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# The Crux

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CONTENTS



## Editorial

Malaria, a curse for humanity, is still playing havoc with human lives. With the emergence of drug resistant strains, the problem has been gravely complicated. Diagnosis with correct speciation is thus very, very important because therapeutic regimens differ significantly. *Seeing is believing* is still the biblical truth-vis-à-vis malaria diagnosis. However, rapid speciating diagnostic formats are available now. Some detect antibodies to malaria antigen (even those that may have been formed months/years back with no current infection) and some detect malarial antigens (those that are actually existing and in circulation in blood). It doesn't require much grey matter to understand these RDTs, that detect circulating antigens are OBVIOUSLY the scientifically preferred choice. It is of no use to detect what happened months/years back and was taken care of adequately! History is after all History!
TROUBLE SHOOTING signifies the importance and relevance of using antigen detection RDTs for malaria diagnosis. In not so distant future, we are likely to have RDTs that identify all four species separately and discretely by utilizing antigen detection.

A not so common disease, that is often difficult to diagnose-Brucellosis-finds entry into the DISEASE DIAGNOSIS segment of this issue. Virtually all human infections derive directly from exposure to infected animals or their products. It has varied names like-Undulant fever, Mediterranean fever, Malta fever and Bang's disease. With variable onset and variable severity of signs and symptoms- the disease can often be missed by even the most qualified clinicians. An in depth study of Brucellosis - its Cardinal features, Epidemiology, Differential Diagnosis, Signs and Symptoms, Clinical aspects, and Investigative approaches - are presented. How to interpret the results obtained is made sufficiently clear.
As Brucellosis can be a biohazard to all laboratory professionals, its therapeutic measures are also covered. Prognosis, complications have not been forgotten and omitted. Our next issues shall delve deeply into a couple of other infective diseases.

An upcoming branch in diagnostic world is Neonatal screening. Though rare, these diseases add up to a sizable number when added up worldwide. What does neonatal screening entail, which diseases are important and how does one screen for these diseases along with the normal reference ranges is the heart of this issue's INTERPRETATION portion. Ideally all infants should have their blood test taken when they are about 48 hours old. A problem caught early enough can prevent morbidity as well as mortality in the affected children. Like immunization, neonatal screening must become a routine practice in time to come.

In BOUQUET we'll laugh at ourselves. Answer the brain teasers and hear what wisdom whispers. Nothing has changed.

TÜV MANAGEMENT SERVICE ISO 9001

Tulip Group

# DISEASE DIAGNOSIS

## BRUCELLOSIS

### Description
- Brucellosis is a zoonotic disease of wild and domestic animals that is transmittable to humans. Virtually all human infections derive directly or indirectly from exposure to infected animals
- Caused by infection with small, Gram-negative bacteria of the genus *Brucella*
- Onset of disease can be acute or insidious, generally beginning within 2-4 weeks after inoculation but can be highly variable and may develop several months after exposure
- Clinical manifestations are nonspecific and can include fever, sweats, malaise, anorexia, depression, headache, and back pain
- Serious complications can include meningitis, endocarditis, and osteomyelitis

### Synonyms
Undulant fever /Mediterranean fever /Malta fever /Bang's disease.

### Clinical Alert
The practitioner should be alerted to the possibility of *Brucella* endocarditis, a potentially life-threatening complication, if:
- A new or changing murmur is detected
- A predisposing valvular lesion is present
- Relapse of bacteremia occurs after completed therapy

### Cardinal Features
- Virtually all human infections derive directly or indirectly from exposure to animals that are infected with Gram-negative bacteria of the genus *Brucella*
- Sheep, goats, cattle, and their products are the main source of outbreaks
- Routes of infection include direct contact with infected animals or their secretions through cuts and abrasions in the skin, infected aerosols inhaled or inoculated into the conjunctival sac, ingestion of unpasteurized contaminated dairy products
- Aerosol transmission is highly infectious
- Most cases worldwide occur in men with occupational exposure to animals, e.g. farmers, ranchers, veterinarians, abattoir workers
- Incubation period can vary from less than one week to several months but the majority of patients become ill within 2-4 weeks of exposure
- Symptoms are nonspecific and include fever, sweats, malaise, anorexia, headache, back pain, and depression
- Physical abnormalities are generally few and include mild lymphadenopathy (10-20% of cases), splenomegaly/hepatomegaly (20-30% of cases)
- Therapy is based on tetracyclines usually with rifampin and/or aminoglycosides
- Relapses are not uncommon because of prematurely discontinued therapy
- Chronic brucellosis, arbitrarily defined as disease that persists for more than 12 months, is caused by persisting deep foci of infection and is characterized by persistently high titers of IgG antibodies in serum and objective signs of disease, e.g. fever
- Some patients experience delayed convalescence after treatment, with persisting nonspecific complaints of ill health, in particular, fatigue
- Serious complications include meningitis, endocarditis, and osteomyelitis

