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The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

ISO 9001

Editorial

The last decade has witnessed a sea change in the operations of medical diagnostic laboratories. Some labs have been relegated to the status of post-offices or clearing and forwarding agencies. The benefits of technological advancements have not percolated to the B and C grade towns and cities. These cities still have to look towards Megapolitan cities. As cost of investigation is a function of number of tests being performed at a time, the Giant Metro Labs have drained the work away from medium and small labs by pooling samples from multiple cities. Consequently, medium sized labs, which might have upgraded, have now been forced to freeze at haemograms, clinical biochemistry and basic microbiology. The trend has both, good and bad aspects to it. Good because, the system evolved has brought sophisticated investigations to remote regions and bad because the growth of most labs in B, C and even A grade towns has been stunted. However, all is not well! Consider the following gray areas:

1. Proper patient preparation.
2. Proper sample collection
   - Right volume withdrawal / Right anti-coagulant usage
   - Right preservative addition / Right sample storage till dispatch.
3. Proper sample transportation
   - Container for transportation / Maintenance of cool chain / Time taken to reach destination
   - Delay between receipt of sample at destination and sample processing
   - Proper sample storage at the destination laboratory.
4. Result generation after investigation is completed.
5. Dispatch and communication of the result to the referring lab.
6. Result generation by the primary referring lab with attendant duplication of typographical errors.
7. Availability of report to the patient.

This process inevitably leads to delays between sample procurement and report delivery. The above chain of events, inherently, has scope for multiple result affecting variables on which the primary and reporting laboratories have no control over.
Who can deny the advantage of providing a thyroid profile within 2 hours as compared to 48 hours.

Most analytes have limited half-lives and if a delay is apprehended, some can be preserved by using appropriate stabilizers and by storing the sample at the desirable temperature. Thermal and cryogenic shocks deteriorate the sample. To minimize errors, it would be better to use the services of laboratory in your vicinity or neighbourhood. Best-case scenario is to perform the test in the laboratory of sample collection.

IT IS IMPERATIVE ON OUR PART TO NOT ONLY GIVE ACCURATE REPORTS BUT TO ALSO GIVE THEM ON TIME.
Like justice, a report delayed is report denied. Of what use is a report that reaches after a patient has expired. Can we put ourselves in the shoes of the helpless patients and rethink the whole problem. Isn't it strange that samples travel from Metro A to Metro B and vice-versa? Are medical samples gathering frequent flier miles! TROUBLE SHOOTING section gives an analyte stability calendar and recommends correct storage temperatures and preservatives. It is still advised that a situation where one needs to refer to this calendar should ideally not arise. DO THAT SPECIAL TEST IN YOUR LABORATORY, IF NECESSARY, UPGRADE YOUR SERVICES.
This Issue discusses an important autoimmune disease - Rheumatoid arthritis under the DISEASE DIAGNOSIS section. INTERPRETATION defines microalbuminuria and emphasizes its significance as an early marker for Diabetes or Cardiovascular complications. As CRUX has gone global, BOUQUET too has acquired an international flavor.

DISEASE · DIAGNOSIS

RHEUMATOID ARTHRITIS

Disease Background

Arthritis literally means "inflammation of a joint". Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory disease that chiefly affects the synovial membranes of multiple joints in the body. As the disease is systemic, extra-articular manifestations are seen. Remissions and exacerbations of symptoms occur. Prevalence is 1-2% of general population, worldwide. Females to males ratio is 3:1. Usual age of onset is 40-60 years; although it can occur at any age. There is no known cure for RA or means of preventing it. Optimal management requires early diagnosis and timely introduction of agents that reduce the probability of irreversible joint injury.

Etiology of Rheumatoid Arthritis

Exact cause is still uncertain. Several factors may play a role in the development of RA.

