VOLUME - II
ISSUE - VII
JAN / FEB 2005

The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

Editorial

With this issue starts the second year of existence of "THE CRUX". First of Volume II, seventh in line, this issue too retains the old flavor and fervour that has been highly appreciated by our readers. We have been taking up topics of current relevance under the three fixed heading articles, viz., DISEASE DIAGNOSIS, INTERPRETATION AND TROUBLE SHOOTING.

Many a times we have covered topics as suggested by our readers. Trust our efforts proved to be of use to you. We assure you, in times to come, these volumes will ease your anxiety in future on many occasions. It is almost impossible to convey all aspects related to a particular investigation to a clinician and there will be times when the clinician may not agree with the result of the investigation provided by you. Classical book-picture results are hard to come by now a days, so, when you would need to explain the likely variables these issues would certainly come in handy and relieve your pain. One such investigation that has many variables is Prothrombin time (INR); the results depend upon a host of method and patient related factors. The INR may vary vastly in the same patient without any obvious reason. Hitherto hidden reasons would become obvious once you go through the TROUBLE SHOOTING section of this issue.

INTERPRETATION folios discuss the clinical application of doing Immunoglobulin assays. A brief resume is given about the immunoglobulins followed by clinical situations where their levels are altered.

The DISEASE DIAGNOSIS segment considers Malaria in this communication. All clinico-diagnostic aspects have been discussed in detail. The pros and cons of the newer platforms that are now commercially available have been discussed threadbare. The propensity of the disease dictated that we take up the matter in length and we have done so.

Having gone through the printed matter you would be crystal clear about the diagnostic approaches towards malaria especially in context to the immunochromatographic techniques. These kits detect malaria at field level, they differentiate species and do not require a highly trained manpower (even primary health center staff can perform these tests) to perform the test AND WHAT'S MORE they even tell you that the disease has been cured!

To comfort you from the day-to-day tensions, BOUQUET has not been forgotten.

DISEASE DIAGNOSIS

MALARIA

Introduction

(Mal=Bad, Aria=Air as in marshy/swampy regions).
Malaria is widely prevalent in the hot and humid tropical and sub-tropical countries that fall between 23.5 North Latitude and 23.5 South Latitude. This includes regions of Africa, Asia, South America, Central America, the Caribbean and some isolated parts of North America. Four *Plasmodium* species infect the humans, viz., *P. vivax* (temperate zone), *P. falciparum* (tropical zone), *P. ovale* (East Africa, West Africa and the Philippines in Asia), *P. malariae* (sub-tropics). Climatic changes, human migration and the drug resistance are the key factors that have contributed to the spread of the disease. As the global warming proceeds, more and more regions are likely to fall under the malaria belt. The human malaria closely resembles the primate malarial species and perhaps the human forms have evolved from the simian forms. The first cases were recorded in 1700 BC (China) and 1570 BC (Egypt). Today 300-500 million new cases are seen annually and about 2-3 million of these turn out to be fatal. Usually, wherever malaria is rampant, the Governments have created specific cells or institutions that are concerned only with malaria and its control. In India it is NMEP.

Malaria, Life Cycle

Two hosts are required to complete the life cycle. Females of about a dozen Anopheles species transmit the human malaria. Female mosquito bites an infected person and sucks up the gametozoites along with the blood. The gametes mature and in the stomach of the mosquito they fuse forming a zygote. The zygote enters the wall of the mosquito's gut and develops into an oocyst. Oocyst produces thousands of sporozoites, which migrate to the salivary gland of the mosquito; these are transmitted to humans with the bites to spread the disease. The mosquito cycle takes about 10-14 days to complete.
Through the blood, the sporozoites reach the liver and in 1-2 weeks they divide to form 30,000 - 40,000 merozoites. At this point they leave the liver and enter the blood stream to infect the RBCs and multiply within them to form another lot of merozoites and burst the infected red cells. This is the time when the clinical symptoms are experienced. Some merozoites are converted into gametocytes (male and female) and the cycle repeats itself all over again. *P. vivax* and *P. ovale* have exo-erythrocytic cycles and hence require radical cures with drugs like primaquin.

