National Institute of Mental Health & Neuro Sciences

(Institute of National Importance)
Bengaluru - 560029, India.

SCREENING FOR INBORN ERRORS OF METABOLISM

Inborn errors of metabolism are a group of rare genetic disorders that affect one or more of the hundreds of biochemical pathways in the human body. Patients with these disorders are unable to utilize or synthesize fatty acids, amino acids, organic acids, or macromolecules, because of defects in the enzymes or other components of various metabolic pathways. These conditions are frequently identified in infants and young children with acute or chronic symptoms. When possible, early diagnoses with timely and effective intervention are essential for preventing adverse clinical outcomes such as permanent neurologic damage, disabilities, and even death in affected babies.

Screening for inborn errors of metabolism involves measurement of the levels of specific metabolites present in the dried blood spot (DBS) specimens. Abnormal levels of certain metabolites (eg. amino acids and acylcarnitines) suggest the presence of a particular metabolic disorder. For measuring the concentration of these compounds, discs are punched out of the DBS and analysed by a validated bioanalytical **LC-MS/MS (Tandem Mass Spectrometry)** method. Tandem Mass Spectrometry is a technique that has been shown to be suitable for the reliable detection of inborn errors of metabolism. It is highly accurate and able to measure multiple compounds simultaneously.

DBS are whole blood samples that are spotted on a filter paper**.** CDC (Centers for Disease Control and Prevention) guidelines for Newborn Screening program recommend the use of S&S 903(Schleicher and Schuell) filter paper.

SAMPLE COLLECTION

Blood samples collected on filter paper can be obtained when the child is symptomatic or asymptomatic. If possible, obtaining the sample prior to treatment is preferable, since this interferes with the test results. Instructions for sample collection are mailed when requested by the clinician ordering the test and also explained to the patients when they come personally to collect the sample collection cards.

METHOD

D O ’S

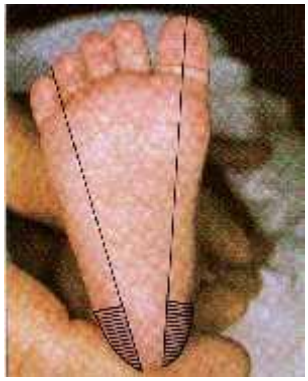
- Blood is collected by heel-prick on the sample collection cards. Three spots, collected in full circle (diameter- 15 mm) are required.
  NOTE: For children >1year and infants >5m of age, finger or toe pricks can be done for collecting the blood sample.
- Specimen should be air- dried thoroughly for 3-4 hours at room temperature before sending the sample or storing it.

DONT'S

- Samples should not be layered or spotted.
- EDTA blood should not be used.
- Wet specimens should not be stacked on one another.
- In case of blood transfusion, sample should be collected after a minimum of 3 weeks after transfusion. Capillary tubes or other devices should not be used for spotting the blood on the filter paper.
- The blood sample should not be exposed to heat or direct sunlight.

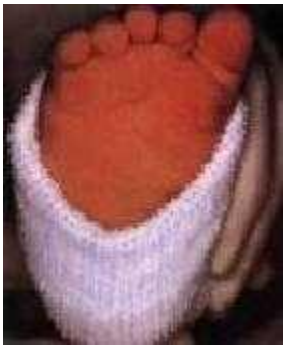
Procedure for Sample Collection (Instructions *might also be given on some cards.*)
***[Reference: CDC** (http://www.cdc.gov) and **Perkin Elmer** (http://www.perkinelmer.com)websites]*

1. Ensure heel is warm.
2. Wipe infant's heel with spirit/alcohol.
3. Allow heel to air dry.
4. Heel puncture should be performed on the plantar surface.
5. The length of the puncture should not exceed 2.4 mm.
6. Gently wipe off the first drop with cotton.
7. Wait for the formation of second large hanging drop.
8. Gently touch the center of the filter paper to the blood drop and fill each printed circle with a single application of blood.
9. Specimen should be air-dried thoroughly for **4 hours at room temperature.**



