VOLUME - I
ISSUE - I I
MAR / APR 2004

The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

ISO 9001

Editorial

Hope you appreciated the contents, format and presentation in the first communiqué.
It brought to you, Dengue Fever, one of the emerging arthropod borne diseases that can be tackled easily if timely and correct diagnosis can be provided to the clinician. It discussed in length about the diagnostic problems faced by the laboratarians and the remedial measures too. The write up asserted the significance of the afebrile phase after the initial febrile episode when clinicians and diagnosticians take the case lightly for that is the phase of "THE LULL BEFORE THE STORM". Laxity at that juncture is usually fatal for the patient and the reputation of the clinician and the laboratory involved. This issue highlights another emerging infectious disease – Leptospirosis – this is also fatal if not diagnosed in its early stages. These manageable diseases have threatened to spare no part of our country. Diagnostic tools usable at the field settings can significantly lower the morbidity and mortality statistics of the diseases mentioned. All relevant clinical information (except therapeutics) and correct diagnostic approach are talked about. In any DISEASE DIAGNOSIS, the CRUX is early and correct diagnosis.

Following in the footsteps of the previous issue, which eased the pain of obtaining blood samples, this issue takes up problems encountered while dealing with multipoint non-linear curves that are so often employed in ELISA AND TURBIDIMETRY based diagnostic platforms. Where the manufacturer provides multiple standards/controls, they must be used in order to achieve accurate and reportable results. The TROUBLE SHOOTING section amply clarifies the reason why.

CK is an important analyte that is requisitioned for by the clinicians. Sometimes just CK-MB is desired by the physician to arrive at a diagnosis of myocardial infarction. This is a grossly unscientific approach to such an important diagnosis where the right diagnosis can mean the difference between life and death. INTERPRETATION elucidates as to why it is mandatory to estimate both, CK-NAC and CK-MB simultaneously. It also enumerates the entities where various CK isoenzymes have altered values.

Here again, we present to you a BOUQUET of varied colours and fragrances. Make fun IN LIGHTER VEIN, think about what WISDOM WHISPERS and don't worry about the BRAIN TEASERS (the answers are provided!).

Trust you shall communicate to us your views about these transmissions from the TULIP GROUP. In case you would want us to discuss about any topic of relevance or importance to the diagnostic community as a whole, we shall be too pleased to do so in one of the forthcoming issues. As this is your forum, you have an inherent right to dictate terms! You can write to us, fax us or E-mail us anything that you wish to say.

We await your responses eagerly.

Tulip Group

DISEASE · DIAGNOSIS

LEPTOSPIROSIS

Leptospirosis is a widely prevalent but clinically under suspected, environmentally acquired and geographically widespread zoonotic disease (not reported only from Antarctica). Congested living, changing environmental conditions and changing human/animal behaviour account for a widespread sporadic case incidence. It is also an important occupational hazard. First Indian case was reported from Andaman Islands but now it is reported from almost every part of the country.

Causative agent: Is a spirochete. Order: Spirochaetalis Family: Leptospiraceae Genus: Leptospira
Species: L. *interrogans* (pathogenic), has 24 serogroups and more than 240 serovars L. *biflexa* (saprophytic), L. *parva* (intermediate).
Leptospires are tightly coiled spirochetes, usually 0.1μm by 6μm to 0.1μm by 20 μm. The helical amplitude is about 0.1μm to 0.15μm and the wavelength is approximately 0.5 μm. The cells have pointed ends, either or both of which are bent into a distinctive hook. They are actively motile rotating/spinning along the long axis bending and flexing sharply. They stain best with silver dyes. Narrow diameter makes visualization possible by dark field microscopy, phase contrast microscopy, or electron microscopy. They are obligate anaerobes; surviving at pH ranges of 6.8 to 7.8 (optimum being 7.2 to 7.6) Leptospires produce various enzymes – catalase, oxidase, lipase, phospholipase, hyaluronidase, aminopeptidase etc.

Mode of Infection

Occurs through urine of an infected animal. Transmission usually occurs when there is direct contact between urine droplets or urine contaminated water and the mucous membranes of the eyes, nose and mouth (as in swimming in contaminated water) or through abraded or intact skin (rarely). Humans can also drink contaminated water and acquire the disease. Carnivores can get the disease by eating an infected carcass. Leptospiriuria occurs in animals for months after initial infection. In humans it may go on for two months. Humans and non-adapted animals are incidental hosts. With rare exceptions, man represents a dead end in the chain of infection, as person-to-person spread is extremely rare. Leptospire organisms can survive outside the body if environmental conditions are favourable. The bacteria prefer moist, slightly alkaline soil, stagnant ponds, and low-flow, slow moving slightly alkaline streams.