PrevalenceRates

Globally, approximately 43% cases are caused by *P. falciparum,* 50% by *P. vivax,* 7-8% by *P. malariae* and the rest by *P. ovale.* In India, *P. vivax* accounts for about 60% infections, *P. falciparum* for 40% and *P. malariae* for about 1% of the infections. Approximately 4-8% infections are of a mixed origin. *P. falciparum* (because of high mortality and morbidity associated with it) and *P. vivax* (due to morbidity and high infection and relapse rate) are still considered to be "The Big Two"; and have therefore become the targets for prevention, eradication, diagnosis and treatment.

Clinical Malaria

Incubation time: The time between the bite and development of malaria is 8-14 days except in *P. malariae* (35 days).

Classical Presentation: To begin with, the symptoms resemble those of a minor viral illness such as lack of sense of well being, headache, fatigue, abdominal discomfort & myalgia followed by fever and nausea/vomiting. These may be followed by typical malaria picture such as fever spikes (sudden rise and fall of temperature), chills and rigors.

Cold stage: With pyrexia, there is intense headache and muscular discomfort. The patient feels cold, clutches blankets and curls up shivering and uncommunicative (the Rigor). Within minutes the limbs begin to shake and teeth chatter, and the temperature hikes rapidly to a peak. This phase usually lasts for 10-30 minutes but can extend up to 90 minutes.

Hot stage: At the end of the rigor there is vasodilatation and the skin feels hot and dry. The temperature, though, is high. Profuse sweating which lasts for up to 2-4 hours follows the hot stage. During this period the patient is soaked in sweat and the temperature falls. The blood pressure too falls on account of vasodilatation. The patient feels exhausted and may sleep. Defervescence usually takes 4-8 hours. Fever is irregular at first with temperature exceeding 39°C, however, it may rise up to 40°C.

Left Untreated:

- Fever recurs every third day in *P. vivax* and *P. ovale* infection establishing a two-day (tertian) cycle.
- Spike occurs every three days (quartan) in *P. malariae* infection i.e., Fever occurs every fourth day.
- The fever pattern in *P. falciparum* infection is erratic. Paroxysms with rigors are more common in *P. vivax* and *P. ovale* than in *P. falciparum* and *P. malariae* malaria.True rigors are unusual in naturally acquired falciparum malaria.
- As the infection continues there is hepatosplenomegaly with concurrent development of anemia. The patient loses weight. If no treatment is given, the natural infection stabilizes for several weeks or months and then gradually resolves.

Relapse: Relapse is the return of the disease after apparent cessation. Both *P. vivax* and *P. ovale* have a tendency to relapse after resolution of a primary infection. Relapse occurs weeks or months after the primary infection.

Recrudescence:This is the recurrence of symptoms after temporary abatement. While recrudescence occurs after a few days or weeks, a relapse occurs after some weeks or months. *P. falciparum* is the usual cause of recrudescence infection and these arise 2-4 weeks following treatment.

Complications in Malaria

Severe falciparum malaria: Fatal malaria is nearly always caused by *P. falciparum*. It can manifest as

- Cerebral malaria: The clinical symptoms of cerebral malaria are coma, severe anemia (especially in children), increased or diminished muscle tone, fever, jaundice (adults), hepatosplenomegaly and retinal hemorrhages. Left untreated, it is nearly always fatal. Overall fatality rate is 15% in children and 20% in adults (but is up to 50% in pregnant women).
- Hypoglycemia: May be asymptomatic or may aggravate coma further.
- Pulmonary edema: Hyperventilation (respiratory distress).
- Acute renal failure: Is a common complication of malaria in adults living areas of low or unstable transmission. There can be oliguria or polyuria. May be associated with jaundice and bleeding tendency.
- Metabolic acidosis: Associated with hyperventilation with increased respiratory effort and/or hypotension.
- Blackwater fever: This is a condition where:
  After several episodes of falciparum malaria, particularly if there has been inadequate treatment, there is occasionally an abrupt onset of massive intravascular hemolysis with fever, chills and prostration. There is hemoglobenemia and hemoglobinuria. Hemoglobin in acidic urine gives it a blackish coloration and hence the term Blackwater fever.
- Algid malaria: Algid malaria is a condition where:
  There may be subnormal temperature, weakness, prostration, feeling cold, vomiting, loose motions, rapid respiration and oliguria. Death may occur but the patient is conscious till the end. The reasons could be adrenal crisis, absorption of endotoxin from the gut or cachetin-tumor necrosis factors from endotoxin Activated macrophages.
- Malaria in pregnancy: There is increased risk of severe falciparum malaria in the

in regions of reduced or diminished transmission, it is an important cause of fetal death and results in high maternal immortality. In regions of intense transmission it may be associated with low birth weight. The infected mothers may be asymptomatic.

