still providing urinalysis test results comparable to those achieved with fresh specimens or those stored under refrigerated conditions. Commonly used preservatives for chemical urinalysis specimens include tartaric acid, boric acid, chlorhexidine, ethyl paraben, thymol and sodium propionate (and 'cocktails' of these). Preservation times are typically within the range of 24 to 72 hours. Claims for the duration of stability for specific analytes should be obtained from the manufacturer.

Culture and Antibiotic Susceptibility Preservatives: Preservatives for culture and antibiotic susceptibility testing are designed to maintain the specimen in a state equivalent to that which would be achieved with refrigeration by deterring the proliferation of organisms that could result in a false positive culture or bacterial overgrowth. Careful attention must be given to the formulation of these preservatives to achieve this objective. There is evidence to suggest that non-pH buffered boric acid may be harmful to certain organisms and that buffered boric acid preservatives can reduce the harmful effects of the preservative on the organisms. Preserved urine specimens can be stored at room temperature until the time of testing. Product claims regarding duration of preservative potency should be obtained from the manufacturer.

Labels: If the collection container is used for transport, the label should be placed on the container and not on the lid, since the lid can be mistakenly placed on a different container. Note that some labels are unsuitable for specimens stored under refrigerated conditions because of a lack of adhesion at low temperatures.

Volume: It is important for specimen collection personnel to ensure there is sufficient volume to perform the required tests. For specimens in preservative tubes, the fill volume must be correct. As above, under-filling or over-filling these tubes may adversely affect test result accuracy.

Collection Time and Date: Collection time and date must be shown on the specimen label. For timed specimens, both the start and stop times of the collection must be shown. The time at which the specimen was received in the laboratory must also be documented for verification of proper handling and transport after collection.

Collection Method: The method of collection should be confirmed when the specimen is received in the laboratory to ensure the type of specimen submitted meets the needs of the required test(s). An example of an optimum specimen/test match would be a first morning specimen for urinalysis and microscopic examination.

Specimen Preservation: If the specimen is not received within two hours of collection, specimen reception personnel must confirm that a tube containing an appropriate preservative has been used. Confirmation that the specimen is received within the allowable time for the particular preservative tube used is required.

Light Protection: Specimens submitted for testing of light-sensitive analytes must be collected in containers that protect the specimen from light.

Stool Analysis

A stool analysis is a series of tests done on a stool (feces) sample to help diagnose certain conditions affecting the digestive tract. These conditions can include infection (such as from parasites, viruses, or bacteria), poor nutrient absorption, or cancer.

For a stool analysis, a stool sample is collected in a clean container and then sent to the laboratory. Laboratory analysis includes microscopic examination, chemical tests, and microbiologic tests. The stool will be checked for color, consistency, amount, shape, odor, and the presence of mucus. The stool may be examined for hidden (occult) blood, fat, meat fibers, bile, white blood cells, and sugars called reducing substances. The pH of the stool also may be measured. A stool culture is done to find out if bacteria may be causing an infection.

Why it is done

Stool analysis is done to:

- Help identify diseases of the digestive tract, liver, and pancreas. Certain enzymes (such as trypsin or elastase) may be evaluated in the stool to help determine how well the pancreas is functioning.
- Help find the cause of symptoms affecting the digestive tract, including prolonged diarrhea, bloody diarrhea, an increased amount of gas, nausea, vomiting, loss of appetite, bloating, abdominal pain and cramping, and fever.
- Screen for colon cancer by checking for hidden (occult) blood.
- Look for parasites, such as pinworms or Giardia.
- Look for the cause of an infection, such as bacteria, a fungus, or a virus.
- Check for poor absorption of nutrients by the digestive tract (malabsorption syndrome). For this test, all stool is collected over a 72-hour period and then checked for fat (and sometimes for meat fibers). This test is called a 72-hour stool collection or quantitative fecal fat test.

How to Prepare

Many medicines can change the results of this test. You will need to avoid certain medicines depending on which kind of stool analysis you have. You may need to stop taking medicines such as antacids, antidiarrheal medicines, antiparasite medicines, antibiotics, laxatives, or nonsteroidal anti-inflammatory drugs (NSAIDs) for 1 to 2 weeks before you have the test. Be sure to tell your doctor about all the nonprescription and prescription medicines you take.

Be sure to tell your doctor if you have:

- Recently had an X-ray test using barium contrast material, such as a barium enema or upper gastrointestinal series (barium swallow). Barium can interfere with test results.
- Traveled in recent weeks or months, especially if you have traveled outside the country. This helps your doctor look for the parasites, fungi, viruses, or bacteria that may be causing a problem.

If your stool is being tested for blood, you may need to avoid certain foods for 2 to 3 days before the test. This depends on what kind of stool test you use. And do not do the test during your menstrual period or if you have active bleeding from hemorrhoids. If you aren't sure about how to prepare, ask your doctor. Do not use a stool sample for testing that has been in contact with toilet bowl cleaning products that turn the water blue.

How it is Done

Stool samples can be collected at home, in your doctor's office, at a medical clinic, or at the hospital. If you collect the samples at home, you will be given stool collection kits to use each day. Each kit contains applicator sticks and two sterile containers.

You may need to collect more than one sample over 1 to 3 days. Follow the same procedure for each day.

Collect the samples as follows:

- Urinate before collecting the stool so that you do not get any urine in the stool sample.
- Put on gloves before handling your stool. Stool can contain germs that spread infection. Wash your hands after you remove your gloves.
- Pass stool (but no urine) into a dry container. You may be given a plastic basin that can be placed under the toilet seat to catch the stool.
  - Either solid or liquid stool can be collected.
  - If you have diarrhea, a large plastic bag taped to the toilet seat may make the collection process easier; the bag is then placed in a plastic container.
  - If you are constipated, you may be given a small enema.
  - Do not collect the sample from the toilet bowl.
  - Do not mix toilet paper, water, or soap with the sample.
- Place the lid on the container and label it with your name, your doctor's name, and the date the stool was collected. Use one container for each day's collection, and collect a sample only once a day unless your doctor gives you other directions.

Take the sealed container to your doctor's office or the laboratory as soon as possible. You may need to deliver your sample to the lab within a certain time. Tell your doctor if you think you may have trouble getting the sample to the lab on time.

If the stool is collected in your doctor's office or the hospital, you will pass the stool in a plastic container that is inserted under the toilet seat or in a bedpan. A health professional will package the sample for laboratory analysis.

You will need to collect stool for 3 days in a row if the sample is being tested for quantitative fats. You will begin collecting stool on the morning of the first day. The samples are placed in a large container and then refrigerated.

You may need to collect several stool samples over 7 to 10 days if you have digestive symptoms after traveling outside the country.

Samples from babies and young children may be collected from diapers (if the stool is not contaminated with urine) or from a small-diameter glass tube inserted into the baby's rectum while the baby is held on an adult's lap.

Sometimes a stool sample is collected using a rectal swab that contains a preservative. The swab is inserted into the rectum, rotated gently, and then withdrawn. It is placed in a clean, dry container and sent to the lab right away.

How it feels

There is no pain while collecting a stool sample. If you are constipated, straining to pass stool may be painful.

If your health professional uses a rectal swab to collect the sample, you may feel some pressure or discomfort as the swab is inserted into your rectum.

Risks

Any stool sample may contain germs that can spread disease. It is important to carefully wash your hands and use careful handling techniques to avoid spreading infection.

Results

A stool analysis is a series of tests done on a stool (feces) sample to help diagnose certain conditions affecting the digestive tract.
The normal values listed here-called a reference range-are just a guide. These ranges vary from lab to lab, and your lab may have a different range for what's normal. Your lab report should contain the range your lab uses. Also, your doctor will evaluate your results based on your health and other factors. This means that a value that falls outside the normal values listed here may still be normal for you or your lab.
Stool analysis test results usually take at least 1 to 3 days.

Stool analysis

Normal:	The stool appears brown, soft, and well-formed in consistency.
	The stool does not contain blood, mucus, pus, undigested meat fibers, harmful bacteria, viruses, fungi, or parasites.
	The stool is shaped like a tube.
	The pH of the stool is 7.0–7.5.
	The stool contains less than 0.25 grams per deciliter (g/dL)[less than 13.9 millimoles per liter (mmol/L)] of sugars called reducing factors. The stool contains 2–7 grams of fat per 24 hours (g/24h).
Abnormal:	The stool is black, red, white, yellow, or green.
	The stool is liquid or very hard.
	There is too much stool.
	The stool contains blood, mucus, pus, undigested meat fibers, harmful bacteria, viruses, fungi, or parasites.
	The stool contains low levels of enzymes, such as trypsin or elastase.
	The pH of the stool is less than 7.0 or greater than 7.5.
	The stool contains 0.25 g/dL (13.9 mmol/L) or more of sugars called reducing factors.
	The stool contains more than 7 g/24h of fat (if your fat intake is about 100 g a day).

Many conditions can change the results of a stool analysis. Your doctor will talk with you about any abnormal results that may be related to your symptoms and past health.

Abnormal values

- High levels of fat in the stool may be caused by diseases such as pancreatitis, sprue (celiac disease), cystic fibrosis, or other disorders that affect the absorption of fats.
- The presence of undigested meat fibers in the stool may be caused by pancreatitis.
- A low pH may be caused by poor absorption of carbohydrate or fat. Stool with a high pH may mean inflammation in the intestine (colitis), cancer, or antibiotic use.
- Blood in the stool may be caused by bleeding in the digestive tract.
- White blood cells in the stool may be caused by inflammation of the intestines, such as ulcerative colitis, or a bacterial infection.
- Rotaviruses are a common cause of diarrhea in young children. If diarrhea is present, testing may be done to look for rotaviruses in the stool.
- High levels of reducing factors in the stool may mean a problem digesting some sugars.
- Low levels of reducing factors may be caused by sprue (celiac disease), cystic fibrosis, or malnutrition. Medicine such as colchicine (for gout) or birth control pills may also cause low levels.

What Affects the Test

- Reasons you may not be able to have the test or why the results may not be helpful include:
- Taking medicines such as antibiotics, antidiarrheal medicines, barium, bismuth, iron, ascorbic acid, nonsteroidal anti-inflammatory drugs (NSAIDs), and magnesium.
- Contaminating a stool sample with urine, blood from a menstrual period or a bleeding hemorrhoid, or chemicals found in toilet paper and paper towels.
- Exposing the stool sample to air or room temperature or failing to send the sample to a laboratory within 1 hour of collection.

