Oregon Newborn Bloodspot Screening Practitioner’s Manual

Northwest Regional Newborn Screening Program

PUBLIC HEALTH DIVISION
Oregon State Public Health Laboratory
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Acknowledgment

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Recommended citation

Welcome! The purpose of this manual is to provide useful information to health care providers about the Oregon Newborn Bloodspot Screening (NBS) Program. The Oregon NBS Program is part of the Oregon State Public Health Laboratory (OSPHL). Specimens are received and tested by the OSPHL and abnormal results are referred to the NBS Follow-up Team.

This manual describes the process of newborn bloodspot screening from collection through reporting and newborn screening follow-up. It outlines the roles and responsibilities of the NBS Program, medical practitioners, and parents. It also discusses newborn screening practice standards, common problems that can occur during screening, and links to helpful resources. We invite practitioners to contact us with any questions or concerns, or with suggestions on improving this manual. Contact information and additional resources are available at www.healthoregon.org/nbs.

NBS programs attempt to identify infants affected by specific medical conditions in time to prevent impairment. Infants with these conditions often appear normal at birth. Only with time does the medical condition affect the infant’s health and development. Although each screening condition is rare, when combined, approximately one in 250 infants is affected.

The chance that a screening condition will impact any single infant is remote. However, the cost of not detecting an affected infant is immense, both in human suffering and financial terms. Some of the reasons that newborn screening is so important are:

- Approximately 20 disorders can kill or severely harm an infant in the first two weeks of life.
- Approximately 20% of infants with a screening condition will be symptomatic within one week of birth.
- Approximately 10% of infants with a screening condition could die within one week of birth, if untreated.
- Affected infants may lose significant IQ points, leading to lifelong impairment, if some screening conditions are not treated within 2 weeks of birth.

The goal of NBS is to detect treatable medical conditions within the first two weeks of life.
Newborn screening is changing rapidly and will continue to change in the future. While states are trying to develop standard newborn screening recommendations, variation continues from state to state and practitioners must be aware of the newborn screening practice that applies to their patients. Practitioners who are licensed in Oregon or treat Oregon residents must orient to the newborn screening rules and regulations that apply.

Oregon began newborn screening for PKU in 1963. Since then, newborn bloodspot screening has expanded to include other metabolic conditions, cystic fibrosis, sickle cell disease, severe combined immunodeficiency (SCID), and as of 2018, some lysosomal storage disorders. In 2019, the OSPHL screens for 44 medical conditions listed in this manual. An additional 25 secondary conditions may be identified.

Practitioners are integral to newborn bloodspot screening. Most parents agree to screening when properly counseled by their practitioner about the importance of detecting newborn screening conditions early. Early detection can result in the infant’s normal growth and development.

You are responsible for the proper, timely collection and handling of specimens for every infant in your care and prompt action in response to abnormal results. Your decisions and actions in response to an abnormal screening result to ensure rapid evaluation, accurate diagnosis and treatment can have lifelong implications for the infant and the family.

Newborn screening is not intended to diagnose an infant’s medical condition. Newborn screening is only intended to identify infants that should have further medical follow-up. Not all infants affected by these medical conditions will be identified by newborn screening.

Effective communication is essential for newborn bloodspot screening to succeed.
“Abnormal Result” means a result of a laboratory examination that meets the screening criteria for a newborn screening panel condition requiring additional testing and medical follow-up.

“Facility” means:
   a) Hospitals and freestanding birth centers; and
   b) Health care clinics and offices where practitioners and other health care professionals provide direct medical care to newborns or infants six months or younger.

“Freestanding birthing center” has the meaning given that term in ORS 442.015.

“Hospital” has the meaning given that term in ORS 442.015.

“Kit” means: the filter paper collection device, attached demographic form, and other items provided by the Oregon State Public Health Laboratory for the purposes of collection or submission of specimens for newborn screening testing.

“Practitioner” means: the person who takes responsibility for the delivery or health care of an infant born in Oregon and is one of the following:
   a) A physician licensed under ORS 677;
   b) A naturopathic physician licensed under ORS 685;
   c) A nurse practitioner or advanced practice registered nurse licensed under ORS 678;
   d) A chiropractic physician licensed under ORS chapter 684; or
   e) A direct entry midwife licensed under ORS 687.

“Preterm” means: an infant born prior to the start of the 37th week of pregnancy.

“Specimen” means: a blood specimen obtained from an infant by means of capillary puncture or skin puncture (heel-stick) and spotted onto a newborn screening kit and allowed to air dry.
Newborn screening requires coordinated efforts from:

- **Practitioners:** In addition to being responsible for the medical care of their patients, practitioners are legally responsible for collecting and handling screening specimens and providing prompt follow-up in the event of an abnormal result. They should also provide education for parents regarding newborn screening.

- **Oregon State Public Health Laboratory (OSPHL) and NBS Follow-up Team:** The laboratory is responsible for testing, record keeping, ensuring quality of laboratory methods, notifying providers of results, tracking abnormal and unresolved results, and providing educational materials.

- **Oregon Health & Science University (OHSU) subspecialty programs:** These partners are responsible for providing consultation services to practitioners and the OSPHL.

Oregon statute (ORS 433.285) requires every infant to be tested, and the Oregon Administrative Rule (OAR) 333-024-1020 and 333-024-1025 define who is responsible for specimen collection. The definition of “practitioner” includes physicians, nurses and midwives who deliver or care for infants in hospitals, birth centers or homes. Parents share the responsibility for ensuring their infants are tested.

Per OAR 333-024-1030, practitioners have a responsibility to determine the screening status of every infant under their care. If an infant under six months of age enters a practice and the practitioner is unable to determine whether the infant has been tested, a specimen must be collected and sent to the NBS Follow-up Team within two weeks of the first visit to the practitioner.

Practitioners are responsible for ensuring that newborn bloodspot screening results are received and reviewed. Per OAR 333-024-1080(4), the practitioner must communicate abnormal results to the parent or guardian of the infant and recommend appropriate medical care.
Medical conditions on the newborn bloodspot screening panel

Oregon newborns are screened for the following medical conditions recommended by the College of Medical Genetics and Discretionary Advisory Committee on Heritable Disorders in Newborns and Children. More information on these medical conditions is available at the end of this manual and at:

- Baby’s First Test: [http://babysfirsttest.org/](http://babysfirsttest.org/)
- The Oregon State Public Health Laboratory: [www.healthoregon.org/nbs](http://www.healthoregon.org/nbs)
- The American College of Medical Genetics (ACMG): [https://www.acmg.net](https://www.acmg.net).