- Malaria in Children: The majority of malaria infections present with fever and malaise. In addition to the clinical features mentioned for adults, malaria in children may lead to convulsions, coma, hypoglycemia, metabolic acidosis and severe anemia.
- In highly endemic areas, the patient may be infected with one, two or even more species of the malarial parasite.
- Chronic malaria often leads to Tropical Splenomegaly Syndrome (TSS), which has its own attendant complications.

Differentiating Features of The "Big Two"
(*P. vivax* and *P. falciparum* malaria)

Sr. No.	*P. vivax*	*P. falciparum*
1	*P. vivax* is relatively **benign** and rarely produces serious complications or death.	*P. falciparum* on the other hand, is associated with **serious complications** e.g. cerebral malaria, jaundice, renal failure etc. including high mortality.
2	In *P. vivax* , **relapse occurs** due to persistence of inactive forms (Hypnozoites) in liver tissues which periodically invade blood stream producing clinical malaria.	*P. falciparum* does not have any dormant form in liver and once the infection is cured, there is **no relapse**.
3	In *P. vivax* malaria, less than **1% of RBC's** are parasitised.	In *P. falciparum,* the number of **RBC's** involved may go up to **35%.**
4	Mainly the young RBC's are infected.	Infects young and old erythrocytes alike.
5	The **gametocytes** (sexual stage) -male and female mature in **peripheral blood** and are sucked up by the female Anopheles mosquito for completion of their life cycle.	For maturation of gametocytes, *P. falciparum* **must invade deeper circulation**. The blood capillaries of internal organs get clogged with infected RBC's thus obstructing flow of blood. Also some biochemical changes take place which damage the organs.
6	Gametocytic stage persists in the peripheral blood for **2** days.	Gametocytes persists in the blood for **30-60** days or more.

Drug Resistant Malaria

Literally, it means malaria that does not respond to the usual anti-malarial medicines. *P. falciparum* accounts for 70% of these cases. Resistance to chloroquine, sulphadoxine + pyrimethamine combination and quinine have been reported.

Clinical and Laboratory Diagnosis of Malaria

- Clinical diagnosis is still the commonly used approach, particularly in the rural setups. Primary health care centers with provision of peripheral smear examination are gradually spreading even in the third world. One must, however, remember that malaria symptoms can mimic other febrile illnesses. Clinical laboratory diagnosis should be mandatory.
- Microscopy of thin and thick smears for detecting malarial parasites with routine hematology stains is an established method for confirmation of malaria. An expert can detect as low as 10 parasites per l of blood. Apart from being helpful in speciation, morphological alterations can be read to assess the parasitemia, as well as response to chemotherapy. While microscopy is relatively cheap, it has its attendant shortfalls:

  In field conditions, typical microscopic procedures may not detect parasitemia under 100/ l of blood.

  Microscopy is labor intensive and each slide may take up to 60 minutes from sample collection to provision of results

  Microscopist's expertise and the efficacy of stains used come into play.