STEP BY STEP PROCEDURE FOR SAMPLE COLLECTION THROUGH HEEL PRICK

1. Warm site with soft cloth, moistened with warm water up to 41°C, for three to five minutes.



2. Cleanse site with cotton dipped in isopropyl alcohol .Wipe DRY with sterile gauze pad.

3. Puncture heel. Wipe away first blood drop with sterile gauze pad.

4. Allow another LARGE blood drop to form.

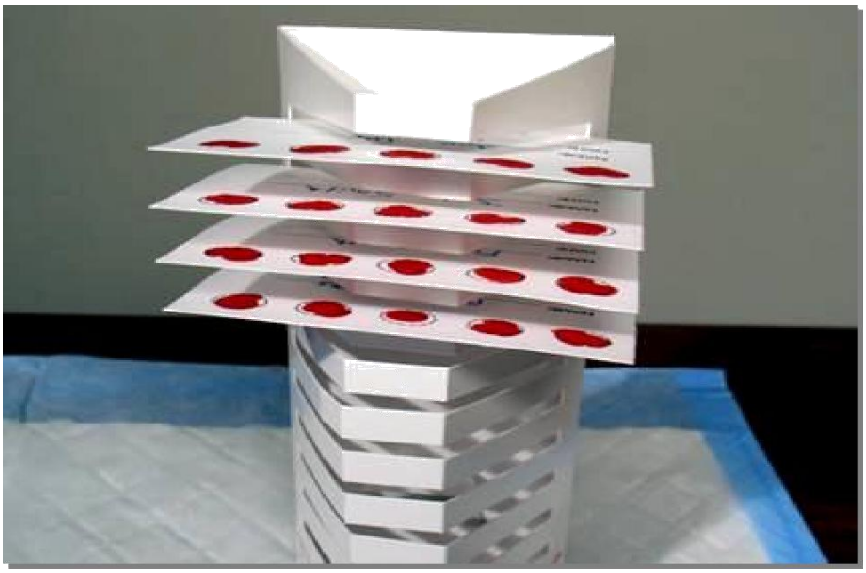
5. Lightly touch filter paper to LARGE blood drop. Allow the blood to soak through and completely fill the circle with a SINGLE application to LARGE blood drop.

6. Fill all required circles with blood.

7. Apply blood to one side of filter paper only.

- The specimen should be allowed to air dry in a horizontal position for at least 3 hours before mailing.
- Neither side of the blood spots may touch a surface.
- Specimens are to be kept away from direct sunlight and heat sources during the drying process.
- Heat must not be used to facilitate drying.

CORRECT PROCEDURE TO DRY BLOOD SAMPLES



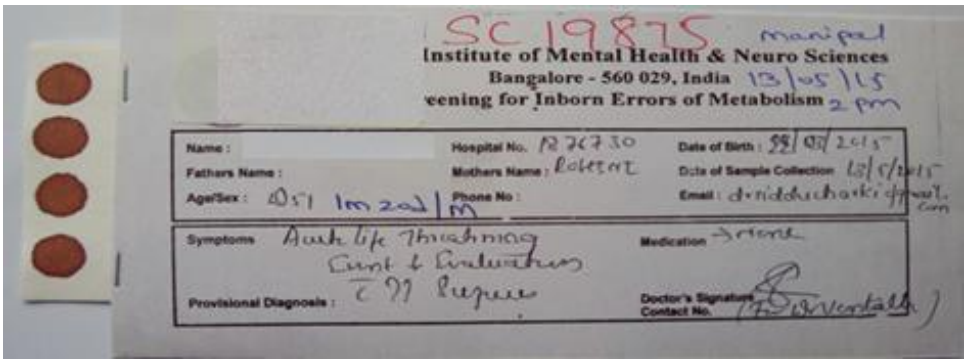
NOTE :

ALL SPECIMENS MUST BE SENT TO THE LABORATORY IF POSSIBLE ON THE SAME DAY OF SAMPLE COLLECTION.

