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The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

Editorial

Are you aware of a disease that can assist HIV/ AIDS to creep into a body! Not a big bomb in itself but can definitely bring in others if given a chance!! These organisms at various times have been classified as protozoans, as viruses but are finally termed as bacteria. Indeed, we are talking about Chlamydiae. Chlamydia is a genus of bacteria, several of which are pathogenic. Notably, chlamydia infections are the most common bacterial sexually transmitted diseases in humans, as well as the leading cause of infectious blindness worldwide. Chlamydia are unusual bacteria: obligate intracellular organisms, they must infect host cells to mature and reproduce. Chlamydiae have the ability to establish long-term associations with host cells. The differential diagnosis can be as varied as Gonorrhea, Vaginal yeast infection, Bacterial vaginosis, Trichomoniasis, Cervical inflammation, Vaginal allergies, Spermicide allergies, Vaginal hygiene product allergies, Detergent allergies, Fabric softener allergies, Genital herpes, Molluscum contagiosum, Genital warts, Pelvic inflammatory disease, and other Sexually transmitted diseases. A proper diagnosis, hence, is a must for appropriate therapy institution. The DISEASE DIAGNOSIS segment clarifies all related clinico-diagnostic mysteries of Chlamydiae.

INTERPRETATION portion delves deep into understanding amylase-lipase in relation to pancreatic disorders, which can more often than not be lethal with a very high mortality rate. All amylase isoenzymes and clinical utility of lipase estimation are considered at length.

The simplest of things can sometimes be very tricky for the untrained and the less educated ones. While overseas, phlebotomists do just venepunctures and obtain the red liquid connective tissue of the human body to be further investigated by Laboratarians. However, in India and elsewhere, this job is performed by anyone – be it nurses or the laboratory technicians. The whole process and the technique along with its related complications are discussed under TROUBLESHOOTING section of this issue. The article will be carried over to the next issue also as it is neither simple nor short. Due seriousness must be given to this simple but important process.

BOUQUET, this time, has brainteasers in a pictorial format. Wisdom whispers talks about love. Amongst all this, a few jokes to lighten your heart are not omitted at all.

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DISEASE DIAGNOSIS

CHLAMYDIA

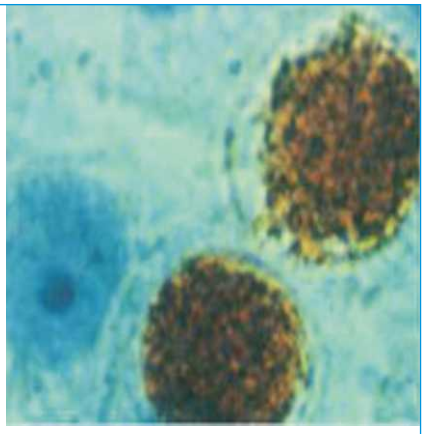
Chlamydia trachomatis

Scientific classification

Kingdom : Bacteria
Phylum : Chlamydiae
Order : Chlamydiales
Family : Chlamydiaceae
Genus : Chlamydia

Species

Chlamydia muridarum
Chlamydia suis
Chlamydia trachomatis



C. trachomatis inclusion bodies.

Introduction

Chlamydia is a genus of bacteria, several of which are pathogenic. Notably, chlamydia infections are the most common bacterial sexually transmitted diseases in humans, as well as the leading cause of infectious blindness worldwide. *Chlamydia* are unusual bacteria: obligate intracellular organisms, they must infect host cells to mature and reproduce.

The three *Chlamydia* species include *Chlamydia trachomatis* (a human pathogen), *Chlamydia suis* (affects only swine), and *Chlamydia muridarum* (affects only mice and hamsters). Prior to 1999, the **Chlamydia** genus also included the species that are presently in the genus *Chlamydophila*: Two clinically relevant species, *Chlamydophila pneumoniae* and *Chlamydophila psittaci* were moved to the *Chlamydophila* genus.

Physiology

Chlamydia are unusual bacteria - unusual enough that they were originally classified as protozoans (and then as viruses), before 16S ribosomal RNA analysis placed them as members of the Eubacteria domain. *Chlamydia* are obligate intracellular parasite bacterial pathogens, and are thus unable to replicate outside of a host cell. However, to disseminate effectively, these pathogens have evolved a unique biphasic life cycle wherein they alternate between two functionally and morphologically distinct forms:

- The elementary body (EB) is infectious, but metabolically inert (much like a spore), and can survive for limited amounts of time in the extracellular milieu. Once the EB attaches to a susceptible host cell, it mediates its own internalization through pathoen-specified mechanisms (via type III secretion system) that allows for the recruitment of actin with subsequent engulfment of the bacterium.