Pathophysiology

On gaining entry in the body, the organism multiplies in blood and tissues. The resulting leptospiremia can spread to any part of the body but particularly affects liver and kidneys.
In the kidneys, organisms migrate to renal tubules, and tubular lumen and cause interstitial nephritis and tubular necrosis. Renal failure is due to tubular necrosis but hypovolemia from dehydration and from altered capillary permeability can also contribute to renal failure. Liver involvement is seen as centrilobular necrosis with Kupffer cell hyperplasia. Jaundice may occur because of hepatocellular dysfunction. Leptospires also may invade skeletal muscle, causing oedema, vacuolization of myofibrils, and focal necrosis. Muscular microcirculation is impaired and capillary permeability is increased with resultant fluid leakage and circulatory hypovolemia. In severe disease, a disseminated vasculitis syndrome may result from damage to the capillary endothelium. Leptospires may invade the aqueous humor of the eye, where they may persist for many months, occasionally leading to chronic or recurrent uveitis.
Despite the possibility of severe complications, the disease is most often self-limited and non fatal. Over a time, a systemic immune response may eliminate the organisms from the body, but it may lead to a symptomatic reaction that can produce secondary end-organ injury.

Clinical Aspects

Incubation period is 7-12 days (range is 2-20 days). Approximately 90% of the cases manifest a mild anicteric form of the disease and 5-10% have the severe form with jaundice, otherwise known as Weil disease. Natural course of the disease falls into two distinct phases, septicemic and immune. Between these phases is an intermediate phase of 1-3 days when the patient shows some clinical improvement.

First stage

This stage is called the septicemic or leptospiremic stage because the organism may be isolated from the blood cultures, CSF and most other tissues. This stage lasts for 4-7 days; patient develops a non-specific flu-like illness of varying severity. Chills, weakness, and myalgias primarily affecting the calves, back and abdomen characterize it. Other symptoms are sore throat, cough, chest pain, hemoptysis, rash, frontal headache, photophobias, mental confusion, and other symptoms of meningitis. Because of abrupt onset, the patient can often tell exactly when the symptoms started.

Intermediate stage

During the 1-3 days period of improvement that follows the first stage, the temperature drops and the patient may even become afebrile and relatively asymptomatic. The fever then recurs indicating the onset of the second stage when clinical or subclinical meningitis occurs.

Second stage

This stage is called the immune or leptospiriuric stage because circulating antibodies may be detected or the organism may be isolated from the urine of the patient; it may not be recoverable from the blood or CSF. This stage occurs as a consequence of the body's immunologic response to infection and lasts up to 30 days or more. Disease referable to specific organs is seen. These organs are meninges, liver, eyes and kidneys. Non-specific symptoms such as fever and myalgia may be less severe than in the first stage and last a few days to a few weeks. Many patients experience headache that is intense and poorly controlled by analgesics; this heralds the onset of meningitis.

Anicteric disease: Aseptic meningitis is the most important clinical syndrome observed in the immune anicteric stage. Meningeal symptoms develop in 50% of cases. Cranial nerve palsies, encephalitis, and changes in consciousness are less common. Mild delirium also may be seen. Symptoms may be nonspecific and a viral etiology may be suspected. Meningitis usually lasts a few days, but occasionally can last a couple of weeks. Death is extremely rare in the anicteric cases.

Icteric disease: Leptospires may be isolated from the blood for 24-48 hours after jaundice appears. Abdominal pain with diarrhoea or constipation, hepatosplenomegaly, nausea, vomiting, and anorexia are also seen. Uveitis can develop early or late in the disease (even after 1 year) in 2-10% of cases. Iridocyclitis and chorioretinitis are the other late complications and may persist for years. They manifest 3-4 weeks after exposure. Subconjunctival haemorrhage is the most common ocular complication of leptospirosis occurring in as many as 90% of cases. Leptospires may be present in aqueous humor. Pulmonary manifestations occur in 20-70% of cases, adenopathy, rashes and muscular pain are also seen.