What to Think About

- Stool may be checked for hidden (occult) blood. Testing for faecal Occult Blood confirms.
- A stool culture is done to find the cause of an infection, such as bacteria, a virus, a fungus, or a parasite.
- A bowel transit time test is done to help find the cause of abnormal movement of food through the digestive tract.
- The D-xylose absorption test is done to help diagnose problems that prevent the small intestine from absorbing nutrients in food. This test may be done when symptoms of malabsorption syndrome (such as chronic diarrhea, weight loss, and weakness) are present.
- A stool analysis to measure trypsin or elastase is not as reliable as the sweat test to detect cystic fibrosis.

Gram Stain

Gram staining is an empirical method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls. The method is named after its inventor, the Danish scientist Hans Christian Gram (1853-1938), who developed the technique in 1884 (Gram 1884). The importance of this determination to correct identification of bacteria cannot be overstated as all phenotypic methods begin with this assay.

The Basic Method

1. First, a loopful of a pure culture is smeared on a slide and allowed to air dry. The culture can come from a thick suspension of a liquid culture or a pure colony from a plate suspended in water on the microscope slide. Important considerations:
   - Take a small inoculum—don't make a thick smear that cannot be completely decolorized. This could make gram-negative organisms appear to be gram-positive or gram-variable.
   - Take a fresh culture—old cultures stain erratically.
2. Fix the cells to the slide by heat or by exposure to methanol. Heat fix the slide by passing it (cell side up) through a flame to warm the glass. Do not let the glass become hot to the touch.
3. Crystal violet (a basic dye) is then added by covering the heat-fixed cells with a prepared solution. Allow to stain for approximately 1 minute.
4. Briefly rinse the slide with water. The heat-fixed cells should look purple at this stage.
5. Add iodine (Gram's iodine) solution (1% iodine, 2% potassium iodide in water) for 1 minute. This acts as a mordant and fixes the dye, making it more difficult to decolorize and reducing some of the variability of the test.
6. Briefly rinse with water.
7. Decolorize the sample by applying 95% ethanol or a mixture of acetone and alcohol. This can be done in a steady stream, or a series of washes. The important aspect is to ensure that all the color has come out that will do so easily. This step washes away unbound crystal violet, leaving Gram-positive organisms stained purple with Gram-negative organisms colorless. The decolorization of the cells is the most "operator-dependent" step of the process and the one that is most likely to be performed incorrectly.
8. Rinse with water to stop decolorization.
9. Rinse the slide with a counterstain (safranin or carbol fuchsin) which stains all cells red. The counterstain stains both gram-negative and gram-positive cells. However, the purple gram-positive color is not altered by the presence of the counter-stain, it's effect is only seen in the previously colorless gram-negative cells which now appear pink/red.
10. Blot gently and allow the slide to dry. Do not smear.

What's Going On?

Bacteria have a cell wall made up of peptidoglycan. This cell wall provides rigidity to the cell, and protection from osmotic lysis in dilute solutions. Gram-positive bacteria have a thick mesh-like cell wall, gram-negative bacteria have a thin cell wall and an outer phospholipid bilayer membrane. The crystal violet stain is small enough to penetrate through the matrix of the cell wall of both types of cells, but the iodine-dye complex exits only with difficulty (Davies et al. 1983). The decolorizing mixture dehydrates cell wall, and serves as a solvent to rinse out the dye-iodine complex. In Gram-negative bacteria it also dissolves the outer membrane of the gram-negative cell wall aiding in the release of the dye. It is the thickness of the cell wall that characterizes the response of the cells to the staining procedure. In addition to the clearly gram-positive and gram-negative, there are many species that are "gram-variable" with intermediate cell wall structure (Beveridge and Graham 1991). As noted above, the decolorization step is critical to the success of the procedure. Gram's method involves staining the sample cells dark blue, decolorizing those cells with a thin cell wall by rinsing the sample, then counterstaining with a red dye. The cells with a thick cell wall appear blue (gram positive) as crystal violet is retained within the cells, and so the red dye cannot be seen. Those cells with a thin cell wall, and therefore decolorized, appear red (gram negative). It is a prudent practice to always include a positive and negative control on the staining procedure to confirm the accuracy of the results (Murray et al 1994) and to perform proficiency testing on the ability of the technicians to correctly interpret the stains (Andserson, et al. 2005).

Excessive Decolorization

It is clear that the decolorization step is the one most likely to cause problems in the gram stain. The particular concerns in this step are listed below (reviewed in McClelland 2001)

1. Excessive heat during fixation: Heat fixing the cells, when done to excess, alters the cell morphology and makes the cells more easily decolorized.
2. Low concentration of crystal violet: Concentrations of crystal violet up to 2% can be used successfully, however low concentrations result in stained cells that are easily decolorized. The standard 0.3% solution is good, if decolorization does not generally exceed 10 seconds.
3. Excessive washing between steps: The crystal violet stain is susceptible to wash-out with water (but not the crystal violet-iodine complex). Do not use more than a 5 second water rinse at any stage of the procedure.
4. Insufficient iodine exposure: The amount of the mordant available is important to the formation of the crystal violet – iodine complex. The lower the concentration, the easier to decolorize (0.33% – 1% commonly used). Also, QC of the reagent is important as exposure to air and elevated temperatures hasten the loss of Gram's iodine from solution. A closed bottle (0.33% starting concentration) at room temperature will lose >50% of available iodine in 30 days, an open bottle >90%. Loss of 60% iodine results in erratic results.
5. Prolonged decolorization: 95% ethanol decolorizes more slowly, and may be recommended for inexperienced technicians while experienced workers can use the acetone-alcohol mix. Skill is needed to gauge when decolorization is complete.
6. Excessive counterstaining: As the counterstain is also a basic dye, it is possible to replace the crystal violet—iodine complex in gram-positive cells with an over-exposure to the counterstain. The counterstain should not be left on the slide for more than 30 seconds.

Alternatives to the Gram Stain

Gram's staining method is plainly not without its problems. It is messy, complicated, and prone to operator error. The method also requires a large number of cells (although a membrane-filtration technique has been reported; Romero, et al 1988). However, it is also central to phenotypic microbial identification techniques. This method, and it's liabilities, are of immediate interest to those involved in environmental monitoring programs as one of the most common isolates in an EM program, Bacillus spp., will frequently stain gram variable or gram negative despite being a gram-positive rod (this is especially true with older cultures). The problems with Gram's method have lead to a search

for other tests that correlate with the cell wall structure of the gram-positive and the gram-negative cells. Several improvements/ alternatives to the classical gram stain have appeared in the literature.

KOH String Test

The KOH String Test is done using a drop of 3% potassium hydroxide on a glass slide. A visible loopful of cells from a single, well-isolated colony is mixed into the drop. If the mixture becomes viscous within 60 seconds of mixing (KOH-positive) then the colony is considered gram-negative. The reaction depends on the lysis of the gram-negative cell in the dilute alkali solution releasing cellular DNA to turn the suspension viscous. This method has been shown effective for food microorganisms (Powers 1995), and for Bacillus spp (Carlone et al 1983, Gregersen 1978), although it may be problematic for some anaerobes (Carlone et al 1983, but also see Halebian et al 1981). This test has the advantage of simplicity, and it can be performed on older cultures. False negative results can occur in the test by using too little inoculum or too much KOH (DNA-induced viscosity not noticeable). False positive results can occur from too heavy an inoculum (the solution will appear to gel, but not string), or inoculation with mucoid colonies. This can serve as a valuable adjunct to the tradition gram stain method (von Graevenitz and Bucher 1983).

Aminopeptidase Test

L-alanine aminopeptidase is an enzyme localized in the bacterial cell wall which cleaves the amino acid L-alanine from various peptides. Significant activity is found almost only in Gram-negative microorganisms, all Gram-positive or Gram-variable microorganisms so far studied display no or very weak activity (Cerny 1976, Carlone et al. 1983). To perform the test, the reagent is used to make a suspension (with the bacteria). Aminopeptidase activity of the bacteria causes the release of 4-nitroaniline from the reagent, turning the suspension yellow. The test is especially useful for non-fermenters and gram-variable organisms, and is a one step test with several suppliers of kits. Results of the test are available in 5 minutes.

Fluorescent Stains

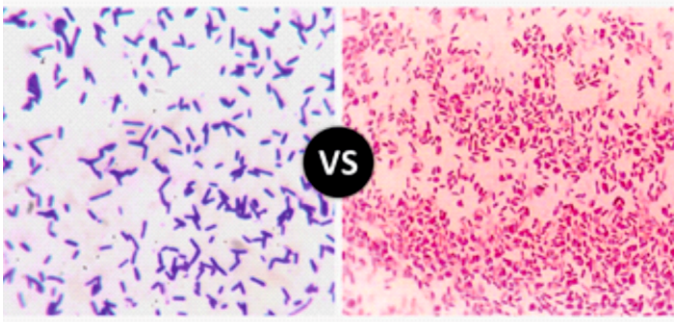
A popular combination of fluorescent stains for use in gram staining (particularly for flow-cytometry) involves the use of the fluorescent nucleic acid binding dyes hexidium iodide (HI) and SYTO 13. HI penetrates gram-positive but not gram-negative organisms, but SYTO 13 penetrates both. When the dyes were used together in a single step, gram-negative organisms are green fluorescent by SYTO 13 while gram-positive organisms are red-orange fluorescent by HI which overpowers the green of SYTO 13 (Mason et al 1998). There are commercial kits available for this procedure, which requires a fluorescent microscope or a flow cytometer. Sizemore et al (1990) developed a different approach to fluorescent labeling of cells. Fluorescence-labeled wheat germ agglutinin binds specifically to N-acetylglucosamine in the outer peptidoglycan layer of gram-positive bacteria. The peptidoglycan layer of gram-negative bacteria is covered by a membrane and is not labeled by the lectin. A variant of this method has also been used to “gram stain” microorganisms in milk for direct measurement by flow cytometry.

LAL-based Assay

Charles River Laboratories has just released a product to be used with their PTS instrument – the PTS Gram ID (Farmer 2005). This methodology makes use of the same reaction used for the chromogenic LAL test. Gram-negative organisms, with bacterial endotoxin, initiate the LAL coagulase cascade which results in activation of the proclotting enzyme, a protease. In the LAL test, this enzyme cleaves a peptide from the horseshoe crab coagulen, resulting in a clot. It can also cleave a peptide from a synthetic substrate, yielding a chromophore (p-nitroaniline) which is yellow and can be measured photometrically at 385 nm (Iwanaga 1987). Gram-positive organisms, lacking endotoxin, do not trigger the color change in this method, while gram-negative organisms do trigger it. Results are available within 10 minutes.