- Genetic factors. Certain genes have been identified that play a role in the immune system and which are associated with a tendency to develop RA.
- Environmental factors. There may be an as-yet-unidentified viral or bacterial agent which triggers the disease process. In autoimmune diseases this often occurs because the antibody which attacks the virus or bacteria "cross-reacts" with part of the body, in this case the joints. However, rheumatoid arthritis is not contagious.
- Other factors. Other factors are known or suspected to play a role in the development of autoimmune disease. These include: A) Food allergies. Numerous studies have demonstrated improvement in RA symptoms with identification and removal of allergenic foods. B) Abnormal bowel permeability. Individuals with RA have increased intestinal permeability to dietary and bacterial antigens. Increased bowel permeability can lead to absorption of antigens which contributes to increased levels of circulating endotoxins and immune complexes characteristic of RA. Food allergies may contribute greatly to the increased intestinal permeability. Nonsteroidal anti-inflammatory drugs may also play a role. C) Altered bacterial flora and small bowel overgrowth are common in people with RA and the degree of imbalance is associated with severity of symptoms and disease activity. D) Decreased DHEA (adrenal hormone) levels. Defective androgen synthesis has been proposed as a possible factor in the development of RA. E) Low cortisol levels. Cortisol is the body's natural anti-inflammatory hormone. It is produced by the adrenal glands and its production may be decreased by chronic stress or use of steroid medications. F) Heavy metal toxicity with metals such as mercury, cadmium and lead have been associated with RA. These metals may interfere with collagen synthesis. G) Chronic stress affects the immune system and is linked to disease onset and worsening in people with RA.

Risk Factors

1) Female gender. Females are affected 3 times more than the male gender.
2) Genetic factors. First-degree relatives of affected individuals have a 4-6 times greater risk of developing RA as compared to population in general. The presence of HLA-DR4 antibody in 70% of patients with RA supports genetic predisposition.
3) Heavy metal exposure.
4) Food allergies and intolerances.
5) Altered gut flora, which can be due to chronic use of antibiotics or acid blocking medications.
6) High stress level.
7) Family history of RA or other autoimmune conditions.
8) Cigarette smoking.

Symptoms

A) These include inflammation of joints, swelling, difficulty moving and pain.
Other symptoms include-
B) Loss of appetite.
C) Fever, usually of low grade.
D) Loss of energy, malaise, asthenia.
E) Anemia.
F) Can affect other parts of the body. And
G) Other features include lumps under the skin in areas subject to pressure like back of elbows (rheumatoid nodules).

Criteria	Definition
1. Morning stiffness	Morning stiffness in and around joint lasting for at least 1 hour before maximal improvement
2. Arthritis of three or more joint areas	At least three joints simultaneously have soft tissue swelling or fluid (not bony overgrowth alone) observed by clinician (the 14 possible joints are PIP, MCP, wrist, elbow, knee, ankle and MTP joints)
3. Arthritis of hand joints	At least one area swollen in a wrist, MCP or PIP joint
4. Symmetric arthritis	Simultaneous involvement of joint areas on both sides of the body (bilateral involvement of PIP, MCP, or MTP joints is acceptable without absolute symmetry)
5. Rheumatoid nodules	Subcutaneous nodules over bony prominences or extensor surfaces, or in juxta-articular regions, observed by clinician
6. Serum rheumatoid factor	Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in < 5% of normal control subjects
7. Radiological changes	Radiological changes typical of rheumatoid arthritis on the posterior hand and wrist radiographs, which must include erosions or unequivocal bony calcification localized in, or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify)

Interpretation: For classification purposes, patient is said to have RA if they satisfyfour of the seven criteria. Criteria 1-4 must have been present for at least 6 weeks. Patients with two clinical signs are not excluded. Designation as classic, definite, or probable RA is not to be made.

