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.

<table>
<thead>
<tr>
<th>Analyte (assay platform)</th>
<th>ACMG Recommended Conditions</th>
<th>Additional Conditions/Treatment Detectable by MS/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amino Acids (MS/MS)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phe</td>
<td>PKU</td>
<td>TPN</td>
</tr>
<tr>
<td></td>
<td>BS</td>
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<tr>
<td></td>
<td>HPA</td>
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<tr>
<td></td>
<td>REG</td>
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<tr>
<td>Leu/Ile, Val</td>
<td>MSUD</td>
<td>TPN</td>
</tr>
<tr>
<td>Met</td>
<td>HCY</td>
<td>Met</td>
</tr>
<tr>
<td></td>
<td>TPN, nonspecific liver disease</td>
<td></td>
</tr>
<tr>
<td>Cit, Arg, ASA</td>
<td>ASA</td>
<td>ARG</td>
</tr>
<tr>
<td></td>
<td>CIT</td>
<td>CIT-II</td>
</tr>
<tr>
<td>Tyr</td>
<td>TYR-I</td>
<td>Nonspecific liver disease</td>
</tr>
<tr>
<td></td>
<td>TYR-II</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TYR-III</td>
<td></td>
</tr>
<tr>
<td>GUAC</td>
<td>GERM</td>
<td>GAMT</td>
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<tr>
<td><strong>Acylcarnitines (MS/MS)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C0</td>
<td>CUD</td>
<td>Maternal CUD, maternal GA-I, maternal MCAD</td>
</tr>
<tr>
<td>Test</td>
<td>C3</td>
<td>C4</td>
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<tr>
<td></td>
<td>CblA, Cbl B</td>
<td>IBDH</td>
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<tr>
<td></td>
<td>MUT</td>
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</table>
Test Definition: SNS
Supplemental Newborn Screen, BS

<table>
<thead>
<tr>
<th>m/z 470 (C16:1OH)</th>
<th>Cefotaxime metabolite</th>
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<tbody>
<tr>
<td>Succinylacetone</td>
<td>TYR-I</td>
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</table>

**Genetics Test Information**
Panel includes all disorders recommended by the American College of Medical Genetics detectable by Tandem Mass Spectrometry (MS/MS)(1)

**Testing Algorithm**
See Informative Markers for Supplemental Newborn Screening at Mayo Clinic in Special Instructions.

The following algorithms are available in Special Instructions:

- Newborn Screening Follow-up for Elevations of C8, C6, and C10 Acylcarnitines (also applies to any plasma C8, C6, and C10 acylcarnitine elevations)
- Newborn Screening Follow-up for Isolated C4 Acylcarnitine Elevations (also applies to any plasma C4 acylcarnitine elevation)
- Newborn Screening Follow-up for Isolated C5 Acylcarnitine Elevations (also applies to any plasma C5 acylcarnitine elevation)

**Special Instructions**
- Request for Original Newborn Screening Card
- Newborn Screening Follow-up for Isolated C4 Acylcarnitine Elevations (also applies to any plasma or serum C4 acylcarnitine elevations)
- Newborn Screening Follow-up for Elevations of C8, C6, and C10 Acylcarnitine Elevations (also applies to any plasma or serum C8, C6, and C10 acylcarnitine elevations)
- Newborn Screening Follow-up for Isolated C5 Acylcarnitines Elevations (also applies to any plasma or serum C5 acylcarnitine elevations)
- Informative Markers for Supplemental Newborn Screening at Mayo Clinic
- Blood Spot Collection Card-Spanish Instructions
- Blood Spot Collection Card-Chinese Instructions

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

**NY State Available**
Yes

**Specimen**

**Specimen Type**
Whole blood

**Additional Testing Requirements**
A repeat specimen is required within 1 week of birth for infants tested before they are 12 hours old.

**Specimen Required**
Patient must be older than 12 hours and less than 1 week of age.
**Test Definition: SNS**  
Supplemental Newborn Screen, BS

**Supplies:** Card-Blood Spot Collection Filter Paper (T493)

**Preferred:** Blood Spot Collection Card (T493)

**Acceptable:** Whatman Protein Saver 903 Paper, Ahlstrom 226 filter paper

**Specimen Volume:** 3 Blood spots

**Collection Instructions:**

1. Do not use device or capillary tube containing EDTA to collect specimen.

2. Do not expose specimen to heat or direct sunlight.

3. Do not stack wet specimens.

4. Keep specimen dry.

5. If collection of a new specimen is necessary, let blood dry on the Blood Spot Collection Card (T493) at ambient temperature in a horizontal position for 3 hours.