### Causes
#### Common causes
- Infection with *Brucella abortus, B. melitensis, B. suis*; brucellosis caused by *B. melitensis* is the most important clinically apparent disease
- Routes of infection include direct contact with infected animals or their secretions through cuts and abrasions in the skin
- Infected aerosols may be inhaled or inoculated into the conjunctival sac
- Ingestion of unpasteurized contaminated dairy products

#### Rare causes
- Ingestion of contaminated meat products (meat is not usually eaten raw and the numbers of organisms in muscle tissue are low)
- Infection with *B. canis*
- Human-to-human transmission is rare but a few cases in which sexual transmission is suspected have been reported
- Transfusion of infected blood
- Transplantation of infected tissue
- At least one case of presumed intrauterine transmission has been reported
- Veterinarians are occasionally inoculated with live vaccine during occupational accidents
- Laboratory accidents

#### Contributory or predisposing factors
- Occupational exposure to infected animals
- Consumer exposure to unpasteurized milk products
- Exposure while traveling in countries where brucellosis is endemic
- Disease is worse in chronically ill, immunosuppressed, or malnourished individuals
- Iron deficiency increases susceptibility (may be associated malnutrition)
- Antacids and drugs that decrease gastric acidity have been implicated in foodborne brucellosis

### Epidemiology
#### Incidence and prevalence
True incidence and prevalence are unknown.

#### Demographics
**Age**
- All ages, but especially 20-60 years of age (mainly occupational exposure
- Less than 10% of reported cases occur in individuals less than 19 years of age
- Brucellosis in children is frequently a mild, self-limited disease compared with the more chronic disease seen in adults
- In areas where *Brucella melitensis* is the endemic species, disease in children can be severe

**Gender**
- Males more likely to contract disease by occupational exposure than females
- Females more likely to contract disease from ingestion of contaminated dairy products than males

**Geography**
- Disease has a limited geographic distribution but is a major problem in Mediterranean countries, Western Asia, and parts of Africa and Latin America

**Socioeconomic status**
- Brucellosis is an occupational risk for individuals who work closely with animals, e.g. farmers, ranchers, veterinarians, abattoir workers
- Laboratory personnel may contract the disease from contaminated tissue samples

### Differential Diagnosis

| | |
|---|---|
| Infectious mononucleosis | Tuberculosis |
| Influenza | Systemic lupus erythematosus |
| Typhoid fever | Lymphoma, non-Hodgkin's |

### Signs & Symptoms
#### Signs
- Undulant fever pattern (increased in the afternoon and evening) - maximum 101-104°F
- Transient, nonspecific rashes have been described
- Purpura from thrombocytopenia
- Mild lymphadenopathy, especially cervical and inguinal, is reported in 10-20% of patients
- Splenomegaly in 20-30% of cases
- Hepatic dysfunction (abnormal liver function test in 30-60% of cases)
- Orchitis, epididymitis in 2-40% of patients
- Cystitis
- Nephritis, prostatitis are rare manifestations

#### Symptoms
- Weakness, headache, sweating, chills, generalized aching are commonly reported
- Arthralgia occurs in up to 90% of patients
- Weight loss, irritability also common
- Depression is common and is often out of proportion to the severity of other symptoms

- Cough or other pulmonary symptoms (X-ray may be normal)
- Visual disturbances, eye pain
- Chronic fatigue syndrome and various neuropsychiatric symptoms have been described
- Occasionally, patients describe a malodorous sweat and a peculiar taste in the mouth

### Associated Disorders

Uveal tract lesions.

### Diagnostic Decision

- Diagnosis of brucellosis should be considered when an individual who has had contact with domestic animals presents with evidence of localized or systemic infection
- Diagnosis is confirmed when *Brucella* is cultured from blood, bone marrow, or other tissues
- A presumptive diagnosis may be made by serologic testing
- A single titer is not diagnostic but the majority of patients with active infection have titers of 1:160 or greater
- If agglutination test is positive even in the absence of bacteriologic evidence, a diagnosis of brucellosis is likely if there is a history of exposure to infected animals or animal products, relevant epidemiologic data, and characteristic clinical findings

### Clinical Hallmarks

- Brucellosis is one of the most challenging diseases for the clinician since the organism can be present in a multitude of different forms and can test the diagnostic acumen of even the most seasoned clinician
- Brucellosis should be considered whenever a difficult diagnostic problem develops that appears similar to tuberculosis but when work-up for tuberculosis is unrevealing
- The remarkably slow and fastidious growth characteristics (up to 6 weeks) of *Brucella* spp. complicates clinical diagnosis and increases the need for serodiagnosis and nucleic acid-based assays to confirm this diagnosis. Regrettably, PCR-based diagnostic systems have yet to reach widespread clinical use for the diagnosis of brucellosis
- If clinically suspected, it is important to inform the laboratory handling potentially infected cultures so that appropriate biosafety precautions can be instituted. Appropriate precautions (gloves, mask, goggles) should also be used when handling respiratory secretions or other body fluids
- If diagnosed in a cluster of subjects, or in a patient without epidemiologic risk, government authorities may be notified to investigate possibility of a bioterrorist attack