### Table 1: Medical conditions on the Oregon Newborn Screening Panel.

<table>
<thead>
<tr>
<th>Medical Condition</th>
<th>Analyte(s) tested for</th>
<th>Incidence in NW region</th>
<th>Symptoms if not treated</th>
<th>Common Medical Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organic Acid Disorders</strong></td>
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<tr>
<td>Propionic acidemia (PA)*</td>
<td>C3, C3/C2</td>
<td>1 per 271,000</td>
<td>Vomiting; lethargy; acidosis possibly resulting in death</td>
<td>Protein-restricted diet; medical formula; carnitine therapy</td>
</tr>
<tr>
<td>Methylmalonic acid (MMA)*</td>
<td>C3, C3/C2</td>
<td>1 per 95,000</td>
<td>Vomiting; lethargy; acidosis possibly resulting in death</td>
<td>Protein-restricted diet; medical formula; carnitine therapy and hydroxocobalamin therapy</td>
</tr>
<tr>
<td>Isovaleric acidemia (IVA)</td>
<td>C5</td>
<td>1 per 148,000</td>
<td>Vomiting; lethargy; acidosis possibly resulting in coma, death</td>
<td>Protein-restricted diet; carnitine and glycine therapy</td>
</tr>
<tr>
<td>3-methylcrotonyl CoA carboxylase deficiency (3MCC)</td>
<td>C5OH</td>
<td>1 per 51,000</td>
<td>Most have been asymptomatic</td>
<td>None, except carnitine therapy if deficient</td>
</tr>
</tbody>
</table>

