Overview

Useful For

Presymptomatic identification of disorders to allow for early initiation of treatment and consequent improvement in the long-term prognosis of affected patients

The conditions identifiable by amino acid and acylcarnitine analysis are detected by supplemental newborn screening using tandem mass spectrometry (MS/MS) as described here.

Analyte (assay platform)	**ACMG recommended conditions**		**Additional conditions/treatment detectable by MS/MS**
	Core condition	Secondary targets	
Amino acids (MS/MS)			
Phe	PKU	BS HPA REG	TPN
Leu/Ile, Val	MSUD		TPN
Met	HCY	Met	TPN, nonspecific liver disease
Cit, Arg, ASA	ASA CIT	ARG CIT-II	
Tyr	TYR-I	TYR-II TYR-III	Nonspecific liver disease
GUAC	GAMT		
Acylcarnitines (MS/MS)			
C0	CUD		Maternal CUD, maternal GA-I, maternal MCAD
C3	CblA, Cbl B MUT PA	Cbl C, Cbl D	
C4		IBDH SCAD	FIGLU
C5	IVA	SBCAD	Antibiotics containing pivalic acid
C5-OH	BKT HMG MCC MCD	MGA-I MHBD	Maternal MCC, biotinidase deficiency
C8	MCAD	GA-II MCKAT M/SCHAD	

C3-DC		MAL	
C10:2		DR	
C5-DC	GA-I		
C14:1, C16, C18:1	VLCAD	CACT CPT-I CPT-II	
C16-OH	LCHAD TFP		
m/z 225<399<473			Dextrose infusion
m/z 342 (C8:1)			Artifact often observed in premature neonates
m/z 470 (C16:1OH)			Cefotaxime metabolite
Succinylacetone	TYR-I		

This test is **not appropriate for** metabolic screening of symptomatic patients.

Genetics Test Information

This screening test includes all disorders recommended by the American College of Medical Genetics detectable by tandem mass spectrometry.(1)

Testing Algorithm

For more information see:

-Newborn Screen Follow-up for Elevated C5-DC
-Newborn Screen Follow-up for Isolated Elevation of C3-DC
-Newborn Screen Follow-up for Elevated C5-OH
-Newborn Screen Follow-up for Isolated Elevation of C3
-Newborn Screen Follow-up for Elevated C14:1 +/- Other Long-Chain Acylcarnitine
-Newborn Screen Follow-up for Decreased Free Carnitine (C0)
-Newborn Screen Follow-up for Elevated C16 +/- C18:1 Acylcarnitines
-Newborn Screen Follow-up for Elevated C8 Acylcarnitine with Lesser Elevations of C6 and C10 Acylcarnitines
-Newborn Screen Follow-up for Elevated C0; Elevated C0/(C16+C18)
-Newborn Screen Follow-up for Elevated C16-OH +/- C18-OH
-Newborn Screen Follow-up for Elevated C4-OH Acylcarnitine
-Newborn Screen Follow-up for Elevated C4 and C5 Acylcarnitine +/-Other Elevated Acylcarnitines

Special Instructions

- Request for Original Newborn Screening Card
- Blood Spot Collection Card-Spanish Instructions
- Blood Spot Collection Card-Chinese Instructions
- Blood Spot Collection Instructions
- Newborn Screen Follow-up for Isolated Elevation of C3-DC
- Newborn Screen Follow-up for Elevated C5-DC
- Newborn Screen Follow-up for Elevated C5-OH
- Newborn Screen Follow-up for Isolated Elevation of C3
- Newborn Screen Follow-up for Decreased Free Carnitine (C0)
- Newborn Screen Follow-up for Elevated C14:1 +/- Other Long-Chain Acylcarnitine

- Newborn Screen Follow-up for Elevated C16 +/- C18:1 Acylcarnitines
- Newborn Screen Follow-up for Elevated C8 Acylcarnitine with Lesser Elevations of C6 and C10 Acylcarnitines
- Newborn Screen Follow-up for Elevated C0; Elevated C0/(C16+C18)
- Newborn Screen Follow-up for Elevated C16-OH +/- C18-OH
- Newborn Screen Follow-up for Elevated C4 and C5 Acylcarnitine +/-Other Elevated Acylcarnitines
- Newborn Screen Follow-up for Elevated C4-OH Acylcarnitine

Method Name
Flow Injection Analysis Tandem Mass Spectrometry (FIA-MS/MS)

NY State Available
Yes

Specimen

Specimen Type
Whole blood

Additional Testing Requirements
If an infant is tested before 24 hours of life, **repeat testing on another specimen collected within 1 week of birth is required**.

Specimen Required
Patient must be older than 24 hours and less than 1 week of age.