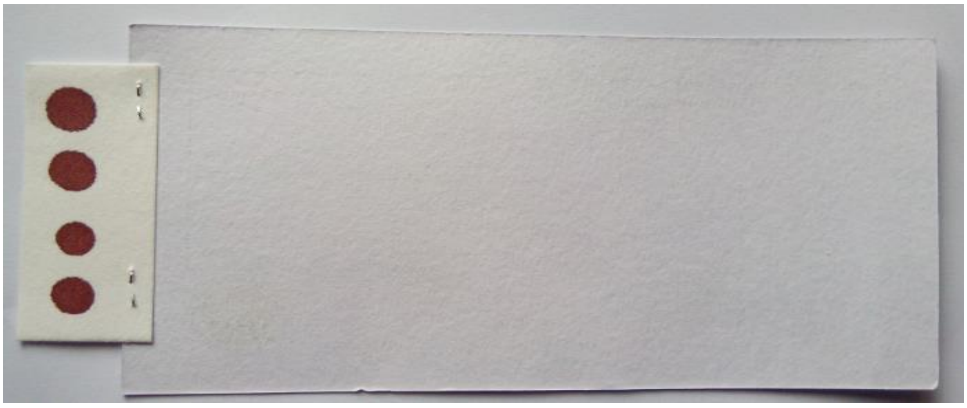
SPECIMENS SHOULD NOT BE PLACED IN PLASTIC COVERS PRIOR TO MAILING!

The picture given below indicates a blood sample for TMS that was appropriately collected:

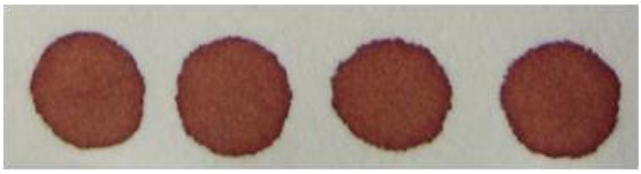
FRONT SIDE OF FILTER PAPER



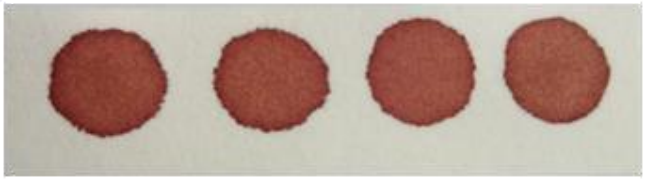
BACK SIDE OF FILTER PAPER



FRONT SIDE OF THE FILTER PAPER



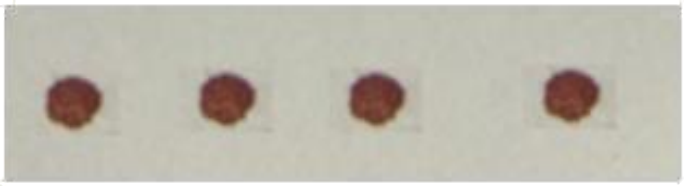
BACK SIDE OF THE FILTER PAPER



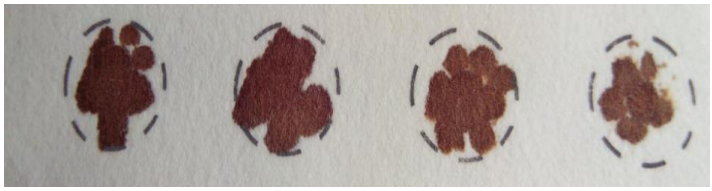
The pictures given below indicate samples that are improperly collected

SUCH IMPROPER SAMPLES WILL BE REJECTED AS THE ANLAYSIS WILL GIVE INCORRECT RESULTS

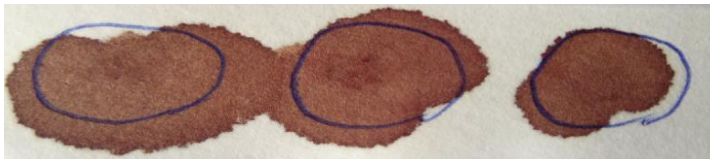
INSUFFICIENT SPOTS FOR ANALYSIS



SAMPLE OVERLAY(MULTIPLE BLOOD SPOTS COLLECTED ON TOP OF THE OTHER)