Microscopy Interpretation Chart for Malaria Species

Stage (or period of infection)	*P. vivax*	*P. malariae*	*P. falciparum*	*P. ovale*
Early trophozoites	1/3 diameter of RBC; prominent vacuole; heavy chromatin	Single chromatin dot; vacuole less common than other species; cytoplasm "heavy"	1/5 diameter of RBC; small chromatin; marginal forms frequent	Similar to *P. vivax* and *P. malariae*
Late trophozoites	Large amount of chromatin; hemozoin almost fills cell	Cytoplasm dense, round oval or band shape; nearly fill cell	Not usually seen in peripheral blood	Compact cytoplasm; small (if any vacuole)
Hemozoin	Short rods, scattered irregularly; yellowish brown in color	Rounded; large, darker than in *P. vivax;* often peripheral	Granular; coarse in gametocytes	Lighter than in *P. malariae;* similar to *P. vivax*
Erythrocytes	Large than normal irregular shaped; Schuffner's dots apparent in all but earliest stages; multiple infections common	About normal; stippling and multiple infections are rare	Normal size; Maurer's dots often in late trophozoites (late troph's rarely seen in peripheral blood)	Schuffner's dots often present; red cell often enlarged and shaped irregularly
Schizont	12-24 merozoites; hemozoin clumped; often fills cell	8-10 merozoites in a rosette or cluster	8-24 merozoites (rarely seen in peripheral blood)	4-16 merozoites
Microgameto-cytes (usually smaller and less common than macrogameto-cytes)	Rounded or oval; almost fill cell; hemozoin evenly distributed; chromatin clumped minimal cytoplasm; no vacuoles	Similar to *P. vivax* but smaller; pigment more conspicuous	Crescent shaped; about 50% larger than blood cell; chromatin diffuse; hemozoin central pale blue cytoplasm	Similar to *P.vivax* but smaller
Macrogameto-cytes	Similar to microgametocyte but cytoplasm darker blue; chromatin more compact and red	Prominent pigment; round, dark brown granules; course than in *P. vivax*	Similar in size and shape to microgameto-cyte; chromatin red more compact hemozoin concentration	———
Exoerythrocytic cycle	8 days	13 days	6 days	9 days
Prepatent Period (minimum)	11-13 days	15-16 days	9-10 days	10-14 days
Schizogonic cycle	48 hours (tertain)	72 hours (quarten)	36-48 hours (tertian)	48 hours (tertian)
Development in mosquito	10 days	25-28 days	10-12 days	14 days

Rapid Diagnostic Tests (RDTs) for Malaria

These are ideal devices that can be used even at the field level and now they can even differentiate amongst various species. Immunological tests employ interplay of antigens and antibodies. The two most important antigenic markers targeted for detection by the RDTs are: a) Histidine Rich Protein-II (HRP-II) and b) Parasite Lactate Dehydrogenase (pLDH). To date, the following monoclonal antibodies have been raised to detect malarial antigens in human blood:

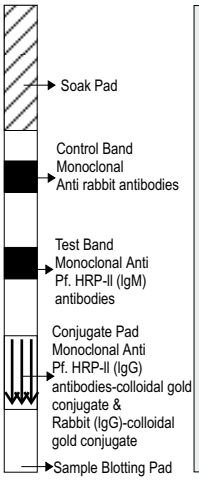
1) Antibodies to HRP-II specific for *P. falciparum.* 2) Antibody to pLDH specific *P. falciparum* and *P. vivax* malarial parasites and 3) Antibodies to pan malaria pLDH which is common to all four species of interest to man. While HRP-II based tests can detect malaria even after a complete cure has been affected, the pLDH-based tests detect an ongoing current infection only. HRP-II antigen takes 2-3 weeks to clear off from the patients system but pLDH antigen clears off within 2-3 days after a complete cure has been affected.

Rapid Diagnostic Tests for Malaria: Formats Pros and Cons

Rapid Diagnostic Tests (RDTs) have now come to the rescue of Laboratarians and the Clinicians alike. They provide quick and reliable reports, can be used even by untrained staff (even at field level) and what's more, they provide exact speciation too. The malaria diagnosis has never been so simple before as these RDTs detect even low parasitemia cases too. Need one ask for more!

First Generation

Pf. HRP II stand alone RDT



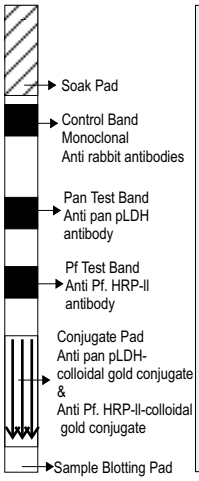
Pros

- Sensitivity and specificity > 98% for *P. faliciparum* malaria
- Results comparable with Gold Standard
- No cross reactivity with other species
- Correlates with on-set of clinical symptoms

Cons

- Stand alone test for *P. falciparum* malaria
- Cannot be used for monitoring therapy
- Cannot identify "Carriers" due to absence of HRP II in gametocytes