Summary

The differentiation of bacteria into either the gram-positive or the gram-negative group is fundamental to most bacterial identification systems. This task is usually accomplished through the use of Gram's Staining Method. Unfortunately, the gram stain methodology is complex and prone to error. This operator-dependence can be addressed by attention to detail, and by the use of controls on the test. Additional steps might include confirmatory tests, of which several examples were given. As with all microbiology assays, full technician training and competent review of the data are critical quality control steps for good laboratory results.



Gram Positive Bacteria vs. Gram Negative Bacteria

Gram Stain Procedure

Procedure:

A. Slant Cultures

1. Prepare and heat-fix smears.
2. Prepare the smears of *S. epidermidis* and *N. sicca* on a second slide. Heat-fix.
3. Stain the slides as follows:
   a. Flood the crystal violet for one minute.
   b. Pour off excess dye and wash gently in tap water and drain the slide against a paper towel.
   c. Expose the smears to Gram's iodine for one minute by washing with iodine, then adding more iodine and leaving it on the smear until the minute is over.
   d. Wash with tap water and drain carefully. (Do not blot.)
   e. Wash with 95% alcohol for 30 seconds.
   f. Wash with tap water at the end of the 30 seconds to stop the decolorization. Drain.
   g. Counterstain with 0.25% safranin for 30 seconds.
   h. Wash, drain, blot, and examine under oil.
   i. Draw the cells showing morphology, grouping, and relative sizes. Color a few of the cells of each bacterial species to show the Gram reaction.
   j. Save these slides and the ones from parts B & C of this exercise to use at the next lab period.

B. Broth Cultures

1. Because the smear made from the broth will be a thin smear and nearly invisible to the naked eye even after staining, it may be advisable to draw a ring with a felt open on the under side of the slide to mark the area in which the broth smear will be made.

Also, when making a smear from broth do not add a drop of water to the slide.
2. Hear-fix the smears, Gram stain them with the above procedure, and examine them. When focusing the broth smear use the technique suggested for thin smears.
3. Compare the appearance of the cells in the two smears.

Microorganism Measuring Procedure:

Procedure:

1. Place a slide micrometer on the stage of the microscope with the ruled area directly over the center of the condenser lens.
2. Look through the eyepiece with the low power objective in place and identify the ocular micrometer, check by turning the eyepiece. Note the number of gradations between numbers.
3. Focus the stage micrometer scale and note the gradations that are 0.01 mm apart and those which are 0.1 mm apart (if present). Adjust the two scales so that they are parallel and partially superimposed.
4. Align an ocular micrometer line with a stage micrometer line at the left side of the field. Look for another ocular micrometer line toward the right side of the field which is aligned similarly with a stage micrometer line.
5. Count the number of graduations between the two aligned marks. Count the number of ocular micrometer divisions which span the observed number of mm.
6. Divide the distance by the number of divisions to determine the distance between each two ocular micrometer divisions. Record the results on the chart provided.
7. Repeat the procedure using the high dry objective and the oil immersion objective. Note that with the higher magnifications the stage micrometer lines look thick. It is necessary at these magnifications to align an ocular micrometer line along one edge of a stage micrometer line and choose a second ocular micrometer line also lying along the corresponding edge of a stage micrometer line.
8. Record on the board the calibration of your assign microscope using the oil immersion objective.
9. Measure the diameter of the microscope field on low power, high dry, and oil immersion using the stage micrometer.
10. Return the stage micrometer to its case after carefully wiping off the oil.
11. Measure a rod-shaped bacterium, a coccus, a yeast cell, a protozoan, and a human red blood cell. Record the results.

Catalase and Porphyrin Detection Procedure:

Catalase Test

Materials:

Bacterial culture 24-48 hrs old, 3-5% H_2O_2

Procedure:

A. On a plate/slant
   1. To a plate or slant add 2 drops of H_2O_2
   2. Observe for the evolution of bubbles (catalase positive reaction)
B. On a slide
   1. Pick a colony from a plate with a wooden applicator stick (do not use a loop)
   2. Smear colony on a clean, dry slide and apply 5 drops of H_2O_2
   3. Observe for the evolution of bubbles (catalase positive reaction)

Acid-Fast Procedure

1. Prepare smears of organisms to be stained.
2. Heat fix the smears.
3. Cut or tear absorbent paper (bibulous paper) to fit the slide leaving one end for handling. Do not allow the paper to protrude beyond the slide, but the smears must be covered.
4. Place the slide on wire gauze on a ring stand.
5. Saturate the paper with carbolfuschin.
6. Heat the slides with a hand-held bunsen burner until steam can be seen rising from the surface. Alternately remove the burner and reheat the slide to maintain steaming for 3-5 minutes. As the paper begins to dry during the staining process add a drop or two of carbolfuschin to keep the slide moist. Adding too much stain will cool the slide (and drip on the bench). Overheating the slide or letting it dry will distort the cells. Under heating the slide will fail to stain acid-fast cells.
7. At the end of staining remove the paper with tweezers and wash the slide thoroughly.
8. Drain the slide.
9. Decolorize with acid-alcohol for 30 seconds.
10. Rinse, drain, and counterstain with methylene blue for 45 seconds.
11. Rinse, blot, and examine. First observe each organism on its separate smear. Then examine the mixed smear.
12. Acid-fast organisms will appear red and non-acid-fast organisms will be blue.

Simple Stain Procedure:

Procedure:

1. Clean and dry microscope slides thoroughly.
2. Flame the surface in which the smear is to be spread.
3. Flame the inoculating loop.
4. Transfer a loop full of tap water to the flamed slide surface.
5. Reflame the loop making sure the entire length of the wire that will enter the tube has been heated to redness
6. Remove the tube cap with the fingers of the hand holding the loop.
7. Flame the tube mouth.
8. Touch the inoculating loop to the inside of the tube to make sure it is not so hot that it will distort the bacterial cells; then pick up a pinhead size sample of the bacterial growth without digging into the agar.
9. Reflame the tube mouth, replace the can, and put the tube back in the holder.
10. Disperse the bacteria on the loop in the drop of water on the slide and spread the drop over an area the size of a dime. It should be a thin, even smear.
11. Reflame the inoculating loop to redness including the entire length that entered the tube.
12. Allow the smear to dry thoroughly.
13. Heat-fix the smear cautiously by passing the underside of the slide through the burner flame two or three times. Test the temperature of the slide after each pass against the back of the hand. It has been heated sufficiently when it feels hot but can still be held against the skin for several seconds. Overheating will distort the cells.
14. Stain the smear by flooding it with one of the staining solutions and allowing it to remain covered with the stain for the time designated below.
    Methylene blue - 1 minute
    Crystal violet - 30 seconds
    Carbol fuchsin - 20 seconds
    During the staining the slide may be placed on the rack or held in the fingers.
15. At the end of the designated time rinse off the excess stain with gently running tap water. Rinse thoroughly.
16. Wipe the back of the slide and blot the stained surface with bibulous paper or with a paper towel.
17. Place the stained smear on the microscope stage smear side up and focus the smear using the 10X objective.
18. Choose an area of the smear in which the cells are well spread in a monolayer. Center the area to be studied, apply oil directly to the smear, and focus the smear under oil with the 100X objective.
19. Draw the cells observed.

H and E: Method, Tips and Troubleshooting

The complete method for H&E staining is contained in the tables below, but we'll take a look at each of the stages in turn and explain the process which should help you should you need to troubleshoot.

Dewax and rehydrate

The first stage in any histological staining or immunohistochemistry method is to dewax your sections on the slides. The paraffin needs to be removed to enable the aqueous solutions to penetrate the fixed tissue. This is usually done using either xylene or 'Histoclear'. After the wax is removed (typically in three separate 5 minute immersions in dewaxing solution), the tissue needs to be rehydrated which is done through a decreasing series of alcohol solutions. In these first stages of an H&E protocol (or any immunohistochemical method) there's not much to troubleshoot- you should ensure that most of the xylene is drained from the rack and slides before placing them in the 100% alcohol and also ensure that the slides are exposed to the air for the minimum amount of time between solutions. For the tap water stages of this protocol, I would recommend using a container within the sink with constantly running water- this helps to contain the excess stains from the slides which would otherwise stain the whole sink blue and pink!

Got the blues

As mentioned in the previous article, haematoxylin is used to dye the nucleus and binds to the histones with the aid of the metal salt, otherwise known as the 'mordant'. A five minute submersion in haematoxylin is standard- this relatively long time ensures that all of the available nuclear binding sites are saturated. This method of staining is known as 'regressive' staining. The tissue is incubated for a long period to allow for deliberate over-staining and then some of the stain is removed in a step called 'differentiation'. The other method is 'progressive' staining where the solution of haematoxylin may not be as concentrated and the tissue is checked at regular intervals until the desired intensity is reached. Personally, I have always used the former method as it's probably the easiest and in the end is less time-consuming as you don't need to keep taking the slides from the haematoxylin, submersing them in tap water and checking the intensity using a microscope. When you take your slides out of the haematoxylin, all will be blue! Initially, the nuclei will be deep purple/blue, but the differentiation stage will render the stained nuclei dark blue. If you over-differentiate, fear not! Most of the stages of the H&E method can be repeated and the final colour can be adjusted.

Differentiation and Bluing

To achieve the blue coloured nuclei, there are two stages. The first is the differentiation stage. After the tap water rinse, the slides are submerged for a short period of time in an acid-alcohol solution which removes the excess staining. At this stage, the nuclei will be very dark and deep purple in colour. To achieve the classic H&E blue colour, the slides are rinsed then submerged in a solution called 'Scotts Tap Water'. This second stage is known as 'bluing'. The use of tap water (as opposed to distilled water) is important in these stages and bluing can be achieved with tap water alone (depending on the intensity of the staining following the haematoxylin stage). Tap water is slightly acidic (pH of around 6.0 to 6.8), but this is more alkaline than the pH of the haematoxylin which tends to be around pH 2.7. Two to five minutes in running tap water will remove most of the excess mordant giving sharp blue nuclear staining.