### Investigations

### Body fluids

#### 1. Culture of Brucella

**Description**

- *Brucella* can be recovered from blood, bone marrow, or other tissues (lymph nodes, liver, spleen)
- Lysis-centrifugation may shorten time necessary to isolate organisms
- Cultures of bone marrow have a higher yield than blood
- Rate of isolation from blood can vary from 15-70% depending upon methods used and incubation period
- Laboratory personnel should use proper precautions for protection against laboratory-acquired infection

**Disadvantages**

- Relatively slow-growing organism; usually requires 1-3 weeks of growth time
- Laboratory must maintain cultures for minimum of 4 weeks if brucellosis is suspected

**Normal:** *Brucella* not detected in isolate.

**Abnormal:** *Brucella* detected in isolate.

**Cause of abnormal result-Brucellosis.**

- Drugs, disorders and other factors that may alter results
- Some *Brucella* isolates have been misidentified as *Moraxella Phenylpyruvica*.

#### 2. Serum agglutination test ( SAT)

**Description**

In the absence of bacteriologic confirmation, a presumptive diagnosis may be made on the basis of high or rising titers of specific antibodies.

**Advantage**

- If test is positive even in the absence of bacteriologic evidence, diagnosis of brucellosis is likely if there is a history of exposure to infected animals

**Disadvantages**

- False-negative reactions can result from a prozone (excess antigen) phenomenon
- False-positive reactions can result from cross-reactions with antibodies to *Yersinia*, *Vibrio cholerae*, or *Francisella tularensis*
- False-positive and -negative results can be avoided by routinely diluting serum beyond 1:320
- False-negative results caused by blocking antibodies have been reported with blocking antibody titers as high as 1:640
- If a strong clinical suspicion of brucellosis exists, dilutions as high as 1:1280 should be made
- Specialized serologic studies are required to detect *Brucella canis* antibodies

**Normal:** True SAT titers below 1:160 are strong evidence against active brucellosis.

**Abnormal:**

- No single titer is always diagnostic, but most cases of active infection have titers equal to or greater than 1:160
- Four-fold or greater rise in titer of antibody in serum samples drawn 1-4 weeks apart
- Most patients develop a rise in titer within 1-2 weeks of illness, with virtually all patients showing seroconversion by 3 weeks
- Keep in mind the possibility of a false-positive result

**Cause of abnormal result**

- Titers equal to or greater than 1:160 indicates past or present exposure to *Brucella* or antigens that cross-react with *Brucella*
- A 4-fold increase is indicative of recent exposure to *Brucella* or *Brucella*-like organisms
- **Drugs, disorders and other factors that may alter results**
- *Yersinia, Vibrio cholerae*, and *Francisella tularensis* antibodies cross-react with anti-*Brucella* antibodies and can produce a false-positive result.

#### 3. Enzyme Immunoassay (EIA)

**Description:**

- Widely used serologic diagnostic test for brucellosis
- Sensitive method for detecting IgG and IgM anti-*Brucella* antibodies
- Until better standardization is established, EIA should be used for suspected cases with negative or equivocal SAT titers or for evaluation of patients with suspected relapse or reinfection

**Advantages/disadvantages:**

Disadvantage: not well standardized yet.

**Normal:** IgG and IgM anti-*Brucella* antibodies not detected

**Abnormal:**

- IgM antibody titers rise early in brucellosis (usually first week of infection), peak at about 3 months, then fall gradually
- High titers may persist for years
- IgG antibodies appear 2-3 weeks after illness onset, peak in approximately 8 weeks, and persist as long as infection is active
- IgG antibodies disappear rapidly with resolution of disease and usually disappear within one year
- Persistence of IgG antibody (titer >1:160) indicates continuing active infection
- With relapse, both IgM and IgG titers increase

**Cause of abnormal result :** Brucellosis.

- **Drugs, disorders and other factors that may alter results**
- *Brucella* skin test may cause a rise in antibody titers, confusing the interpretation of the results and should be avoided.

**Immediate Action**

Immediate treatment not normally required unless physician detects life - threatening complication (e.g. Endocarditis).