Weil Syndrome

This severe form of leptospirosis primarily manifests as profound jaundice, renal dysfunction, hepatic necrosis, pulmonary dysfunction, and haemorrhagic diathesis. It occurs at the end of first stage and peaks

in the second stage, but the patient's condition can deteriorate at any time. Often the transition between the stages is obscured. Fever may be marked during the second stage. Criteria to determine who will develop Weil disease are not well defined. Pulmonary manifestations include cough, dyspnoea, chest pain, blood stained sputum, hemoptysis, and respiratory failure. Vascular and renal dysfunction accompanied by jaundice develop 4-9 days after the onset of disease, and jaundice may persist for weeks. Patients with severe jaundice are more likely to develop renal failure, haemorrhages and cardiovascular collapse. Hepatomegaly and tenderness in the right upper quadrant may be present. Oliguric or anuric acute tubular necrosis may occur during the second week due to hypovolemia and decreased renal perfusion. Multiorgan failure, rhabdomyolysis, adult respiratory distress syndrome, haemolysis, splenomegaly (20%), congestive heart failure, myocarditis, and pericarditis also may occur. Weil syndrome carries a mortality rate of 5-10%. The most severe cases of Weil syndrome with hepatorenal involvement and jaundice carry a case fatality of 20-40%. Mortality rate is higher for older patients.

Leptospirosis may present with a macular or maculopapular rash, abdominal pain mimicking acute appendicitis, or generalized lymph node enlargement resembling infectious mononucleosis. It also may present as aseptic meningitis, encephalitis, or PUO. Leptospirosis should be considered when a patient has a flu like disease with aseptic meningitis or disproportionately severe myalgias.

Signs And Symptoms

First stage: Fever; subconjuctival suffusion; pharyngeal injection; hepatosplenomegaly; mild jaundice; muscle tenderness; lymphadenopathy, and a macular, maculopapular, erythematous, urticarial, or haemorrhagic rash.

Second stage: *General:* Adenopathy, rash, fever, bleeding, and signs of hypovolemic/cardiogenic shock. *Icteric:* Jaundice, hepatomegaly, abdominal tenderness, signs of coagulopathy. *Pulmonary:* Cough, hemoptysis, dyspnoea, respiratory distress. *Neurologic:* Cranial nerve palsies, confusion, changes in consciousness, delirium, other signs of meningitis. *Ocular:* Subconjunctival haemorrhage, uveitis, signs of iridocyclitis and chorioretinitis. *Haematologic:* Bleeding, petechiae, purpura, ecchymosis, splenomegaly, and abdominal tenderness. *Cardiac:* Signs of congestive heart failure, pericarditis.

Differential Diagnosis

Endemic areas

Dengue fever, Rickettsiosis (Q fever, Typhus), Malaria, Pulmonary tuberculosis, Viral hepatitis, Bacterial/viral meningitis, Influenza, Brucellosis, Ehrlichiosis, Tularemia, Syphilis, HIV, Sepsis, Yellow fever.

Non Endemic areas

Pyelonephritis/UTI, Overwhelming adenovirus infection, Acute abdomen, Gastroenteritis, Atypical pneumonia, Viral haemorrhagic fever, Lung-renal syndrome – as connective tissue disorder or vasculitis.

Immunity in Leptospirosis

IgM antibodies appear early, may remain detectable for months or even years but at lower titers. IgG antibodies detection is variable. May not be detected at all or be detected for short periods only but may persist for years. The antibodies are directed against: a) common antigens (so called genus-specific antigens) that are shared by all leptospires, both pathogenic and saprophytic and b) serovar specific and serogroup specific antigens. Patients with leptospirosis may produce antibodies that react with several serovars. This phenomenon is called cross-reactivity and is observed in the initial phases of the disease. After the acute disease, cross-reactive antibodies gradually disappear as the immune response "matures", usually in the course of weeks or months, while serogroup- and serovar-specific antibodies persist for years. Therefore, genus-specific antibodies are usually detectable for months while serovar-specific antibodies are detectable for years. It is generally believed that serovar-specific antibodies are protective and that a patient is immune to re-infection with the same serovar as long as the concentration (titer) of specific antibodies is high enough. Antibodies provoked by an infection with a particular serovar do not necessarily protect against infection with other serovars.

Laboratory Diagnosis

General Investigations:

Blood: Leucocytosis with neutrophilia with or without shift to the left. Raised ESR.

Elevated aminotransferases (up to 200 U/L). Serum bilirubin and serum alkaline phosphatase may also be raised.

Urine: Proteinuria ±, pyuria ±, RBCs, hyaline and granular casts may also been seen.

CSF: In early phase polymorphs are seen while later in the disease monocytes are observed. CSF protein is normal or raised while glucose remains normal. CSF pressure is normal but a lumbar tap can relieve the headache.

