INDIRECT IMMUNOFLUORESCENCE IN THE DIAGNOSIS OF AUTOIMMUNE DISORDERS

Appropriate clinical correlation and diagnostic support in the arena of Autoimmune disorders is achieved at Dr.Lal Path labs with an array of tests available with reference to the vast spectrum of antibodies involved in the pathogenesis of autoimmune disorders.

Overview :

Systemic Lupus Erythematosus comprises a vast majority of Autoimmune disorders spanning a wide array of clinical features ranging from skin rashes to joint related symptoms (Arthritis) to further complicated pictures like Serositis, renal disorders, neurologic & hematologic disorders (leucopenia).

Antinuclear antibodies (ANA) are positive in >98% of patients during the course of disease; repeated negative tests suggest that the diagnosis is not SLE, unless other autoantibodies are present. High-titer IgG antibodies to double-stranded DNA and antibodies to the Sm antigen are both specific for SLE and, therefore, favor the diagnosis in the presence of compatible clinical manifestations.

The presence in an individual of multiple autoantibodies without clinical symptoms should not be considered diagnostic for SLE, although such persons are at increased risk since clinical SLE begins in most patients years after autoantibodies appear.

Antinuclear antibodies (ANA) is a term which describes a variety of autoantibodies against constituents of cell nuclei including DNA, RNA and various nuclear proteins.

These ANA are found in high frequency in patients with connective tissue diseases like rheumatic disorders and SLE. There is a high correlation between ANA positivity and SLE, and a negative ANA essentially rules out the disease; however at times repeated testing might be required to firmly establish the diagnosis.

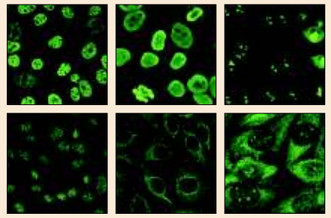
Frozen sections of rat liver were historically the most popular substrate for demonstration of ANAs but these have now been replaced by various types of cell lines. We at LPL have acquired HEp-2 cells manufactured by a UK based cell culture firm for reporting various patterns of ANA which has been found extremely helpful by clinicians in correlating with the specific autoimmune diseases, as the process is rapid and cheap.

When used in ANA screening HEp-2 cells show three distinct advantages over rat tissue, namely, they are a standardized antigen substrate with less batch to batch variability than rodent tissue; HEp-2 cells are larger enabling easier visualization of cell morphology with a consequent increase in assay sensitivity over rat sections, and finally, many HEp-2 cells are actively dividing, exposing antigens not normally expressed in the resting cells of rat liver sections.

We use an indirect immunofluorescense technique wherein patient sera and appropriate controls are incubated with the HEp-2 substrate. After repeated washings and tagging with fluorescent labeled conjugate followed by washing off the excess; the slides are viewed with a fluorescent microscope for detecting the characteristic apple green fluorescence which corresponds to areas of the HEp-2 cell where antibody has bound.

Brief summary between microscopic and clinical interpretation of HEp-2 fluorescence patterns in ANA screening :

PATTERN	APPEARANCE	ANTIGEN INVOLVED	MAIN DISEASE ASSOCIATIONS
Homogeneous	Solid nucleus staining	dsDNA Ss DNA Histones Scl-70 (may give a speckled pattern)	High titres : SLE Low titres : SLE or other connective tissue dis.
Speckled	Fine or coarse, usually without staining of the nucleoli	SS-A SS-B Sm RNP + others	High titres: Sjogrens-Sicca syndrome complex SLE MCTD Scleroderma Low titres : Miscl conn. Tissue dis.
Nucleolar	Large coarse speckles(usually less than 6 per nucleus, with or without fine speckles)	Pm-Scl Nucleolin Ku And other nuclear antigens	Scleroderma ; Sjogren's syndrome
Centromere	Discrete speckles (usually in multiples of 46)	Chromosomal centromere	CREST Syndrome
Mitochondrial	Coarse granular filamentous cytoplasmic speckled pattern extending around the nucleus and throughout the cytoplasm	M2	Primary biliary cirrhosis; Scleroderma
Jo-1	Fine speckled staining concentrated around the nucleus	Histyl-tRNA synthetase	Polymyositis; dermatomyositis associated with interstitial pulmonary disease



Images Clockwise: Nuclear coarse speckled ; nuclear homogeneous; nucleolar clumpy; centromere autoantibody; mitochondrial pattern; ribosomal pattern

Dr. Chirantan Bose MD.