## Management Issues

### Goals

- Relieve symptoms
- Shorten duration of illness
- Reduce incidence of complications, some of which may be life-threatening

## Summary Of Therapeutic Options

### Choices

- Prolonged therapy is imperative for achieving a cure
- Combination therapy is necessary for achieving a cure
- Optimum antibiotic therapy for brucellosis is disputed
- Treatment recommended by the World Health Organization for acute brucellosis in adults is **rifampin** (600-900mg/day) and **doxycycline** (200mg/day) for a minimum of 6 weeks
- **Tetracycline** (30-40mg/kg/day; maximum 2g/day in four divided doses) or **trimethoprim-sulfamethoxazole** (10mg/kg/day; maximum 480mg/day) in combination with rifampin has also been recommended
- Intramuscular **streptomycin** (20mg/kg/day in two divided doses; maximum 1g/day intramuscularly) with an oral tetracycline (or **trimethoprim-sulfamethoxazole**) for the first 7-14 days of therapy in addition to **rifampin** (20mg/kg/day) may give fewer relapses
- **Gentamicin** (5mg/kg/day in three divided doses) can be substituted for streptomycin
- **Fluoroquinolones**, e.g. **ciprofloxacin**, may have a role in adjunctive therapy but should never be used as monotherapy

### Clinical Hallmarks

- Abortions and infertility are a significant problem for domesticated farm animals since *Brucella* spp. are concentrated in the reproductive tissues as a result of high concentrations of erythritol, a growth factor for brucellosis. This gave the organism its name, *Brucella abortus*. This is a significant economic loss for some agricultural businesses in endemic countries such as the Middle East
- Brucellosis in women is not associated with a high incidence of abortion as human placental tissues do not produce erythritol. Abortions have been ascribed to brucellosis in women during pregnancy but not at a significantly greater rate than other systemic infectious diseases in pregnancy
- Brucellosis is an extreme biohazard in the laboratory as infectious aerosols can be generated leading to laboratory-acquired inhalation forms of brucellosis. Warn the laboratory that you are considering this diagnosis before attempting to culture this organism. The laboratory should perform all transfers of cultured materials in a biosafety cabinet to avoid accidental inhalation

### Prognosis

- Prior to the advent of antibiotic therapy, most patients recovered from brucellosis within 3 months
- Less than 2% of untreated cases are fatal
- Majority of cases resolve with appropriate antimicrobial therapy; long illnesses and complications are rare
- If morbidity exceeds 1-2 months, other diagnoses or complications of brucellosis should be considered

### Clinical Hallmarks

- Brucellosis is a systemic infection that often produces a plethora of symptoms with a paucity of physical findings; consider brucellosis in patients with subacute illness and profound weakness, malaise, nausea, loss of weight, and low-grade fever if the epidemiology fits a history compatible with exposure to brucellosis
- Brucellosis has virtually been eliminated from milk and other dairy products by the use of pasteurization (this rapidly kills *Brucella* species in milk). Unfortunately, some soft cheeses are less tasty to some refined palates if pasteurized milk is used and therefore brucellosis continues to occur as a result of unpasteurized dairy products, particularly in southern France, and other Mediterranean countries. There are also case reports of brucellosis infection originating from the ingestion of unpasteurized goat cheese ('queso blanco') imported from Mexico
- Eradication of brucella organisms necessitates cell-mediated immune responses; it was speculated that this infection would become a significant opportunistic infection for patients living in endemic regions with AIDS, but while this remains a potential risk, it has not become a clinically important problem

### Therapeutic failure

- Prolonged therapy is imperative for achieving a cure
- Therapeutic failure or relapses are generally not caused by development of resistance to antimicrobial therapy but by premature discontinuation of therapy
- Patients who relapse are usually cured with retreatment using the same antimicrobial agents

### Recurrence

- Recurrence of brucellosis may result from a persistent focus of infection that requires surgical drainage in addition to antibiotic therapy.

### Deterioration

- Use of multiple antibiotics is recommended
- Addition of a fluoroquinolone, e.g. ciprofloxacin, is appropriate in cases of deterioration

## Complications

- Alimentary tract complications are found in up to 70% of patients. Anorexia, abdominal pain, nausea, vomiting, diarrhea, or constipation are not uncommon
- Acute ileitis has been reported
- Rarely, brucellosis is complicated by acute cholecystitis, pancreatitis, and spontaneous bacterial peritonitis
- Liver is frequently involved to differing degrees, depending upon infecting species. Ranges from insignificant aggregates of mononuclear cells surrounding foci of necrosis scattered through the parenchyma to a diffuse nonspecific inflammation resembling viral hepatitis
- Osteoarticular complications in 20-60% of cases. Sacroiliitis is the most common complication
- Arthritis, spondylitis, osteomyelitis (usually vertebral), tenosynovitis, and bursitis have been reported
- Hips, knees, and ankles are involved more often than small joints
- Direct invasion of the nervous system occurs in less than 5% of patients
- Meningitis is the most frequent central nervous system complication
- Encephalitis, myelitis, radiculoneuritis, brain abscess, epidural abscess, demyelinating syndromes, and meningovascular syndromes have been reported
- Endocarditis occurs in less than 2% of cases but accounts for the majority of brucellosis-related deaths
- Pericarditis can occur
- Bronchitis, bronchopneumonia, lung nodules, abscesses, miliary lesions, and pleural effusions may be associated with airborne transmission of brucellosis
- Hematologic complications include anemia, leukopenia, thrombocytopenia, and clotting disorders
- Cutaneous lesions including rashes, papules, ulcers, erythema nodosum, petechiae, purpura, and vasculitis have been reported
- Erythematous macular, papular, or pustular rash may appear on the hands and arms of veterinarians after removing placentas from infected animals
- Epididymitis occurs in 10% of cases in men

## Risk Factors

- Exposure to animals infected with Brucella: virtually all human infections derive directly or indirectly from exposure to animals. Establishment of a Brucellosis Eradication Program to eliminate the disease from the country can be a tremendous advantage.
- Ingestion of unpasteurized contaminated dairy products: a cause of brucellosis.