Subtypes of Rheumatoid Arthritis

	Type I	Type II	Type III
Type of polyarthritis	Self limited disease	Minimally progressive disease	Progressive disease
% Rheumatoid Factor positive	<5%	60-90%	60-90%
Odds of HLA-DR4	1:1	3-5:1	3-5:1
Meets RA criteria 3-10 yrs later	0%	90-100%	90-100%
Response to treatment approach	Long term treatment not needed	Good response to treatment although some progression usually seen	Disease progression continues despite treatment

Extra-articular Manifestations of Rheumatoid Arthritis

Lesion	Description
1. Rheumatoid nodules	20-30% of RA patients, rarely symptomatic subcutaneous nodules on extensor surfaces of arms and legs common locations: olecranon bursa, proximal ulna, Achilles tendon, occiput
2. Rheumatoid vasculitis	May manifest as polyneuropathy and mononeuritis multiplex, cutaneous ulcerations and dermal necrosis, digital gangrene, and visceral infarctions
3. Pleuropulmonary manifestations	Commoner in males, include pleural disease, interstitial fibrosis, pleuropulmonary nodules, pneumonitis and arteritis
4. Cardiac manifestations	Rarely symptomatic, pericarditis may progress to constrictive pericarditis
5. Neurological manifestations	Peripheral neuropathy (due to vasculitis), nerve entrapment (median, ulnar, radial, or anterior tibial nerve)
6. Felty's syndrome	Consists of chronic RA, splenomegaly, neutropenia (neutrophils <1500/cu.mm) and occasionally anemia and thrombocytopenia
7. Sjogren's syndrome	Keratoconjunctivitis sicca (eye dryness), dry mouth and other mucous membranes
8. Osteoporosis	Common, may be aggravated by steroid therapy

Differential Diagnosis of RA

A. Other rheumatic diseases:-Rheumatic fever, Systemic lupus erthematosus, Ankylosing spondylitis, Polymyositis or dermatomyositis, Vasculitic syndromes, Scleroderma, Psoriatic arthritis, Reiter's syndrome, Sjogren's syndrome, Mixed connective tissue disease (MCTD), Behcet's disease. B. Infectious arthritis C. Inflammatory bowel disease D. Neoplastic diseases including leukemia E. Non-rheumatic conditions of bones and joints F. Hematologic diseases G. Psychogenic arthralgia H. Miscellaneous: Sarcoidosis, Hypertrophic osteoarthropathy, Chronic active hepatitis, Familial Mediterranean fever.

Criteria for Determining Clinical Remission

5 or more of the following present for at least two consecutive months

Morning stiffness = 15 minutes

No fatigue

No joint pain

No joint tenderness or pain on motion

No soft tissue swelling in joints or tendon sheaths

ESR (Westergren's method) = 30 mm/Ist hour for a female, or = 20 mm/Ist hour for a male

Exclusions: Clinical manifestations of active vasculitis, pericarditis, pleuritis or myositis, and unexplained recent weight loss or fever attributable to RA will prohibit a designation of complete clinical remission.

Pathophysiology

Normal synovium is 1-3 cell layer thick. It provides nutrients for cartilage (cartilage itself is avascular) and also synthesizes hyaluronic acid (joint lubricant) as well as collagens and fibronectin (supporting framework). The subintimal area of synovium has its blood vessels and normally has very few cells.

What are the triggers for RA synovial membrane changes? There are two theories: - 1) T cell alongwith an unidentified antigen initiates the chronic inflammatory process. This theory is based upon the known association of RA with class II major histocompatibility antigens, the large number of CD4+ T cells and skewed T cell receptor gene usage in the RA synovium. 2) This theory holds that while T cells may be important in initiating the disease, chronic inflammation is self-perpetuated by macrophages and fibroblasts in a T-cell independent manner. This theory is based upon the relative absence of activated T cell phenotypes in chronic RA and the preponderance of activated macrophage and fibroblast phenotypes.