Weil Disease: Marked leucocytosis with neutrophilia. Raised INR. Throbocytopenia (50% of normal), which is accompanied by renal failure. Azotemia and renal failure are the other prominent characteristics. CPK is increased in 50% of the cases. Jaundice in Weil disease is associated with very high CPK, but transaminases are only modestly elevated.

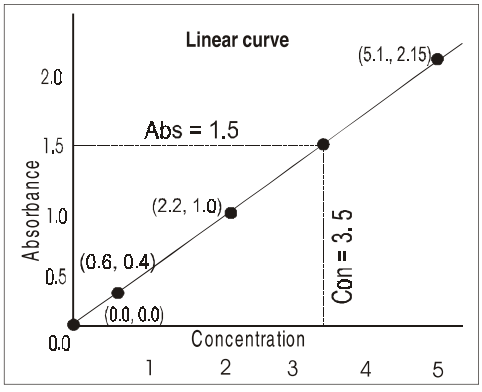
Special Investigations:

- Culture. Laboratories take up to 2 months (urine being acidic gives lower yields)
- Dark field microscopy of body fluids, plasma, bronchoalveolar lavage. Require high level of expertise and yield lower positive results.
- Complement fixation test.
- Latex agglutination test.
- Immunohistochemical stains (gastrocnemius, kidney, lung)
- Counter immunoelectrophoresis.
- One point micro capsule agglutination test.
- Quantitative buffy coat analysis.
- Indirect haemagglutination assay (IHA)
- Sensitised erythrocyte lysis test
- Macroscopic slide agglutination test.
- PCR conducted on urine, serum, aqueous humor, and CSF
- MAT or microscopic agglutination test. MAT is still the diagnostic norm. A fourfold rise in titer between acute and convalescent sera is widely accepted as indicating infection, though in some places a single titer may be considered diagnostic above a minimum value of 1:50 to 1:100. In India it is 1:80. Convalescent titers can remain as high as 1:800 for 13 months and 1:192 for seven years after infection. Such persistence of agglutinins together with the fact that antibodies may delay or lessen the immune response and that some individuals may be seronegative despite being positive for blood cultures, emphasize the need for a means of diagnosis that is more specific to acute infection.
- Immunchromatographic tests – dipstick and device tests. These are IgM antibody based tests that employ broadly reactive Leptospira genus specific antigen. Useful in detecting a current/recent disease in its early phases. Serum, plasma or even whole blood can be used even in field settings and provide accurate results within fifteen minutes. Due to inadequate production of IgM antibodies initially, a second test must always be carried out after a few days if the clinical picture warrants the same.

TROUBLE SHOOTING

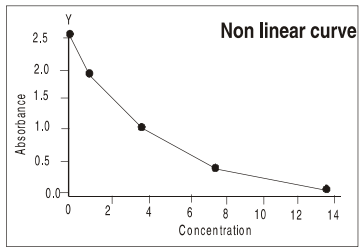
Non Linear Multi Point Assays

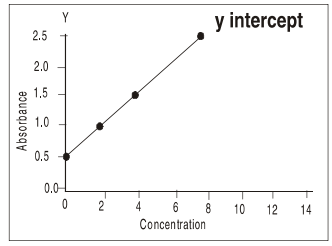
Medical laboratory investigations can be qualitative or quantitative. Qualitative assays are reported as positive or negative, while quantitative assays provide an exact value of the analyte tested. A minute error can change the course of diagnosis and hence the treatment. Most of the instruments we use react to optical alterations caused by the test reactions (referred to as optical densities/absorbances or ODs at specified wavelengths of light). Before the machine can tell the value of an unknown sample, it must have ODs of known standards or calibrators stored in its memory. For linear reactions (till their linearity limits) a single standard/control is good enough, however, for non-linear reactions; multiple standards are mandatory. Most biochemical assays employ linear reactions, while ELISAs and turbidimetric assays utilize non-linear curves.



Understanding graphs and curves: Each graph has two axes 1) x (horizontal) and 2) y (vertical) axes. The point of their intersection is called the origin. In medical diagnostic laboratory parlance x is usually the concentration and y the absorbance axis. The values assigned on the axes are called the co-ordinates. In linear curves, the absorbance changes proportionately to the concentration. There is a constant factor between absorbance and concentration that the curve follows. This is called a linear curve. Extrapolating values are easy, i.e., if one co-ordinate is known, the other can be easily found.