**Additional Information:**

1. For collection instructions in Spanish, see Blood Spot Collection Card-Spanish Instructions (T777) in Special Instructions.

2. For collection instructions in Chinese, see Blood Spot Collection Card-Chinese Instructions (T800) in Special Instructions.

**Forms**

If not ordering electronically, complete, print, and send an Inborn Errors of Metabolism Test Request (T798) with the specimen.

**Specimen Minimum Volume**

Blood Spots: 1

**Reject Due To**

<table>
<thead>
<tr>
<th></th>
<th>NA</th>
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<tbody>
<tr>
<td>Hemolysis</td>
<td>NA</td>
</tr>
<tr>
<td>Lipemia</td>
<td>NA</td>
</tr>
<tr>
<td>Icterus</td>
<td>NA</td>
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<tr>
<td>Other</td>
<td>Blood spot specimen that shows serum rings or has multiple layers</td>
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</tbody>
</table>

**Specimen Stability Information**

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
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<tbody>
<tr>
<td>Whole blood</td>
<td>Ambient (preferred)</td>
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</tr>
<tr>
<td></td>
<td>Frozen</td>
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Clinical and Interpretive

Clinical Information

Newborn screening as a public health measure was initiated in the early 1960s for the identification of infants affected with phenylketonuria (PKU). Since then, additional genetic and nongenetic conditions were 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 usually 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, which is 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 1 separate test for each disorder. In Mayo's experience, the combined incidence of the disorders identifiable by MS/MS in a single blood spot analysis is approximately 1 in 1,700 newborns.

Supplemental newborn screening by MS/MS as described here does not replace current state screening programs, because MS/MS does not allow primary screening for galactosemia, congenital hypothyroidism, congenital adrenal hyperplasia (CAH), cystic fibrosis, biotinidase, sickle cell disease, Mucopolysaccharidosis type II, Adrenoleukodystrophy, Pompe disease, severe combined immune deficiency (SCID), critical congenital heart disease, and congenital hearing loss.

The Secretary's Advisory Committee on Heritable Disorders in Newborns and Children (SACHDNC) recommends all programs screen for 34 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 which in most cases are not specific for a particular disease. This is the reason why the HHS Secretary also recognizes more than 25 additional
conditions as secondary targets that do not meet all inclusion criteria but are identified nevertheless because most of them 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 the patient's families because genetic counseling and prenatal diagnosis can be offered.

Although not currently in the recommended uniform screening panel, guanidinoacetate methyltransferase (GAMT), a disorder of creatine synthesis, is a condition included in the Mayo Clinic Laboratories' supplemental newborn screen. When untreated, this disorder results in a depletion of cerebral creatine leading to global developmental delays, intellectual disability, severe speech delays, and seizures. Patients with GAMT exhibit behavioral problems and features of autism. Treatment consists of lifelong supplementation with creatine monohydrate, ornithine, and dietary protein restriction to decrease cerebral GAA levels. Individuals with GAMT who are treated before the appearance of symptoms may exhibit normal neurodevelopmental outcomes.

**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 within Mayo Clinic’s Division of Laboratory Genetics.

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 1 week of age as part of prospective newborn screening.

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

Test is not appropriate for metabolic screening of symptomatic patients.

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 positives. These data correspond to a false positive rate of 0.024% and a positive predictive value of 69%.

Newborns discharged before 12 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
Supportive Data

The performance of Mayo'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 1 affected case in 1,735 babies screened (n=742,449).

Clinical Reference


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 (MS/MS). 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 newborn screening for succinylacetone, amino acids, and acylcarnitines in dried blood spots. Clin Chem 2008;54:657-664)
Test Definition: SNS
Supplemental Newborn Screen, BS

No

Day(s) and Time(s) Test Performed
Monday through Saturday; 9 a.m.

Analytic Time
2 days

Maximum Laboratory Time
3 days

Specimen Retention Time
1 year

Performing Laboratory Location
Rochester

Fees and 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 Regional Manager. 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. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information
83789

LOINC® Information

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Test Order Name</th>
<th>Order LOINC Value</th>
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<tbody>
<tr>
<td>SNS</td>
<td>Supplemental Newborn Screen, BS</td>
<td>54089-8</td>
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<table>
<thead>
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<td>82594</td>
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