RA injury makes the synovial lining membrane to proliferate (becomes 8-10 cell layer thick). The subintimal area becomes vascularised and is infiltrated by T and B lymphocytes, macrophages and mast cells. The intense cellular infiltrate is accompanied by new blood vessel growth or angiogenesis. This hypertrophied synovium is called as Pannus and it invades and erodes contiguous bone and cartilage. In RA, the integrity, resilience and water content of the cartilage are impaired (because of elaboration of proteolytic enzymes). Neutrophils in the synovial fluid may aggravate this process. The invading synovium causes erosion of the contiguous bone via release of prostaglandins and proteases by synovial cells and, possibly by osteoclasts. Synovial cavity normally contains about 1-2 ml of highly viscous fluid with few cells. In RA, large collections of fluid ("effusions") occur which is, in effect, filtrates of plasma (and, therefore, exudative i.e., high protein content). The synovial fluid is highly inflammatory, but unlike the synovial tissue that is infiltrated by lymphocytes and macrophages but not neutrophils, the predominant cell in the synovial fluid is the neutrophil.

Diagnosis of Rheumatoid Arthritis

There are no laboratory tests that are diagnostic for rheumatoid arthritis. Laboratory tests useful in assessing patients with this disease fall into four categories: hematological, acute phase reactants, rheumatoid factor (RF), and synovial fluid analysis.

Hematological evaluation: Normocytic, normochromic anemia (anemia of chronic disease), mild leucocytosis with a normal differential count. In Felty's syndrome leukopenia is present. Eosinophilia in severe systemic disease is sometimes noted. Thromobocytosis however is frequently noted and reflects ongoing inflammation. Complications of taking NSAIDs may change the hematological picture.

Acute Phase Reactants: ESR and CRP are the most useful tests. Both may be elevated in RA with active inflammation (ESR ⩾ 30 mm/hr by the Westergren method and CRP of > 7 mg/dL), but in about 40% cases these values may be normal. ESR can correlate with severity of inflammation and, if so, can be used to follow the course of inflammation. CRP correlates better with clinical disease activity, radiological progression, and response to therapy.

Rheumatoid Factor: Discovered in 1940, RF is an autoantibody (usually IgM) against the Fc portion of IgG. RF can be detected by latex agglutination test, ELISA, nephelometrically or by turbidimetry. RF is found in 60-90% cases of RA; however, it is not specific for RA. It has also been identified in patients with chronic bacterial infections, viral disease, parasitic disease, chronic

Inflammatory disease of uncertain etiology, mixed `cryoglobulinemia, hypergammaglobulinemic purpura, pulmonary fibrosis, SLE, and transiently after immunization. In addition RF can be found in up to 5% of normal population. One must, however, remember that a negative RF test does not rule out RA. Despite these limitations, RF is an excellent prognostic indicator as high titers have been shown to be associated with extra-articular lesions and with severe unremitting disease. Additionally Anti-nuclear antibody (ANA) is found to be positive in 20-30% cases of RA

Synovial fluid analysis: Synovial fluid helps by confirming and differentiating from other types of arthritis (OA, crystal induced`arthropathy, septic arthritis). In RA the joint aspirate is straw coloured, turbid, has reduced viscosity, increased protein content, and decreased or normal glucose. WBC counts may vary from 2000 to >50000/µL with neutrophils predominating (up to 85% on differential). Crystals indicate gout or calcium pyrophosphate dihydrate deposition disease (CPPD) and should not be seen in RA. Cultures of the synovial fluid should be done to rule out septic arthritis in patients with monoarticular or oligoarticular presentation. Lately, an enzyme called MMP-3 has also been associated with RA and is found in the synovial fluid.

Other markers: HLA-DR4 typing, presence of other antibodies seen in RA (antikeratin and antiperinuclear), and cytokine (TNFα, IL1, IL6) measurements are used currently only in research and have no clinical role at the moment.