Pf. HRP-II / Pan pLDH combination RDT



Pros

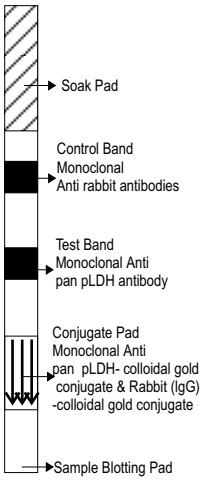
- Pan band specific for all 4 malarial species
- High sensitivity for *P. falciparum*

Cons

- Differentiation between stand alone *P. falciparum* & mixed infections is not possible
- In endemic areas of high prevalence of mixed infection, need for speciation under microcopy for correct therapy
- Presumption of causative species based on the presence of pan band and species prevalence rate

First Generation

Pan pLDH stand aloneRDT



Pros

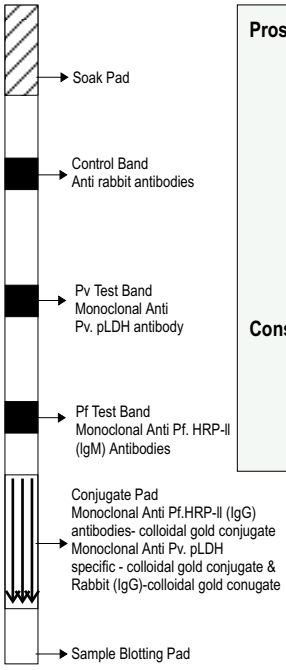
- Pan band specific for all 4 malarial species
- Employed for screening of collected blood bags and potential donors
- Monitoring success of antimalarial therapy

Cons

- Cannot speciate and differentiate between *P. falciparum, P. vivax, P.malariae and P.ovale*

Second Generation

Pf. HRP-II / Pv pLDH combination RDT



Pros

- Can speciate and differentiate between the "Big Two"
- Good clinical sensitivity and specificity > 98% for *P. falciparum* malaria, due to HRP-II based detection system
- Overcomes limitations associated with stand alone HRP-II based assays
- Suited for detection of mixed infections

Cons

- Cannot speciate and differentiate *P.malariae* and *P.ovale*

It is imperative the the second generation RDTs for malaria should meet all the assay design requirements and help to speciate between the "**Big Two"**.
Third generation tests likely to be available in the not too distant future, would be able to conserve all advantages of the second generation tests but also improve upon the same being able to differentiate between all the malarial species namely; *P. falciparum, P. vivax, P. ovale* and *P. malariae.*

INTERPRETATION

INTERPRETATION IMMUNOGLOBULINS (Ig)
(Immunoglobulins IgG, IgM and IgA)

Immunocompetent persons have an immune system that can be divided into the following two functionally cooperative but developmentally independent ways:

- Thymus (T) lymphocyte system; it represents a functionally heterogenous group of cells concerned with immune regulation and antigen elimination.
- Bursa or bone marrow (B) lymphocyte system; B lymphocytes differentiate into plasma cells which synthesize and secrete antibodies after an antigenic stimulus

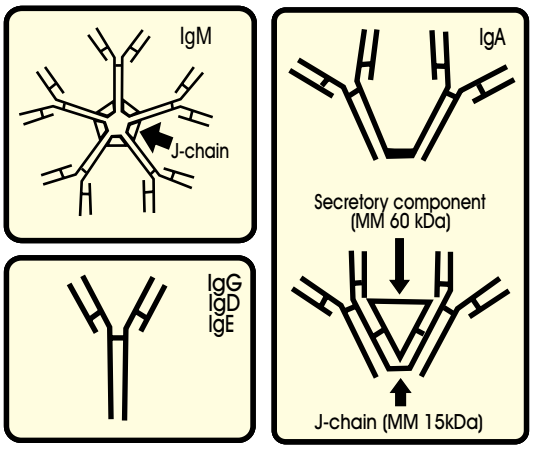
Immunoglobulins represent a heterogenous group of proteins with antibody function, i.e. they are capable of binding antigen. The structure of antigen binding site is made according to the configuration of the antigen with which the antibody reacts. Immunoglobulins have following effector functions:

- Formation of immune complexes with antigens
- Binding the membrane receptors of defense cells and their activation
- Reaction with plasma proteins, e.g. with complement components, and activation of these proteins in order to eliminate the antigen

Ig Classes

IgG, IgA, IgM, IgD, and IgE are present in descending order of concentration. IgG has subclasses from IgG_1 to IgG_4. IgA and IgM have two subclasses each namely 1 and 2.