JOINT / SUPERSATURATED SPOTS



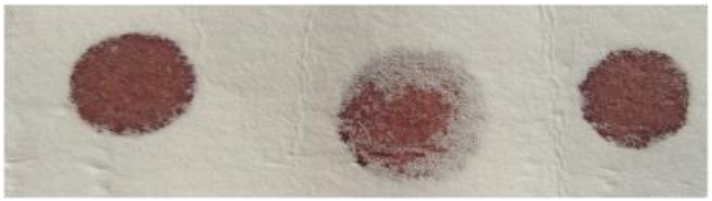
CLOTS/LAYERS SEEN IN THE CENTER OF THE DRIED BLOOD SPOT

DOUBLE BLOOD SPOT RINGS SEEN AFTER DRYING

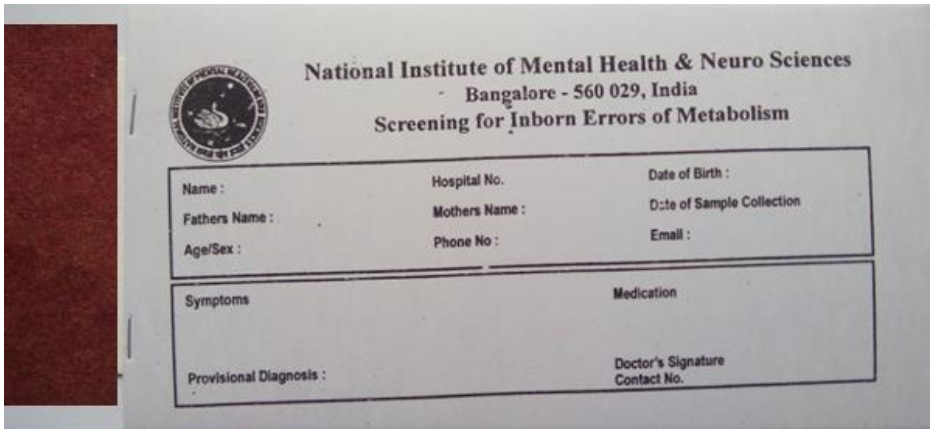
DILUTED/WASHED OUT /DISCOLOURED/CONTAMINATED SAMPLE

SERUM RINGS SEEN

SAMPLE NOT BLOTTED PROPERLY ON BOTH SIDES OF THE FILTER PAPER



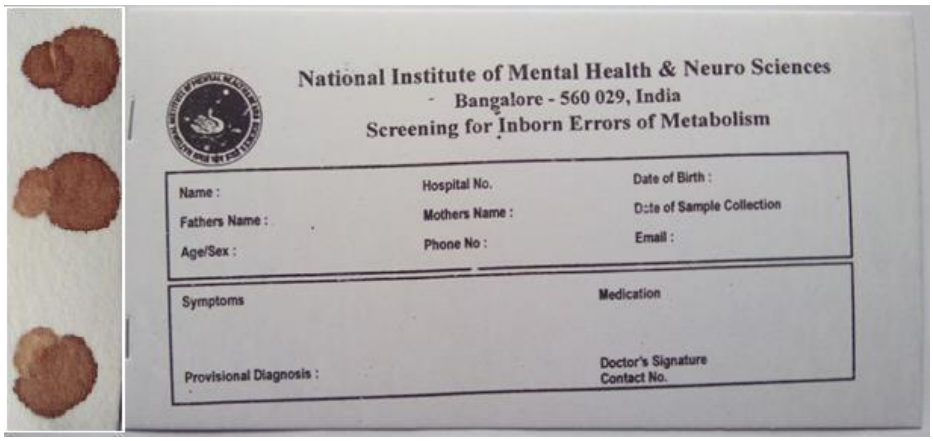
BLOOD SPREAD ON THE ENTIRE FILTER PAPER


National Institute of Mental Health & Neuro Sciences
Bangalore - 560 029, India
Screening for Inborn Errors of Metabolism

Name :
Fathers Name :
Age/Sex :
Hospital No.
Mothers Name :
Phone No :
Date of Birth :
Date of Sample Collection
Email :

Symptoms
Medication
Provisional Diagnosis :
Doctor's Signature
Contact No.