- The internalized EB, within a membrane-bound compartment, immediately begins differentiation into the reticulate body (RB). RBs are metabolically active but non-infectious, and in many regards, resemble normal replicating bacteria. The intracellular bactera rapidly modifies its membrane-bound compartment into the so-called chlamydial inclusion so as to prevent phagosome-lysosome fusion. According to published data, the inclusion has no interactions with the endocytic pathway and apparently inserts itself ito the exocytic pathway as it retains the ability to intercept sphingomyelin-containing vesicles.

To date, no one has been able to detect a host cell protein that is trafficked to the inclusion through the exocytic pathway. As the RBs replicate, the inclusion grows as well to accommodate the increasing numbers of organisms. Through unknown mechanisms, RBs begin a differentiation program back to the infectious EBs, which are released from the host cell to initiate a new round of infection. Because of their obligate intracellular nature, *Chlamydia* have no tractable genetic system, unlike *E. coli*, which makes *Chlamydia* and related organisms difficult to investigate.

Pathophysiology

Chlamydiae have the ability to establish long-term associations with host cells. When an infected host cell is starved for various nutrients such as amino acids (e.g. tryptophan), iron, or vitamins, this has a negative consequence for *Chlamydiae* since the organism is dependent on the host cell for these nutrients.

The starved chlamydiae enter a persistent growth state wherein they stop cell division and become morphologically aberrant by increasing in size. Persistent organisms remain viable as they are capable of returning to a normal growth state once conditions i the host cell improve.

There is much debate as to whether persistence has in vivo relevance. Many believe that persistent chlamydiae are the cause of chronic chlamydial diseases. Some antibiotics such as β-lactams can also induce a persistent-like growth state, which can contribute to the chronicity of chlamydial diseases.

Chlamydia clinical features

Most people have no symptoms. Women with symptoms may have: abnormal vaginal discharge, burning when urination.

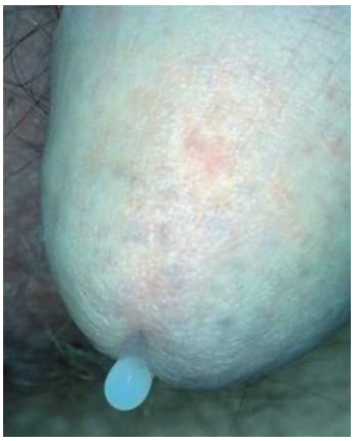
Infections that are not treated, even if there are no symptoms, can lead to: lower abdominal pain, low back pain, nausea, fever, pain during sex, bleeding between periods.

Note: If not treated, *chlamydia* can lead to serious, long-term problems such as pelvic inflammatory disease (PID) and infertility.

Signs & Symptoms of Chlamydia

Some of the common signs and symptoms of Chlamydial infections:
Anal discharge, Cloudy urine, Light vaginal bleeding, Lower abdominal pain, Lower abdominal pain during menstruation, Lower back pain, Lower back pain during menstruation, Pain while urinating, Pain with urination, Painful urination, Pelvic pain, Penile discharge, Smelly vaginal discharge, Spotting, Spotting after sex, Vaginal bleeding, Vaginal discharge yellow vaginal discharge.

Clinical complications/ associated risks



One of the most common sexually transmitted diseases (STDs) is *chlamydia* that is caused by the bacteria *chlamydia trachomatis*. The symptoms of *chlamydia* are usually mild or absent and because of this, many infected persons remain untreated and have potential to spread the disease to others. But serious complications that cause irreversible damage can occur "silently"before a person ever recognizes a problem.
Men with untreated chlamydia infections can become infertile, have chronic pelvic pain, and can become more easily infected with HIV/AIDS. In women, the infection can spread in the uterus or fallopian tubes and cause pelvic inflammatory disease (PID). This can cause permanent damage to the fallopian tubes, uterus, and surrounding tissues. The damage can lead to chronic pelvic pain, infertility, and potentially fatal ectopic pregnancy (pregnancy outside the uterus). Women infected with chlamydia are up to five times more likely to become infected with HIV, if exposed.

In pregnant women, there is some evidence that untreated chlamydial infections can lead to premature delivery. Babies who are born to infected mothers can get *chlamydia* infections in their eyes and respiratory tracts. *Chlamydia* is a leading cause of early infant pneumonia and conjunctivitis (pink eye) in newborns.

Women, in general, are less likely to get sufficient symptoms for them to seek medical care than men. About 75% of women and 50% of men with *Chlamydia* infections have no symptoms. With that in mind, women are more likely to suffer complications and lasting damage than men.

Chlamydia in men can cause urethritis, an inflammation of the urethra, with or without discharge. The discharge in *chlamydia* tends to be subtle in presentation than gonorrhea – this means that the discharge from *chlamydia* may look white, cloudy and watery compared to the discharge from gonorrhea which is more thick, yellowish pus. Other symptoms in men may be pain during sex or burning sensation during urination. Men might also have burning and itching around the opening of the penis.