FROM THE EDITOR'S DESK

To screen or not to screen -- that is the question. Or is it?

Actually, the use of screening in medicine is essential. It is defined as "sifting for the presence of disease." The arguments about screening typically range from laws that require testing of newborns for inborn errors of metabolism, to analyzing urine of school kids for illegal drugs, to searching for the early onset of Alzheimer's disease.

These arguments will never cease, but not about whether to screen, rather about what to screen for, in whom, why, when, and how.

Should screening be random or systematic; indicated or unindicated; voluntary or mandatory; announced or by surprise; covered by insurance or not; with or without true informed consent; invasive or noninvasive; and should it lead to punitive, or redemptive, or lucrative next steps?

When you wake up in the morning and crawl out of bed, you are screening yourself for overnight strokes; when your spouse looks at your skin, she is screening you for melanoma; when a mother combs her child's hair, she is screening for head lice; when a nurse takes your temperature, he is screening for infections; and when you take your own blood pressure, you are screening for asymptomatic hypertension. And so forth.

Why the fuss? The fuss is over the details, like what is being looked for, how much will it cost, whose privacy is invaded, whether there is a helpful action to take if you find something, and what the consequences of screening will be to your health, your family, your job, your insurability, your freedom. This is a topic worth some serious thinking.

In this issue we have highlighted Tandem mass spectrometry, a technological advance, giving rise to the potential for a major expansion in newborn screening for IEM.

Newborn screening is on the verge of a major revolution. Screening for inborn errors of metabolism (IEM) commenced in the 1960s, with the introduction of testing for phenylketonuria. Subsequently, programmes for diagnosis of a number of other conditions in newborn infants, with neonatal bloodspots, have been developed.

Recent advances in technology and clinical intervention have enabled clinical laboratories providing newborn screening services to improve testing and to expand testing to include additional treatable disorders. One of the major technical advances in newborn screening is the use of an analytical instrument known as a tandem mass spectrometer.

Screening for antinuclear antibodies is performed for various systemic autoimmune diseases with various different techniques. A more recent approach to ANA screening is the use of enzyme immunoassays. The assay shows improved sensitivity in ANA determination and a similar performance, compared to the standard method of IFA.

It is indeed encouraging to have received a tremendous response to the previous issue of INSIGHT and we continue to look forward to your valuable feedback and suggestions to guide us in addressing the various issues in need of diagnostic service.

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Volume 13 Sept. 2009 News Letter

60 Years of serving the Nation

Dr Lal PathLabs

INSIGHT

ARMY CONFERS HONORARY BRIGADIER'S RANK ON DR. ARVIND LAL

Dr. Arvind Lal, Chairman and Managing Director of Dr. Lal PathLabs, has been conferred the Honorary Rank of Brigadier in the Army Medical Corps.

President Pratibha Patil, the Supreme Commander of the Armed Forces, granted this award to Dr. Lal in recognition to his outstanding contribution in the field of health care. The Brigadier's rank was conferred on him at a 'Pip-In' ceremony by Lt. Gen. N. K. Parmar, Director General Armed Forces Medical Services.

Dr. Arvind Lal is a pioneer in bringing laboratory services in India at par with the West. Born on August 22, 1949, Dr. Lal is an alumnus of the Armed Forces Medical College (AFMC), Pune, having received his graduate and post-graduate medical degrees from the prestigious institute. Later he worked as a Demonstrator (Lecturer) in the Department of Pathology in AFMC. In 1977 Dr. Lal took charge of the Pathology Laboratory founded in 1949 by his late father.