**Newborn bloodspot screening is not diagnostic. Both false negative and false positive results may occur. Confirmatory testing is required for diagnosis.**
<table>
<thead>
<tr>
<th>Medical Condition</th>
<th>Analyte(s) tested for</th>
<th>Incidence in NW region</th>
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</tr>
</thead>
<tbody>
<tr>
<td>3-hydroxy-3-methylglutaryl CoA lyase deficiency (HMG)</td>
<td>C5OH</td>
<td>Rare, less than 1 per 300,000</td>
<td>Hypoglycemia; acidosis possibly resulting in death; may be asymptomatic</td>
<td>Protein restriction</td>
</tr>
<tr>
<td>Multiple carboxylase deficiency (MCD)</td>
<td>C3, C5OH</td>
<td>Rare, less than 1 per 300,000</td>
<td>Hypotonia; seizures; skin rash; alopecia; lactic acidosis; brain damage</td>
<td>Biotin therapy</td>
</tr>
<tr>
<td>Beta-ketothiolase deficiency (BKT)</td>
<td>C5:1, C5OH</td>
<td>Rare, less than 1 per 1 million</td>
<td>Severe bouts of acidosis possibly resulting in intellectual and developmental disability or death</td>
<td>IV support during episodes; bicarbonate supplement</td>
</tr>
<tr>
<td>Glutaric acidemia, type 1 (GA-1)</td>
<td>C5DC</td>
<td>1 per 85,000</td>
<td>Often asymptomatic in newborn; sudden metabolic crisis damages basal ganglia</td>
<td>IV support during intercurrent illness; protein restriction; carnitine therapy</td>
</tr>
<tr>
<td>Malonic acidemia (MAL)</td>
<td>C3DC</td>
<td>Rare, less than 1 per 300,000</td>
<td>Intellectual disability</td>
<td>Carnitine therapy; MCT oil therapy; long chain fat restriction; avoidance of fasting</td>
</tr>
<tr>
<td>Isobutyrl-CoA dehydrogenase deficiency (IBD)</td>
<td>C4</td>
<td>Rare, less than 1 per 300,000</td>
<td>None to severe cardiomyopathy</td>
<td>Carnitine therapy; protein restriction; avoid fasting</td>
</tr>
<tr>
<td>2-methylbutyryl CoA dehydrogenase deficiency (2MBC)</td>
<td>C5</td>
<td>1 per 181,000 (Hmong have higher incidence)</td>
<td>Hypoglycemia; intellectual and developmental disability; Hmong infants are often asymptomatic</td>
<td>None or avoid fasting</td>
</tr>
<tr>
<td>3-methylglutaconyl CoA hydratase deficiency (3MGH)</td>
<td>C5OH</td>
<td>Rare, less than 1 per 1.3 million</td>
<td>Hypoglycemia; acidosis; may be asymptomatic</td>
<td>Protein restriction; avoid fasting</td>
</tr>
<tr>
<td>2-methyl-3-hydroxybutyryl CoA dehydrogenase deficiency (2M3HBA)</td>
<td>C5:1, C5OH</td>
<td>1 per 541000</td>
<td>Rarely symptomatic; Common among Hmong population</td>
<td>Protein restriction</td>
</tr>
<tr>
<td><strong>Fatty Acid Oxidation Disorders</strong></td>
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<tr>
<td>Carnitine uptake deficiency (CUD)</td>
<td>C0, C16, C18</td>
<td>1 per 116,000</td>
<td>Hypoglycemia; cardiomyopathy</td>
<td>Carnitine therapy</td>
</tr>
<tr>
<td>Medium chain acyl-CoA dehydrogenase deficiency (MCAD)*</td>
<td>C6, C8, C10, C8/C10</td>
<td>1 per 19,000</td>
<td>Hypoglycemia possibly resulting in coma, death; may be asymptomatic</td>
<td>Avoid fasting; carnitine therapy if deficient</td>
</tr>
<tr>
<td>Medical Condition</td>
<td>Analyte(s) tested for</td>
<td>Incidence in NW region</td>
<td>Symptoms if not treated</td>
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<tr>
<td>Very long chain acyl-CoA dehydrogenase deficiency (VLCAD)*</td>
<td>C14, C14:1, C16, C16:1, C18, C18:1</td>
<td>1 per 62,500</td>
<td>Hypoglycemia with or without cardiomyopathy; muscle fatigue</td>
<td>Avoid fasting; low fat diet with MCT oil supplement; carnitine therapy</td>
</tr>
<tr>
<td>Long chain 3 hydroxyacyl-CoA dehydrogenase deficiency (LCHAD)*</td>
<td>C14:1, C16, C16OH, C18, C18OH</td>
<td>1 per 541,000</td>
<td>Hepatic dysfunction; hypoglycemia; failure to thrive</td>
<td>Long chain fatty acid restriction; medium chain triglycerides (MCT) oil supplement; carnitine therapy; avoid fasting</td>
</tr>
<tr>
<td>Trifunctional protein deficiency (TFP)</td>
<td>C14:1, C16, C16OH, C18, C18OH</td>
<td>Very rare. Incidence unknown</td>
<td>Feeding difficulties; lethargy; hypoglycemia; low muscle tone; liver problems</td>
<td>Long chain fatty acid restriction; medium chain triglycerides (MCT) oil supplement; carnitine therapy; avoid fasting</td>
</tr>
<tr>
<td>Short chain acyl-CoA dehydrogenase deficiency (SCAD)</td>
<td>C4</td>
<td>1 per 81,000</td>
<td>Most asymptomatic; hypotonia, intellectual and developmental disability</td>
<td>None</td>
</tr>
<tr>
<td>Glutaric acidemia type II, also known as Multiple acyl-CoA dehydrogenase deficiency (MADD)</td>
<td>C4, C5, C6, C8, C10, C14, C16, C18:1</td>
<td>1 per 541,000</td>
<td>Multiple congenital abnormalities; acidosis; hypoglycemia</td>
<td>Low fat diet; avoid fasting,</td>
</tr>
<tr>
<td>Carnitine palmitoyl transferase deficiency, type I (CPT-I)</td>
<td>C0/C16+C18</td>
<td>1 per 812,000</td>
<td>Hypoketotic hypoglycemia, brought on by fasting or intercurrent illness; Average age at presentation: birth to 18 months</td>
<td>Avoid fasting and long chain fatty acids; MCT oil supplement</td>
</tr>
<tr>
<td>Carnitine palmitoyl transferase deficiency, type II (CPT-II)*</td>
<td>C0, C4, C5, C6, C14, C16, C16:1, C18, C18:1</td>
<td>1 per 400,000</td>
<td>Muscle weakness; pain; myoglobinuria leading to renal failure in 25%. Average age at presentation: 15 to 30 years; severe neonatal form is usually lethal with multiple congenital anomalies</td>
<td>Avoid fasting and severe exercise; MCT oil supplement</td>
</tr>
<tr>
<td>Carnitine acylcarnitine translocase deficiency (CACT)</td>
<td>C16; C18:1</td>
<td>Very rare. Incidence unknown.</td>
<td>Fatigue; irritability; poor appetite; fever; diarrhea; vomiting; hypoglycemia; seizure; hypotonia</td>
<td>Avoid fasting and severe exercise; MCT oil supplement; L-carnitine supplement</td>
</tr>
<tr>
<td>Medical Condition</td>
<td>Analyte(s) tested for</td>
<td>Incidence in NW region</td>
<td>Symptoms if not treated</td>
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<tr>
<td><strong>Amino Acid Disorders</strong></td>
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<tr>
<td>Arginoinosuccinate lyase deficiency</td>
<td>ASA/citrulline</td>
<td>1 per 125,000</td>
<td>Hyperammonemia; intellectual and developmental disability; seizure; death</td>
<td>Protein-restricted diet; medical formula; medication</td>
</tr>
<tr>
<td>(Arginosuccinic aciduria; ASA)*</td>
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<tr>
<td>Citrullinemia, type I (CIT)*</td>
<td>Citrulline</td>
<td>1 per 325,000</td>
<td>Hyperammonemia; intellectual and developmental disability; seizure; death</td>
<td>Protein-restricted diet; medical formula; medication</td>
</tr>
<tr>
<td>Maple syrup urine disorder (MSUD)*</td>
<td>Leucine</td>
<td>1 per 271,000</td>
<td>Vomiting; lethargy; acidosis possibly resulting in death</td>
<td>Protein-restricted diet; and medical formula</td>
</tr>
<tr>
<td>Homocystinuria (HCY)</td>
<td>Methionine</td>
<td>1 per 203,000</td>
<td>Intellectual and developmental disability; dislocation of lenses; marfanoid body habitus; strokes</td>
<td>Pyridoxine; protein-restricted diet; medical formula; Foltanx</td>
</tr>
<tr>
<td>Phenylketonuria (PKU)</td>
<td>Phenylalanine</td>
<td>1 per 28,500</td>
<td>Profound intellectual and developmental disability; seizures</td>
<td>Protein-restricted diet; medical formula; Kuvan if responsive</td>
</tr>
<tr>
<td>Tyrosinemia, type I and type II</td>
<td>Succinylacetone and tyrosine</td>
<td>1 per 812,000</td>
<td>Vomiting; lethargy; liver disease; coagulopathy; renal tubular acidosis</td>
<td>Protein-restricted diet; medical formula; medication</td>
</tr>
<tr>
<td>Tyrosinemia, type II and type III</td>
<td>Succinylacetone and tyrosine</td>
<td>1 per 652,000</td>
<td>Corneal thickening; developmental delay; hyperkeratosis of palms and soles</td>
<td>Protein-restricted diet; medical formula; medication</td>
</tr>
<tr>
<td>Arginase deficiency (ARG)</td>
<td>Arginine</td>
<td>1 per 1.6 million</td>
<td>Irritability; developmental delay; spastic tetraplegia</td>
<td>Protein-restricted diet; medical formula; medication</td>
</tr>
<tr>
<td><strong>Endocrine Disorders</strong></td>
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<tr>
<td>Primary congenital hypothyroidism</td>
<td>Thyroid hormone T4 and Second tier TSH</td>
<td>1 per 2,300</td>
<td>Intellectual and developmental disability; other brain damage; growth delay</td>
<td>Thyroid hormone</td>
</tr>
<tr>
<td>Congenital adrenal hyperplasia (CAH)*</td>
<td>17-OH-progesterone</td>
<td>1 per 12,700</td>
<td>Addisonian crisis/ salt wasting in 3/4 infants; dehydration; shock; hyperkalemia; virilization of females</td>
<td>Glucocorticoid and/or mineralocorticoid therapy</td>
</tr>
<tr>
<td>Medical Condition</td>
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<tr>
<td><strong>Pulmonary Disorders</strong></td>
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<tr>
<td>Cystic fibrosis (CF)</td>
<td>Immunotrypsinogen (IRT) Second tier genotyping</td>
<td>1 per 6,500</td>
<td>Lung disease; growth failure</td>
<td>Pulmonary therapy; prevent infection; replace digestive enzymes</td>
</tr>
<tr>
<td><strong>Other Metabolic Disorders</strong></td>
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</tr>
<tr>
<td>Biotinidase deficiency</td>
<td>Biotinidase</td>
<td>1 per 1.05 million</td>
<td>Intellectual and developmental disability; seizures; skin rash; alopecia; hearing loss</td>
<td>Biotin therapy</td>
</tr>
<tr>
<td>Classic galactosemia (GALT)*</td>
<td>Galactosemia enzyme (GALT)</td>
<td>1 per 95,000</td>
<td>Neurodevelopmental impairment; liver disease; cataracts; Gram-negative sepsis in newborns</td>
<td>Galactose-restricted diet</td>
</tr>
<tr>
<td><strong>Hemoglobin Disorders</strong></td>
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<tr>
<td>Sickle cell disease</td>
<td>Hemoglobin patterns</td>
<td>1 per 6,900 (1 per 2,050 in African Americans)</td>
<td>In sickle cell disease: death by sepsis or splenic sequestration anemia; sickling crisis</td>
<td>Penicillin and comprehensive care</td>
</tr>
<tr>
<td><strong>Immunology Disorders</strong></td>
<td></td>
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<tr>
<td>Severe combined immunodeficiency (SCID)</td>
<td>T-cell receptor excision circles (TRECs)</td>
<td>1 per 50,000 to 1 per 100,000</td>
<td>Severe respiratory infection; poor growth; rashes appear like eczema; chronic diarrhea; recurrent oral thrush</td>
<td>Bone marrow transplant</td>
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<tr>
<td><strong>Lysosomal Storage Disorders</strong>**</td>
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<tr>
<td>Pompe* (glycogen storage disease Type II)</td>
<td>Alpha-glucosidase (GAA)</td>
<td>1 per 28,000</td>
<td>Generalized muscle weakness; respiratory failure; cardiomegaly; enlarged liver; hearing loss</td>
<td>Enzyme replacement therapy</td>
</tr>
<tr>
<td>Mucopolysaccharidosis Type I (MPS I)*</td>
<td>Alpha-L-iduronidase (IDUA)</td>
<td>Between 1 per 87,000 and 1 per 185,000</td>
<td>Skeletal abnormalities; cognitive impairment; heart disease; cloudy corneas; deafness; reduced life expectancy</td>
<td>Bone marrow transplant; enzyme replacement therapy</td>
</tr>
<tr>
<td>Medical Condition</td>
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<tr>
<td>Fabry (alphaglactosidase A deficiency)</td>
<td>Alpha-galactosidase (GLA)</td>
<td>Between 1 per 1500 and 1 per 13,000</td>
<td>Renal failure; Hypertrophic cardiomyopathy; Pain in hands and feet; poor sweating; irritable bowels; proteinuria; hearing loss</td>
<td>Enzyme replacement therapy</td>
</tr>
<tr>
<td>Gaucher* (glucocerebrosidase deficiency)</td>
<td>Beta-glucocerebrosidase (GBA)</td>
<td>1 per 57,000</td>
<td>Enlarged spleen and liver; low platelets; anemia; bone disease; Type III have eye tracking issues as well</td>
<td>Enzyme replacement therapy</td>
</tr>
</tbody>
</table>

* Infants may have severe neonatal presentation.