### Modify Risk Factors

**Lifestyle and wellness**

**Diet**

Proper heat treatment of milk products is important for effective prevention of brucellosis in humans.

**Environment**

Meat workers, veterinarians, farmers, and other workers whose occupation puts them at risk for brucellosis infection should appropriately bandage wounds, wear gloves, and goggles.

**Immunization**

Vaccination of livestock can reduce incidence of domestically acquired brucellosis in humans.

# INTERPRETATION

## NEONATAL SCREENING TEST

Ideally all neonates should have a blood test taken when they are about 48 hours old (usually on day 2). This test is the Neonatal Screening Test, also called the Newborn screening test, 'Guthrie' test, or 'heel prick' test. The test can tell if a baby has one of many rare but serious health problems before any harm is done and even before there are signs that there is a health problem.

Finding these problems early means that the children can have treatment which can lessen, or prevent, harm from the problems.

### How is the test done?

Routine Neonatal screening: Ideally all babies should have a neonatal screen. One can collect either cord blood or blood by heel prick after 48 hours of birth. By heel prick, the blood should be dispensed on special filter papers approved for the purpose of neonatal screening -S or S 903. After the collection, air dry for 2 hours and then preserve in a plastic envelop or paper envelop at 2 to 8 degrees centigrade in refrigerator. Process the samples as soon as possible and communicate the reports to the concerned physicians. If this test suggests that there may be a problem the parents are contacted and further tests should be arranged. Great care should be taken to make sure that each test which shows that there may be a problem is followed up. Most of the children who are found to have these problems will need specialised support, usually through a major children's hospital.

Over 30 different health problems can be detected using these blood spots.

### How often are these problems found?

Each of these health problems is rare, but added together, about one in 2000 newborn babies will be found to have one of these health problems. It can vary geographically.

Incidence of Inborn Errors of Metabolism (I.E.M.) in India (approximate):

(1). Generalised aminoaciduria 1 in 1,605, (2). Congenital Hypothyroidism 1 in 3,500, (3). Galactosemia 1 in 4,000, (4). Tyrosinemia 1 in 6,234, (5). Maple Syrup Urine Disease (M.S.U.D.) 1 in 10,215, (6). Phenyl ketonuria 1 in 18,728, (7). Hyperglycinemia 1 in 26,053, (8). Histidinemia 1 in 37,456, (9). Methioninemia 1 in 112,369.

Incidence of Aminoacid metabolism disorders in general poupulation:

(1). Phenylketonuria 1 in 11,5000, (2). Maple Syrup Urine Disease (M.S.U.D.). 1 in 220,000, (3). Hypermethioninemia & Homocystinuria 1 in 220,000, (4). Histidinemia 1 in 24,000, (5). Hyperprolinemia ?( 6). Hyperlysinemia 1 in 245,000, (7. Hereditary Tyrosinemia ? (8) . Non ketotic - Hyperglycinemia 1 in 245,000 Ketotic - Hyperglycinemia 1 in 245,000, (9). Arginiosuccinic aciduria 1 in 245,000, (10). Hyper ornithinemia 1 in 245,000, (11). Hartnup disease 1 in 26,000, (12). Cystinuria 1 in 7,000, (13). Iminoglycinuria 1 in 20,000, (14). Fanconi's syndrome 1 in 245,000.

Incidence of other disorders in the world:

(1). Cystic fibrosis 1 in 2,500, (2). Cystinuria 1 in 7,000, (3). Alfa - 1 - Anti trypsin deficiency 1 in 8,000, (4). Iminoglycinuria 1 in 20,000, (5). Hartnup disease 1 in 26,000, (6). Hyperprolinemia 1 in 40,000, (7). Biotidinase def. 1 in 60,000, (8). Adenosine deaminase deficiency 1 in > 100,000.

The most commonly accepted diseases that need neonatal screening are:

(1). Hypothyroidism, (2). Phenylketonuria, (3). Maple Syrup Urine Disease (M.S.U.D.), (4). Biotinidase deficiency, (5). Sickle cell disease, (6). Galactosemia, (7). Tyrosinemia, (8). Histidinemia, (9). G6 PD deficiency, (10). Cystic fibrosis.