One can not forget the importance of imaging sciences in diagnosing RA

FOR ESTABLISHING DIAGNOSIS AND MONITORING THERAPY OF RHEUMATOID ARTHRITIS, QUANTIFICATION OF RF AND ACUTE PHASE REACTANTS IS MANDATORY. EXACT QUANTIFICATION IS NOW MADE POSSIBLE BY TURBIDIMETRIC ASSAYS. RELIABILITY OF TURBIDIMETRIC ASSAYS DEPENDS UPON USE OF MULIPLE CONTROLS/ STANDARDS THAT ARE PROVIDED WITH THE KITS.

BOUQUET

In Lighter Vein

Linguistic lapses

- In a Japanese hotel: You are invited to take advantage of the chamber -maid
- In a Bangkok dry cleaner's shop: Drop your trousers here for best results.
- In an advertisement by a Hong Kong dentist: Teeth extracted by the latest Methodists.
- From a brochure of a car rental firm in Tokyo: When passenger of foot heave in sight, tootle the horn. Trumpet him melodiously at first, but if still obstacles your passage then tootle him with vigour.
- Two signs from a Majorcan shop entrance: English well talking. Here speeching American.
- In a Bangkok temple: It is forbidden to enter a woman, even a foreigner, if dressed as man.
- In a Rhodes tailor shop: Order your summers suit. Because is big rush we will execute customers in strict rotation.
- Advertisement for donkey rides in Thailand: Would you like to ride on your own ass?
- In a Belgrade hotel elevator: To move cabin, push button for wishing floor. If the cabin should enter more persons, each one should press a number of wishing floor. Driving is then going alphabetically by national order.

Wisdom Whispers

- ✓ Promise only what you can deliver,then deliver more than you promise.
- ✓ Great minds discuss ideas

Medium minds discuss events

Small minds discuss people.

- ✓ Good manners sometimes means simply putting up with other people's bad manners.
- ✓ Never fear shadows; they simply mean that there is light shining somewhere nearby.
- ✓ Your name is your trademark, protect it as if it is your life.

Brain Teasers

1. What is the chief source of LDH1 ?
   A) Cardiac muscle B) Liver C) Lymph nodes D) Endocrine glands
2. Hypoglycemia by definition is blood sugar less than what level?
   A) 60 mg/dL B) 55 mg/dL C) 50 mg/dL D) 45 mg/dL
3. What is the weight of active bone marrow in an adult?
   A) 500 gm B) 1000 gm C) 2000 gm D) 4000 gm
4. Which normal human tissue can metastasize?
   A) Chorionic villi B) Intestinal villi
   C) Osteoid tissue D) Chondroid tissue
5. Afternoon tide refers to..
   A) CO_2 B) Neutrophil count C) O_2 D) Platelet count
6. Pregnancy test can be positive in a male in..
   A) Testicular choriocarcinoma B) Hepatocellular carcinoma
   C) Osteosarcoma D) Prostatic carcinoma
7. Of the following, which is the largest in size?
   A) Megakaryoblast B) Promegakaryocyte
   C) Megakaryocyte D) Myelocyte
8. Which of the following hepatitis is known to occur?
   A) Hepatitis G B) Hepatitis H C) Hepatitis I D) Hepatitis J

Answers: 1. A., 2. D., 3. B., 4. A., 5. B., 6. A., 7. C., 8. A.

TROUBLE SHOOTING

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 once 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 (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 20 l/ml serum or 20 l 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 l/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				
-Amylase (AMYL) — Total	1 year	7 days	7 days		Affected by hemolysis and lipemia Contaminated by saliva while pipetting - ↑
-Amylase (AMYL) — Pancreatic	1 year	7 days	7 days		
Androstenedione	1 year	4 days	1 day		↓ with storage at 22 °C more than 1day
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		
1- 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, C3c 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		

(To be continued in the next issue)

INTERPRETATION

MICROALBUMINURIA

Almost all kidney diseases are associated with selective rise in urinary excretion of plasma proteins or tubular structural proteins. Proteinurias can be differentiated by various marker proteins. Minimal selective glomerular proteinuria is seen in diabetic nephropathy (stage III), hypertensive nephropathy (early phase) and SLE-nephropathy (early phase). In these conditions, the total protein loss is 30-300 mg/L, or 25-200 mg/g creatinine. Most of the protein lost is albumin. Detection of microalbuminuria is an important diagnostic criterion for diabetic and hypertensive nephropathy.