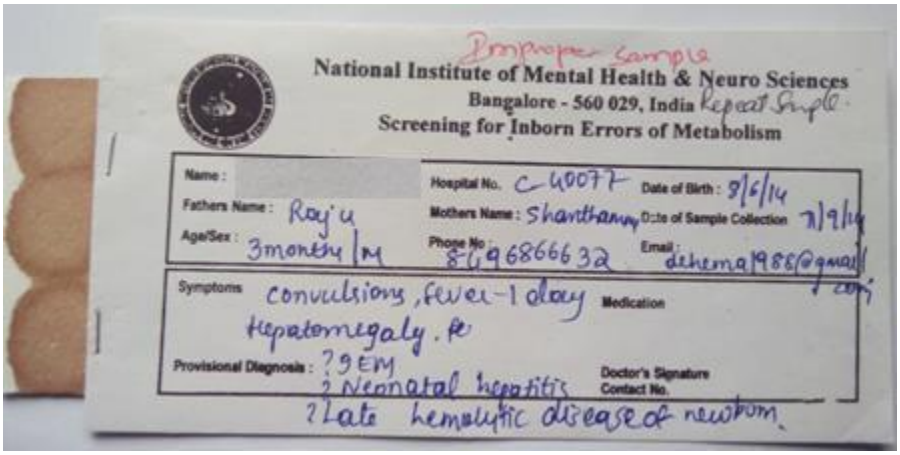
SAMPLE WITHOUT ANY CLINICAL DETAILS


National Institute of Mental Health & Neuro Sciences
Bangalore - 560 029, India
Screening for Inborn Errors of Metabolism

Name :
Fathers Name :
Age/Sex :
Hospital No.
Mothers Name :
Phone No :
Date of Birth :
Date of Sample Collection
Email :

Symptoms
Medication
Provisional Diagnosis :
Doctor's Signature
Contact No.

ATTACHED SPOTS WITH DILUTION


Improper sample
National Institute of Mental Health & Neuro Sciences
Bangalore - 560 029, India Repeat Sample
Screening for Inborn Errors of Metabolism

Name :
Fathers Name : Raju
Age/Sex : 3months/M
Hospital No. C40077
Mothers Name : Shanthamma
Phone No : 8696866632
Date of Birth : 8/6/14
Date of Sample Collection 7/9/14
Email : dehema1988@gmail.com

Symptoms convulsions, fever-1 day
hepatomegaly. PE
Medication
Provisional Diagnosis : ?IEM
?Neonatal hepatitis
?Late hemolytic disease of newborn
Doctor's Signature
Contact No.

DISPATCH OF SAMPLES

Sample collection card should be put inside a paper envelop after it is thoroughly dried and this envelope should be placed in a second paper envelop marked **'Sample for IEM screening'** along with the **request/consent form**, sealed and dispatched by courier/speed post, to :

Dr Rita Christopher
Officer In charge, Metabolic Laboratory
Professor, Department of Neurochemistry
NIMHANS, Post Box 2900,
Hosur Main Road,
Bengaluru- 560029.
Tel:80-26995163, Fax: 080-26564830
Email: rita@nimhans.kar.nic.in
nimhans08@gmail.com

Other Contacts :

1. Metabolic Laboratory

Archana Natarajan
Junior Scientific Officer
RoomNo101,Neuro Biology
Research Centre Building,
NIMHANS, , Post Box 2900,
Hosur Main Road,
Bangalore 560029.
Tel :080-26995029
Email: nimhans08@gmail.com