### Which are the high risk group patients?

Two high risk groups are important:

(1). Critically ill newborns: Many of whom may be having Galactosemia, Maple Syrup Urine Disease (M.S.U.D.), Propionic acidemia, Multiple Carboxylase deficiencies, Urea cycle defects etc.

(2) . Families with a history of:

a. Previous child with mental retardation or Cerebral Palsy (C.P.) or congenital anomaly .
b. Past history of recurrent abortions .
c. History of sudden infant death in previous siblings .
d. Mother with significantly low intelligence and microcephaly .
e. Family history of haemoglobinopathy .
f. Significant degree of consanguinity

For the high risk group one should consider :

(1). Genetic counselling .

(2). Antenatal diagnostic facilities if they are willing

a. TORCH titre.
b. USG with doppler and anomaly scan.
c. Chorionic villous biopsy with karyotyping.
d. Amniotic fluid studies for -
- Karyotype / Fluorescent in situ Hybridisation (F.I.S.H.)
- A.F.P. & beta - HCG
- Biochemical analysis
- Gas Chromatography-Mass Spectrometry (G.C.M.S.)
- SOS enzyme assay or DNA studies

(3). Haemoglobinopathy workup.

(4). Neonatal Screening and Neonatal management in NICU with regular follow up.

### Neonatal screening in NICU for Inborn Errors of Metabolism (I.E.M.)

Though individually IEMs are rare, collectively they are quite common. If we investigate thoroughly all the sick newborns, we may find quite a significant number of cases amongst them. The word IEM carries such a notorious stigma that most of us are still quite allergic to such disorders and we do not attempt to diagnose these conditions in quite early stages when they are really quite manageable and we can definitely prevent mortality and reduce the morbidity to a certain extent.

**Genetic Screening** is defined as search in the population for persons with genetic characteristics likely to be harmful to themselves or their descendants.

A. History of Neonatal screening :
(1) . Guthrie dried blood spot test for phenylketonuria. It is a bacteriological test first described in 1962.
(2). Thyroid hormones test: on dried blood spot by R.I.A. for congenital hypothyroidism.

B. Goals of neonatal screening :
(1). Medical intervention.
(2). Genetic counselling.
(3). Prenatal diagnosis.

C. Criteria for disease selection :
(1). Treatable disease, (2). Difficult to diagnose, (3). Requires immediate therapy to prevent disability and mortality, (4). Reasonably frequent in population.

D. Recommended screening for :
(1). Hypothyroidism, (2). Galactosemia, (3). Phenyl ketonuria, (4). Maple Syrup Urine Disease, (5). Tyrosinemia , (6). Other aminoacidopathies, (7). Sickle cell disease.

E. Other recommended disorders :
(1). Congenital adrenal hyperplasia : (C.A.H.), (2). Histidinemia, (3). Methioninemia, (4). Thalassemia, (5). Duchenne Muscular Dystrophy: (D.M.D.)

Prerequisites for Screening :

The tests should be:

(1). Simple, (2). Specific,( 3). Sensitive, (4). Cost effective, (5). Easy for collection and transport.

### Test Methods

A) B.I.A. Bacterial Inhibition Test :
(1). Phenylalanine, (2). Leucine, (3).Methionine, (4). Tyrosnine, (5). Histidine,

(6). Lysine, (7). Glutamine,( 8). Glycine, (9). Proline.

B) E.A.A. Enzyme Auxotrophic Assay :
(1). Argininosuccinic aciduria, (2). Citrullinemia, (3). Argininemia, 4. Ornithinemia, (5). Orotic Aciduria, (6). T.L.C. Thin Layer Chromatography, (7). A.R.G. Auto Radio Graphy, (8). P.C.R. Polymerase Chain Reaction.

C) F.S.T. Fluorescence Spot Test :
(1). Gal - 1 - Put enzyme, (2). Alfa - 1 - Antitrypsin enzyme, (3). Adenosine Deaminase enzyme, (4). G - 6 - PD enzyme,( 5). C - 1 - Esterase inhibitor (for Angioneurotic edema).

D) R.I.A. Radio Immuno Assay :
(1). T3, T4, T.S.H., (2). Trypsin for Cystic Fibrosis, (3). Pregnandiole - (for adrenogenital syndrome).

E) E.P.P. Electro Phoretic Pattern :
()1. Haemoglobinopathies, ( 2). Thalassemia, (3). Sickle cell anemia.

F) TLC : Thin Layer Chromatography : (single & two dimensional).
(1). Aminoacids in urine, blood, C.S.F. etc., (2). Sugars, ( 3). Organic acids.

G) A.G.R. : Auto Radio Graphy :
(1). Enzyme Hypoxanthine-Guanine Phosphoribosyl Transferase (H.G.P.R.T.) (for Lesch Nyhan Syndrome).

H) Colorimetric Method :
(1). Biotidinase enzyme for Multiple Carboxylase Deficiency Syndrome.

I) Tandem Mass Spectrophotometry :
(1). Organic acid analysis, Medium Chain Acyl-CoA Dehydrogenase Deficiency (M.C.A.D.).
(2). Fatty acid oxidation defects .
(3). Aminoacid metaboism disorders .