Eosin

The aim of H&E staining is to achieve a contrast between the deep blue nuclei and red/pink of the other cellular components. Eosin is used to stain cytoplasm, muscle fibres and so on. This pink counterstain also helps to differentiate between nuclei and non-nuclear components in cells. Don't be tempted to over-stain at this stage- always err on the side of caution. Over-staining can result in cells which are not immediately easy to differentiate. You can remove a certain amount of over-stain in running tap water. Some methods suggest that Eosin is diluted in ethanol, but I would recommend a solution in water as the ethanol has the potential to remove the haematoxylin staining from the previous stages.

Method

This is a basic method for H&E staining in which I have split into two sections: the dehydration/rehydration section (for rehydration following the eosin/tap water rinse, simply follow Step 9 to Step 1 before mounting the slides).

Dehydration/Rehydration

Step	Solution	Time	Notes
1	Xylene	5 mins	These steps should be carried out in a fume hood or downflow bench
2	Xylene	5 mins	
3	Xylene	5 mins	Drain off excess xylene before (4)
4	Absolute ethanol	20 secs	
5	Absolute ethanol	20 secs	
6	Absolute ethanol	20 secs	
7	95% ethanol	20 secs	
8	80% ethanol	20 secs	
9	70% ethanol	20 secs	
10	Tap water	2 mins	Running tap water in a sink

H&E Staining

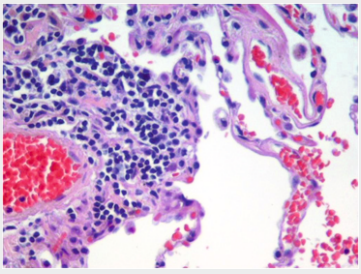
Step	Solution	Time	Notes
1	Harris' Haematoxylin	5 mins	Run control slides first to check times and staining.
2	Tap water	20 secs	Running tap water in a sink
3	1% Acid alcohol	5 secs	5 secs is maximum time in this solution
4	Scotts Tap Water	2 mins	Until tissue sections turn blue
5	Eosin	2 mins	
6	Tap water	20 secs	Running tap water in a sink, then check slides.

The nuclei in the cells should be dark blue whereas the cytoplasm will be pink. Fibrin and muscle will be deep pink and collagen will stain pale pink. Any erythrocytes will be red. Remember to allow any excess fluid to drain back into the staining dish before proceeding to the next step. Slides are mounted directly from the last xylene step.

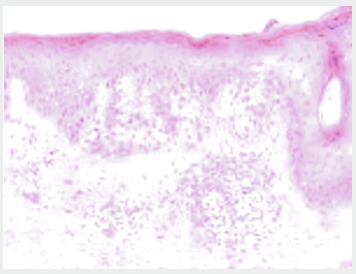
H & E Staining

H & E STAINING		
PROBLEM	**CAUSE**	**SOLUTION**
White spots are seen in the section after deparaffinization step. If they are not recognized at this point, spotty or irregular staining will be seen microscopically on the stained section.	**A.** The section was not dried properly before beginning deparaffinization. **B.** The slide did not remain in xylene long enough for complete removal of the paraffin.	**A.** The slides must be treated with absolute alcohol to remove the water and then retreated with xylene to remove the paraffin. If incomplete drying is severe, the sections may loosen from the slides. **B.** The slides should be returned to xylene for a longer time.
The nuclei are too pale (the hematoxylin is too light).	**A.** The sections were not stained long enough in hematoxylin. **B.** The hematoxylin was overoxidized and should not have been used. **C.** The differentiation step was too long. **D.** Pale nuclei in bone sections may be the result of over decalcification.	**A.** The section must be restained. When sections have been placed in an extremely acidic fixative such as Zenker solution, the ability to stain the nucleus may be impaired and the time in the hematoxylin may have to be increased, or a method to increase tissue basophilia may be needed. **B.** Discard hematoxylin and replace with fresh. **C.** Run back and restain **D.** No solution
The nuclei are overstained (the hematoxylin is too dark), or diffuse hematoxylin staining of the cytoplasm has occurred.	**A.** The sections were stained too long in hematoxylin. **B.** The sections are too thick. **C.** The differentiation step was too short.	**A.** Decolorize the section and restain, making appropriate adjustments in the staining time of hematoxylin. **B.** Recut the section. **C.** Decolorize the section and restain, making appropriate adjustments in the differentiation times.
Red or red-brown nuclei.	**A.** The hematoxylin is breaking down. **B.** The sections were not blued sufficiently.	**A.** Check the oxidation status of the hematoxylin. **B.** Allow a longer time for bluing of the sections; it is impossible to over blue the sections.
Pale staining with eosin.	**A.** The pH of the eosin solution may be above 5.0, possibly caused by carryover of the bluing reagent. **B.** The sections may be too thin. **C.** Slides may have been left too long in the dehydrating solutions.	**A.** Check the pH of the eosin solution, and adjust it to a pH of 4.6 to 5.0 with acetic acid if necessary. Be sure the bluing reagent is completely removed before transferring the slides to the eosin. **B.** Check the thickness of the section. **C.** Restain with eosin and do not allow the stained slides to stand in the lower concentrations of alcohols.
Cytoplasm is overstained, and the differentiation is poor.	**A.** The eosin solution may be too concentrated, especially if phloxine is present. **B.** The section may have been stained for too long. **C.** The sections may have been passed through the dehydrating alcohols too rapidly for good differentiation of the eosin to occur.	**A.** Dilute the eosin solution. **B.** Decrease the staining time. **C.** Allow more time in each of the dehydrating solutions for adequate differentiation of the eosin. (Also, check the section thickness.)

H & E STAINING		
PROBLEM	**CAUSE**	**SOLUTION**
Blue—black precipitate on top of the sections.	The metallic sheen that develops on most hematoxylin solutions has been picked up on the slide.	Filter the hematoxylin solution daily before staining slides.
Water bubbles are seen microscopically in the stained sections.	The sections were not completely dehydrated, and water is present in the mounted section.	Remove the cover glass and mounting medium with xylene. Return the slide to fresh absolute alcohol (several changes). After the sections are dehydrated, clear with fresh xylene and mount with synthetic resin. All dehydrating and clearing solutions should be changed before staining any more sections.
Difficulty bringing some areas of the tissue in focus with light microscopy.	Mounting medium may be present on top of the cover glass.	Remove the cover glass and remount with a clean cover glass. Review the method used for mounting sections, and modify if needed.
The mounting medium has retracted from the edge of the cover glass.	**A.** The cover glass is warped. **B.** The mounting medium has been thinned too much with xylene.	**A.** Remove the cover glass and apply a new cover glass. **B.** Apply a new cover glass with fresh mounting medium. Keep the mounting medium container tightly capped when not in use. Use a small container for the mounting medium and discard when it becomes too thick.
The water and the slides turn milky when the slides are placed in the water following the rehydrating alcohols.	Xylene has not been removed completely by the alcohols.	Change the alcohols, back the slides up to absolute alcohol, and rehydrate the sections.
The slides are hazy or milky in the last xylene rinse prior to coverslipping.	Water has not been completely removed from the sections before being placed in the xylene.	Change the alcohol solutions, especially the anhydrous or absolute reagents. Redehydrate the sections and clear in fresh xylene.
The mounted stained sections do not show the usual transparency and crispness when viewed by light microscopy.	The mounting medium may be too thick, causing the cover glass to be held too far above the tissue.	Remove the cover glass and mounting medium with xylene. Remount the section with fresh mounting medium.



Good H&E staining



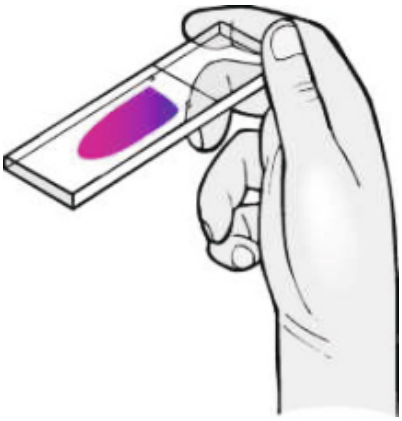
Under stained H&E stain

The Pap Stain

PROBLEM	POSSIBLE REASON	REMEDY
DARK NUCLEI	Too much time in Harris' Hematoxylin. Not enough time in HCl or HCl concentration less than recommended.	Reduce time in HTX by 10,15,20,30 sec intervals. Increase time in acid by 5,10 sec.
PALE NUCLEI	Polyethylene glycol coating not removed from cells prior to Hematoxylin. Concentration of HCl greater than recommended or too much time in HCl. Not enough time in Hematoxylin. Hematoxylin diluted by water (if water not properly drained from slides). Stain not changed frequently enough resulting in Hematoxylin exhausted.	Extend prestaining soak with aqueous ethanol. Reduce time in acid by 5,10 sec and ensure correct amount of acid is added to the solution. Increase time in HTX by 10,15,20,30 sec intervals. Ensure the arm of the staining machine is operating correctly. Ensure a set amount of slides are stained and then stains are changed.
CYTOPLASMIC COLOUR NOT CONSISTENT	Air drying prior to fixation. Polyethylene coating inadequately removed from cells. Solutions not at proper level within staining dishes. Excessive time in Hematoxylin or Hematoxylin not removed prior to OG and EA dyes. Slides left too long in ethanol rinses or clearing solutions following OG and EA. Inadequate rinsing of slides between solutions. Insufficient rinsing following staining solutions. pH of tap and distilled water not sufficiently alkaline. pH of EA needs to be controlled (pH 4.5 to 5 achieves maximum results).	Report the findings to the referring clinician. Extend prestaining soak in aqueous ethanol. Check staining solution level. Reduce time in HTX by 10,15,20,30 sec intervals. Reduce ethanol rinse time. Check if ethanol is changed regularly. Increase ethanol rinse time. Check pH. Check pH. Ensure a set amount of slides are EA dye exhausted stained and then stains are changed.
MACROSCOPICALLY ALL SLIDES ARE PINK, ORANGE OR YELLOW	Slide drying oven temperature too high.	If this happens there is nothing that can be done to obtain a well-stained sample.
DULL PINK AND DEGENERATE APPEARANCE	This usually occurs to smears that accompany histology specimens. It is usually due to formalin fixation.	Ensure formalin pot and smear is transported in separate bags.
DULL, GREYISH APPEARANCE OF CELLS	Water contamination of dehydrating and clearing solutions. Polyethylene glycol coating not removed from cells prior to staining of filter.	Ensure dehydrating and clearing solutions are changed regularly. Extend the prestaining soak time.
OPAQUE/WHITE COLOUR ON BACK OF SLIDE	Bluing agent not rinsed from slides. Water rinses following Scott's Tap.	Use two separate but thorough water substitute. (For progressive Pap staining)
STAIN DEPOSIT	Staining dyes not changed or filtered properly.	Ensure staining dyes changed or filtered regularly.
FUNGAL CONTAMINANTION	Slides contaminated by fungus during the staining process.	Change staining solutions regularly and ensure the staining containers are disinfected with a dilute bleach solution.