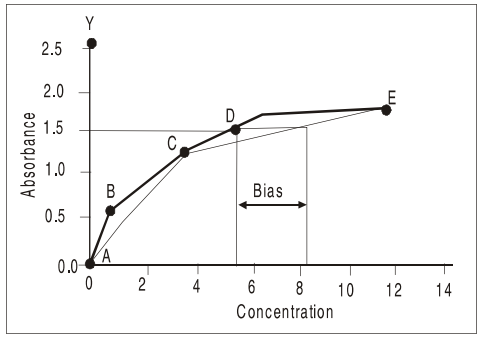
Non-linear curves also follow a relationship/pattern, but it is not constant throughout the curve (it changes between every two values of absorbance and concentration). The factor which defines the direction of curve is called the *slope*. Sometimes when the concentration is zero, the absorbance may not be zero. It may have value; say 0.5 (This minimum absorbance in the absence of any analyte is known as non-specific binding or absorbance due to matrix effect). So when x=0 and the lowest value of y is not, this value is called the y-*intercept*. Likewise, one may have *x-intercept* too.



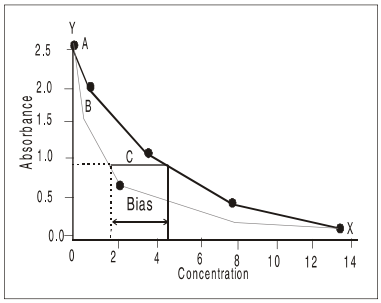


Importance of slope and intercept: Slope gives the direction of curve (in a non-linear curve it changes from point to point). Intercept gives valuable information about non-specific binding, absorbance due to matrix effect, background absorbance due to the substrate/stop solution etc. The terms slope, calibrate and the calibration of the slope and intercept refer to the procedures used to determine the slope adjustment of diagnostic kits to account for changes in such factors as new reagent lots, temperature, pH etc. If there is no kit deterioration and other factors (instrument optics and electronics, working temperatures, pipetting volumes, and test protocol adherance) are kept constant, a onetime plotting of the curve is enough (as with turbidimetric assays) for a particular lot of calibrators and reagents on a particular instrument. Usually, multiple absorbances are plotted against preset values. The values are adjusted or calibrated so that the slope and intercept of the resulting curve conforms to the proper functioning of the kit. In doing so, the manufacturer's instructions for the procedures for each particular kit be strictly adhered to.

A standard curve is a line plotted on graph paper that reflects the pattern of values from a test procedure or instrument in response to variations in the concentration of the analyte being tested. The curve is usually established by using several known values (calibrators) of the analyte under investigation. Most *immunoassays* and *turbidimetric* assays are non-linear curves and hence it is not possible to predict the slope of the curve at a given point without actually plotting the point on the graph. If all given calibrators/dilutions are not used to plot the standard curve, it can affect the slope and hence give rise to bias in the results. Bias is the term used to define the difference in results of measured value from the true/actual value. The dark curve is plotted using all the 5 calibrators while the light curve is plotted using only three calibrators. The slope changes for both the curves between points A-C and C-E. Any

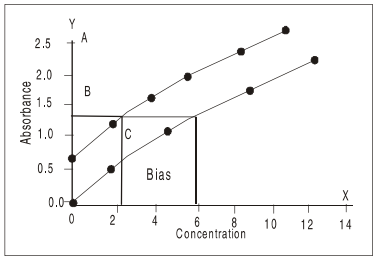


value between these points will show considerable bias in the result. In the example provided the result for dark curve is 5.5 while the result from light curve is 8.2. This can change the interpretation entirely. A single point calibration for non-linear curves gives erroneous results, thereby throwing the hitherto good reputation earned from years of hard labour into disarray. Such a technique upsets diagnosis as well as the prognosis.



Shift of the curve due to change in slope: There can be a shift in the curve due to change in reagent lots, changes in assay procedure which will affect the slope of the curve. If the slope of the curve changes, then, being non-linear it is difficult to predict what will be the changed slope at various points in the curve. Therefore, one has to re-calibrate and establish a new slope for the curve. If one does not re-calibrate, it may cause considerable bias in the assay results from lot to lot and from assay to assay.

Shift in the curve due to change in the intercept: Shift in the curve due to common factor affecting all the components of the kit will cause only a shift in the intercept without changing the slope of the curve. Most of the time such shifts are due to aging of the kit due to continuous storage and usage. Here also there will be a considerable bias in the results and one has to ensure correct results. The advantage of a shift in the intercept without changing the slope is that one can predict the slope of the curve at any point using the shift in the intercept. One can also calculate the change in intercept using just one or two calibrators.However, for accurate results it is always recommended to plot all the calibrators.