** Lysosomal Storage Disorders were added to the panel in October 2018. Published incidence rates are provided.

Newborn screening may identify other medical conditions that are not listed above. These other findings are referred to by the RUSP as “secondary conditions”. Secondary conditions or traits that are identified by newborn screening will be reported as described in this manual. It is within the discrimination of the infant's healthcare provider and legal guardian to determine what, if any, medical follow-up is needed for a secondary condition that is identified.
Newborn screening kits must be ordered from the Oregon State Public Health Laboratory (OSPHL). Visit the NBS Kit Order website at www.bitly.com/nbs-kits or call 503-693-4100 and ask for NBS Kit Orders.

Kits may be ordered as double, triple, or single kits depending on the needs of the facility. The kits are considered a medical collection device. They must be stored according to the manufacturer instructions and not tested after the expiration date.

Double Kits

Double kits are used for most births. Each specimen in the kit has a barcode and kit number that allow the “2nd specimen” to be matched easily by the screening lab to the data from the “1st specimen”. This matching system helps to link the data from newborn screening testing services to ensure records for each infant are complete and easily accessible by providers.

Triple Kits

Three-part kits are intended to be used for neonatal intensive care unit (NICU) infants. Each specimen in the kit has a barcode and a kit number that allow the “2nd specimen” and “3rd specimen” to be matched easily by the screening lab to the data from the “1st specimen”. This matching system helps to ensure that newborn screening testing services and records for each infant are complete and easily accessible by providers.

Single Kits

Single kits must be used when the remaining specimen from a double or triple kit has been lost, damaged, or an infant is born out of state. If known, the kit number from the “1st specimen” should be written on the single kit to help with matching the data for the infant. These kits will also be used when the OSPHL requests a repeat specimen.
If you suspect an infant may have a screening condition, based on symptoms or family history, contact the NBS Follow-up Team or NBS medical consultant for information about appropriate diagnostic testing.

Newborn bloodspot screening should be collected as described below:

**Routine births**

For routine births use a newborn screening double kit. The first specimen should be collected as soon as possible after 24 hours of age but before 48 hours of age and a second specimen must be collected between 10 and 14 days of age as shown in table 3.

After the first specimen is collected, the “2nd specimen” in the double kit must be routed to the provider who will collect this specimen by the facility or provider who collected the “first specimen”. Many hospitals choose to send the second part of the kit with the parent to give to the follow up provider.

If the primary care provider does not receive a “2nd specimen” collection card to perform a collection between 10 and 14 days, or the kit may expire before testing can be performed, a single kit should be used to collect a specimen. The kit must be tested at the lab prior to the expiration date on the card.

**Infants admitted to the NICU**

For babies that require admission to a neonatal intensive care unit, collect the first specimen as soon as possible after 24 hours of age but before 36 hours of age unless the infant is being transfused. In this case, collect the specimen prior to transfusion regardless of the age of the infant. If an infant is transfused prior to 24 hours of age the second specimen must be collected at 48-72 hours of age. If the infant is not transfused prior to 24 hours of age the second specimen must be collected between 10 and 14 days of age (11 and 15 days of life). A third specimen must be collected at approximately 1 month, but no sooner than 28 days after birth.
For infants that are discharged or transferred after the first specimen (or second specimen) is collected, the remaining collection cards from the triple kit must be routed to the provider who will collect these specimens by the facility or provider who collected the “first specimen”.

If the remaining collection cards are not received by the primary care provider or the provider who will be collecting the subsequent specimen(s) to perform the second (or third) collection, or if these cards will expire before testing can be completed, a single kit should be used to collect a 10-14 day specimen and a specimen at approximately 1 month, as needed. If a double kit is used for a premature or low birthweight infant, a single kit should be used for the third collection.

**Transfer between medical facilities prior to 24 hours of age**

Whenever possible, if an infant is transferred between medical facilities prior to 24 hours of age, the discharging facility should ensure that a specimen is collected before the infant is transferred. The remaining cards should be sent with the infant to the receiving facility. If additional specimens will be collected on the remaining cards by the receiving facility, the submitter information on the card should be updated to accurately reflect the receiving facility’s location.

If the provider believes specimen collection for newborn bloodspot screening prior to transfer would pose a risk to the welfare of the child, then the reason for the decision to not collect should be documented in the medical record and the transferring hospital should clearly communicate to the receiving facility that the first specimen collection was not performed. The receiving facility should then ensure that a newborn screen is collected and submitted for testing.

**Early discharge**

If a family is requesting an early discharge, collect the “1st specimen” before they leave your care. Some infants may not return for routine postnatal care.

**Baby expires**

In many cases, blood spot specimens from an infant who expired are a valuable resource for the family. If an infant is likely to die, we recommend that you collect a newborn screening specimen.

**Older infants**

The Oregon State Public Health Laboratory has established procedures for testing specimens from newborns and infants up to 6 months of age. The Oregon State Public Health Laboratory cannot perform newborn screening testing for children older than 6 months of age.

If an infant expires, please notify the NBS Follow-up Team by:

- Calling 503-693-4174 or
- Faxing the infant’s information to 503-693-5601
Table 2 — Age of infant at specimen collection

<table>
<thead>
<tr>
<th></th>
<th>Collection Kit</th>
<th>First specimen</th>
<th>Second specimen</th>
<th>Third specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine Birth</td>
<td>Double Kit</td>
<td>As soon as possible after 24 hours of age but before 48 hours of age</td>
<td>10-14 days</td>
<td>Not Collected</td>
</tr>
<tr>
<td>NICU infants transfused prior to 24 hours of age</td>
<td>Triple Kit</td>
<td>Prior to transfusion</td>
<td>48-72 hours after birth</td>
<td>~ 1 month, no sooner than 28 days</td>
</tr>
<tr>
<td>NICU infants not transfused prior to 24 hours of age</td>
<td>Triple Kit</td>
<td>As soon as possible after 24 hours of age but before 36 hours of age and prior to transfusion</td>
<td>10-14 days of age (11-15 days of life)</td>
<td>~ 1 month, no sooner than 28 days</td>
</tr>
</tbody>
</table>
Incomplete demographic information may result in your specimen not being tested.

Be sure to use the correct part of the double or triple kit: “1st Specimen” for the first specimen and “2nd Specimen” for the second specimen, and for NICU infants, “3rd Specimen” for the third specimen. If the specimen collection cards are not used in the correct order, the infant’s results may not link correctly within the laboratory information system. This could delay testing for hemoglobinopathy, cystic fibrosis, and SCID screening, which are routinely only performed on the first specimen.

Accurate patient and provider information must be provided on every collection card to allow for rapid follow-up if results are abnormal. The specimen demographic form must be filled in completely with the requested patient information. This information is required by Clinical Laboratory Improvement Amendments of 1988 (CLIA) and must be legible.