Preparing Peripheral Blood Smears

The Peripheral Blood Smear



A peripheral blood smear (peripheral blood film) is a glass microscope slide coated on one side with a thin layer of venous blood. The slide is stained with a dye, usually Wright's stain, and examined under a microscope. Microscopic examination of the peripheral blood is used to supplement the information provided by automated hematology analyzers ("blood cell counters"). Hematology analyzers provide accurate quantitative information about blood cells and can even identify specimens with abnormal cells. However, the precise classification of abnormal cells requires a trained microscopist, a well-made peripheral blood smear, and a light microscope with good optical characteristics. In practice, hematology analyzers of varying sophistication are used for cell counting in all but the smallest hematology laboratories. In addition to providing cell counts and graphical displays of the information recovered, these instruments also provide a warning ("flag") that atypical cells were found and provide a presumptive identification of the abnormality. The instrument operator reviews the information from each specimen and decides if smear preparation and light microscopy are necessary. If not, the information is released to the clinician.

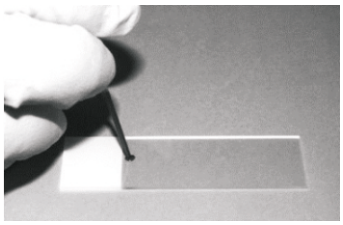
Peripheral Blood Smear Preparation

The wedge slide ("push slide") technique developed by Maxwell Wintrobe remains the standard method for the preparation of peripheral blood smears (films). The following procedure (Fig. 1) is utilized to prepare a peripheral smear.

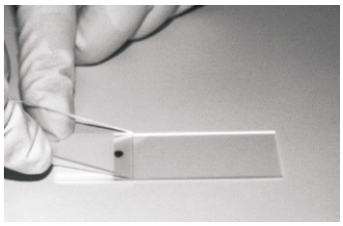
- Place a 1" x 3" glass microscope slide with a frosted end on a flat surface (usually the counter top of a laboratory bench).
- Attach a label on the slide or write the patient name, specimen identification number, and date of preparation on the frosted surface.
- Place a 2 - 3 mm drop of blood approximately 1/4" from the frosted slide, using a wooden applicator stick or glass capillary tube.
- Hold the slide by the narrow side between the thumb and forefinger of one hand at the end farthest from the frosted end.
- Grasp a second slide ("spreader slide") between the thumb and forefinger of the other hand at the frosted end.
- Place the edge of the spreader slide on the lower slide in front of the drop of blood (side farthest from the frosted end).
- Pull the spreader slide toward the frosted end until it touches the drop of blood. Permit the blood to spread by capillary motion until it almost reaches the edges of the spreader slide.
- Push the spreader slide forward at a 30° angle with a rapid, even motion. Let the weight of the slide do the work.

Table 1: Preparation of Peripheral Blood Smear

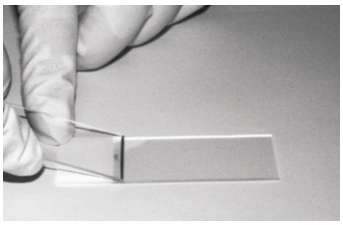
Step 1. Placing a small drop of venous blood on a glass microscope slide, using a glass capillary pipette. A wooden applicator stick can also be used for this purpose.



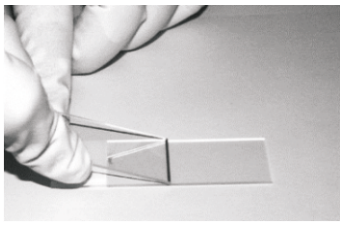
Step 2. A spreader slide has been positioned at an angle and slowly drawn toward the drop of blood.



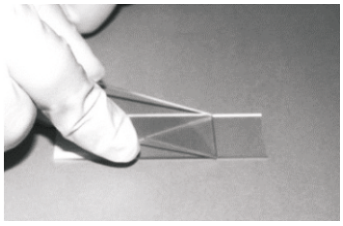
Step 3. The spreader slide has been brought in contact with the drop of blood and is being drawn away. Note layer of blood at the edge of the spreader slide.



Step 4. The spreader slide is further pulled out, leaving a thin layer of blood behind.



Step 5. The blood smear is nearly complete.



Step 6. End result. A glass slide with a well-formed blood film. After drying for about 10 minutes, the slide can be stained manually or placed on an automated slide stainer.

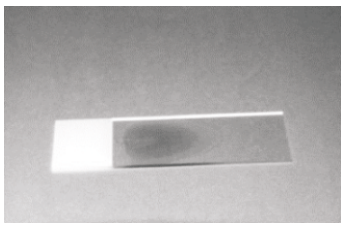


Fig. 1. Wedge slide technique for preparation of a peripheral blood smear.

A well-made peripheral smear is thick at the frosted end and becomes progressively thinner toward the opposite end. The "zone of morphology" (area of optimal thickness for light microscopic examination) should be at least 2 cm in length. The smear should occupy the central area of the slide and be margin-free at the edges (Fig 2).

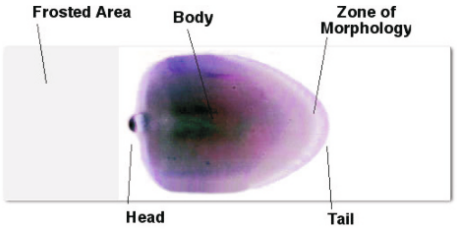


Fig. 2. Photograph of the peripheral blood smear prepared above. The arrow points to the zone of morphology.

Motility Testing

Hanging Drop Procedure

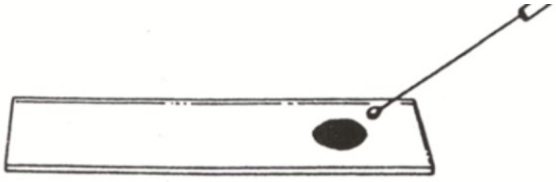
1. Hold a clean coverslip by its edges and carefully dab Vaseline on its corners using a toothpick. If too much Vaseline is used, it will be squeezed toward the center and mix with the drop or squeeze out the edges and get on the objective lens of the microscope.
2. Place a loopful of the culture to be tested in the center of the prepared coverslip.
3. Turn the clean concavity slide upside down (concavity down) over the drop on the coverslip so that the Vaseline seals the coverslip to the slide around the concavity.
4. Turn the slide over so the coverslip is on top and the drop can be observed banging from the coverslip over the concavity.
5. Place the preparation in the microscope slide holder and align it using the naked eye so an edge of the drop is under the low power objectives.
6. Turn the objective to its lowest position using the coarse adjustment and CLOSE THE DIAPHRAGM.
7. Look through the eyepiece and raise the objective slowly using the coarse adjustment knob until the edge of the drop is observed as an irregular line crossing the field.
8. Move the slide to make that line (the edge of the drop) pass through the center of the field.
9. Without raising or lowering the tube, swing the high dry objective into position (Be sure the high dry objective is clean).
10. Observe the slide through the eyepiece and adjust the fine adjustment until the edge of the drop can be seen as a thick, usually dark line.
11. Focus the edge of the drop carefully and look at each side of that line for very small objects that are the bacteria. The cells will look either like dark or slightly greenish, very small rods or spheres. Remember the high dry objective magnifies a little less than half as much as the oil immersion objective.
12. Adjust the light using the diaphragm lever to maximize the visibility of the cells.
13. Observe the cells noting their morphology and grouping and determine whether true motility can be observed.
14. Brownian movement should be visible on slides of all the organisms, but two should also show true motility.
15. Wash the depression slide and after soaking in lysol buckets.

NOTE: The bacteria are still alive in a hanging drop slide. Slides made from possible pathogens should be soaked in lysol for 5-10 minutes with the coverslip pulled aside to expose the drop before they are washed.

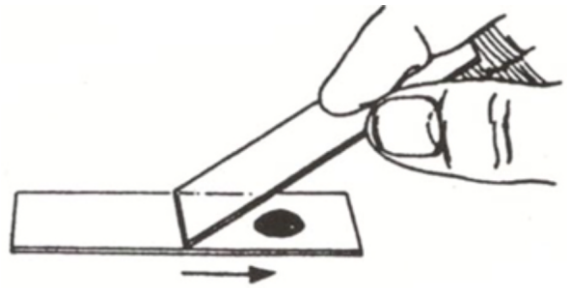
Negative Stain Procedure

To conduct a proper negative stain the following procedure should be followed:

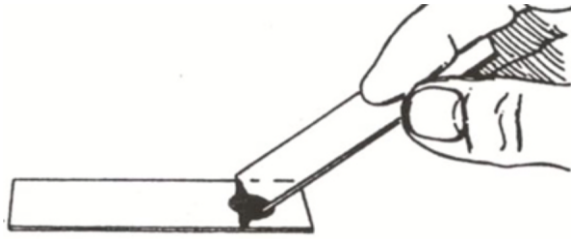
1. Place a very small drop (more than a loop full--less than a free falling drop from the dropper) of nigrosin near one end of a well-cleaned and flamed slide.
2. Remove a small amount of the culture from the slant with an inoculating loop and disperse it in the drop of stain without spreading the drop.

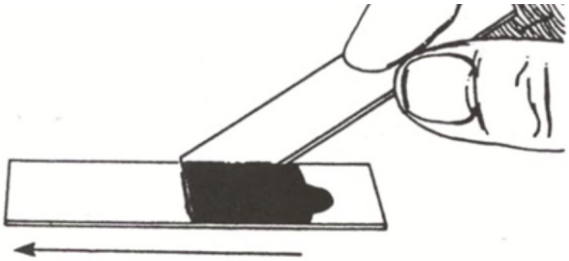


3. Use another clean slide to spread the drop of stain containing the organism using the following technique.



4. Rest one end of the clean slide on the center of the slide with the stain. Tilt the clean slide toward the drop forming an acute angle and draw that slide toward the drop until it touches the drop and causes it to spread along the edge of the spreader slide. Maintaining a small acute angle between the slides, push the spreader slide toward the clean end of the slide being stained dragging the drop behind the spreader slide and producing a broad, even, thin smear.