Under his expert guidance and leadership, Dr. Lal PathLabs, the 60 years old Laboratory service provider has become one of the most reputed laboratories in Asia, having to its credit the highest number of eight accreditations from the National Accreditation Board for Testing and Calibration Laboratories (NABL, Ministry of Science and Technology, Govt. of India). He brought International recognition to the Indian Healthcare Industry in the form of accreditation from the College of American Pathologists (CAP - USA).

Dr. Lal has revolutionized laboratory medicine by introducing the maximum number of new tests, instruments and ICT (Information, Communications, Technology) systems i.e. his R & D unit is recognized by the Department of Science and Technology, Government of India. This unit has done India proud by developing the latest tests in Molecular Diagnostics like the Real Time PCR for HIV Viral Load Studies, Tuberculosis - India's number one infectious disease and Genotyping for Hepatitis C Virus-the biggest killer of all liver diseases and the newly introduced Swine Flu test. The aim of the R&D division is to provide highly specialized tests at low cost to the weaker sections of the society.

Dr. Lal is a member of many expert and advisory bodies constituted by the central and the state governments. He is accredited by having the first PPP (Public Private Partnership) in the field of laboratory testing with the Government of Tripura since 2004. Dr. Lal is also an active member of the Confederation of Indian Industries (CII) Healthcare Committee and has played a significant role in promoting the Indian Healthcare sector abroad and also in promoting PPP initiatives with the union government. He is the founder Vice President of ACRO (Association of Clinical Research Organizations) for furthering the cause of Clinical Research in India. Dr. Lal was appointed as the Honorary Physician to the President of India in 2001. He has also been serving as a pathologist to many present and past Prime Ministers of India. His laboratory is the nodal laboratory testing the largest number of government employees from CGHS, ECHS, Ministry of Railways, Delhi Government and many others.

Conferred with the Padma Shri award by the President this year, Dr. Lal has been honoured with Lifetime Achievement Award in Medicine, the Delhi Ratan Award and many others for his extraordinary contribution in the field of Laboratory Medicine. Under his leadership, in July 2008, his laboratory was bestowed, with the prestigious CNBC TV 18 and ICICI 'Emerging India Award' presented in London, U.K.

NEWBORN SCREENING

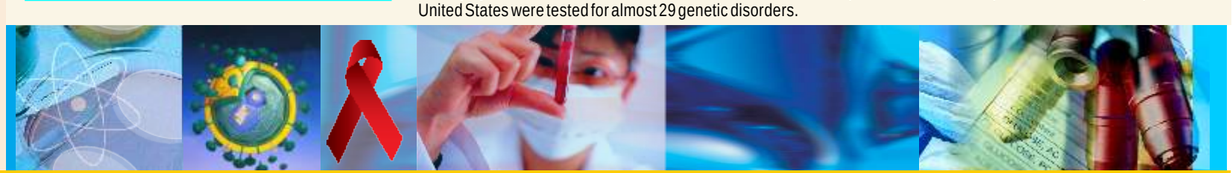
What is Newborn Screening?

Newborn screening is a set of blood tests to look for evidence of certain endocrine and genetic disorders. Newborn screening has the potential to identify risk of certain diseases before the appearance of clinical symptoms. Early detection and early treatment can prevent mental retardation, spasticity, serious illness, and premature death.

World Statistics

According to World Health Organisation (WHO), 140 million children are born every year, of which 5 million children die in the first month of life in the developing countries and 4 million children are born with some genetic abnormality. Whenever a child dies due to unknown cause, it is termed as Sudden Infant Death Syndrome (SIDS). It is estimated that 25-30% of such children are unable to thrive due to metabolic disorders. In other words, 25-30% of SIDS are a result of treatable metabolic disorders. These children suffer from life threatening complications occurring as a result of disturbance in the metabolic pathway. The diagnosis can be preempted & the complications well controlled by newborn screening.