J) Micro - chip technology :
1 cm x 1 cm chip ; DNA sequencing for about 30 disorders.

K) ELISA :
(1). Phenylalanine , (2). Methionine, (3). Homocystine, (4). Lucine & isoleucine, (5). T4, (6). GALT (The GALT test is an adaptation of the qualitative visual assay of Bentler and Baluda that is being currently used worldwide).

**False Negative Results may occur:**

(1). When child has not been fed with milk for a prolonged time.
(2). When the neonate is on intravenous fluid therapy.
(3). When the neonate has just received blood transfusion from a healthy donor.

**False Positive Results:**

(1). Antibiotics.
(2). Contamination with urine and stools samples.
(3). Prematurity.
(4). Baby on ventilator.
(5) . Blood transfusion with blood obtained from an affected donor.

**Precautions to be exercised:**

(1). Collect cord blood ( best method )
(2). Collect urine after 48 hrs. after initiation of milk feeding .
(3). Collect blood and urine sample before blood transfusion or 48- 72 hours after blood transfusion .
(4). Use standard Whatman filter paper no. 3 or (S & S - 903).
(5). Diameter of each spot should be 6 mm minimum.
(6). Blood should soak through to the other side of the paper.
(7). Proper details of the baby.
(8). Repeat the test after 1 - 2 wks. , if the results are abnormal
(9). Use better methods like, High Precision Thin Layer Chromatography (H.P.T.L.C.), High Performance Liquid Chromatography (H.P.L.C.), Gas Liquid Chromatography (G.L.C.), Gas Chromatography-Mass Spectrometry (G.C.M.S., R.I.A.), and D.N.A. / R.N.A. study for confirmatory diagnosis.
(10).Use specific enzyme assays for the diagnosis and carrier state studies
(11).Use specific mutation studies for the confirmed diagnosis and carrier state studies.

**Positive Consequences of undertaking these exercises:**

(1). Early intervention, prevents disability and deaths.
(2). Family counselling possible.
(3). Prenatal diagnosis for next pregnancy.
(4). Avoids frustration for both parents and physicians.

**Negative consequences of undertaking these exercises:**

(1). Parental agony and anxiety.
(2). Over protection or neglect of the child.
(3). Feeling of guilt.
(4). Family breakup and divorce.

Neonatal screening which was considered as a part of research in the past, should now be applied to the field of health as preventive programme just like immunization.

(To be continued.)

## BOUQUET

### IN LIGHTER VEIN

- A SHORT HISTORY OF MEDICINE: "Doctor, I have an ear ache."
  2000 B.C.- "Here, eat this root."
  1000 B.C. - "That root is heathen, say this prayer."
  1850 A.D. - "That prayer is superstition, drink this potion."
  1940 A.D. - "That potion is snake oil, swallow this pill."
  1985 A.D. - "That pill is ineffective, take this antibiotic."
  2000 A.D. - "That antibiotic is artificial. Here, eat this root!"

- A pipe burst in a doctor's house. He called a plumber. The plumber arrived, unpacked his tools, did mysterious plumber-type things for a while, and handed the doctor a bill for $600.
  The doctor exclaimed, "This is ridiculous! I don't even make that much as a doctor!."
  The plumber quietly answered, "Neither did I when I was a doctor."

- A doctor said to his car mechanic, "Your debit is several times more per hour then we get paid for medical care."
  "Yeah, but you see, doc, you have always the same model, it hasn't changed since Adam; but we have to keep up to date with new models coming every year."

- If it is dry - add moist; if it is moisten - add dryness. Congratulations, now you are a dermatologist.

- A veterinarian was feeling ill and went to see her doctor. The doctor asked her all the usual questions, about symptoms, how long had they been occurring, etc., when she interrupted him: "Hey look, I'm a vet -- I don't need to ask my patients these kind of questions: I can tell what's wrong just by looking. Why can't you?"
  The doctor nodded, looked her up and down, wrote out a prescription, and handed it to her and said, "There you are. Of course, if that doesn't work, we'll have to have you put down."

- A lady rushes into the veterinarian and screams, "I found my dog unconscious and I can't wake him -- do something."
  The vet lays the dog on the examination table and after a few simple tests he says, "I'm sorry, I don't feel a pulse, I'm afraid your dog is dead".
  The lady can't accept this and says, "No, no, he can't be dead -- do something else."
  The vet goes into the other room, and comes back with a little cat. The cat jumps up on the table and starts sniffing the dog from head to toe. It sniffs and sniffs up and down the dog, then all of a sudden just stops and jumps off the table and leaves. "Well, that confirms it," the vet says, "your dog is dead."
  The lady is very upset but finally settles down. "Okay, I guess you're right. How much do I owe you?"
  The vet says, "That will be $340."
  The lady has a fit and asks, "Why is it so much? After all the vet didn't do anything for the dog."
  "Well", the vet replied, "it's $40 for the office visit and $300 for the CAT SCAN!"