5. Allow the smear to dry without heating.

6. Focus a thin area under oil immersion and observe the unstained cells surrounded by the gray stain.

Flagella Stain Procedure:

Procedure:

A. Bacterial Suspension

From an agar slant culture: Suspend a loopful of bacteria in 2 ml distilled water to obtain an opalescent suspension. Allow the suspension to stand undisturbed for 15 to 20 minutes while flagella are regenerated and extended.

B. Slides

Select new or unscratched slides. Clean slides with cleaning powder as directed. Flame one of the slides and allow it to cool. Make a heavy wax line on the flamed side along the margin of one end, along both sides to within about one inch of the other end, and across the slide to complete the rectangle. Handle the slide by the unmarked end only. The wax line creates a retaining wall to allow a pool of stain to surround the cells during the 15 to 15 minute staining period.

C. Preparation of smear

Handling the suspension carefully remove a large loopful of the suspension and place it at one end of the rectangular area. Tilt the slide to permit the suspension to run down to the other end of the slide. If the drop fails to run, add another drop. Air dry the film. Do not heat. Place the slide on a horizontal staining rack.

D. Staining procedure

Add about 1 ml. of the Flagella Stain solution (one dropper full) to the smear and allow to stain for 10-15 minutes. The solution should not be allowed to flow outside the waxed area. Flood off the stain by adding tap water to the slide while it remains on the rack. Do not tip the slide before this is done. Drain and flood the slide with carbol fuchsin for one minute. Rinse by flooding. Drain and air dry. Do not blot.

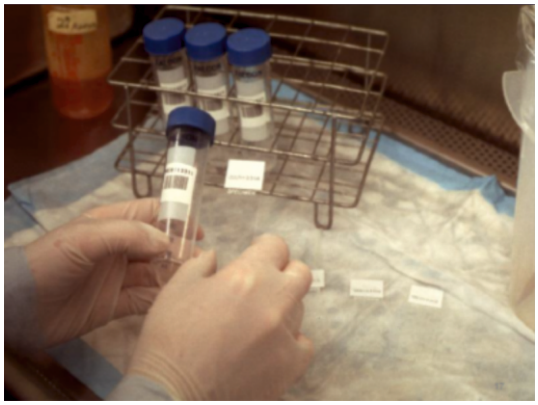
E. Examination of slide

With the naked eye identify the line made by the drop as it ran down the slide. Position the slide so that the edge of the run is under the objective. Focus the edge of the run under low power, as oil, and examine it using the oil immersion objective. Identify cells as distinguished from dye and debris which will adhere to the slide because of the mordant. Cells will be small rods and have regular outlines. They will be more plentiful near the lower end of the run and may look larger than usual because of the mordant. Once cells are identified follow along the edge of the run, examining cells until some are found with flagella attached. Flagella are much longer than the cell and often look like faint hairs. Ideally, cells should be located which are isolated enough to determine unequivocally the arrangement of the flagella and, for the Pseudomonas, the number of flagella per cell. Draw the cells and their flagella.

Steps in Performing AFB Microscopy

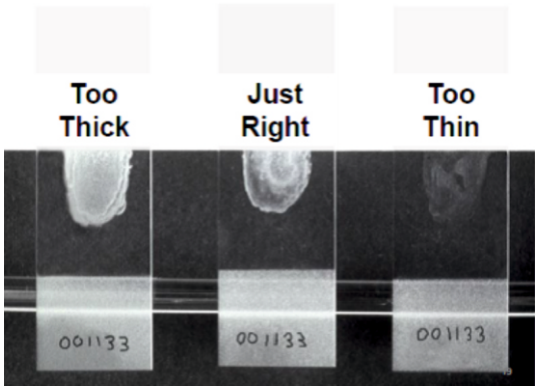
1. Preparing and Fixing Smears
2. Staining Smears
3. Examining Smears
4. Recording and Reporting Results

- New, clean, greaseless, and unscratched slides should be used.
- Match identifiers on slide with clinical specimens.
- Labeling should be performed with material that stays permanently affixed to the slide during the staining procedure (e.g., graphite or diamond tip pencil).



Preparing Smears

- After processing and concentrating the specimen, 1 to 2 drops of material should be smeared on the slide.
- Smear material in an area of approximately 2-cm^2.



Fixing Smears

- Prior to heat fixing, smears should be allowed to air dry completely within the biological safety cabinet
- Acceptable methods for heat fixing
- Flame fixing by passing over flame 2–3 times for a few seconds smear side up – Avoid scorching
- 2 hrs minimum at 65-75°C on an electric slide warmer
- 15 min at 80°C
- 5% phenol in 70% EtOH for 5 min^2 – also kills AFB

Considerations:

- Flame fixing may aerosolize organisms from smear
- Insufficient heat or time can lead to smear washing off
- Slide warmers may not heat evenly
- Viable AFB remain with some fixing methods.

Heat Fixing Smear

Safety Concerns:

- Wear gloves at all times
- Work inside the BSC
- Use open flame for shor periods of time

AFB Staining Principles

- Primary stain penetrates cell wall
- Intense decolorization does not release primary stain from the cell wall of AFB
- Color of AFB-based on primary stain
- Counterstain provides contrasting background

Stains Used in Fluorescence Microscopy

- Primary Stains
  - Auramine O
  - Auramine O-Rhodamine-B
- Counter Stains
  - Potassium Permanganate
  - Acridine Orange

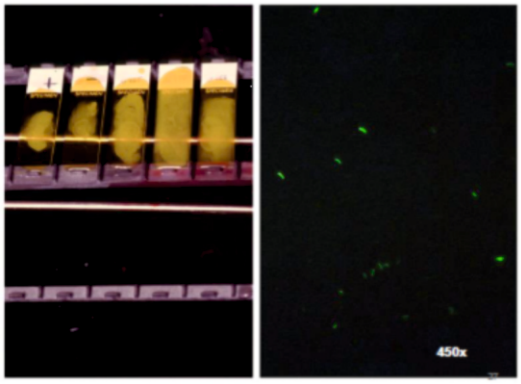
Fluorescence AFB Microscopy

- Primary fluorochrome
  –Auramine O
  –Auramine O-Rhodamine B
- Counter Stain
  –Potassium permanganate

Fluorescence AFB Microscopy

- **Primary Fluorochrome**
  –Auramine O
  –Auramine O/ Rhodamine B
  –Acridine Orange

- **AFB Fluorescence**
  –Green
  –Yellow/Orange



Water Quality is Key

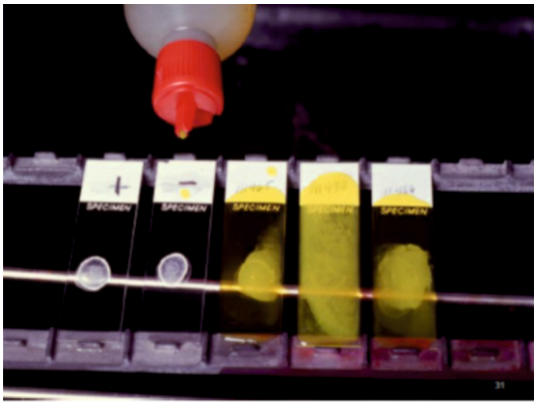
- AFB microscopy is not specific
  –Acid fast environmental contaminants as well as NTM and MTB presence in the specimen will be detected
- Introduction of an environmental contaminant during wash steps and in reagent preparation must be avoided
- Use of a negative control slide essential for detecting potential environmental contaminants
- Avoid using large containers of reagents and carboys of water
- Use filtered distilled or deoinized water
- Water filtration and distribution systems should be monitored.

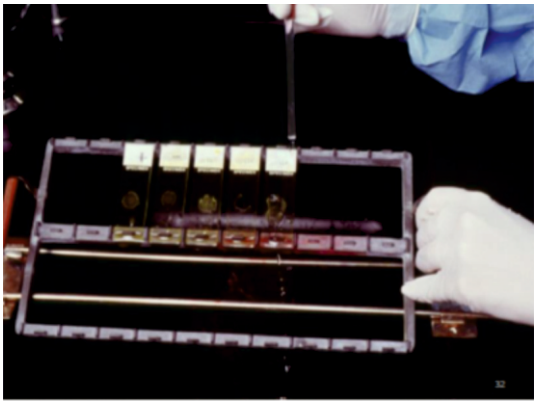
Staining Reagents

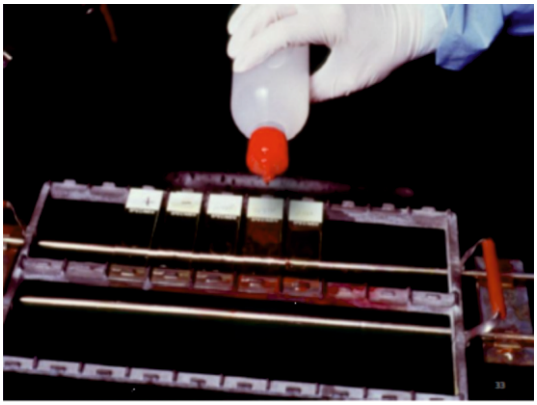
- Commercial products are available or reagents can be prepared in-house
  –If prepared in-house, proper precautions must be taken when handling dyes including appropriate PPE and the use of a fume hood
- Reagents containing fluorescence stains should be stored to protect from light exposure.

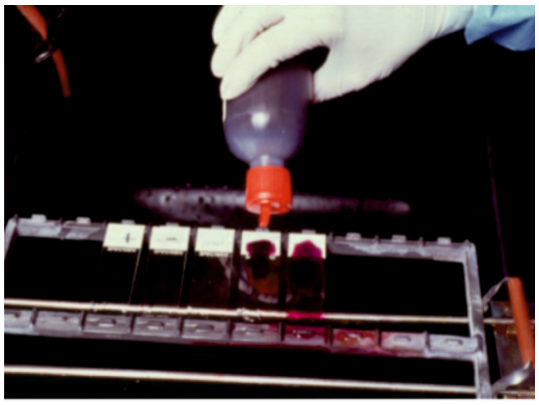
Steps in the Fluorescent Staining Procedure

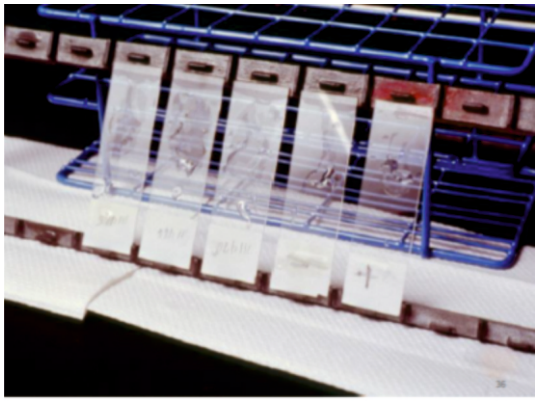
1. Place slides on staining rack; slides should not touch.
2. Flood slides with fluorochrome stain; no heating. Follow protocol or package insert for timing.
3. Rinse with water; aim flow at edge of slide.
4. Decolorize with 0.5% acid-alcohol solution, Follow protocol or package insert for timing.
5. Rinse with water; drain excess.
6. Flood slide with counterstain; Follow protocol or package insert for timing.
7. Rinse with water; drain excess.
8. Air-dry stained slide; do not blot.

