NEWBORN SCREENING SECOND TIER TESTS (2TT) IN DRIED BLOOD SPOTS

Newborn screening second tier tests (2TT) are confirmatory tests performed when primary screens, either by tandem mass spectrometry or another method, yield an equivocal result. The 2TT functions as a confirmatory test in the original dried blood spot by measuring a disease specific analyte or an analyte profile, but for various reasons, including cost, time, and complexity, are not suitable to be used as primary screening assays. Benefits of 2TT include increases in testing sensitivity, specificity, and positive predictive value. Moreover, reductions in the false-positive rate are observed and as a result of utilizing the same specimen there is no additional patient contact which thereby avoids unnecessary parental anxiety and follow-up efforts and costs.

Below is information regarding available 2TT through Mayo Medical Laboratories. When these 2TT results are normal, they override the primary screening results and the newborn screen is reported as normal.

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<th>MAYO ID: ALLOI</th>
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**ANALYTES MEASURED**

Allo-isoleucine, leucine, isoleucine, valine, and hydroxyproline

**METHOD**

Liquid chromatography tandem mass spectrometry

**INDICATION TO ORDER**

Elevated/reduced branched chain amino acids (BCAA) by primary screening

**DISORDERS**

Maple Syrup Urine Disease, Branched chain ketoacid dehydrogenase kinase (BCKDK) deficiency

**CLINICAL UTILITY**

BCAAS are a frequent cause of false positive newborn screening results that are preventable through the use of this 2TT. Mayo experience (2004-2013): positive predictive value (PPV) for Isoleucine-Leucine was 43%. In addition, this test is useful to determine interference by hydroxyproline and quantification of individual BCAAs when evaluating a case with low levels found by primary screening.

**REFERENCES**


### MAYO ID: CAH2T

**ANALYTES MEASURED**  
17-Hydroxyprogesterone (17OHP), androstenedione, cortisol, 11-deoxycortisol, and 21-deoxycortisol

**METHOD**  
Liquid chromatography tandem mass spectrometry

**INDICATION TO ORDER**  
Elevated 17-OHP by primary screening

**DISORDERS**  
Congenital adrenal hyperplasia (CAH) and related disorders

**CLINICAL UTILITY**  
Newborn screening for CAH typically uses immunoassays to quantify 17-OHP as a marker for CAH; however, cross-reactivity of the antibodies with other steroids yields a high false-positive rate. Tandem mass spectrometry allows for more specific determination of 17-OHP and other steroids. Application of this technology in newborn screening significantly enhances the correct identification of patients with CAH and reduces the number of false-positive screening results when implemented as a 2TT performed prior to reporting of initial newborn screen results. Mayo experience (2004-2012): positive predictive value (PPV) and false positive rate (FPR) for 17OHP were 7% and 0.096%, respectively. Without the 2TT, the FPR of the primary screening would have been 0.99%.

**REFERENCES**


### MAYO ID: GPSY

**ANALYTES MEASURED**  
Glucopsychosine

**METHOD**  
Liquid chromatography tandem mass spectrometry

**INDICATION TO ORDER**  
Reduced beta-glucosidase activity by primary screening

**DISORDER**  
Gaucher disease

**DESCRIPTION**  
An elevated concentration of glucopsychosine is suggestive of a biochemical diagnosis of Gaucher disease.

**REFERENCES**


Mayo Clinic Experience - Manuscript in preparation

### MAYO ID: HCMM

**ANALYTES MEASURED**  
Homocysteine (Total), methylmalonic acid, and methylcitric acid

**METHOD**  
Liquid chromatography tandem mass spectrometry

**INDICATION TO ORDER**  
Elevated propionylcarnitine (C3), elevated/reduced methionine by primary screening

**DISORDERS**  
Homocystinuria, Disorders of propionate metabolism, Remthylation disorders, Maternal vitamin B12 deficiency

**CLINICAL UTILITY**  
C3-acylcarnitine and methionine are frequent causes of false positive results that are preventable by a normal result of this test. Mayo experience (2004-2013): positive predictive value (PPV) for C3 and methionine was 49% and 31%, respectively.