Complications among men are rare. Infection sometimes spreads to the epididymis, causing pain, fever, and, rarely, sterility. Moreover, genital chlamydial infection can rarely cause arthritis that can be accompanied by skin lesions and inflammation of the eye and urethra (Reiter's syndrome).

Chlamydia can be transmitted during vaginal, anal, or oral sex. Chlamydia can also be passed from an infected mother to her baby during vaginal childbirth.

Any sexually active person can be infected with *chlamydia*. The greater the number of sex partners, the greater the risk of infection. Because the cervix of teenage girls and young women is not fully matured and is probably more susceptible to infection, they are at particularly high risk for infection if sexually active. Since *chlamydia* can be transmitted by oral or anal sex, men who have sex with men are also at risk for chlamydial infection.

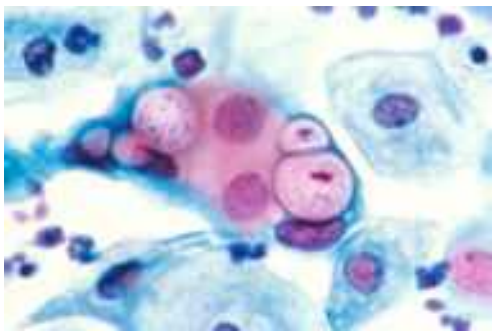
Chlamydia can be easily treated and cured with antibiotics. An appropriate specialist should be consulted and self medication be avoided. All sex partners should also be evaluated, tested, and treated. Persons with *chlamydia* should abstain from sexual intercourse until they and their sex partners have completed treatment, otherwise re-infection is possible.

Women whose sex partners have not been appropriately treated are at high risk for re-infection. Having multiple infections increases a woman's risk of serious reproductive health complications, including infertility. Retesting should be encouraged for women three to four months after treatment. This is especially true if a woman does not know if her sex partner received treatment.

The surest way to avoid transmission of STDs is to abstain from sexual contact, or to be in a long-term mutually monogamous relationship with a partner who has been tested and is known to be uninfected. Latex male condoms, when used consistently and correctly, can reduce the risk of transmission of *chlamydia.*

Pathology & incidence

Chlamydia infection



Pap smear showing *C. trachomatis* (H&E stain)

Chlamydia infection is a common sexually transmitted infection (STI) in humans caused by the bacterium *Chlamydia trachomatis*. The term *Chlamydia* infection can also refer to infection caused by any species belonging to the bacterial family *Chlamydiaceae*. *C. trachomatis* is only found in humans. *Chlamydia* is a major infectious cause of human genital and eye disease.

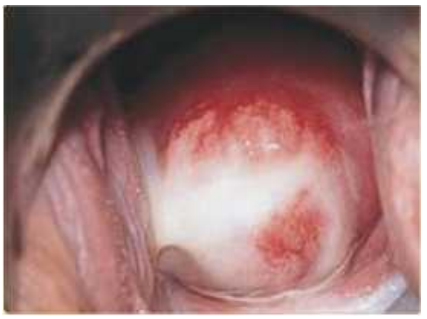
Chlamydia infection is one of the most common sexually transmitted infections worldwide (even in advanced countries)?about 2.8 million cases of chlamydia infection occur in the United States each year. It is the most common bacterial STI in humans.

C. trachomatis is naturally found living only inside human cells. *Chlamydia* can be transmitted during vaginal, anal, or oral sex, and can be passed from an infected mother to her baby during vaginal childbirth. Between half and three-quarters of all women who have a chlamydial cervicitis have no symptoms and do not know that they are infected. In men, infection of the urethra (urethritis) is usually symptomatic, causing a white discharge from the penis with or without pain on urinating (dysuria). Occasionally, the conditions spreads to the upper genital tract in women (causing pelvic inflammatory disease) or to the epididymis in men (causing epididymitis). If untreated, chlamydial infections can cause serious reproductive and other health problems with both short-term and long-term consequences. *Chlamydia* is easily treated with antibiotics.

Chlamydia conjunctivitis or trachoma is a common cause of blindness worldwide. The World Health Organization estimates that it accounted for 15% of blindness cases in 1995, but only 3.6% in 2002.

Related conditions:

Genital disease

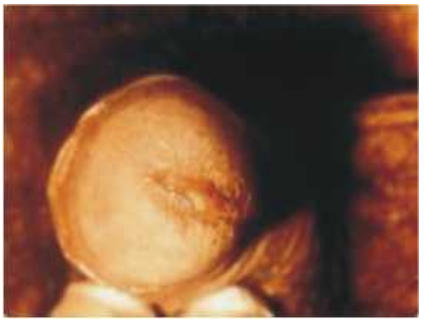


Females

Chlamydial cervicitis in a female patient characterized by mucopurulent cervical discharge, erythema, and inflammation.