A study by March of Dimes has revealed that in 2007, almost 90 percent of newborns (about 4 million babies) in the United States were tested for almost 29 genetic disorders.



Indian Statistics

People of India constitute more than one-sixth of the World's population, but statistically they have been under-represented in newborn screening. About 4 percent of the population in India suffers from mental retardation and 5-15% of the sick newborns have a metabolic problem. It is estimated that 1 in 2000 Indian newborns suffer at birth from some kind of metabolic disorder. The World ratio for the same category is 1 in 3600. An estimated 3,90,000 children with G6PD deficiency and 9760 with aminoacid disorders are born in India each year (Dr IC Verma, Community Genetics 2002;5:192-196). Dr Radha Rama Devi, in her study (Newborn Screening in India. Indian J Pediatr 2004;71:157-160) observed a high prevalence of inborn errors of metabolism (metabolic genetic disorders) to the extent of 1 in every thousand births. A prevalence of congenital hypothyroidism 1 in 1700 , congenital adrenal hypothyroidism 1 in 2575 and aminoacid disorders 1 in 3600 was observed. Dr Mamta Muranjan from Mumbai detected 13.85% of confirmed cases of organic disorders in four year study from 1995-1998 (Indian Pediatrics 2001;31:518-524).

Even if we consider a meager 2% of live births in India to develop metabolic genetic disorders, then 500,000 affected children are born every year.

Disorders Included in Newborn Screening Panel

- Biotinidase Deficiency
- Congenital Primary Hypothyroidism
- Cystic Fibrosis
- Congenital Adrenal Hyperplasia
- G6PD Deficiency
- Galactosemia
- 30 Disorders of Aminoacids, Organic acids & Fatty acid oxidation by Tandem Mass Spectrometry

Biotinidase Deficiency:

Biotinidase deficiency is a disorder caused by the lack of enzyme biotinidase. Babies with partial or total deficiency need more biotin than normally found in the diet. Biotin is a water soluble vitamin of the B complex group.

Clinical manifestations: Children with biotinidase deficiency may present with clinical symptoms as early as first week of life, but usually begin to show clinical symptoms between 3 to 6 months of age. If untreated, they develop a variety of cutaneous and neurological abnormalities. Affected children usually have myoclonic seizures,hypotonia, seborrheic or atopic dermatitis, partial or complete alopecia.

Laboratory findings include ketolactic acidosis, organic aciduria, mild hyperammonemia. Metabolic acidosis can result in coma and death.

Treatment if initiated sufficiently early can prevent the occurence of clinical symptoms. Once neurological symptoms appear, it is not possible to reverse the damage with treatment. Sensorium & hearing loss is common with profound biotinidase deficiency and is usually irreversible.

Screening is done by assessment of biotinidase activity on whole blood spotted on filter paper. Cases detected as profound or partially deficient, are confirmed by testing biotinidase activity in serum.

Benefit of Screening: Observation at LPL is that biotinidase deficiency is the most common disorder. Once symptoms have occurred, some of the findings particularly neurologic are not reversible with therapy.

Congenital Adrenal Hyperplasia:

Congenital adrenal hyperplasia (CAH) is a disorder of adrenal cortex. Majority of CAH cases are attributable to 21-hydroxylation defect in the adrenal cortex. This results in low concentration of aldosterone & cortisol and elevated 17-hydroxyprogesterone (17OHP). Screening for CAH measures the level of 17-hydroxyprogesterone (17OHP).

Types

1. "Classic severe" salt wasting (SW) form
2. "Classic, less severe" simple-virilizing (SV) form
3. "Mild" non classic form

Clinical Manifestations

Neonates affected with SW form are at risk of adrenal crisis. Crisis can manifest as poor feeding, vomiting, loose stools or diarrhoea, weak cry, failure to thrive, dehydration and lethargy. These symptoms may not be evident until serum sodium concentrations are below 125mEq/L. Some affected infants may suffer from brain injury or learning disabilities. Female newborns affected with SW forms have ambiguous genitalia. Affected male infants do not exhibit any physical signs at birth. Therefore, without newborn screening & in the absence of a positive family history, all affected males and few females remain undiagnosed until adrenal crisis.