The technical presentation concluded amply clarifies as to why it is imperative to use all the required calibrators/dilutions for ELISAs and Turbidimetric assays as per the protocols provided by the manufacturers.

BOUQUET

In Lighter Vein

· A policeman bitten by a dog came for treatment to a government run hospital. He asked the pharmacist, "Arey bhai! Kuttey katne kee dawa dena" – brother give me medicine for dog bite."
The pharmacist asked him, "Santree jee! Aap ko bhee kuttey nay kaat liya – how did a dog bite a policeman?"
The constable replied, "To tell you the truth, I was not wearing my uniform at the time."

· After its debacle in the recent World Cup cricket, the Indian team returned home. Scared of the public ire against its performance, the players decided to stay indoors for some days. Ultimately Srikkant decided to venture out, disguised himself wearing a veil. A lady came and greeted him. Srikkant ran back into hiding. The next day he went out disguised in another costume. Once again the same lady accosted him. Frustrated he asked, "How the hell, did you recognize me?" The lady replied, "Come on, Sri, that is very easy. I am Ravi Shastri!"

· Two friends Santa Singh and Banta Singh, were always boasting of their parents' achievements to each other.
Santa Singh: "Have you heard of the Suez Canal?"
Banta Singh: "Yes I have."
Banta Singh: "Well my father dug it."
Banta Singh: "That's nothing. Have you heard of the Dead Sea?"
Santa Singh: "Yes. I have."
Banta Singh: "Well, my father killed it."

Wisdom Whispers

· I have no yesterdays,
Time took them away,
Tomorrow may not be,
But I have today

· For any beauty you possess at sixteen, be very grateful. Of the beauty you have at sixty, you may be proud – it is your own achievement.

· Before you get upset, ask yourself: does it really matter.

· Sow an act, reap a habit.
Sow a habit, reap a character.
Sow a character, reap a destiny.

· Some of us are like tea leaves.
We don't know our real strength until we are in hot water.

· Avoid people who make long speeches. The less a man knows, the longer he takes to tell it.

· Try loving your enemies. If nothing else, you will confuse them.

· Take a chance. The turtle only makes progress when it sticks its neck out.

Brain Teasers

1. With which cells are Chloromas associated?
A) Myeloblasts B) Plasma cells C) Lymphoblasts
D) Reticulum cells.
2. According to the revised FAB classification of AMLs what does M_6 stand for?
A) Myelomonocytic leukemia B) Promyelocytic leukemia
C) Monoblastic leukemia D) Eryhtroleukemia.
3. In which of the following diseases intraerythrocytic parasites are not seen?
A) Malaria B) Babesiosis C) Bartonelosis D) Loasis
4. In megaloblastic anemia, which of the following is not seen on peripheral smear examination?
A) Howell-Jolly bodies B) Heinz bodies C) Cabot rings
D) Basophilic stippling.
5. Chronic disease of which organ leads to presence of stomatocytes in the peripheral blood?
A) Kidneys B) Thyroid C) Liver D) Spleen.
6. In which leukemia are smudge cells usually seen?
A) AML B) CML C) ALL D) CLL.
7. Of the following, which Plasmodium species shows a crescent shaped macrogametocyte?
A) ovale B) vivax C) falciparum D) malariae.
8. Which of following cytochemical stains is positive in ALL?
A) PAS B) Myeloperoxidase C) Sudan black D) Non specific esterase.

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

INTERPRETATION

CREATINE KINASE (CK)

The two enzymes CK and adenylate kinase (AK) play a decisive role in the synthesis of ATP, the immediate energy source of the muscle, the CNS and many proliferating tissues. Human creatinine kinase is synthesized by a number of different genes. The respective gene products are called CK-M (muscle), CK-B (brain) and CK-Mi (mitochondria). The total CK activity measurable in serum is composed of the activities of the cytoplasmic, dimeric isoenzymes (CK-MM, CK-MB, CK-BB) and their postsynthetically modified forms, and the activities of the macro creatinine kinase (macro CK).

CK Isoenzymes

Isoenzyme	% of total CK
CK-BB (brain)	0–3 (found mainly in brain, also in smooth muscle, thyroid, lungs and prostate)
CK-MB (heart)	0–6 (found mainly in myocardium, also in tongue, diaphragm and skeletal muscle)
CK-MM (muscle)	90-97 (found mainly in the skeletal muscle).

Normal Values (at 37°C)

CK

Adult males 24-195 U/L, Adult females 24-170 U/L

Children: Umbilical cord 175-402 U/L,

Newborns ⩽ 5 days 195-700 U/L,

< 6 months 41-330 U/L, > 6 months 24-229 U/L.