Stages of diabetic nephropathy and characteristic findings

Stage	Time course	Symptoms
I Hypertrophy, hyperfunctional state	At time of DM diagnosis	Enlarged kidneys, RPF and GFR increased
II Alterations in kidney histology but no clinical symptoms	2-5 years	Thickening of capillary basement membrane, expansion of the mesangium
III Early nephropathy	10-15 years	Microalbuminuria, possible rise in blood pressure
IV Clinically apparent nephropathy	10-25 years	Persistent proteinuria, RPF and GFR diminished, hypertension in 60% of cases
V Renal insufficiency	15-30 years	Increased serum creatinine, hypertension in 90% of cases

(RPF= renal plasma flow, GFR= glomerular filtration rate)

Classification and cut off limits of albuminuria

	µg/min*	mg/24 h**	mg/L***	mg/g creatinine****
Normal	< 20	< 30	< 30	< 24
Microalbuminuria	20-200	30-300	30-300	24-200
Macroalbuminuria	> 200	> 300	> 300	> 200

Albumin as a marker protein of diabetic nephropathy

The main goal in prevention of diabetic nephropathy is the early detection of patients who are at risk of diabetic nephropathy at an early stage when adequate therapy can still prevent or reduce the progressive loss in renal function. Determination of urinary albumin excretion is indicated for early detection and for course and therapeutic monitoring. Diagnosis of diabetic nephropathy should only be made if increased urinary albumin excretion has been confirmed by two out of three independent examinations within a period of six months and the presence of diabetes mellitus has been reliably established. It is, however, essential to rule out other causes of kidney disease UTI, tubular damage etc. By regularly estimating urinary albumin and proper treatment, the development of nephropathy can be halted and even reversed. Success of treatment is reflected by a decrease or lack in further rise of urinary albumin.

Microalbuminuria in conjunction with generalized vascular sclerosis and hypertension

A relationship between microalbuminuria and cardiovascular risk factors such as obesity, hyperlipidemia, cigarette smoking, alcohol consumption, and mean systolic blood pressure has been found. Now microalbuminuria, is itself considered to be yet another cardiovascular risk factor.

Indications for testing microalbuminuria

Diabetic subjects

IDDM: Annually in all patients suffering from diabetes mellitus for 5 years or more and over 12 years of age

NIDDM: As soon as diabetes mellitus is diagnosed, and at regular intervals (1-2 times) annually, thereafter in case of negative test results.

Non diabetic subjects

All patients with potential disease involvement of the kidneys for early diagnosis

Patients at risk of cardiovascular complications.

Methods available for determining microalbuminuria are

1. Urine dipsticks: Very coarse indicators, usable as screening tools only
2. Semiquantitative latex agglutination assays, can be used as screening tools at best
3. Biochemical (dye-binding reactions) estimations (these estimate other proteins also)
4. Immunoturbidimetric/ nephelometric assays, radioimmunoassays and ELISA based platforms are ideal and give exact values.

Remarks

- Microalbuminuria also occurs in response to acute inflammatory conditions such as ischaemia, trauma and thermal injury, surgery, pancreatitis and inflammatorty bowel disease. In many of these conditions the albumin excretion increases within minutes or hours of the initiating stimulus and only lasts for about 24-72 hours. Urinary tract infections also lead to albuminuria.
- As the name indicates, only urine can be used as a sample.
- Albumin excretion is increased after physical activity. It is therefore recommended that sample be collected from a resting patient
- As albumin excretion is subject to physiological variations it is mandatory to take two measurements on consecutive days, in case of contradictory results three measurements on three different days must be done preferably within a week.
- Liquid intake of the patient must be in the normal range i.e., 1.5 to 2 litres/day.
- The patient should not have an ongoing non specific febrile disease.