The person performing the collection must:

1. Verify that the collection kit will not expire before all parts of the kit can be tested by the laboratory. If a double kit will expire within 1 month of the collection, please use a different kit. The expiration date is on the spine and the back of the kit as well as on the top of the filter paper portion.

2. Identify the infant and match with the correct screening kit. Make sure to select the correct kit part (1st, 2nd or 3rd) depending on the specimen being collected.
3. **ALL** demographic fields must be filled in before collecting the specimen (see figure 2).

   a. If the birth mother will not be maintaining custody of the infant, provide the name, address and phone number for the infant’s guardian in the “Mother” fields. This information may be used to locate the infant for follow-up.

   b. Labels may be used to provide demographic information. They must be included on all layers of the screening kit. They must not cover demographic information fields that will be hand-written.

---

**Figure 2: Newborn Bloodspot Screening Specimen Collection Card**

Provide the name and contact information for the provider who is responsible for the infant’s medical care and treatment *after discharge* in the “Send report to PCP / Clinic” field. This practitioner will be sent screening results and follow-up information. Do not use the resident, attending, or on-call provider!
Each facility or medical provider must establish a procedure for staff performing newborn bloodspot screening specimen collections. Resources are available from the Clinical Laboratory Standards Institute that can help with creating or updating your procedure.

The preferred newborn bloodspot screening specimen is capillary blood obtained from a heel lance. Specimens obtained from peripheral or central lines are acceptable if they are flushed of parenteral nutrition or antibiotics. Blood from an intravenous stick is acceptable only if it does not clot and is applied to the filter paper directly. Cord blood is not recommended.

1. Use a scalpel bladed lancet manufactured specifically for heel stick collection from an infant. Do not use a lancet longer than 2.0 mm. Do not use capillary tubes or other collection devices.

2. Select a lance site on the infant’s heel (see Figure 3). Cleanse lance site with alcohol and air dry. Do not use betadine, iodine, lotion, or essential oils on the baby prior to collecting the specimen.

* These recommendations conform to CLSI publication NBS01-A6.
3. Perform lancing on the most medial or most lateral portion of the plantar surface of the heel.

4. Lance the heel with the sterile scalpel bladed lancet. Wipe away the first drop of blood to remove tissue fluids. Do not “milk” or squeeze the heel.

5. Allow a drop of blood to collect on the heel that is large enough to fill a collection circle.

6. Touch the filter paper gently to the drop of blood. Only apply blood to one side of the filter paper (it doesn’t matter which side is used).

7. Allow the blood to soak through the filter paper so that the blood spot looks similar on both the front and back of the collection kit.
Complete, even saturation of the filter paper is essential for accurate testing. The filter paper is calibrated to absorb a specific quantity of blood. Incomplete, uneven saturation or layering of the blood alters the quantity of blood used for testing and will lead to inaccurate test results. This figure is also available at: http://bit.ly/nbs-example.

8. Collect the blood in all four circles, repeating instructions 5 through 7. If blood flow is not sufficient, re-lance the heel. It is better to fill three circles completely than to fill four circles inadequately.

9. Air dry specimens at room temperature for between 2 and 4 hours in a horizontal position with the blood spots exposed. Hanging wet specimens vertically will cause heavier red cells to migrate to the dependent end of the circle resulting in uneven saturation.

10. Do not expose the specimen to excess heat or humidity at any time. Do not dry on a heater, in a microwave, with a hair dryer or in sunlight. Do not place in plastic bags, leave in a hot mailbox or in a hot car. These practices can destroy some proteins and enzymes that are required for accurate test results.

11. Ensure that the specimen is completely dry before transporting.
It is critically important that the Oregon State Public Health Laboratory (OSPHL) receive newborn screening specimens as soon as possible after collection and drying. Many of the conditions on the newborn screening panel can cause serious injury or death in the first week of life. Early diagnosis and treatment for these medical conditions must occur rapidly.

**Figure 5: Newborn Screening Process**

Specimens should be sent as soon as they are dried (between 2 and 4 hours) and no later than 24 hours after collection.

1. Keep a record of the specimens that are sent, including the kit numbers. A packing list or manifest should be included with the shipment.

2. Insert the dried specimen(s) into an envelope. Do not put specimens in plastic bags or containers. Do not compress the specimens.

3. Send the specimens no later than 24 hours after collection.

4. All specimens must be sent by express mail, courier, or another timely delivery mechanism. Specimens should be received by the OSPHL within 48 hours of collection.
5. Send the specimens to:
   Oregon State Public Health Laboratory
   Newborn Screening Program
   7202 NE Evergreen Parkway Suite 100
   Hillsboro, OR 97124

6. Maintain a record of each specimen leaving your facility, including the date and time of pick-up and delivery of the specimens.

Prompt transit is essential for identifying infants who may be impacted by a screening condition within 1 week of birth. Some transportation delays are unavoidable, such as holidays, weather events, or road closures. However, most delays in specimen transport are caused by a facility failing to send the specimens promptly. Delays within a facility may be from inefficient internal processes, slow courier services, simple forgetfulness, or, most dangerously, batching specimens.

Batching specimens to reduce facility shipping costs leads to unnecessary and potentially deadly delays in newborn screening.
Results are available online

Newborn screening result reports for infants known to be under your care can be accessed online through the OSPHL reporting website, Secure Remote Viewer (SRV), as soon as they are available. You can find information and the form to request access to SRV here: [www.bitly.com/get-phl-results](http://www.bitly.com/get-phl-results). If you have questions, contact the NBS Follow-up Team at 503-693-4174.

Results reporting

Newborn screening results are available in SRV to the “Hospital or submitter” and the “PCP/ Clinic”, as identified on the specimen kit, after being released by the OSPHL. Results may also be mailed or faxed to these facilities and providers.

Abnormal results that meet the screening criteria for a newborn screening condition require additional testing and medical follow-up by the infant’s provider. The NBS Medical Consultants and the NBS Follow-up Team will provide information to support providers in making medical decisions for these patients. The contact information for these consultants is available at: [www.healthoregon.org/nbs](http://www.healthoregon.org/nbs).

Newborn screening may detect secondary conditions, traits, and carriers. These findings will be reported as described above. It is within the discretion of the infant’s health care provider and parent or legal guardian to determine what, if any, medical follow-up is needed in these circumstances.

The provider named in the “Send Report to PCP/ Clinic” field will be legally responsible for responding to abnormal test results until another provider accepts responsibility by submitting a specimen or by requesting test results.

If diagnostic testing is ordered as a part of newborn screening, results of this testing must be reported to the NBS Follow-up Team by:

Calling 503-693-4174 or

Faxing the infant’s information to 503-693-5601
I did not receive my newborn screening results!

If you have access to SRV, and the results of an infant’s screening tests are not available to you within one week following collection and submission, please report this to the NBS Follow-up Team. Send a fax to 503-693-5601 on your facility letterhead to request a copy of the report. Provide the infant’s full name, date of birth, kit number, and mother’s full name and date of birth.

If the specimen was not received, you will be contacted by the NBS Follow-up Team.