(Conversion of U/L into μKat/L: 1 μ Kat/L=60 U/L)

CK-MB: Normal value ⩽ 24 U/L.

CK-BB: For adults < 2 U/L.

CK-MM: Reference values for total CK activity for adults can be used.

CK-mito: Normal value is < 2U/L

Clinical data, ECG findings and the results of CK determination complement each other with regard to clinical sensitivity and specificity. In spite of determination of CK-MB the differential diagnosis of myocardial infarction / skeletal damage presents problems in the following circumstances: extensive skeletal muscle damage and concomitant small infarction, chronic skeletal muscle disease and myocardial involvement or MI after coronary artery bypass grafting. In these cases determination of one of the cardiospecific troponins is necessary.

Diagnostic Alert

As adenylate kinase interferes with CK estimation and AK is found to a greater extent in the Indian population. It becomes imperative to use reagents that are capable of inhibiting AK so as not to over estimate CK. A report generated by employing inappropriate kits can initiate unnecessary therapy.

Approximate distribution of the CK isoenzymes in human organs

Tissue	U/g	CK-MM	CK-MB	CK-BB	CK-mito
Skeletal muscle	800-4000	++++	(+)	(+)	+
Myocardium	240-800	+++	++	(+)	++
Brain	⩽ 550	-	-	+++	++
Bladder	⩽ 135	-	-	++++	+
Blood	⩽ 0.2	++++	(+)	-	-
Colon	⩽ 200	(+)	(+)	++++	+
Umbilical cord blood	⩽1.0	++++	(+)	+	?
Prostate	⩽ 135	-	-	++++	?
Uterus	⩽400	-	-	++++	+
Vein wall	⩽ 60	-	-	++++	?

(++++:>75%, +++:50-75%, ++:25-50%, +:5-25%, (+):<5% at 37°C)

Total CK and CK-MB trends in acute myocardial infarction

	Total CK	CK-MB
Initial rise:	2-6 hrs after onset of damage	4-8 hrs after onset of damage.
Peak levels:	18-36 hrs after onset of damage	18-24 hrs after the onset of damage.
Return to basal levels:	3-6 days after onset of damage	3 days after onset of damage

6% Rule

The decision criterion is an increase in the total CK activity to > 240 U/L (37°C) within the diagnostic time window and a simultaneous increase in CK-MB activity. A CK-MB fraction more than 6% of the total CK activity is regarded as diagnostic for MI. A fraction < 6% indicates skeletal muscle damage. The clinical specificity of the 6% rule is high as the number of false positive results caused by presence of extracardiac CK-MB is small. However, following this rule, smaller MIs may be missed. False positive values can be caused by Adenylate Kinase, which occurs in large quantities in the liver and in blood cells.

Increased Total CK: Amyotrophic lateral sclerosis, anoxia, atresia (biliary), bowel injury, brain tumour, burns (thermal, electrical), cancer (breast, lung, oat cell, gastrointestinal, prostatic), carbon monoxide poisoning, cardiomyopathy (cobalt-beer), carrier state (for Duchenne's muscular dystrophy), cerebrovascular accident, CNS trauma, coma (hepatic), convulsions, coughing (severe), delirium tremens, dermatomyositis, eosinophilia-myalgia syndrome, exercise, head injury, haemodialysis, hypokalemia (severe), hypothermia, hypothyroidism, infarction (bowel, cerebral, myocardial, prostate), intoxication (alcohol, salicylate), intramuscular injection (recent), labor, leptospirosis, malignant hyperthermia, meningoencephalitis, muscle spasms, muscular dystrophy (Duchenne's, limb-girdle, fascioscapulohumeral), myocarditis, myoglobinuria, myopathy (from alcoholism), myotonic dystrophy, myxedema, necrosis of striated muscle, organ rejection (heart transplant), parturition, polymyositis, pregnancy, prostatic injury, psychosis (acute with agitation), pulmonary edema, pulmonary embolism, renal failure, renal insufficiency (chronic), Reye's syndrome, rhabdomyolysis, Rocky Mountain spotted fever, shock, skeletal muscle disorders, status epilepticus, striated muscle atrophy (acute), subarachnoid haemorrhage, surgery (bowel, cardiac, CNS, prostate), tachycardia, thyrotoxicosis, toxic shock syndrome (day 7), trauma (muscular), typhoid fever, and very muscular people.