Supplies: Card-Blood Spot Collection Filter Paper (T493)
Preferred: Blood Spot Collection Card
Acceptable: Whatman Protein Saver 903 Paper, Munktell, PerkinElmer 226 filter paper, or local newborn screening card
Specimen Volume: 3 Blood spots
Collection Instructions:
1. Do not use device or capillary tube containing EDTA to collect specimen.
2. Completely fill at least 3 circles on the filter paper card (approximately 100 microliters blood per circle).
3. Let blood dry on the Blood Spot Collection Card at ambient temperature in a horizontal position for 3 hours.
4. Do not expose specimen to heat or direct sunlight.
5. Do not stack wet specimens.
6. Keep specimen dry.

Additional Information:
1. For collection instructions, see Blood Spot Collection Instructions.
2. For collection instructions in Spanish, see Blood Spot Collection Card-Spanish Instructions (T777).
3. For collection instructions in Chinese, see Blood Spot Collection Card-Chinese Instructions (T800).

Forms
If not ordering electronically, complete, print, and send a Biochemical Genetics Test Request (T798) with the specimen.

Specimen Minimum Volume

1 Blood spot

Reject Due To

Blood spot specimen that shows serum rings or has multiple layers	Reject
Insufficient specimen	Reject
Unapproved filter papers	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole blood	Ambient (preferred)		FILTER PAPER
	Refrigerated		FILTER PAPER
	Frozen		FILTER PAPER

Clinical & Interpretive

Clinical Information

Newborn screening as a public health measure was initiated in the early 1960s to identify infants affected with phenylketonuria (PKU). Since then, additional genetic and nongenetic conditions have been included in state screening programs. The goal of newborn screening is to detect diagnostic markers of the selected disorders in blood spots collected from presymptomatic newborns. Inherited disorders of amino acid, fatty acid, and organic acid metabolism typically manifest during the first 2 years of life as acute metabolic crises and usually result in severe neurologic impairment or death. These metabolic decompensations are typically triggered by intermittent febrile illness, such as common viral infections leading to prolonged fasting and increased energy demands. Early identification of affected newborns allows for early initiation of treatment to avoid mortality, morbidity, and disabilities due to these disorders.

Tandem mass spectrometry (MS/MS) is a powerful multianalyte screening method ideally suited for population-wide testing. Since the early 1990s, MS/MS has made screening possible for more than 30 genetic disorders affecting the metabolism of amino acids, fatty acids, and organic acids based on the profiling of amino acids and acylcarnitines in blood spots. The simultaneous MS/MS analysis of amino acids, acylcarnitines, and succinylacetone in dried blood spots can be performed in less than 3 minutes per specimen, generating metabolite profiles that allow for the biochemical diagnosis of multiple disorders. This is in contrast to conventional screening techniques traditionally based on the principle of one separate test for each disorder. In Mayo Clinic's experience, the combined incidence of the disorders identifiable by MS/MS in a single blood spot analysis is approximately 1 in 1700 newborns.

Supplemental newborn screening by MS/MS as described here does not replace current state screening programs

because MS/MS does not provide primary screening for galactosemia, congenital hypothyroidism, congenital adrenal hyperplasia, cystic fibrosis, biotinidase deficiency, sickle cell disease, X-linked adrenoleukodystrophy, lysosomal disorders, severe combined immune deficiency, spinal muscular atrophy, critical congenital heart disease, or congenital hearing loss.

The Health and Human Services Secretary's Advisory Committee on Heritable Disorders in Newborns and Children recommends all programs screen for 36 core disorders.
These conditions are considered to fulfill 3 basic principles:
-Condition is identifiable at a period of time (24-48 hours after birth) at which it would not ordinarily be clinically detected.
-Test with appropriate sensitivity and specificity is available.
-Demonstrated benefits of early detection, timely intervention, and efficacious treatment.
*This test does not screen for critical congenital heart disease and congenital hearing loss, both of which are tested in the nursery using methods other than blood spots (audiometry, pulse oximetry).

Screening tests do not conclusively determine disease status but measure analytes that, in most cases, are not specific for a particular disease. This is the reason why the Health and Human Services Secretary also recognizes more than 25 additional conditions as secondary targets that do not meet all inclusion criteria but are identified nevertheless because most are components of the differential diagnosis of screening results observed in core conditions. Even for the secondary conditions, the possibility of making a diagnosis early in life not only helps avoid unnecessary diagnostic testing but is also beneficial to patients' families, as genetic counseling and prenatal diagnosis can be offered.

Reference Values
An interpretive report will be provided.

Interpretation
The quantitative measurements of the various amino acids, acylcarnitines, and succinylacetone support the interpretation of the complete profile but, for the most part, are not diagnostic by themselves. The interpretation is by pattern recognition. Abnormal results are not sufficient to conclusively establish a diagnosis of a particular disease. To verify a preliminary diagnosis, independent biochemical (ie, in vitro enzyme assay) or molecular genetic analyses are required, many of which are offered by Mayo Clinic Laboratories.