Ig Structure



Immunoglobulin G (IgG)

Increased in	Decreased in
• IgG myeloma • Sarcoidosis • Chronic liver disease • Autoimmune diseases • Parasitic diseases • Chronic infection	• Acquire immunodeficiency • Hereditary deficiencies • Protein- losing syndromes • Pregnancy • Non IgG myeloma • Waldenström's macroglobulinemia

Immunoglobulin M (IgM)

Increased in	Decreased in
• Liver disease • Chronic infections • Waldenström's macroglobulinemia	• Hereditary deficiency • Acquired immunodeficiency • Protein losing syndromes • Non IgM myeloma • Infancy, early childhood

Immunoglobulin A (IgA)

Increased in	Decreased in
(in relation to other Ig's) • Gamma-A myeloma (M-component) • Cirrhosis of liver • Rheumatoid arthritis with high titers of rheumatoid factors • SLE (some patients) • Sarcoidosis (some patients) • Wiskott-Aldrich syndrome • Other rare entities	*(alone)* • Normal persons (1:700) • Hereditary telangiectasia (80% of patients) • Type III dysgammaglobulinemia • Malabsorption (some patients) • SLE (occasionally) • Cirrhosis of liver (occasionally) • Still's disease (occasionally) • Recurrent otitis media(occasionally) • Non IgA myeloma • Waldenström's macroglobulinemia • Acquired immunodeficiency *(combined with other Ig's)* • Agammaglobulinemia Acquired Primary Secondary (multiple myeloma, leukemia, nephritic syndrome, protein losing enteropathy) Congenital Hereditary thymic aplasia Type I dysgammaglobulinemia (all, IgG, IgM,and IgA decreased) Type II dysgammaglobulinemia (IgA and IgM absent, IgG has normal levels) Infancy, early childhood.

Serum Immunoglobulin Changes in Various Diseases

Disease	IgG	IgA	IgM
Immunoglobulin disorders			
Lymphoid aplasia	D	D	D
Agammaglobulinemia	D	D	D
Type I dysgammaglobulinemia (selective IgG and IgA deficiency)	D	D	N or I
Type II dysgammaglobulinemia (absent IgA and IgM)	N	D	D
IgA globulinemia	N	D	N
Ataxia Telangiectasia	N	D	N
Haematological neoplasms			
Heavy chain disease	D	D	D
IgG myeloma	I	D	D
IgA myeloma	D	I	D
Macroglobulinemia	D	D	I
ALL	N	D	N
CLL	D	D	D
AML	N	N	N
CML	N	D	N
Hodgkin's disease	N	N	N
Liver disease			
Hepatitis	I	I	I
Laennec's cirrhosis	I	I	N
Biliary cirrhosis	N	N	I
Hepatoma	N	N	D
Miscellaneous			
Rheumatoid arthritis	I	I	I
SLE	I	I	I
Nephrotic syndrome	D	D	N
Trypanosomiasis	N	N	I
Pulmonary tuberculosis	I	N	N

N=normal, I= increased, D=decreased

TROUBLE SHOOTING

QUALITY ASSURANCE FOR ROUTINE HEMOSTASIS LABORATORIES

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

Patient preparation

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

Sample collection techniques

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

Sample preparation

The anticoagulant of choice is buffered sodium citrate (3.2% or 0.109M). Factors V and VII remain stable in it. These factors are more labile in oxalate and heparin neutralizes thrombins action on fibrinogen. Buffered citrate neutralizes CO_2 that is absorbed while sample processing.
The buffered 3.2% Sodium Citrate negates the effects of absorbed CO_2 which happens on centrifugation of the sample for separating the plasma. Using 3.8% citrate would produce unduly prolonged timings. The optimum citrate to blood ratio is 1:9. With apt molarity of citrate, all available calcium is bound and clotting is prevented. A shift in the ratio leads to erroneous results as is explained: **More blood less citrate-** Calcium chelation will be inadequate leading to micro clot formation and consumption of factors which at the end would lead to unduly prolonged timings. **More citrate less blood-** Excess citrate would consume calcium from the reagents and would again give prolonged timings eventually.