Increased CK-BB: Anoxia, atresia (biliary), cancer (breast, gastrointestinal, oat cell, prostatic, widespread malignancies), cerebrovascular accident (hemorrhage, infarction), hemodialysis, hypothermia, intestinal necrosis, labor, malignant hyperthermia, renal failure, shock, surgery (CNS), and uremia.

Increased CK-MB: Anoxia, burns (electrical, thermal), cancer (lung), carbon monoxide poisoning, cardiomyoapthy (cobalt-beer), collagen vascular diseases, congestive heart failure (rare), coronary angiography (rare), coronary insufficiency (rare), hypothermia, hypothyroidism, malignant hyperthermias, muscular dystrophy (Duchenne's), myocardial infarction, myocarditis, myoglobinuria (severe), polymyositis, pulmonary embolism, renal insufficiency (chronic), Reye's syndrome, rhabdomyolysis, Rocky Mountain spotted fever, surgery (cardiac, valve replacement), SLE, and trauma (cardiac).

Increased CK-MM: Cardiac catheterization (with myocardial damage), cardioversion, coronary arteriography (with myocardial damage), hypothyroidism, intramuscular injection, muscle trauma, myocardial infarction, psychosis (acute with agitation), Reye's syndrome, shock, surgery, and trauma (skeletal muscle).

Decreased Total CK: Addison's disease, anterior pituitary hyposecretion, connective tissue disease, hepatic disease (alcoholic), low muscle mass, metastatic neoplasia, and pregnancy (first half). Drugs include steroids.

Decreased CK-BB, CK-MB, CK-MM: Clinically insignificant/ not applicable.

TULIP NEWS

Instruments Division

Influencing the trends in diagnostic industry with innovative reagents and test systems has been the hallmark of Tulip since its inception.

Tulip Instruments Division was launched in the year 2000 to complement the Precision, Accuracy and Reliability of Tulip Reagents, with Analysers that could perform reliably in stringent domestic conditions. The Instruments Division currently has over 500 Installations with more than 30 Service Engineers and Application Specialists in 11 Branches all over India.

SCREENMASTER 3000 sturdy, user friendly, fully open system with 90 programmable locations for performing End-points, Kinetics, Fixed-time Kinetics and Multistandard Assays on Cuvette or aspiration modes. Software for QC includes, mean, SD, CV & Levy Jennings Chart. With thermal printer and 10-position incubator, the Screenmaster 3000 Is a workhorse for medium sized laboratories.

MAPLAB Plus The only Semi Auto Analyser with a True Elisa Reader that can perform End-points, Kinetics, Fixed-time Kinetics, Multistandards, Qualitative, Latex, Elisa and EIA tests. Software for QC includes, mean, SD, CV & Levy Jennings Chart. With thermal printer and 10-position incubator the MapLab Plus is the only clinical analyser of its kind in India.

QUANTIAMATE The only Turbidimetry analyser with standardised reagents that gives the laboratory an optimization of reagents, assay procedures and measuring system to deliver accurate and precise results. Can also be used as a backup chemistry analyser.

FULLY The sturdiest fully automated batch analyser with a stat mode. With a Windows based software that is user friendly, FULLY is the ideal clinical analyser for all routine tests in a busy modern lab.

HEMOSTAR Sleek, user-friendly coagulation analyser based on turbo-photometric detection of clot based assays. Hemostar has 10 in-built incubation positions for sample

HEMOSTAR XF Single channel coagulometer with turbo-photometric clot detection principle, which improves sensitivity of detecting weak fibrin polymers. Hemostar XF has 10 locations included four programmed locations for PT, APTT, TT and quantitative fibrinogen assays. This walk away plug and play system has been designed to make complicated clotting analysis of hemostasis system into a simple error free task.

CoaLAB 6000 Fully automated compact & versatile bench top coagulation analyser for clotting assays based on the patented Turbodensitometric principle of clot detection. CoaLAB 6000 is engineered to operate in random access mode for PT, APTT and Fibrinogen with an option for batch and stat mode. CoaLAB 6000 has the agility for throughput of 130 PT tests per hour. CoaLAB is an ideal coagulation analyser designed to simplify complicated coagulation analysis into a walkway task for laboratories with high and medium throughput.

Printed and published by D.G. Tripathi, Edited by Dr. R.J. Sood M.D. (path.) for and on behalf of Tulip Diagnostics Private Ltd, Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh Alto Santacruz, Bambolim Complex Post Office, Goa - 403202, INDIA. Fax: (0832) 2458544, E-mail: tulip@sancharnet.in. Website: www.tulipgroup.com