The practitioner must communicate abnormal results to the parent or guardian of the infant.
Preterm, low birth weight, or sick infants

Newborn screening for premature, low birth weight (LBW) or sick infants can be complex. The infant’s immaturity or illness may interfere both with the collection of the specimens and the interpretation of results. In addition, some screening conditions may be difficult to identify in a preterm, low birth weight or sick infant. These include:

**Primary Congenital Hypothyroidism (CH)**

Low T4 and an elevated TSH are the classic hallmarks of congenital hypothyroidism, but some infants with primary CH may have a delayed rise in their TSH. Practitioners should not assume that a premature or sick infant with a low T4 only has transient hypothyroxinemia of prematurity (THOP) and not primary CH. Serial screening specimens for T4/TSH are required until the T4 normalizes or the baby is diagnosed with a thyroid dysfunction.

**Lysosomal Storage Disorders (LSD)**

Elevations in the white blood cell counts of sick or premature infants may result in a false negative result for LSDs. First specimen collections that occur before 20 hours of age or on infants born weighing less than 2000 grams will be unsatisfactory for this assay and require a repeat specimen.

**Parenteral nutrition and carnitine therapy**

These are not contraindications to screening, but specimens should not be taken from the line used to deliver nutrition or drugs. High levels of several amino acids can occur during parenteral nutrition and are the most common reason for “mixed elevations.”
Red cell transfusions

NICU infants should have a specimen collected prior to transfusion. Donor cells may cause normal levels of analytes and may result in false normal screening results being reported. It may take as long as 120 days for an affected infant to accumulate abnormal analyte values after a transfusion, significantly delaying diagnosis and treatment.

Pivalic acid antibiotic therapy

Antibiotics containing pivalic acid (e.g., pirampicillin, pimecillinan, cefditorempivoxil) given to mothers during labor or to newborns may cause false elevation of isovaleryl/2-methyl butyryl carnitine.

Maternal conditions may affect newborn screening results

These include:
- Thyroid dysfunction
- Steroids
- Fatty liver of pregnancy or HELLP syndrome (hemolysis, elevated liver enzymes, low platelets)
- Maternal CAH, PKU and 3-MCC deficiencies
- Maternal carnitine deficiency
- Maternal B12 deficiency
- Illicit drug use

Report that a transfusion occurred on the specimen collection card.

Report that Pivalic acid was administered on the specimen collection card.
• If the child is younger than 6 years, request his or her newborn screening records by faxing the child’s full name, date of birth, kit number, and mother’s name (at the time of the child’s birth) and date of birth on your letterhead to 503-693-5601.

• Records that are over 6 years old are outside of their record retention and should have been destroyed. It is unlikely that older records will be located. When requesting records older than 6 years, include a medical record release authorization signed by the patient, if over 18, or the parent or guardian.

• If you are requesting records for a baby who was born in another state, please contact that state’s newborn bloodspot screening program to request results. Contact information for each state can be found on the NewSTEPs at www.newsteps.org.

• Parents or legal guardians may request the infant’s newborn bloodspot screening records by completing the form located at www.bitly.com/get-phl-results.
Use, release, and retention of residual bloodspot specimens

After newborn screening testing is complete, some of the bloodspot specimen may be usable for other purposes. This remaining specimen is called a residual bloodspot specimen.

Residual bloodspot specimens may be used by the Oregon State Public Health Laboratory (OSPHL) for:

- Quality assurance and method development activities as required to maintain compliance with regulatory and accreditation requirements.
- Program evaluation and quality improvement.
- Education activities required by Oregon Statute.

Residual bloodspot specimens will only be released by the OSPHL:

- To perform routine newborn screening testing, if a testing service listed on OAR 333-024-1070 cannot be performed by the OSPHL.
- When required by a court order.
- When a release is requested by the parent or legal guardian of the infant, following the procedure detailed on the Oregon NBS website, www.healthoregon.org/nbs.

Residual specimens are retained by the OSPHL for 18 months. Specimens will be destroyed during the month after the retention time is met using a method that protects patient confidentiality and privacy.
Tips to avoid rejected specimens

Improperly collected specimens compromise the accuracy of test results. When a specimen is rejected, the infant must repeat the collection procedure. This unnecessarily delays the screening of the newborn.

Contact the OSPHL at 503-693-4174 to request more information about specimen collection or to request support from the NBS Education Coordinator.

Tips to avoid “Layered Blood” rejection

Tips to avoid this type of rejection

• Use the proper size lancet (< 2mm length).
• Allow a large drop to form on the heel before touching with the filter paper.
• Collect blood into one circle at a time.
• Do not apply additional blood to an incompletely filled circle.
• Do not apply blood to both sides of the filter paper.
• Do not compress the filter paper.
Tips to avoid “Incomplete Saturation” or “Quantity Not Sufficient” rejection

Tips to avoid this type of rejection

• Use the proper size lancet (< 2mm length).
• Allow a large drop to form on the heel before touching with the filter paper.
• If blood flow is not sufficient, re-lance the infant.
• Watch the blood soak completely through the paper.
• Collect blood into one circle at a time.
• Do not apply additional blood to an incompletely filled circle.

Tips to avoid “Contaminated” rejection

Tips to avoid this type of rejection

• Only use alcohol to clean the heel and then wipe dry with a sterile gauze pad.
• Do not store or dry the specimens near beverages, food, or other contaminates.
• Do not allow specimens to contact alcohol, antiseptic solutions, hand lotion, powders, or essential oils.
• Wipe away the first drop of blood.
• Do not “milk” or squeeze the heel. This may cause dilution with tissue fluids.
• Adequately flush the line, if using a TPN or central line.
Education services

The Oregon NBS program provides education services to improve the quality of newborn screening practices. These include a quality assurance surveillance program, facility site-visits, and comprehensive reviews of screening systems by the NBS Education Coordinator. In addition, education resources are made available to practitioners and parents at www.healthoregon.org/nbs.

Fee exemption

In Oregon, no person is refused service because of the inability to pay the fee for testing (OAR 333-024-1100). A practitioner or parent/legal guardian requesting exemption from fees shall complete a Statement of Fee Exemption. A printable copy of this form can be found here www.healthoregon.org/nbs.

The Oregon State Public Health Laboratory must receive the completed Statement of Fee Exemption within 30 days of the first newborn screening. Upon receipt of the statement and confirmation by the Oregon Health Authority records, the Oregon Health Authority will issue a refund check to the payer of record.

Parent refusal to have the infant screened

A parent may opt not to have their infant screened because of adherence to religious beliefs opposed to this testing. A signed “Religious Objection to Newborn Screening Blood Test (informed dissent)” form found here: www.healthoregon.org/nbs. This form should be included in the infant’s medical record. A copy should be given to the parents and baby’s primary care provider.

A copy must be forwarded to the NBS Follow-up Team within 30 calendar days from the day the infant was born.