For APTT the optimum concentration of $CaCl_2$ is 0.02 M. This replaces the calcium necessary to activate the intrinsic coagulation cascade. This ultimately generates thrombin from the prothrombin via the coagulation cascade. Appropriate volumes of $CaCl_2$ should be aspirated for the days work. Prewarmed $CaCl_2$ should always be discarded at the end of the day.

The anticoagulant to blood ratio of 1:9 is for a normal hematocrit (one that falls within normal range for the age and sex of the patient).

The formula for the anemic and polycythemic patients is $\mathbf{C = 1.85 \times 10^{-3} (100-H) V}$
Here C=volume of sodium citrate in ml. V=volume of whole blood-sodium citrate in ml. H=Hematocrit in percentage. When PCV is higher than 55% the patient blood contains so little plasma that excess unutilized anticoagulant remains and is available to bind reagent calcium leading to prolongation of the results. Contrary to this when PCV is less than 20% the patient blood contains excess plasma but less anticoagulant leading to formation of micro clots with consequent consumption of factors and eventually prolongation of test timings.

Sample Processing and Storage

Ideally containers for collection and processing should be made of plastic or siliconised glass. They should be scrupulously clean and dry. Scratched glass surfaces can activate the clotting mechanism. Leavening agents used by the plastic industry have an inhibitory effect. All containers should be free from detergents, acids and alkalies. These chemicals have an effect on the pH. Change in pH effects factor stability. Detergents inhibit reactive characteristics of the sample/reagent mixture. Nothing is better than using clean disposable labware.
Time: Ideally the samples must be processed immediately. At room temperature (22°-24°C) the tests must be conducted within 2 hours while if held at 2°- 4°C the time available is 3 hours. Plasma samples should not be held at 4°- 8°C for prolonged periods as they can undergo cold activation. Samples obtained for factor and fibrinolysis assays should be stored in crushed ice if delay is anticipated. Citrated blood for platelet aggregation studies should remain in capped tubes at room temperature (20°-25°C) before testing.
Centrifugation : All samples collected must be tightly capped to prevent absorption of atmospheric CO_2, which can shift the pH, and hence the eventual results. Centrifugation speeds are also important. The PT uses platelet poor plasma (PPP) while the APTT uses platelet free plasma (PFP). Excessive centrifugation on account of heat generation can destroy the clotting factors. Under centrifugation would invariably leave platelets in the plasma leading to activation of the clotting mechanism in vitro again leading to erroneous results. Normally centrifugation for 15 minutes approximately 1500 G yields PPP and at 2000 G produces PFP. The "G" is the function of length of rotor head and RPM. Each laboratory should calculate its own ideal centrifugation time and speed.

Calibration of instruments / equipments

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

Storage of Reagents

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

End Point Reading

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

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

Drugs/Clinical Conditions influencing patient results

PT tests are influenced on administration of the following drugs

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

APTT tests are influenced by administration of the following drugs

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

Thrombin test time is prolonged in the following circumstances

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

MNPT and INR

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

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

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

Quality Control Aspects

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

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

AT ALL COSTS ADHERE TO THE MANUFACTURER'S INSTRUCTIONS.

BOUQUET

In Lighter Vein

- I don't know whether there is such a thing as a Day of Reckoning after death. For men of religion, I quote an anecdote. Someone asked a friend, notorious for drinking, if his brother who was a priest raised any objections to his heavy consumption of liquor. He replied, "We have a very good relationship. He prays for me, I drink to his health."

- An American Red Indian went into a bar in Chicago and ordered himself a drink. A white American sitting next to him on the stool asked him, "And how do you like our city?" Fine," replied the Red Indian. "And how do you like our country?"

- Since Gorbachov took over, Vodka, the favourite beverage of the Russians, has become a scarce commodity. There was a mile-long queue outside a liquor store. "I can't take this anymore," said Ivanov. "I am going to get my pistol and shoot Gorbachov."
  Two hours later Ivanov was back to rejoin the queue. "What happened?" asked the others still in the line.
  "I decided to get back here," replied Ivanov. "The queue outside Gorbachov's apartment waiting to kill him is longer than this one."