Chlamydial infection of the CERVIX -cervicitis is an asymptomatic sexually transmitted illness for a significant percentage of the female population. Of those who have an asymptomatic infection that is not detected by their doctor, approximately half will develop pelvic inflammatory disease (PID), a generic term for infection of the uterus, fallopian tubes, and/or ovaries. PID can cause scarring inside the reproductive organs, which can later cause serious complications, including chronic pelvic pain, difficulty becoming pregnant, ectopic (tubal) pregnancy, and other dangerous complications of pregnancy. Women infected with chlamydia are up to five times more likely to become infected with HIV, if exposed.

Chlamydia is known as the "Silent Epidemic" because in women, it may not cause any symptoms and will linger for months or years before being discovered. Symptoms that may occur include: unusual vaginal bleeding or discharge, pain in the abdomen, painful sexual intercourse (dyspareunia), fever, painful urination or the urge to urinate more frequently than usual (urinary urgency).



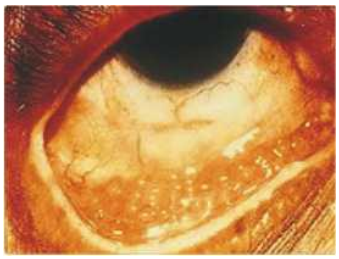
Males

Male patients may develop a white, cloudy or watery discharge (shown) from the tip of the penis.

In men, *Chlamydia* shows symptoms of infectious

urethritis (inflammation of the urethra) in about 50% of cases. Symptoms that may occur include: a painful or burning sensation when urinating, an unusual discharge from the penis, swollen or tender testicles, or fever. Discharge, or the purulent exudate, is generally less viscous and lighter in color than for gonorrhea. If left untreated, it is possible for *Chlamydia* in men to spread to the testicles causing epididymitis, which in rare cases can cause sterility if not treated within 6 to 8 weeks. *Chlamydia* is also a potential cause of prostatitis in men, although the exact relevance in prostatitis is difficult to ascertain due to possible contamination from urethritis.

Eye disease



Conjunctivitis due to *chlamydia*.

Chlamydia conjunctivitis or trachoma was once the most important cause of blindness worldwide, but its role diminished from 15% of blindness cases by trachoma in 1995 to 3.6% in 2002, according to WHO estimates. The infection can be spread from eye to eye by fingers, shared towels or cloths, coughing and sneezing and eye-seeking flies. Newborns can also develop *chlamydia* eye infection through childbirth. Using the SAFE strategy (acronym for surgery for in-growing or in-turned lashes, antibiotics, facial cleanliness, and environmental improvements), the World Health Organisation aims for the global elimination of trachoma by 2020 (GET 2020 initiative).

Rheumatological conditions

Chlamydia may also cause reactive arthritis - the triad of arthritis, conjunctivitis and urethritis (inflammation of the urethra) - especially in young men. About significant percentage of men develop reactive arthritis due to *chlamydia* infection and many of these are permanently affected by it. It can occur in both sexes, though is more common in men.

Perinatal infections

As many as half of all infants born to mothers with *chlamydia* will be born with the disease. *Chlamydia* can affect infants by causing spontaneous abortion; premature birth; conjunctivitis, which may lead to blindness; and pneumonia. Conjunctivitis due to *chlamydia* typically occurs one week after birth (compare with chemical causes (within hours) or gonorrhea (2-5 days)).

Other conditions

Chlamydia trachomatis is also the cause of lymphogranuloma venereum, an infection of the lymph nodes and lymphatics. It usually presents with genital ulceration and swollen lymph nodes in the groin, but it may also manifest as proctitis (inflammation of the rectum), fever or swollen lymph nodes in other regions of the body.

Differential Diagnosis

For a diagnosis of *Chlamydia*, the following list of conditions have been mentioned in sources as possible alternative diagnoses to consider during the diagnostic process for *Chlamydia*:

Gonorrhea
Vaginal yeast infection
Bacterial vaginosis
Trichomoniasis
Cervical inflammation
Vaginal allergies
Spermicide allergies
Vaginal hygiene product allergies
Detergent allergies
Fabric softener allergies
Genital herpes
Molluscum contagiosum
Genital warts
Pelvic inflammatory disease
Sexually transmitted diseases
Other causes of vaginal discharge

Diagnosis

Screening

For sexually active women who are not pregnant, screening is recommended in those under 25 and others at risk of infection. Risk factors include a history of chlamydial or other sexually transmitted infection, new or multiple sexual partners, inconsistent condom use. For pregnant women, guidelines vary: screening women with age or other risk factors is recommended by the appropriate authorities nowadays (which recommends screening women under 25). Most Obstetricians and Gynecologists recommend screening all at risk, while some recommend universal screening of pregnant women. It is accepted that in some communities there may be other risk factors for infection, such as ethnicity. Evidence-based recommendations for screening initiation, intervals and termination are currently not possible. There is no universal agreement on screening men for *chlamydia*.