The reports are in text form only; values for the more than 60 analytes and analyte ratios are not provided. A report for a normal screening result is reported as: "In this blood spot sample, the amino acid and acylcarnitine profiles by tandem mass spectrometry showed no biochemical evidence indicative of an underlying metabolic disorder."

A report for an abnormal screening result includes a quantitative result of the abnormal metabolites, a detailed interpretation of the results, including an overview of the results significance, possible differential diagnoses, recommendations for additional biochemical testing and confirmatory studies (enzyme assay, molecular analysis), and a phone number for a contact at Mayo Clinic if the referring physician has additional questions.

Cautions
Testing is only appropriate for patients less than one week of age as part of prospective newborn screening.

This test is supplemental and not intended to replace state mandated newborn screening.

In a few instances, falsely abnormal results may occur in the analysis of amino acid and acylcarnitine profiles. To keep the number of false-positive and false-negative results to a minimum, results are interpreted based on the metabolite profiles, the information provided on the newborn screening card, and second-tier tests for several nonspecific analytes. In 2013, testing of 71,207 newborns lead to the referral of 55 cases, 38 of them were later confirmed as true-positive results. These data correspond to a false-positive rate of 0.024% and a positive predictive value of 69%.

Newborns discharged before 24 hours of life will need to be retested during the first week of life, eg, at the first well-child examination, as is customary for state-mandated newborn screening programs. This is necessary to avoid false-negative amino acid results due to limited protein intake on the first day of life.

Carrier status (heterozygosity) for inborn errors of metabolism cannot be reliably detected by amino acid and acylcarnitine profiling.

Supportive Data

The performance of Mayo Clinic's supplemental newborn screening program is characterized by a very low false-positive rate of 0.024% and a high-positive predictive value of 69%. The positive detection rate is one affected case in 1735 babies screened (n=742,449).

Clinical Reference

1. Watson MS, Mann MY, Lloyd-Puryear MA, Rinaldo P, Howell RR. Newborn screening: toward a uniform screening panel and system. Genet Med. 2006;8 Suppl 1(Suppl 1):1S-252S
2. Rinaldo P, Zafari S, Tortorelli S, Matern D. Making the case for objective performing metrics in newborn screening by tandem mass spectrometry. Ment Retard Dev Disabil Res Rev. 2006;1294):255-261
3. Matern D, Tortorelli S, Oglesbee D, Gavrilov D, Rinaldo P. 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(4):585-592
4. McHugh DMS, Cameron CA, Abdenur JE, et al. Clinical validation of cutoff target ranges in newborn screening of metabolic disorders by tandem mass spectrometry: a worldwide collaborative project. Genet Med. 2011;13(3):230-254
5. Marquardt G, Currier R, McHugh DMS, et al. Enhanced interpretation of newborn screening results without analyte cutoff values. Genet Med. 2012;14(7):648-655
6. Hall PL, Marquardt G, McHugh DMS, et al. Post-analytical tools improve performance of newborn screening by tandem mass spectrometry. Genet Med. 2014;16(12):889-895

Performance

Method Description

In the United States, every newborn undergoes state-mandated screening on the second day of life or before leaving the hospital. Blood from a heel prick is dripped onto a filter paper card. The blood is left to dry before sending the filter paper card along with pertinent demographic information to the screening laboratory.

Blood for the supplemental newborn screening is collected in the same way and then sent to the Biochemical Genetics Laboratory, after obtaining parental consent. A 1/8-inch (3-mm) disk is punched out of the blood spot onto 96-well plate. Then, the amino acids and acylcarnitines are extracted by the addition of methanol and known concentrations of isotopically labeled amino acids and acylcarnitines as internal standards. The extract is moved to another 96-well plate,

dried under a stream of nitrogen, and derivatized by the addition of *n*-butanol hydrochloric acid. In a parallel process, succinylacetone is extracted from the residual blood spot, derivatized with an acidic hydrazine solution, evaporated and combined with the amino acid and acylcarnitine extract amino acids and acylcarnitines are measured as their butyl esters with the hydrazone derivative of succinylacetone by electrospray tandem mass spectrometry. The concentrations of the analytes are established by computerized comparison of ion intensities of these analytes to that of the respective internal standards.(Turgeon C, Magera MJ, Allard P, et al. Combined newborns screening for succinylacetone, amino acids, and acylcarnitines in dried blood spots. Clin Chem. 2008;54[4]:657-664; Gavrilov DK, Piazza AL, Pino G, et al. The combined impact of CLIR post-analytical tools and second tier testing on the performance of newborn screening for disorders of propionate, methionine, and cobalamin metabolism. Int J Neonatal Screen. 2020;6[2]:33)

PDF Report
No

Day(s) Performed
Monday through Saturday

Report Available
2 to 3 days

Specimen Retention Time
2 years

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Main Campus

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
83789

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
SNS	Supplemental Newborn Screen, BS	54089-8

Result ID	Test Result Name	Result LOINC® Value

82594	Supplemental Newborn Screen Result	54089-8
23727	Reviewed By	18771-6