- The contents of a signboard on the door of a lawyer's chambers which reads: "where there is a will there is a way; where there is a way there is a law; where there is a law there is a rule; where there is a rule there is a loophole; where there is a loophole there is a lawyer, and here I am Mr. So and so… Advocate."

- A kindly gentleman wanting to befriend a family that had moved into his neighborhood spoke to the youngest son: "Son, how many brothers and sisters are you?"
  "Sir, we are nine brothers and three sisters," replied the youngster.
  "And what does your father do?"
  "Sir, this is all that he does."

Wisdom Whispers

- Do not pray for an easy life. Pray instead to be a stronger person
- Acquire knowledge. Acquire skills. They weigh nothing, and you can carry them with you all your life.
- Study the face of nature and you will never be bored.
- It is good that others should succeed. Do not allow their successes to cast a shadow on your own efforts.
- The wisest man is he who doesn't think he is.
- Don't give up. One success will erase many failures.
- Don't let petty-minded people prevent you from doing your thing. Dogs may bark but the caravan moves on.

Brain Teasers

1. In a plasma cell, the peri-nuclear hoff:
   A) Is occupied by bacteria B) Represents an active Golgi complex
   C) Is caused by nuclear degeneration D) Represents the extruded nucleus.
2. "Thrushed breast" or "tigered effect" terms are used in relation to:
   A) Hair B) Skin C) Lungs D) Heart
3. The term autosplenectomy is used in relation to:
   A) Sickle cell anaemia B) Polycythemia vera C) Erythroleukemia
   D) Erythroblastosis fetalis
4. Gumma is found in:
   A) Syphilis B) Cat scratch disease C) Brucellosis D) Schistosomiasis
5. How many subclasses does IgG have?
   A) 1 B) 2 C) 3 D) 4
6. Which set of lymph nodes are involved most commonly in sarcoidosis?
   A) Pre-auricular B) Epitrochlear C) Hilar and mediastinal D) Inguinal
7. In which of the following tumors are you likely to find flame cells?
   A) Multiple myeloma B) Bronchogenic carcinoma
   C) Ewing's sarcoma D) Retinoblastoma
8. Bronze diabetes is associated with:
   A) Diabetes mellitus B) Hemochromatosis
   C) Diabetes insipidus D) Carcinoma head of pancreas

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

TULIP NEWS

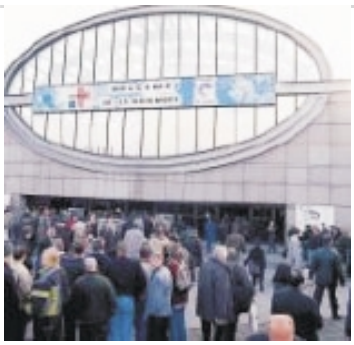
Tulip Group participates in

MEDICA 2004
Düsseldorf 24. - 27. November 2004

Tulip Group of Companies, well-established as the leading manufacturer and marketeer of world class *in vitro* diagnostics reagents and instruments, participated as an exhibitor in MEDICA 2004, the world largest annual trade fair for medicine held at Dusseldorf, Germany.

At the exhibition, the group received an excellent response for its innovative products, with enquiries pouring in from different countries. The group currently exports its products to 60 countries worldwide through its extensive distribution network and plans to penetrate new international market in a big way.

ABOUT MEDICA



MEDICA is the World's forum for Medicine. Exhibitors from 62 countries and visitors from 98 countries attended MEDICA in 2003. MEDICA presents an internationally unequalled broad range of products and companies that extends from global players and medium-sized companies to specialised small firms.
Not only is MEDICA the largest medical trade fair worldwide in terms of its large numbers of visitors and exhibitors, but it is also amongst the 10 largest specialist trade fairs in terms of the number of exhibitors overall.

MEDICA is a well planned trade fair so decision-makers attending MEDICA are well prepared, ready for informative and detailed discussions with exhibitors regarding the new products they have on offer.

By participating at the Medica, Tulip group is rapidly consolidating its image of professionally managed diagnostic manufacturer of International repute internationally

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