2. Sample Collection Room for TMS

Tel- 080-26995160
Email: nimhans08@gmail.com

TMS CHARGES

The lab charges for **Screening of Inborn Errors of Metabolism by Tandem Mass Spectrometry** are as follows:

a) For NIMHANS patients and patients from Government Hospitals - **Rs.1000/-**

b) Samples received from Private Hospitals – Rs.**1500/-**

- The above lab charges may be sent by Demand Draft (DD) in favor of the "**THE DIRECTOR, NIMHANS, Bangalore-560029**".

- **Online (NEFT/RTGS) mode**

Bank details for the payment through Online (NEFT/RTGS)

Account Holder's Name:- **The Director, NIMHANS**
Bank Name:- **State Bank of India, NIMHANS Branch**
Account No:- **64063643613**
IFS Code:- **SBIN0040675**
Bank Address:- **NIMHANS Branch, Hosur Road, Bengaluru – 560029**

NOTE:

1. Universal Transaction Reference (UTR) Number, Date of transaction and Amount Paid should be mentioned in the request form/ through Email for each patient, once the payment is done through NEFT (Online banking).

2. The samples which are not accompanied with the DD and Request form will not be analysed.

ANALYTES MEASURED BY TANDEM MASS SPECTROMETRY

Amino acids: **11 amino acids**

Glycine, Alanine, Valine, Leucine, Methionine, Phenylalanine, Tyrosine, Ornithine, Citrulline, Arginine and Proline.

Acylcarnitines and free carnitine : 30 acylcarnitines and free carnitine.

DISORDERS THAT CAN BE DETECTED

Disorders of amino acid metabolism:

- Phenylketonuria (PKU)
- Biopterin cofactor defects (BH_4 defects)
- Maple syrup urine disease (MSUD)
- Tyrosinemia types I, II and III
- Non-ketotic hyperglycinemia (NKH)
- Hyperargininemia
- Citrullinemias
- Hypermethioninemia
- Homocystinuria (HCU)
- HHH syndrome(Hyperornithinemia, Hyperammonemia, Homocitrullinuria)
- Hyperornithinemia.
- Hyperprolinemias,***etc.***

Fatty acid oxidation defects:

- Medium chain acyl-CoA dehyrogenase deficiency (MCAD)
- Short chain acyl-CoA dehyrogenase deficiency(SCAD)
- Short chain 3-hydroxy acyl-CoA dehydrogenase deficiency (SCHAD)
- Very long chain acyl-CoA dehyrogenase deficiency(VLCAD)
- Long chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD)
- Multiple acyl-CoA dehydrogenase deficiency (MADD /GA Type II)
- Carnitine palmitoyl transferase deficiency I & II
- Carnitine/acylcarnitine translocase deficiency
- Mitochondrial trifunctional protein deficiency
- Carnitine uptake defect ,***etc.***

Organic acidemias:

- Glutaric acidemia Type I
- Methylmalonic acidemia (MMA)
- 3-Ketothiolase deficiency/3-methylacetoacetyl- CoA thiolase deficiency (BKT)
- 3-Methylglutaconyl-Co A hydratase deficiency / 3-methylglutaconic aciduria (MGA)
- Malonic aciduria/ Malonyl CoA decarboxylase deficiency (MA)
- Isovaleric acidemia(IVA)
- Propionic acidemia(PA)
- 3-Methylcrotonyl -CoA carboxylase deficiency(3- MCC)
- Multiple carboxylase defect
- 3-hydroxy 3-methylglutaryl- CoA lyase deficiency(HMG CoA Lyase deficiency)/ Hydroxymethylglutaric acidemia/aciduria
- 2-methyl butyryl-CoA dehydrogenase deficiency (2-MBCD)
- Isobutyryl CoA dehydrogenase deficiency(ICBD)
- Ethylmalonic encephalopathy (EE), ***etc.***