Laboratory detection

The diagnosis of genital chlamydial infections evolved rapidly from the 1990s through 2006. Nucleic acid amplification tests (NAAT), such as polymerase chain reaction (PCR), transcription mediated amplification (TMA), and the DNA strand displacement amplification (SDA) now are the mainstays. NAAT for *chlamydia* may be performed on swab specimens collected from the cervix (women) or urethra (men), on self-collected vaginal swabs, or on voided urine. Urine and self-collected swab testing facilitates the performance of screening tests in settings where genital examination is impractical. At present, the NAATs have regulatory approval only for testing urogenital specimens, although rapidly evolving research indicates that they may give reliable results on rectal specimens.

Because of improved test accuracy, ease of specimen management, convenience in specimen management, and ease of screening sexually active men and women, the NAATs have largely replaced culture, the historic gold standard for chlamydia diagnosis, and the no-amplified probe tests. The latter test is relatively insensitive, successfully detecting only 60-80% of infections in asymptomatic women, and often giving falsely positive results. Culture remains useful in selected circumstances and is currently the onl assay approved for testing non-genital specimens. Presently, RDTs (rapid diagnostic test) formats employing immunochromatographic techniques are available and have immense value in routine and field-level diagnosis.

Treatment

C. trachomatis infection can be effectively cured with antibiotics once it is detected. Currently most therapeutic regimens provide for the following treatments:

Azithromycin1 gram oral as a single dose, or
Doxycycline100 milligrams twice daily for seven days.
Tetracycline
Erythromycin

Untested Treatments

Ciprofloxacin 500 milligrams twice daily for 3 days. (Although this is not an approved method of treatment.)

β-lactams are not suitable drugs for the treatment of chlamydia. While they have the ability to halt growth of the organism (i.e. are microbistatic), these antibiotics do not eliminate the bacteria. Once treatment is stopped, the bacteria will begin to grow once more.

INTERPRETATION

AMYLASE-LIPASE IN PANCREATITIS

INTRODUCTION: Serum amylase and lipase are common tests obtained as biochemical markers for acute pancreatitis. However, the interpretation of these tests can be difficult since several non-pancreatic conditions can present with abnormal serum amylase and lipase levels. In addition, some patients with pancreatitis have normal serum amylase and lipase levels when a blood sample is examined. Several factors can influence serum amylase and lipase levels. The levels depend upon the rate of production from different tissues and the rate of clearance. As an example, serum amylase and lipase levels may be elevated in patients with renal failure. Organs other than the pancreas can produce these enzymes. Alcoholics, for example, may have an elevated serum amylase of salivary origin. The most commonly used amylase assays cannot differentiate between salivary and pancreatic amylase. Certain serum factors influence amylase and lipase enzyme activity. As an example, patients with pancreatitis due to hypertriglyceridemia may appear to have normal amylase levels, most likely due to a circulating factor that inhibits the enzyme's activity. This topic review will present an approach the patient with an elevated serum amylase or lipase.

AMYLASE: Amylase is derived from the Greek word "amylone," which means starch. The main sources of amylase in humans are the pancreas and salivary glands, but it can be found in other tissues in small quantities. The main function of amylase is to cleave starch into smaller polysaccharides at the internal 1 to 4 alpha linkage in the process of digestion. Several isoforms of amylase can be identified by electrophoresis the most abundant are the P form derived from the pancreas and the S form of salivary origin. The molecular weight of the isoforms is relatively small (about 50,000 Daltons) so they can be filtered by the kidneys. There are multiple methods for measuring serum amylase activity. The results are usually reported in Somogyi units (SU) or international units (IU). The normal values depend upon the assay used since the tests used vary by hospital. **α**-Amylase is the major form of amylase found in humans and other mammals. It is also an enzyme present in seeds which reserves are made of starch, or in fungi (baking yeast for instance). The enzyme cuts alpha-bonds of large sugar molecules.

Amylase in human physiology: Although found in many tissues, amylase is most prominent in pancreatic juice and saliva which each have their own isoform of human **α**-amylase. They behave differently on isoelectric focusing, and can also be separated in testing by using specific monoclonal antibodies. In humans, All amylase isoforms link to chromosome 1p21.

Salivary amylase (ptyalin): Amylase is found in saliva and breaks starch down into maltose and dextrin. This form of amylase is also called ptyalin. It will break large, insoluble starch molecules into soluble starches (amylodextrin, erythrodextrin, achrodextrin) producing successively smaller starches and ultimately maltose. Ptyalin acts on linear **α** (1,4) glycosidic linkages, but compound hydrolysis requires an enzyme which acts on branched products. Salivary amylase is inactivated in the stomach by gastric acid. In gastric juice adjusted to pH 3.3, ptyalin was totally inactivated in 20 minutes at 37^0C. In contrast, 50% of amylase activity remained after 150 minutes of exposure to gastric juice at pH 4.3. Both starch, the substrate for ptyalin, and the product (short chains of glucose) are able to partially protect it against inactivation by gastric acid. Ptyalin added to buffer at pH 3.0 underwent complete inactivation in 120 minutes; however, addition of starch at a 0.1% level resulted in 10% of the activity remaining, and similar addition of starch to a 1.0% level resulted in about 40% of the activity remaining at 120 minutes.