NBS Follow-up Team
Fax: 503-693-5601
Cystic Fibrosis (CF)

**CF essentials**

- **Screening test:** Immunoreactive trypsinogen (IRT) > 60 meq/L on two filter paper specimens (trypsinogen is typically elevated in pancreatic insufficient neonates with CF but decreases to low levels by 2–3 months of age). Second-tier DNA screening for 23 common variants (listed below) is done on specimens that meet select criteria.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Mutation</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1717-1G&gt;A</td>
<td>621+1G&gt;T</td>
<td>N1303K</td>
</tr>
<tr>
<td>1898+1G&gt;A</td>
<td>711+1G&gt;T</td>
<td>R1162X</td>
</tr>
<tr>
<td>2184delA</td>
<td>A455E</td>
<td>R334W</td>
</tr>
<tr>
<td>2789+5G&gt;A</td>
<td>ΔF508</td>
<td>R347P</td>
</tr>
<tr>
<td>3120+1G&gt;A</td>
<td>G542X</td>
<td>R560T</td>
</tr>
<tr>
<td>3659delC</td>
<td>G551D</td>
<td>W1282X</td>
</tr>
<tr>
<td>3849+20KbC&gt;T</td>
<td>G85E</td>
<td></td>
</tr>
</tbody>
</table>

- **Confirmatory test:** Sweat chloride testing and DNA mutation analysis
- **Validity:** A small percentage of cases (<10%) will be falsely negative. Most cases should be abnormal on the first screen. IRT may be falsely elevated in premature, stressed, or sick infants. IRT can be falsely low in infants with CF who are born with meconium ileus.
- **Treatment:** Comprehensive, multidisciplinary care, pancreatic enzyme replacement, fat-soluble vitamin replacement, high-calorie/high-fat diet, and airway clearance regimen. Refer to accredited Cystic Fibrosis Center.
- **Outcome:** Early diagnosis improves pulmonary function and nutrition outcomes. With new treatments and ongoing comprehensive care, median survival now extends into the fourth decade.
Cystic fibrosis (CF) is a recessively inherited defect of the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Over 1,800 mutations of the CFTR protein have been identified, but a single mutation (F508del), accounts for over two-thirds of all the mutations worldwide. There are approximately 30,000 adults and children with CF in the United States and approximately 70,000 people affected worldwide. The incidence of CF in the United States is approximately 1:3,500 newborns but varies by ethnicity: 1:3,500 Caucasian Americans, 1:8,500 Hispanic Americans, 1:17,000 African Americans, 1:31,000 Asian Americans.

Clinical features

Mutations in the CFTR gene alter the structure, function or production of the transmembrane chloride channel protein that is critical to the normal functioning of multiple organs. These include the upper and lower respiratory tract, pancreas, liver, sweat glands and genitourinary tract.

The first symptom for 10–15% of infants with CF is meconium ileus, an intestinal obstruction that presents in the first few days of life. Other symptoms of CF develop over time.

For infants without meconium ileus, symptoms during the first few years of life include poor weight gain due to fat malabsorption, chronic cough, wheezing, abdominal pain, malabsorptive / loose stools and/or failure to thrive. Pancreatic insufficiency is present in approximately 85% of CF individuals, and can lead to severe nutritional deficiencies and malnutrition. Respiratory symptoms may be absent in the neonatal period but develop in most individuals by the end of the first year of life. Newborn screening for CF is nationwide, which has led to earlier diagnosis and improved outcomes. Specifically, survival has improved dramatically over the years with median survival now over 40 years of age. Like most inherited disorders, there are milder variants with proportionally fewer symptoms and longer survival.

Causes of CF

CF is a recessively inherited defect in the CFTR protein. CFTR deficiency results in abnormal chloride transport and the formation of excessively viscous mucus, which, in turn, leads to organ dysfunction and failure.

Laboratory tests

The screening test measures trypsinogen, an enzyme produced in the pancreas that is transiently elevated in the blood of most CF infants at birth. This enzyme is detected by immunoreactive trypsinogen (IRT) testing obtained from neonatal dried blood spots. (9)

An elevated IRT on one (if sufficiently high) or both screening specimens triggers 2nd tier DNA analysis (23 ACMG/ACOG mutations). Depending on results, further diagnostic and confirmatory testing will be required.
There are several issues to keep in mind regarding elevated IRT tests:

- Elevated IRT is not diagnostic of CF. Diagnosis must be confirmed with sweat testing and DNA analysis.
- Infants with meconium ileus may not have an elevated IRT, so this diagnosis should lead to more definitive testing for CF, regardless of the IRT result. It is important to remember that all infants with meconium ileus should have routine newborn screening specimens collected even if CF is suspected, as they should be screened for the other conditions on the screening panel.
- A small percentage of infants with CF may not have an elevated IRT. Thus, a normal IRT at birth does not completely rule out CF. Children with recurrent respiratory problems, failure to thrive, or other symptoms consistent with CF, should still be evaluated and undergo sweat chloride testing.

**Confirmatory testing**

CF can be diagnosed by two different methods, sweat chloride testing and/or DNA mutation analysis. Sweat chloride testing remains the gold standard, as it is a concrete marker of CFTR dysfunction. A chloride value in the sweat of ≥60 meq/L confirms the diagnosis, while a value <30 meq/L means that CF is very unlikely. For some infants, sweat chloride values will fall in an intermediate range (30–60 meq/L) and will need further testing to clarify the diagnosis.

DNA mutation analysis of the CFTR gene is another diagnostic method. Approximately 50% of people with CF have two copies of the most common variant, F508del, and most others (~86%) will have at least one copy. There are over 1,800 mutations described in CFTR (see www.cftr2.org), and most are not included in standard multi-array DNA analyses. (10, 11) Confirmation of two CF-causing mutations confirm the diagnosis, while only one may indicate a carrier state, CFTR-related metabolic syndrome (CRMS), or an affected individual with a less common mutation on the second allele.

**Treatment**

Treatment aims to ensure adequate nutrition and growth by supplementing pancreatic enzymes and vitamins and providing a high calorie and high fat diet. Daily airway clearance with nebulized medications are required to loosen secretions and prevent/treat pulmonary exacerbations. People with CF need prompt treatment of any pulmonary exacerbation with antibiotics. Routine immunizations including annual influenza vaccine and a one-time 23-valent pneumococcus vaccine are recommended to help prevent lung infections. Infants should be referred to an accredited CF Center.
Screening practice considerations

- CF infants with meconium ileus or who are pancreatic sufficient may have normal IRT levels.
- IRT levels in affected infants will decline and be in the normal range by 3 months. Thus, older infants or children suspected to have CF should have a sweat chloride test, as the IRT will not be accurate.
- IRT may be falsely elevated in premature, stressed, or sick infants.

<table>
<thead>
<tr>
<th>Results</th>
<th>Likely causes</th>
<th>Actions</th>
</tr>
</thead>
</table>
| 1st and 2nd IRT elevated on filter paper specimens | • Cystic fibrosis probable  
• Possible false positive | • NBS coordinator faxes results.  
• Medical consultant phones practitioner with follow-up recommendations.  
• Sweat chloride testing needed to confirm/clarify diagnosis. |
| One or two CFTR mutations identified on 23-mutation DNA analysis | • If two mutations, CF probable  
• If one mutation, possible CF carrier vs CRMS vs CF with rare 2nd mutation | • NBS coordinator faxes results.  
• Medical consultant phones practitioner with follow-up recommendations.  
• Sweat chloride testing needed to confirm/clarify diagnosis. |

Congenital Adrenal Hyperplasia (CAH)

**CAH essentials**

- **Neonatal emergency:** 3/4 will develop salt wasting crisis, which can be fatal, in the first week to month of life.
- **Incidence:** 1:12,700 newborns; 1:400 Alaskan Natives (Yupik Eskimo)
- **Screening test:** 17-OH-progesterone
- **Validity:** 80% identified on 1st screen  
20% on 2nd screen
- **Causes:** 21-hydroxylase deficiency or other inborn error of cortisol synthesis; recessive inheritance
- **Treatment:** Hydrocortisone and mineralocorticoids
- **False positives:** Occur more frequently in premature, low birth weight or sick infants
- **Outcome:** Early detection and treatment can be lifesaving. Chromosome analysis in infants with ambiguous genitalia will prevent gender misassignment. Ultimate outcome depends on severity of defect, days to treatment and adherence. Refer to pediatric endocrinologist.
CAH is an inherited defect of cortisol synthesis. The adrenal gland cannot make cortisol and overproduces male hormones. Without cortisol, infants are at risk for adrenal crisis and may be unable to regulate salt and fluids, and can die. The most common disorder is 21-hydroxylase deficiency.