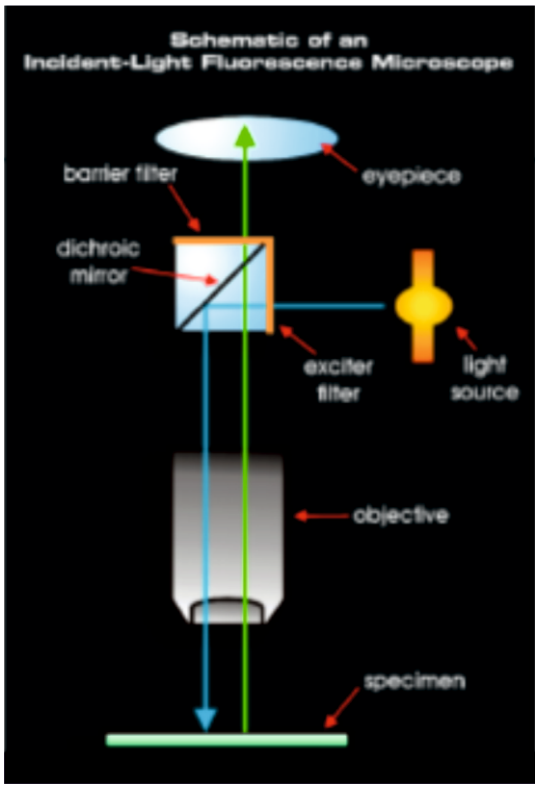








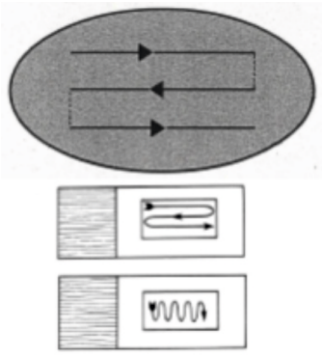




Fluorescence Microscopy

- A fluorescence microscope is required for examining fluorochrome-stained smears:
  - Mercury vapor or halogen bulb light source (about 150 hours of use)
  - Newer mercury bulbs (about 2,000 hours of use)
  - LED Bulbs (about 15,000 hours of use)
  - Excitation and emission (barrier) filters are necessary for visualization of the fluorescently-stained smear (specific to the staining method used.)
- LED-based Fluorescent Microscopy
  - LED modules used to adapt light microscopes for reading fluorescently-stained smears
- May be useful in low income settings
- More research is needed to evaluate performance.

Systematic Examination of Smears



Whichever method you use, BE CONSISTENT!

Number of Fields to Examine

Magnification [a]	Number of Fields [b]
200x	30
250x	30
400x	55
450x	70

[a] This final magnification represents the objective lens magnification multiplied by the eyepiece magnification

[b] The minimum number of fields to examine before reporting a smear as negative for acid-fast organisms.

Examining Smears for AFB

- AFB will be rod-shaped, 1–10 µm in length and 0.2–0.6 µm wide
- Appearance is generally long and slender but may also appear bent
- Bacilli may contain heavily stained areas called beads
- Count a clump of bacilli that are touching as one
- Debris, some species of the genera *Nocardia* and *Corynebacterium*, and some fungal spores may appear acid fast.

ANALYTE STABILITY

TroubleShooting Guidelines

A Compilation from

The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

Analyte Stability

Old friends and old wine are said to be great, however, the same cannot be said about old reports. Every analyte has a limited half-life and therefore, a sample withdrawn from the body cannot be stored indefinitely in any which way. Multiple problems are being faced in the current scenario of centralized sample reporting once (where samples are collected a thousand kilometers away from the place of reporting). The testing laboratory usually absolves itself of all responsibilities. Ideally a sample must be processed immediately after it is obtained. If a particular investigation is available in your city, get it done from there, by doing so you reduce the complications arising out of transportation (time, temperature, contamination related problems). It is better to omit then to commit wrongly. Many a times, if possible, it may be better to refer the patient instead of the sample. A stability calendar of few important analytes is provided for reference to minimize aberrations arising out of limited analyte stabilities.

Analyte	Stability in Serum / Plasma			Stabilizer	Analytical Levels Affected by
	-20 °C	4 - 8 °C	20 - 25 °C		
Acid phosphatase, Prostatic	Without stabilizer			5 mg $NaHSO_4$ /ml serum or 20µl/ml serum or 20µl 10% acetic acid / ml serum	Serum > Plasma Add stabilizer after separation of serum Hemolysis Increase Freezing & Thawing - Increases
	1 day	8 hours	2 hours		
	Stabilized to pH 4-5				
	4 months	8 days	8 days		
Adrenocorticortropic hormone (ACTH)	6 weeks	Unstable	2 days	Aprotinin 400 kU/ml mercaptoethanol 2 µl/ml	Diurnal variation,time of collection important
Alanine aminotransferase (ALAT, ALT)	1 day	7 days	3 days		Does not withstand freeze-thaw cycles Significant reduction on heat exposure 11% loss in 1day at 20°C, 20% in 1 week
Albumin	3 months	3 months	3 months		Affected by hemolysis, lipemia and bilirubin
Aldosterone	4 days	4 days	4 days	EDTA	Does not withstand freeze-thaw cycles
Alkaline phosphatase – Total	2 months	7 days	7 days		Affected by heat exposure, hemolysis
Alkaline phosphatase – Bone	2 months	7 days	7 days		
Aluminium	1 year	2 weeks	1 week		
Ammonia	3 weeks	2 hours	15 minutes	Serine 5 mmol/l and Borate 2 mmol/l	Easily contaminated by sweat Ammonia 3 fold Increase if stored at R.T. for more than 24 hours
	Stabilized by Serine and Borate				
α-Amylase (AMYL) – Total	1 year	7 days	7 days		Affected by hemolysis and lipemia Contaminated by saliva while pipetting -↑
α-Amylase (AMYL) – Pancreatic	1 year	7 days	7 days		
Androstenedione	1 year	4 days	1 day		↓ with storage at 22 °C more than 1 day
Anti-Cardiolipin antibodies	1 month	1 week	2 days		Cannot withstand freeze-thaw cycles -↓
Antinuclear antibodies (ANA)	?	1 week	2 days		
Anti-Phospholipid antibodies	1 month	1 week	1 day		Increases on exposure to heat - negative samples may become positive Affects more IgG than IgM antibodies
Antistreptolysin	?	2 days	2 days		
α1- Antitrypsin	3 months	3 months	7 days	Heparin plasma recommended	EDTA and citrate decreased
Apolipoprotein A-1		3 days	1 day		Do not freeze
Apolipoprotein B		3 days	1 day		Do not freeze
Aspartate Aminotransferase (ASAT, AST)	2 weeks	7 days	4 days		Cannot withstand freeze-thaw cycles Affected by hemolysis and lipemia
Bicarbonate	6 months	7 days	1 day	Keep closed	1 hour after opening the tube
Bilirubin – Conjugated	6 months	7 days	2 days	Darkness required when stored > 8hours	Delay in separation of cells significant reduction Heat exposure, hemolysis, light decrease
Bilirubin – Total	6 months	7 days	1 day		
C-peptide	4 weeks	3 days	5-6 hours		
C-reactive protein (CRP)	3 years	8 days	3 days		
C3 complement	8 days	8 days	4 days		Dependent on antibody during storage, C3 increases, C3 decreases
C4 complement	?	2 days	2 days		
CA 125	3 months	5 days	3 days		
CA 15-3	3 months	5 days	1 day		Increase in patients with renal failure
CA 19-9	3 months	5 days	1 day		Increase in liver transplantation
CA 72-4	3 months	7 days	1 day		
Cadmium	?	?	?	Special tube	Released from red stopper

Analyte		Stability in Serum / Plasma			Stabilizer	Analytical Levels Affected by
		-20 °C	4 - 8 °C	20 - 25 °C		
Calcitonin		?	?	?	Aprotinin 400 kU/ml	
Calcium	Total	8 months	3 weeks	7 days	Use Ca-titrated heparin : Keep tight	pH-dependent , Whole blood recommended only. Affected by hair treatments, Affected by bilirubin, EDTA, hemolysis, lipemia, freeze-thaw cycles
	Ionized (actual)		2 hours			
Carbohydrate deficient transferrin (CDT)		Years	7 days			
Carcinoembryonic antigen (CEA)		6 months	7 days	1 day		Stable upto 3 freeze-thaw cycles False elevation due to HAMA
Catecholamines Noradrenaline Adrenaline Dopamine		4 weeks	2 days	1 day	Glutathione + EDTA, 1.2 mg/ml	Special tube necessary EDTA plasma separated within 15 min and frozen at - 20°C
Ceruloplasmin		2 weeks	1 week	Unstable		Affected by hemolysis
Chloride		Years	3 days	1 day		Affected by glassware if chloride present Affected by pH, hemolysis, lipemia, delay in processing sample, heat
Cholesterol	Total	3 months	7 days	7 days		Affected by EDTA, hemolysis, lipemia
	HDL	3 months	7 days	2 days		
	LDL	3 months	7 days	1 day		
Cholinesterase (CHE)		3 months	17 days	17 days		Affected by benzene, citrate, fluoride, oxlate, pH
Copper		Years	2 weeks	2 weeks	Special tube	Contamination
Cortisol		3 months	4 days	1 day		Reduce on freeze-thaw Affected by heat exposure, hemolysis
Creatine kinase (CK)	Total	4 weeks (Dark)	7 days	2 days	Darkness	Affected by freeze-thaw cycles, heat exposure, hemolysis, lipemia CK-MB not stable without stabilizer including immunoassay
	MB (CK-MB)	4 weeks (Dark)	7 days	2 days	SH reagent	
Creatinine		3 months	7 days	7 days		Affected if not separated immediately
CYFRA 21-1		6 months	4 weeks	2 days		
Dehydroeprandrosterone sulfate (DHEA-s)		Years	2 weeks	1 day		Levels when whole blood stored
Erythropoetin		?	?	2 weeks		Shipped frozen
Estradiol		1 year	3 days	1 day		Decreased if not separated immediately Affected by heat exposure
Estriol		1 year	2 days	1 day		Cross reactivity with estradiol
Ethanol		?	6 months	2 weeks	EDTA / Heparin	Evaporation, closed tubes
Fatty acids,free		2 days	12 hours	30 minutes		Freeze serum immediately
Ferritin		1 year	7 days	3 days		Affected by lipemia and turbidity
α-Fetoprotein (AFP)		3 months	3 days	3 days		Affected by freeze-thaw cycles, lipemia, heat exposure
Follitropin (FSH)		1 year	2 weeks	1 week		Affected by citrate, EDTA and incomplete clotting, 3 freeze thaw cycles
Free Thyroxine (FT4)		3 months	8 days	2 days		Affected by EDTA, heat exposure, heparin, intralipid infusion
Free Triiodothyronine (FT3)		3 months	2 weeks	1 day		Affected by fatty acids & heat exposure
Fructosamine		2 months	2 weeks	3 days		Affected by EDTA, bilirubin
Gastrin		?	?	1 week		Serum frozen as soon as possible
Glucose, blood, (Capillary)		Hemolysate Stabilized			Fluoride, Monoiodoacetate, Mannose	Hemolysis Loss by non-enzymatic glycation (Protein matrix)
		?	2 days	2 days		
Glucose, plasma (Venous)		Stabilized				
		1 day	7 days	1 day		
Glutamate dehydrogenase		2 weeks	7 days	7 days		