## TROUBLE SHOOTING

# ANTIGEN VS ANTIBODY TESTS IN MALARIA DIAGNOSIS

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

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

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

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

### Antibody based RDT's

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

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

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

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

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

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

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

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

### Antigen based RDT's

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

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

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

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

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

## WISDOM WHISPERS

- Do not weep; do not wax indignant. Understand.
- The bird of paradise alights only upon the hand that does not grasp.
- When I find myself fading, I close my eyes and realize my friends are my energy."
- "Don't walk behind me; I may not lead. Don't walk in front of me; I may not follow. Just walk beside me and be my friend."
- "True friends stab you in the front."
- "He who has a thousand friends
  Has not a friend to spare,
  While he who has one enemy
  Shall meet him everywhere."
- "Fear less, hope more;
  Whine less, breathe more;
  Talk less, say more;
  Hate less, love more;
  And all good things are yours."
- "Never be bullied into silence. Never allow yourself to be made a victim. Accept no one's definition of your life; define yourself."
- "In three words I can sum up everything I've learned about life: it goes on."

## BRAIN TEASERS

1. Basophilic stippling in RBCs is seen in which of the following conditions?
   A. Lead Poisoning B. Iron deficiency anaemia C. Spherocytosis D. CML
2. Which of the following doesn't constitute one of the seven Ps of pernicious anemia?
   A. Pancytopenia B. Peripheral neuropathy C. Psychosis D. Psoriasis
3. What can be low in megaloblastic anaemia?
   A. TLC B. LDH C. Serum Iron D. Indirect bilirubin
4. Terminal Deoxynucleotidyl Transferase (TdT) is a marker for which of the following Immature cells?
   A. Lymphoid cells B. Myeloid cells C. Monocytoid cells D. Plasma cells.
5. Basophilia is not seen in which of the following conditions?
   A. Chronic hypersensitivity states B. Systemic mast cell disease
   C. Myeloproliferative disorders D. Parasitic infections
6. Which of the following is an intrinsic defect caused anaemia?
   A. Chemical/toxic induced B. Immune C. Hypersplenism
   D. Unstable haemoglobin

Answers: 1. A, 2. D, 3. A, 4. A, 5. D, 6. D

## TULIP NEWS

For over a decade Tulip Group's products have attained market leadership world-wide mainly due to their reliable and consistent performance levels.

Tulip Diagnostics (P) Ltd's immunohematology range of products have already attained the benchmark status. All this acheivements have motivated Tulip Group's innovative streak to come up with many industry firsts.

Tulip Diagnostics (P) Ltd., is the first Indian company to introduce the Matrix Gel System indigenously. The Matrix Gel System is based on the priniciple of Gel column agglutination technology and offers a beneficial platform of **Enhanced Sensitivity, Specificity and Simplicity** to the immunohaematologist and laboratarians for Bloodgrouping, Typing, Antibody screening and Identification.

The Matrix Gel System has been thoroughly evaluated for its performance and is comparable to other equivalent methods.



## TYPHOID

### Vital Widal

Widal antigen set for tube test with **VITAL STAINING** or **STAIN-WHILE-THEY-GROW TECHNOLOGY** ,which stains the bacterial cell wall, internally ensuring no background color and makes the visualization of weaker agglutination better.

**Presentation: 2x10 ml set / 4x10 ml set**

### Tydal

Widal antigen set for slide and tubes test with innovative **VISIMAX STAINING SYSTEM** that does not mask the immunodominant epitopes & provides optimum antigen-antibody environment for enhanced readability due to high signal-background ratio

**Presentation: 2x5 ml / 4x5ml set/ 4x10 ml set /5 ml each of O, H, AH, AO, BH, BO, CH & CO**

### Typhochek

Conventionally stained Widal Antigen Set, for tube test enhanced with new revolutionary thermostabilizers for robustness under ambient conditions

**Presentation: 4x50 ml set/50 ml each of O, H, AH & BH**

## BRUCELLOSIS

### Brucel-RB

Internationally recommended *Brucella abortus* strain-99 with Rose Bengal stain (standardized against 2nd international preparation) for initial screening of infection caused by *B.abortus*, or *B.melitensis* or *B.suis* on slide.

### Brucel-A

Standardized, specific, smooth *Brucella abortus* stained antigen suspension with thermostabilizers for detection of antibodies to *Brucella abortus* on slide and tube.

### Brucel-M

Standardized, specific, smooth *Brucella melitensis* stained antigen suspension with thermostabilizers for detection of antibodies to *Brucella melitensis* on slide and tube.

**Presentation: 5 ml**

### Brucellosis Positive control

Polyspecific positive control for routine quality control and reagent validation of Brucel-RB, Brucel-A and Brucel-M reagents.

**Presentation: 0.5 ml**

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