MAYO CLINIC
Mayo Medical Laboratories

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.

MAYO ID: ALLOI	
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

Oglesbee D, Sanders KA, Lacey JM, et al. 2nd-tier test for quantification of alloisoleucine and branched-chain amino acids in dried blood spots to improve newborn screening for maple syrup urine disease (MSUD). Clin Chem 2008;54:542-549.

Puckett RL, Lorey F, Rinaldo P, et al. (2010) Maple Syrup Urine Disease: Further evidence that newborn screening may fail to identify variant forms. Mol Genet Metab 100:136-142.

Novarino G1, El-Fishawy P, Kayserili H, et al. Mutations in BCKD-kinase lead to a potentially treatable form of autism with epilepsy. Science 2012;338(6105):394-397.

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

Lacey JM, Minutti CZ, Magera MJ, et al. (2004) Improved specificity of newborn screening for congenital adrenal hyperplasia by second tier steroid profiling using tandem mass spectrometry. Clin Chem 50:621-625.

Minutti CZ, Lacey JM, Magera MJ, et al. (2004) Steroid profiling by tandem mass spectrometry improves the positive predictive value of newborn screening for congenital adrenal hyperplasia. J Clin Endocrinol & Metab 89:3687-3693.

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

Murugeasan V, Chuan WL, Liu J, et al: Glucosylsphingosine is a key biomarker of Gaucher disease. Am J Hematol 2016; 91(11)1082-1089.

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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, Remethylation 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

Matern D, Tortorelli S, Oglesbee D, et al. Reduction of the false positive rate in newborn screening by implementation of MS/MS-based second tier tests: The Mayo Clinic experience (2004-2007). J Inherit Metab Dis 2007:30:585-592.

Tortorelli S, Turgeon CT, McHugh DMS, et al. Two-tier approach to the newborn screening of methylenetetrahydrofolate reductase deficiency and other re-methylation disorders by tandem mass spectrometry. J Pediatr 2010;157:271-275.

Turgeon CT, Magera MJ, Cuthbert CD, et al. Simultaneous determination of total homocysteine, methylmalonic acid, and 2-methylcitric acid in dried blood spots by tandem mass spectrometry. Clin Chem 2010;56:1686-1695.

Huemer M, Kožich V, Rinaldo P, et al. Newborn screening for homocystinurias and methylation disorders: systematic review and proposed guidelines. J Inher Metab Dis 2015;38:1007-1019.

Wong D, Tortorelli S, Bishop L, et al. Outcomes of four patients with homocysteine remethylation disorders detected by newborn screening. Genet Med 2016;18:162-167.

MAYO ID: HGEM	
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 glutarylcarnitine (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 Clinic Experience - Manuscript in preparation

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

Turgeon CT, Orsini JJ, Sanders KA, et al. Measurement of psychosine in dried blood spots: A possible improvement to newborn screening programs for Krabbe disease. J Inher Metab Dis 2015;38:923-929

Carter RL, Wrabetz L, Jalal K, et al. Can psychosine and galactocerebrosidase activity predict early-infantile Krabbe's disease presymptomatically? J Neurosci Res. 2016;94:1084-1093.

Escolar ML, Kiely BT, Shawgo E, et al. Psychosine, a marker of Krabbe phenotype and treatment effect. Mol Genet Metab. 2017;121:271-278.

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

Turgeon CT, Moser AB, Mørkrid L, et al. (2015) Streamlined determination of lysophosphatidylcholines in dried blood spots for newborn screening of X-linked adrenoleukodystrophy. Mol Genet Metab 114:46-50.

Tortorelli S, Turgeon CT, Gavrilov DK, et al. (2016) Simultaneous testing for six lysosomal storage disorders and X-adrenoleukodystrophy in dried blood spots by tandem mass spectrometry. Clin Chem 62:1248-1254.

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

Minter Baerg MM, Stoway SD, Hart J, et al. Precision newborn screening for lysosomal disorders. (submitted)

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 (LSM); cholestane-3 beta, 5 alpha, 6 beta-triol (COT), and/or 7-ketocholesterol (7-KC) may also be elevated.

REFERENCES

Griffiths W, Wang Y, Alvelius G, Liu S, Bodin K, Sjovall. Analysis of oxysterols by electrospray tandem mass spectrometry. J Am Soc Mass Spectrom 2006, 17, 341-362.

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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
DESCRIPTION	The 2TT is based upon a ratio calculated between the creatine (Cre)/creatinine (Crn) ratio as numerator and the activity of acid alpha glucosidase (GAA) as denominator. Using Collaborative Laboratory Integrated Reports (CLIR; https://clir.mayo.edu), this new marker is incorporated in a dual scatter plot that can achieve segregation between Pompe disease and false positive cases all verified by genotyping.

REFERENCES

Tortorelli S, Turgeon CT, Gavrilov DK, et al. Simultaneous testing for six lysosomal storage disorders and X-adrenoleukodystrophy in dried blood spots by tandem mass spectrometry. Clin Chem 2016;62:1248-1254.

Minter Baerg MM, Stoway SD, Hart J, et al. Precision newborn screening for lysosomal disorders. (submitted)

Tortorelli S, Eckerman JS, Orsini JJ, et al. Moonlighting newborn screening markers: The incidental discovery of a second tier test for Pompe disease (submitted)

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

Magera MJ, Gunawardena ND, Hahn SH, Tortorelli S, Mitchell GA, Goodman SI, Rinaldo P, Matern D. (2006) Rapid quantitative determination of succinylacetone in dried blood spots by liquid chromatography tandem mass spectrometry. Mol Genet Metab 88:16-21.

Turgeon C, Magera MJ, Allard P, Tortorelli S, Gavrilov D, Oglesbee D, Raymond K, Rinaldo P, Matern D. (2008) Combined newborn screening for succinylacetone, amino acids, and acylcarnitines in dried blood spots. Clin Chem 54:657-664. (editorial p. 627).