Optimum conditions for ptyalin: Optimum pH - 5.6–6.9. Human body temperature - 37 degrees Celsius. Presence of certain anions and activators: Chlorine and bromine - most effective, Iodine - less effective, Sulfate and phosphate - least effective.

Genetic variation in human ptyalin (salivary amylase): The salivary amylase gene has undergone duplication during evolution, and DNA hybridization studies indicate that many individuals have multiple tandem repeats of the gene. The number of gene copies correlates with the levels of salivary amylase, as measured by protein blot assays using antibodies to human amylase. Perry and coworkers reported that gene copy number is associated with apparent evolutionary exposure to high starch diets. For example, a Japanese individual had 14 copies of the amylase gene (one allele with 10 copies, and a second allele with 4 copies). The Japanese diet has traditionally contained large amounts of rice starch. In contrast, a Biaka individual carried six copies (three copies on each allele). The Biaka are rainforest hunter-gatherers who have traditionally consumed a low starch diet. Perry and colleagues speculated that increased copy number of the salivary amylase gene may have enhanced survival coincident to a shift to a starchy diet during human evolution.

Pancreatic amylase: Pancreatic **α**-amylase randomly cleaves the **α** (1-4) glycosidic linkages of amylose to yield dextrin, maltose or maltotriose. It adopts a double displacement mechanism with retention of anomeric configuration. Normal values in serum/ plasma are upto 53 U/L while for random urine samples they are 350 U/L.

Amylase in human pathology: The test for amylase is easier to perform than that for lipase, making it the primary test used to detect and monitor pancreatitis. Labs will usually measure either pancreatic amylase, or total amylase. If only pancreatic amylase is measured, an increase will not be noted with mumps or other salivary gland trauma. Unfortunately, because of the small amount present, timing is critical when sampling blood for this measurement. Blood should preferably be taken soon after a bout of pancreatitis pain, otherwise it is excreted rapidly by the kidneys. Salivary alpha-amylase has been used as a biomarker for stress that does not require a blood draw.

Interpretation: Increased plasma levels in humans are found in: Salivary trauma (including anaesthetic intubation). Mumps - due to inflammation of the salivary glands. Pancreatitis - because of damage to the cells that produce amylase. Renal failure - due to reduced excretion. Total amylase readings of over 10X the upper limit of normal (ULN) are suggestive of pancreatitis. 5-10X times the ULN may indicate ileus or duodenal disease or renal failure, and lower elevations are commonly found in salivary gland disease.

Genes: Salivary - MY1A, AMY1B, AMY1C. Pancreatic - AMY2A, AMY2B.

LIPASE: Lipases are a group of enzymes that hydrolyze the glycerol esters of long chain fatty acids. Serum lipase is mainly derived from the pancreatic acinar cells, where it is synthesized and stored in granules. Normally, more than 99% of stored enzyme is secreted into the pancreatic duct and less than 1% diffuses across cell membranes and reaches the circulation via lymphatics and capillaries. In acute pancreatitis, acinar cell membranes become more permeable, allowing much more enzyme to reach the circulation. In hemorrhagic pancreatitis, cellular necrosis leads to even more release of enzyme. Lipase is cleared from plasma by glomerular filtration and is subsequently almost totally reabsorbed and metabolized by the renal tubules. The circulating half-life of lipase is between 7 and 14 hours. Serum lipase activity increases within 4 to 8 hours after the onset of acute pancreatitis, peaks at 24 hours and decreases within 8 to 14 days. Lipase levels usually increase from 7 to 11 times the upper limit of normal in acute pancreatitis. Rarely, will lipase stay increased more than 14 days. Prolonged increases signal poor prognosis or the presence of a pancreatic cyst. Initially, the rise in lipase is approximately equal to that of amylase, but after 24 hours lipase has greater clinical sensitivity. Lipase activity remains elevated longer than amylase. Some patients may have elevated lipase and normal amylase activity due to the greater concentration of lipase in the pancreas and its longer serum half-life. Lipase may also be increased in chronic pancreatitis and pancreatic duct obstruction. Pancreatic duct obstruction by fibrous strictures, stones, tumors, or edema increases the secretory pressure and promotes extravasation of lipase into the pericapillary saces. Lipase is not specific for pancreatic disease and may be increased in renal disease, acute cholecystitis, bowel obstruction, intestinal infarction, duodenal ulcers, liver disease, alcoholism, diabetic ketoacidosis, and after endoscopic retrograde chlangiopancreatography. Patients with renal failure may have lipase levels three times the upper limit of normal. Hospitalized patients with nonpancreatic diseases may have lipase values at or slightly above the upper limit of normal, which was established with samples from healthy, nonhospitalized individuals.