IMMUNOTURBIDIMETRIC PLATFORMS ARE IDEAL, AS THEY DO NOT REQUIRE HIGHLY TRAINED MANPOWER, ARE EASILY AVAILABLE, PROVIDE ACCURATE RESULTS WITHIN THIRTY MINUTES. FOR SMALL, PERIPHERAL LABORATORIES LATEX PARTICLE BASED TESTS CAN SERVE AS VITAL SCREENING TOOLS.

TULIP NEWS

TULIP GROUP becomes the first Indian company to get CE accreditation for its breakthrough product RETROCHECK-HIV.

TULIP GROUP becomes the first Indian company to achieve the prestigious CE accreditation for its innovative rapid test **RETROCHECK-HIV** for diagnosis of HIV-1 & HIV-2 infection. This would enable **RETROCHECK-HIV** to be marketed throughout the European Union. **RETROCHECK-HIV** is manufactured in India by **QUALPRO DIAGNOSTICS** a **TULIP GROUP** company. The test detects the presence of antibodies to Human Immunodeficiency Virus-1 (HIV-1) and Human Immunodeficiency Virus-2 (HIV-2). **RETROCHECK-HIV** is a technological breakthrough. It is third generation, rapid, self-performing test for the simultaneous detection of antibodies to the HIV-1 and HIV-2 virus in serum, plasma or even whole blood. It takes only fifteen minutes to complete the test. Because of its simplicity it can be performed by virtually anyone. No specialized equipment or training is required to read the results. **RETROCHECK-HIV** detects HIV infection in the early stage of infection (when the patients do not have symptoms) and is a useful tool in preventing the spread of HIV. Thus, with the CE accreditation, **RETROCHECK-HIV** is poised to become one of the global leaders in the rapid "AIDS test" category.

What is CE Marking?

CE Marking is the symbol as shown above. The letters "CE" are the abbreviation of French phrase "Conformité Européene" which literally means "European Conformity".

CE Marking on a product is a manufacturer's declaration that the product complies with the essential requirements of the relevant European health, safety and environmental protection legislations, in practice by many of the so-called Product Directives. **Product Directive contains "Harmonized Standards" to which the products must conform.*

- CE Marking on a product indicates to governmental officials that the product may be legally placed on the market in their country.
- CE Marking on a product ensures the free movement of the product within the EFTA & European Union (EU) single market (total 28 countries).

PERFORMANCE OF RETROCHECK-HIV AS PER CE EVALUATION

OBJECTIVE	PERFORMANCE	CE-APPROVED LABORATORY
Evaluation with seroconversion samples	Sensitivity: 100% Specificity: 100%	Laboratorie de Virologie, Paris, Laboratorie de Microbiologie, Paris, France
Evaluation with seroconversion samples	At par with ELISA. Recommended for use.	Central Public Health Laboratory, London
Evaluation with HIV-1 subtypes & HIV-2 samples	Sensitivity: 100%	Institute of Tropical Medicine, Antwerp, Belgium
Subtype O sera samples	Sensitivity: 100%	Laboratorie de Virologie, Cedex, France
Evaluation with chronic patient and HIV-1&2 co-infected sera	Sensitivity: 100%	Laboratorie de Virologie, Paris, France
Evaluation of specificity with RF+, E.coli+ and pregnant female samples	Sensitivity: 100%	Groupe Hospitalier Pitie-Salpetriere, Paris, France
Evaluation of specificity with pregnant female samples	Sensitivity: 100%	Servicie de Biologie, France
Evaluation of specificity with potentially interfering infectious disease samples	Specificity: 99.62%	Establissement Francais du Sang, France

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E-mail: tulip@sancharnet.in. Website: www.tulipgroup.com