**REFERENCES**


**MAYO ID: LGEM**

**ANALYTES MEASURED**

2-Hydroxy glutaric acid, 3-hydroxy glutaric acid, glutaric acid, ethylmalonic acid, and methylsuccinic acid

**METHOD**

Liquid chromatography tandem mass spectrometry

**INDICATION TO ORDER**

Elevated butyryl/isobutyrylcarnitine (C4), elevated glutaryl carnitine (C5DC) by primary screening

**DISORDERS**

Glutaric acidemia type I, Glutaric acidemia type II, Short-chain acyl-CoA dehydrogenase (SCAD) deficiency, Isobutyryl-CoA dehydrogenase deficiency, Ethylmalonic encephalopathy

**CLINICAL UTILITY**

This 2TT can aid in the differential diagnosis between glutaric acidemia type I and type II, and prevent the reporting of heterozygotes and patients with benign polymorphisms of the SCAD gene. C4 and C5DC are a frequent cause of false positive results that are preventable by a normal result of this test. Mayo experience (2004-2013): positive predictive value (PPV) for C4 and C5DC was 48% and 53%, respectively.

**REFERENCES**


**MAYO ID: KD2T**

**ANALYTES MEASURED**

Psychosine and 30 kb deletion of GALC gene

**METHOD**

Liquid chromatography tandem mass spectrometry and polymerase chain reaction with gel electrophoresis

**INDICATION TO ORDER**

Reduced galactocerebrosidase activity by primary screening

**DISORDER**

Krabbe disease

**DESCRIPTION**

As a 2TT, psychosine is a useful biomarker for the detection of infantile Krabbe disease. In addition, the common 30-kb deletion which spans intron 10 through the end of the gene accounts for a significant proportion of alleles which contribute to infantile Krabbe disease.

**REFERENCES**


**MAYO ID: LPCBS**

**ANALYTES MEASURED**

C20-C26 lysophosphatidylcholine species

**METHOD**

Liquid chromatography tandem mass spectrometry

**INDICATION TO ORDER**

Abnormal C26:0 lysophosphatidylcholine levels by primary screening

**DISORDER**

X-linked adrenoleukodystrophy

**DESCRIPTION**

Elevations of C24 lysophosphatidylcholine (LPC) and C26 LPC may be indicative of X-ALD.

**REFERENCES**


**MAYO ID: MPSBS**

**ANALYTES MEASURED**

Dermatan sulfate (DS) and heparan sulfate (HS)

**METHOD**

Liquid chromatography tandem mass spectrometry

**INDICATION TO ORDER**

Reduced alpha-L-iduronidase activity or reduced iduronate 2-sulfatase activity by primary screening

**DISORDERS**

Mucopolysaccharidosis type I, Mucopolysaccharidosis type II

**DESCRIPTION**

Elevated concentrations of DS and HS are suggestive of a biochemical diagnosis of Mucopolysaccharidosis type I or type II.

**REFERENCES**

**MAYO ID: PD2T**

**ANALYTES MEASURED**
Six lysosomal enzyme activities, four lysophosphatidylcholines, creatine and creatinine

**METHOD**
Liquid chromatography tandem mass spectrometry

**INDICATION TO ORDER**
Reduced acid-alpha-glucosidase activity by primary screening

**DISORDER**
Pompe disease types A/B

**DESCRIPTION**
Individuals with Pompe disease types A and B typically have elevation of the oxysterol lysophosphomonyelin (LSPM); cholestane-3 beta, 5 alpha, 6 beta-triol (COT), and/or 7-ketocholesterol (7-KC) may also be elevated.

**REFERENCES**

**MAYO ID: SUAC**

**ANALYTES MEASURED**
Succinylacetone

**METHOD**
Liquid chromatography tandem mass spectrometry

**INDICATION TO ORDER**
Elevated tyrosine by primary screening

**DISORDER**
Tyrosinemia type I

**CLINICAL UTILITY**
To prevent reporting of false positive results, especially in premature cases.

**REFERENCES**

**MAYO ID: OXYBS**

**ANALYTES MEASURED**
Cholestane (3-beta, 5-alpha, 6-beta-triol), Lyso-sphingomyelin

**METHOD**
Liquid chromatography tandem mass spectrometry

**INDICATION TO ORDER**
Reduced sphingomyelinase activity by primary screening

**DISORDERS**
Niemann-Pick disease types A/B

**DESCRIPTION**
Individuals with Niemann-Pick disease types A and B typically have elevation of the oxysterol lyso-sphingomyelin (LSPM); cholestane-3 beta, 5 alpha, 6 beta-triol (COT), and/or 7-ketocholesterol (7-KC) may also be elevated.

**REFERENCES**
Mayo Clinic Experience - Manuscript in preparation