Patients affected with SV form manifest adrenal –insufficiency symptoms when subjected to stress and are diagnosed much later when symptoms of virilization, precocious pseudopuberty or growth acceleration occurs. Late discovery of correct gender can cause distress to the family and patient. Mild 21-OH deficiency produces no symptoms at birth and manifest as premature sexual hair, acne and mild growth accleration in childhood and hirsutism, excessive acne, menstrual disorder and infertility later in life. Mild disorders may be missed by newborn screening.

Benefits of newborn screening are:

- Prevent life threatening adrenal crisis. Adrenal crisis can cause shock, brain damage, and death.
- Prevent male sex assignment in virilized female newborns
- Prevent progressive effects of excess adrenal androgens, which can cause short stature and psychosexual disturbances.

Worldwide newborn screening data has shown that screening prompted early diagnosis of CAH even before clinical suspicion in 67% of newborn infants with CAH.

Congenital Hypothyroidism:

Congenital hypothyroidism is caused due to deficiency of thyroid hormone since birth. CH is one of the most common and treatable causes of mental retardation. Some infants are normal at birth due to protection by maternal thyroid hormone.

Clinical Manifestations

Males and females are affected equally. The severity of symptoms and physical findings correlates with the degree of hypothyroidism. Clinical symptoms in the first week of life are usually not apparent. The affected infants suffer from feeding problems, constipation, lethargy, hoarse cry, prolonged jaundice, cool, dry mottled skin, coarse facies with large open fontanelles, umbilical hernia & delayed development. Mental deficiency can be prevented by newborn screening and prompt treatment with thyroid hormones.

Screening is performed by measuring TSH level. Some infants may have a slight increase in TSH. These patients need to be observed and thyroid function test should be repeated after a few months.

Cystic Fibrosis

Cystic fibrosis is a multisystem disease affecting lungs, pancreas, intestine, liver & sweat glands. Cystic fibrosis was thought to be rare in India. However published reports indicate that cystic fibrosis is probably more common than previously thought but is underdiagnosed or missed in majority of the cases (Ahuja AS & Kabra SK. Cystic fibrosis: Indian Experience. Indian Pediatrics.2002:39:813-818). According to Christine Noke, Director Cystic Fibrosis Worldwide Programme,40,000 Indians in United States and 28,000 Indians in United Kingdom suffers from Cystic fibrosis and there is possibility of 100,000 patients in India (www.cfww.org). The total load due to Cystic fibrosis could be more than many European countries (Kapoor V, Shastri SS, Kabra M etal. Carrier frequency of F508del mutation of Cystic fibrosis is noted in Indian population (Journal of Cystic Fibrosis 2006;5:43-46). Cystic fibrosis patients are mostly treated as tuberculosis and the acute exacerbations labeled as 'bronchopneumonia" (Dr Meenu Singh, PGI Chandigarh. www.cfww.org).

Clinical Manifestations

Cystic fibrosis usually presents in infancy. In about 10-20% cases, the first symptom of disease appear soon after birth.Symptoms and severity of disease differ from person to person but the basic problem remains the same i.e. the glands which produce or secrete sweat and mucus do not function properly. This results in unusually thick mucus, which clogs lungs. Mucus also affects the pancreas by blocking digestive enzymes, which are needed for breakdown & assimilation of food. Some patients have both respiratory and digestive problems while some have respiratory problems only.Cystic fibrosis does not affect intelligence.