It is beyond the scope of a forum like this to present stability calendar of all known analytes of importance and relevance to human beings. An effort was made in the last communiqué towards this direction. The remaining commonly tested for analytes are covered in this issue and are presented below.

Analyte	Stability in Serum / Plasma			Stabilizer	Analytical Levels Affected by
	-20 °C	4 - 8 °C	20 - 25 °C		
Lead	?	?	7 days		Special tubes
Lipase	1 year	7 days	7 days		heat exposure,EDTA, hemolysis, bilirubin
Lipoprotein (a) - Lp(a)	3 months	2 weeks	?		Affected by lipemia, turbidity
Lutropin (LH)	1 year	3 days	1 day		Affected by heat exposure, EDTA, HAMA
Magnesium	1 year	7 days	7 days		Affected by hair treatments
Myoglobin	3 months	1 day	2 hours		Affected by EDTA, lipemia
Neuron-specific enolase	3 months	3 days	?	Heparin Plasma	Serum > plasma (Platelets, hemolysis)
Osmolarity	3 months	1 day	3 hours		Gycolysis, citrate
Osteocalcin	Stabilized 14 days	?	Unstable	EDTA (5 mmol/l) and aprotinin (2500 KU/ml)	Bilirubin, citrate, EDTA, Freeze-thaw cycle, hemolysis
Parathyroid hormone (PTH)	?	1 day	6 hours	EDTA	Delay in freezing, separation of cells
Phosphate, inorganic	1 year	4 days	1 day		Bilirubin, fluoride, lipemia
Potassium	1 year	1 week	1 week		Serum>plasma
Progesterone	1 year	3 days	1 day		Blood collection tubes, Cross reactivity with hydroxyprogesterone & lysophosphatidylcholine
Prolactin	1 year	3 days	1 day		Bilirubin, heat exposure Cross reaction with Growth hormone
Prostatic-specific antigen (PSA)	3 months	2 days	1 day		Affected by rectal examination, transrectal biopsy
Protein electrophoresis	3 weeks	3 days	1 day		
Protein, total	Years	4 weeks	6 days		Plasma> serum (fibrinogen)
Rheumatoid factor (RF)	4 weeks	3 days	1 day		
Selenium	1 year	2 weeks	1 week		Contamination
Sodium	1 year	2 weeks	2 weeks		Bilirubin, heparin, lipemia, pH
Testosterone	1 year	3 days	1 day		SHBG, Androstenedione, DHT, Heat exposure, delay in separation of cells
Thyroglobulin	4 weeks	3 days	1 day		
Thyrotropin (TSH)	3 months	3 days	1 day		Alkaline phosphatase, EDTA, HAMA Freeze-thaw cycles, heat exposure
Thyroxine (T4)	4 weeks	7 days	2 days		Freeze-thaw cycles, Citrate, hemolysis, oxalate
Transferrin	6 months	8 days	8 days		Bilirubin, Urea, heat exposure
Triglycerides	Years	7 days	2 days		Decrease of triglycerides, of ↑ free glycerol, but only minor ↑ of total glycerol
Triiodothyronine (T3)	3 months	8 days	2 days		Freeze-thaw cycles, Citrate, protein, heat exposure
Troponin T	3 months	1 day	?		Citrate, heparin, EDTA
Urea	1 year	7 days	7 days		
Uric acid	6 months	7 days	3 days		Bilirubin, cyanide, EDTA, formaldehyde, hemolysis, lipemia, fluoride
Vitamin A	2 years	4 weeks	?		Light (decrease)
Vitamin B1 (thiamin)	1 year	?	?		
Vitamin B2 (riboflavin)	4 weeks	?	?		Light (decrease)
Vitamin B6 (pyridoxal phosphate)	Days	Hours	30 minutes	EDTA plasma darkness	Light (decrease)
Vitamin B	128 weeks	4 hours	15 minutes	EDTA, Darkness	Light (decrease)
Vitamin C	3 weeks	3 hours	?	Metaphosphate 63 mg/ml deproteinized	
	Only with stabilizers				
Vitamin D - 1,25 Dithydroxy cholecalciferol	?	?	2 days		
Vitamin E (tocopherol)	1 year	4 weeks			
Vitamin K (transphyllochinone)	3 months	Unstable	?		UV Light (decrease), use extraction
Zinc	1 year	2 weeks	1 week	Special tubes	Contamination from stoppers

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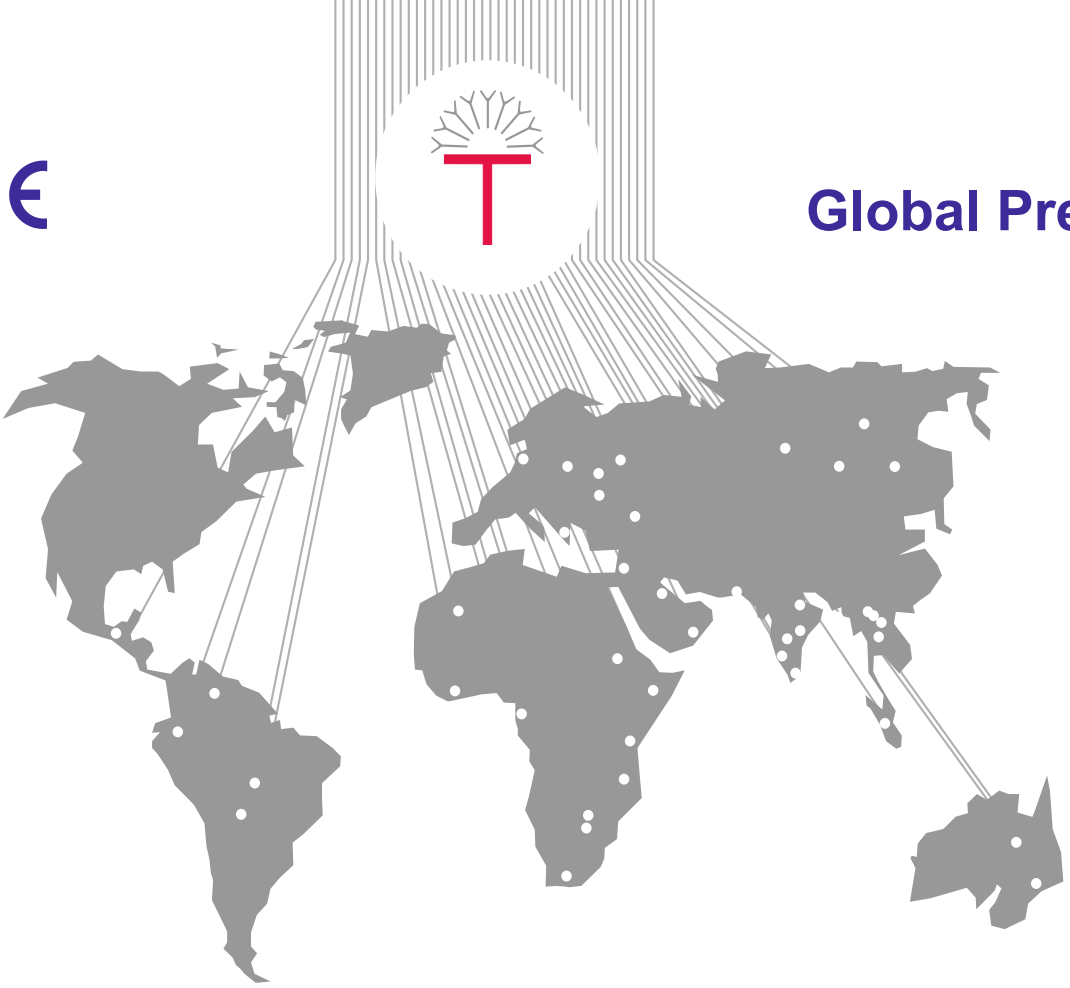
Global Presence



Africa	Cambodia	Belgium	Russia	Papua New Guinea
Angola	China	Denmark	Ukraine	Santiago
Burkina Faso	Indonesia	France	Middle East	Solomon Islands
Cameroon	Iran	Germany	Jordan	Venezuela
Egypt	Japan	Greece	Lebanon	
Ethiopia	Laos	Hungary	Muscat	
Ghana	Malaysia	Ireland	UAE	
Kenya	Mongolia	Italy	Qatar	
Mauritius	Myanmar	Netherlands	South America	
Morocco	Nepal	Poland	Brazil	
Mozambique	Philippines	Portugal	Colombia	
Nigeria	Sri Lanka	Romania	Guyana	
South Africa	Taiwan	Spain	Ecuador	
Tanzania	Thailand	Switzerland	El Salvador	
Zambia	Vietnam	Turkey	Mexico	
Zimbabwe	Europe	UK	Peru	
Asia	Albania	Russia & CIS	Oceania	
Bangladesh	Austria	Kazhakastan	Australia	

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