Mildly elevated lipase levels (400 to 500 U/L) result fro the release of pancreatic enzyme in response to nearby organ disease. Lipase values between 2 and 5 times the upper limit of normal (600 to 1500 U/L) should be used as a cutoff value for diagnosing acute pancreatitis. Some individuals may have persistently elevated lipase and normal amylase activities. This combination of results should suggest the presence of macrolipase, which is an immune complex between lipase and IgG. Increased enzyme activity is due to the inability of the kidney to excrete the lipase complex. Reference range is 40 to 300 U/L. Reference values for lipase determination are laboratory- and method-specific. In general, normal results are usually less than 200 units/L (triolein methods by titration or turbidimetry).

How is it used? The blood test for lipase is ordered, often along with an amylase test, to help diagnose and monitor acute pancreatitis (inflammation of the pancreas), chronic pancreatitis, and other disorders that involve the pancreas. Lipase testing is also occasionally used in the diagnosis and follow-up of cystic fibrosis, celiac disease, and Crohn's disease.

When is it ordered? A lipase test may be ordered when a patient has symptoms of a pancreatic disorder, such as severe abdominal pain, fever, loss of appetite, or nausea. It may also be ordered at intervals when a doctor wants to monitor a patient with a pancreas condition to evaluate the effectiveness of treatment and to determine whether the lipase levels are increasing or decreasing over time. In acute pancreatitis, lipase levels are frequently very high, often 5 to 10 times higher than the highest reference value (often called the upper limit of normal). In acute pancreatitis, lipase concentrations rise within 24 to 48 hours of an acute pancreatic attack and may remain elevated for about 5 to 7 days. Concentrations may also be increased with pancreatic duct obstruction, pancreatic cancer, and other pancreatic diseases. Moderately increased lipase values may occur in other conditions such as kidney disease (due to decreased clearance from the blood), salivary gland inflammation, a bowel obstruction, or peptic ulcer disease, although the lipase test is not usually used to monitor these conditions. Decreased lipase levels may indicate permanent damage to the lipase-producing cells in the pancreas. Since the reference values for lipase will vary from laboratory to laboratory, depending on the test method used, there is no universally accepted number that can be called normal or high.

One must know: In acute pancreatitis, elevated lipase levels usually parallel blood amylase concentrations, although amylase levels tend to rise and fall a bit sooner than lipase levels. Drugs that may increase lipase levels include codeine, indomethacin, and morphine.

Interpretation: The diagnosis of acute pancreatitis can be difficult to make, especially when mild, late in its course or when multiple other disorders are present. Testing: Amylase and lipase should be tested simultaneously. Results for both may be expressed as multiples of the upper limit of normal. Acute pancreatitis is highly likely if: (1) The serum lipase is > 5 times the upper limit of normal. (2) Increases then decreases in serum amylase and lipase are in concert. (3) Serum lipase values show significant change over time. Elevations in amylase and lipase less than 3 times the upper limit of normal can be seen in conditions other than pancreatitis, including drug effect or acute abdomen with bowel infarction or perforation. Very high elevations in amylase (greater than 25 times the upper limit of normal) can be seen in metastatic cancers. In increase in ALT to 3 or more times normal suggests gallstone-induced pancreatitis.

BOUQUET

In Lighter Vein

A man decides to take off early from work and go drinking. He stays until the bar closes at 2am, at which time he is extremely drunk. When he enters his house, he doesn't want to wake anyone, so he takes off his shoes and starts tip-toeing up the stairs. Half-way up the stairs, he falls over backwards and lands flat on his rear end. That wouldn't have been so bad, except that he had couple of empty pint bottles in his back pockets, and they broke, and the broken glass carved up his buttocks terribly. But, he was so drunk that he didn't know he was hurt.

A few minutes later, as he was undressing, he noticed blood, so he checked himself out in the mirror, and, sure enough, his behind was cut up something terrible. Well, he repaired the damage as best he could under the circumstances, and he went to bed.

The next morning, his head was hurting, and his rear was hurting, and he was hunkering under the covers trying to think up some good story, when his wife came into the bedroom.

"Well, you really tied one on last night," she said. "Where'd you go?"

"I worked late," he said, "and I stopped off for a couple of beers."

"A couple of beers? That's a laugh," she replied. "You got plastered last night. Where the heck did you go?"

"What makes you so sure I got drunk last night, anyway?"

"Well," she replied, "my first big clue was when I got up this morning and found a bunch of band-aids stuck to the mirror."

There were three people stranded on an island, a brunette, a redhead, and a blonde. The brunette looked over the water to the mainland and estimated about 20 miles to shore. So she announced, "I'm going to try to swim to shore." She swam out five miles, and got really tired. She swam out ten miles from the island, and she was too tired to go on, so she drowned.