Most common symptoms are:

- Meconium ileus
- Poor growth
- Breathing difficulties
- Nasal polyps
- Pneumothorax (rupture of lung tissue and trapping of air between the lungs and chest wall)
- Hemoptysis (coughing of blood)
- Liver disease, inflammation of the pancreas
- Malnutrition
- Frequent respiratory infections
- Lung damage
- Rectal prolapse
- Abdominal pain and discomfort
- Infertility

Screening: Detection of Cystic fibrosis in newborns depends on the presence of immunoreactive trypsinogen (IRT). IRT levels tend to remain raised for several months in babies with CF, whereas in false positive cases, values usually return to normal within first few weeks of life (Wilcken B, Brown ARB, Urwin R, Brown DA. Cystic fibrosis screening by dried blood trypsin assay results in 75,000 infants. J Pediatr 1983;102:383-387). In babies with meconium ileus, the IRT levels may not be elevated. High frequency of heterozygotes have been reported among neonates with elevated IRT and normal sweat chloride level (CastellanicC, PicciL, ScarpaM, DecheechiMC etal. Cystic fibrosis carriers have higher neonatal immunoreactive trypsinogen values than non-carriers. Am J Med Genet A2005;135(2):142-144).

Benefit of Screening: CF babies are normal with no evidence of lung disease or pancreatic insufficiency. Symptoms develop over first 3-6 months of life. By the time children are diagnosed clinically with CF, they are already seriously ill with lung disease and significant malabsorption problems. Before screening test was introduced, the average age of children without known sibling with CF was about 18 months (Wilcken B,Towns SJ,Mellis CM. Diagnostic delay in cystic fibrosis:lesson from newborn screening. Arch Dis Child 1983;58:863-866). Better treatment methods developed over the past 20 years have increased the life span of CF patients. Early diagnosis helps in improved height and weight due to early initiation of therapy which includes pancreatic enzyme, fat soluble vitamin and salt supplementation (Farrell PM, Kosorok MR, Rock MJ etal. Early diagnosis of cystic fibrosis through neonatal screening prevents severe malnutrition and improves long term growth. Wisconsin Cystic Fibrosis neonatal screening study group. Pediatrics 2001;107:1-13)

Galactosemia

Galactosemia is a disorder in which galactose cannot be broken down in the body. Galactose is found in breast milk, many formulas and milk products. Under normal conditions, galactose is released by the digestion of lactose and is converted to glucose as well as fructose in the body. In galactosemia, there is genetic defect in conversion of galactose to glucose. Increased concentration of galactose in blood can harm the baby's eyes, liver and brain causing cataract & mental retardation. Affected infants may die in the neonatal period due to Escherichia coli sepsis or later due to cirrhosis of liver.

Newborn screening tests: Test for galactose depends on the infant's diet; therefore it is important that the infant is receiving galactose-containing formula or breast milk before testing.

Benefit of Screening: Exclusion of galactose from the diet can prevent cataract, mental retardation and other life threatening complications.

TANDEM MASS SPECTROMETRY

Disorders of Aminoacids, Organic acids & Fatty acid oxidation can be tested by Tandem Mass Spectrometer (TMS).

"That some infants are not being caught and treated when possible is a national tragedy and tandem mass spectrometry should be standard care for all newborns, much as blood pressure test is a part of every medical checkup"- Harry Hannon, Director New Born Screening Services, CDC

Source: The Wall Street Journal, 2004

Why test with Tandem Mass Spectrometer?

Before TMS technology	With TMS
One disease - One test	Many diseases- only one test
Many diseases- Many tests	All tests performed on same blood sample
	Newborn screening easy

- More cases of inborn errors of metabolism are diagnosed by screening with tandem mass spectrometry than are diagnosed clinically. (New England Journal of Medicine 2003; 348: 2304-12).
- SIDS or Metabolic Disorder?: TMS can differentiate

3-6% of SIDS are due to inherited disorders of Fatty Acid Oxidation, (Clinical Chemistry; June 2001:47 (7)1166-1182)

What Analytes are measured by Tandem Mass Spectrometry?