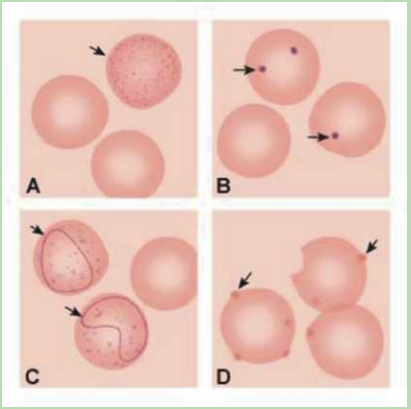
The second one, the redhead, said to herself, "I wonder if she made it. I guess it's better to try to get to the mainland than stay here and starve." So she attempts to swim out. The redhead had a lot more endurance than the brunette, as she swam out 10 miles before she even got tired. After 15 miles, she was too tired to go on, so she drowned.

The blonde thought to herself, "I wonder if they made it! I think I'd better try to make it, too." So she swam out 5 miles, ten miles, 15 miles, NINETEEN miles from the island. The shore was just in sight, but she said, "I'm too tired to go on!" So she swam back.

Wisdom Whispers

- "Absence sharpens love, presence strengthens it." Thomas Fuller
- "Put your hand on a hot stove for a minute, and it seems like an hour. Sit with a pretty girl for an hour, and it seems like a minute. THAT'S relativity." Albert Einstein
- "Love is life. All, everything that I understand, I understand only because I love." - Leo Tolstoy
- "The first duty of love - is to listen." - Paul Johannes
- "Love is the greatest refreshment in life." - Pablo Picasso
- "A heart that loves is always young." - Greek Proverb
- "Love looks through a telescope; envy through a microscope." - Josh Billings
- "My bounty is as boundless as the sea, my love as deep. The more I give thee, the more I have, For both are infinite." - William Shakespeare

Brain Teasers



Match the pictures above: 1.Heinz bodies 2. Cabot's rings 3. Basophilic stippling 4. Howell Jolly body

Answers: A-3, B-4, C-2, D-1.

TROUBLESHOOTING

PHLEBOTOMY

BLOOD COLLECTION:

ROUTINE VENIPUNCTURE AND SPECIMEN HANDLING

Objectives of this exercise: Describe and perform the venipuncture process including: Proper patient identification procedures. Proper equipment selection and use. Proper labeling procedures and completion of laboratory requisitions. Order of draw for multiple tube phlebotomy. Preferred venous access sites, and factors to consider in site selection, and ability to differentiate between the feel of a vein, tendon and artery. Patient care following completion of venipuncture. Safety and infection control procedures. Quality assurance issues. Identify the additive, additive function, volume, and specimen considerations to be followed for each of the various color coded tubes. List six areas to be avoided when performing venipuncture and the reasons for the restrictions. Summarize the problems that may be encountered in accessing a vein, including the procedure to follow when a specimen is not obtained. List several effects of exercise, posture, and tourniquet application upon laboratory values.

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. Several essential steps are required for every successful collection procedure: Identify the patient. Assess the patient's physical disposition (i.e. diet, exercise, stress, basal state). Check the requisition form for requested tests, patient information, and any special requirements. Select a suitable site for venipuncture. Prepare the equipment, the patient and the puncture site. Perform the venipuncture. Collect the sample in the appropriate container. Recognize complications associated with the phlebotomy procedure. Assess the need for sample recollection and/or rejection. Label the collection tubes at the bedside or drawing area. 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

Patient Name: _____

Patient ID: _____

Patient Birthdate: _____ **Sex:** _____

Source of Specimen: _____

Date Collected: _____ **Time:** _____ **Phleb:** _____

Physician: _____ **Location:** _____

Diagnosis: _____

Tests Requested:

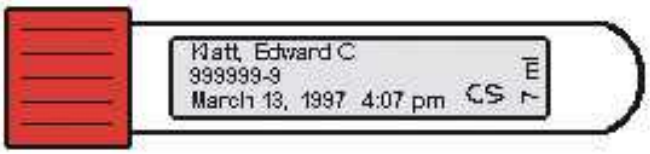
_____ **Electrolyte Panel** _____ **Complete Blood Count**

_____ **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. An example of a simple requisition form with the essential elements is 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 vacutainer tubes is: **First** - blood culture bottle or tube (yellow or yellow-black top). **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. **Third** - non-additive tube (red top). **Last draw** - additive tubes in this order: SST (red-gray or gold top). Contains a gel separator and clot activator. Sodium heparin (dark green top). PST (light green top). Contains lithium heparin anticoagulant and a gel separator. EDTA (lavender top). ACDA or ACDB (pale yellow top). Contains acid citrate dextrose. 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 for confirmation. **DO NOT DRAW BLOOD IF THE ARMBAND IS MISSING.** 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. 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.

(to be continued...)

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