- *Aminoacids:* building blocks of proteins
- *Carnitine:* transportation system for fats in and out of mitochondria
- *Acylcarnitine:* Acylcarnitines are identified by the size of fat molecule attached to it and categorised simply as short, medium amd long chain. The important medium sized fat attached to carnitine that is measured in MCAD is eight carbon fatty acid known as Octanoylcarnitine and is abbreviated as C8

Disorders detected by TMS

I. Aminoacid Disorders:

- Urea cycle disorders
- Hypermethioninemia
- Hyperammonemia, Hyperornithinemia,Homocitrullinuria
- MSUD
- Homocystinuria
- Non-ketotic hyperglycinemia
- PKU
- Tyrosinemia

II. Organic Acid Disorders:

- Glutaric acidemia type 1
- 3-ketothiolase deficiency
- 3-Methylglutaconyl CoA hydratase deficiency
- Multiple carboxylase deficiency
- Methylmalonic acidemia
- Propionic acidemia
- Isovaleric acidemia
- 3-MethylcrotonylC0A carboxylase deficiency
- Beta-Ketothiolase deficiency
- Methylmalonic acidemia with homocystinuria

III. Fatty acid Oxidation Disorders:

- Shortchain acyl CoA dehydrogenase deficiency (SCAD)
- Medium chain acyl CoA dehydrogenase deficiency (MCAD)
- Mitochondrial trifunctional protein deficiency
- Longchain 3-hydroxy acyl CoA dehydrogenase deficiency (LCHAD)
- Very long chain acyl CoA dehydrogenase deficiency (VLCAD)
- Carnitine transport defect
- Carnitine palmitoyl transferase deficiency type 1 (CPT 1)
- Carnitine palmitoyl transferase deficiency type 2 (CPT 2)
- Glutaric acidemia type 2
- Carnitine acylcarnitine translocase deficiency

Sensitivity, Specificity, Positive Predictive Value of TMS

- Sensitivity close to 100% with high positive predictive value (PPV)
  - Classical MSUD
  - UCD
  - Tyrosinemia
  - PKU
  - Earlyonset organic acidemias
  - Fatty acid oxidation disorders (Ref:J Inherit Metab Dis (2007)30:129-133)

False Positive Rate (TMS)

- Low
- 0.2-0.33 % of babies screened needed further testing (Ref:J Inherit Metab Dis (2007)30:129-133)

What is the Screening sample?

5 drops of blood are taken from the baby's heel. Baby's heel is pricked with a lancet and drops of blood are collected on special filter paper.

When should the newborn screening sample be collected?

Infant must be 48 hours of age or older in order to obtain a satisfactory sample for screening. Ideal time is 4 days of age. Sample should be taken prior to administering antibiotics or transfusing blood or blood products.

What is the cutoff age for an infant when accepting sample for newborn screening?

Till one month of age except for disorders by TMS. Test methodologies for other tests are set to obtain valid values till 30 days of age. Result values of tests carried out beyond 30 days may overlap considerably making it difficult to distinguish presence or absence of disorders.

Factors which affect Result & Interpretation:

- Size of blood spot
- Intravenous fluid hydration with dextrose prior to collection of sample can flush out short lived active acylcarnitine metabolite from the intravascular blood stream
- Low Protein diet
- Drugs such as valporic acid, MCT Oil, antibiotics containing a derivative of pivalic acid
- Blood for test aspirated from iv catheter
- IV fluid
- Blood transfusion
- Supplemented with aminoacids

How long to wait to screen an infant who has been transfused?

Given the presumption that the mean age of RBC's of the transfused blood is 60-80 days, it is suggested that sample should be collected 60 days after discontinuation of transfusion.

What to do for an infant who needs antibiotics prior to 24 hours of age?

Sample taken from infants receiving antibiotics at the time of collection may yield false results. Sample should be collected prior to starting antibiotic therapy or 24 to 36 hours after discontinuation of antibiotic therapy.

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