**Clinical features (12)**

Infants may be symptomatic at birth. By 4 to 5 months’ gestation, diminished cortisol production stimulates the fetal pituitary gland to produce ACTH resulting in excessive adrenal androgens. The androgens virilize female external genitalia, but ovaries and uterus are unaffected. Male infants may have increased scrotal pigmentation or may be asymptomatic.

In 75% of cases, the 21-hydroxylase deficiency causes reduced production of mineralocorticoids. This reduction leads to a hypotensive, hyperkalemic, salt-losing crisis with rapid onset of adrenocortical failure within 7–28 days of birth, which can be fatal. In 25% of cases, the infant has a “non-salt losing” or “simple virilizing form.” If untreated, females have progressive postnatal virilization, males develop premature adrenarche, and both sexes have rapid growth with advanced skeletal age, early puberty and short stature as adults. In adulthood, there is hirsutism and acne. Women have irregular menses and infertility. Males have testicular masses (adrenal rests) with increased risk of infertility.

**Causes of CAH**

The term “congenital adrenal hyperplasia” or “adrenogenital syndrome” covers a group of disorders. All are due to an inborn error of steroid hormone synthesis, which blocks the production of cortisol. The low level of cortisol stimulates ACTH, causing adrenal hyperplasia and increased secretion of steroid precursors. Different enzyme defects block the metabolic pathway at different sites and result in different clinical features. There are variants to this disorder, which have later onset. All forms of CAH are inherited as autosomal recessive disorders.

**Laboratory tests**

Screening is based on an immunoassay for a precursor steroid, 17-hydroxyprogesterone (17-OHP). Affected infants have high levels of 17-OHP. Infants with milder disorders have intermediate levels. False positives may occur in preterm, low birth weight and sick infants.

**Confirmation**

Confirmation is by measurement of serum 17-OHP and if salt wasting is suspected, sodium, potassium and plasma renin activity. Chromosome analysis to confirm gender if genitalia are ambiguous.
**Treatment**

Infants should be treated with hydrocortisone and mineralocorticoids in consultation with a pediatric endocrinologist.

**Screening practice considerations**

- This disorder kills quickly and is a neonatal emergency. In both sexes, salt wasting and shock may develop rapidly within 7–28 days of birth. Collect specimens between 24–48 hours of life. Transport all specimens 4–12 hours after collection and no later than 24 hours.
- Female infants who are virilized or infants with ambiguous genitalia should be considered at risk for this condition, tested at birth and monitored for electrolyte abnormalities until the diagnosis is excluded.
- Male infants are not usually recognized at birth.
- About 10% of infants will be detected only on a second screen. (13–15)

<table>
<thead>
<tr>
<th>Results</th>
<th>Likely causes</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated 17 OHP (17-hydroxyprogesterone)</td>
<td>• CAH probable</td>
<td>Neonatal emergency; NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.</td>
</tr>
<tr>
<td></td>
<td>• False positive</td>
<td></td>
</tr>
</tbody>
</table>

**Primary Congenital Hypothyroidism (CH)**

**CH essentials**

- **Incidence:** 1:2,300 newborns
- **Screening test:** T4 (thyroxine) and TSH (thyroid stimulating hormone)
- **Validity:** 90% identified on 1st screen, 10% on 2nd screen
- **Causes:** Thyroid dysgenesis: 85%; hereditary inborn error of thyroid hormone biosynthesis: 15%
- **Treatment:** L-thyroxine normalize T4 by 2 weeks of age; TSH by 1 month
- **False positives:** Early collection within 24 hours of birth; premature or ill infants
- **Outcome:** Can be normal, but depends on severity of thyroid deficit, days to treatment and adherence to treatment. Severely affected infants with just a 2-week delay in reaching a serum T4 >10 ug/dL may have up to a 10-point drop in IQ. (16)
Primary congenital hypothyroidism (CH) occurs in infants who are born without the ability to produce adequate amounts of thyroid hormone. Thyroid hormone is important for normal function of all of the body’s organs and is essential for normal brain development. The incidence of congenital hypothyroidism is 1:2,300. CH is more common in Hispanic and Native American populations (1:700–2,000). There is a 2:1 female/male ratio, explanation unknown. Infants with Down’s syndrome have increased risk of CH (1:140 newborns).

**Clinical features**

Deficiency of thyroid hormone in an infant may result in intellectual and developmental disability and other signs of brain damage if it is not diagnosed and corrected by 3–6 weeks of life. Many infants with CH may appear clinically normal before 3 months of age, by which time some brain damage has usually occurred. Laboratory test results are the only reliable means of diagnosing CH in the newborn.

When symptoms or signs are present, they may include prolonged neonatal jaundice, constipation, lethargy and poor muscle tone, feeding problems, a large tongue, puffy face, large fontanels, distended abdomen and umbilical hernia. Approximately 10% of cases will have other congenital abnormalities, usually cardiac defects. Long-term neurologic damage includes intellectual and developmental disability, ataxia, fine and gross motor delay, slow growth, speech disorders and hearing deficits in 20%. Since thyroid deficiency can occur at any age, normal tests in the newborn period do not exclude deficiency in an older infant or child.

**Causes of primary congenital hypothyroidism**

The most common causes are total or partial failure of the thyroid gland to develop (aplasia or hypoplasia), its development in an abnormal location (an ectopic gland) or a defect in thyroid hormone production (dyshormonogenesis). Less commonly, hypothyroidism is induced by medications (antithyroid drugs or excess iodine) in the mother, or maternal autoimmune thyroid disease with transfer of a maternal TSH receptor antibody that blocks fetal thyroid development.

Some cases of central or secondary (hypopituitary) hypothyroidism may also be detected (see Table, next page). These newborns often have clinical features of other pituitary hormone deficiencies, such as hypoglycemia or small penis and undescended testes in male infants.

**Laboratory tests**

The initial screening test is the T4 assay. Infants with T4 results of <10% are further tested by a screening TSH assay. Different combinations of results are possible; see table on next page.
When the infant’s physician is notified that screening results are abnormal, blood should be collected by venipuncture as soon as possible to confirm the abnormal screening results. In the case where the T4 is low and TSH is elevated, treatment can be started as soon as the serum is obtained, pending final confirmation. If the serum thyroid function tests confirm hypothyroidism, further diagnostic studies, such as a thyroid ultrasound examination or radionuclide scan and X-ray to assess skeletal maturation, may be performed to determine the type, age of onset and severity of hypothyroidism. Generally, these studies do not change management and thus are optional.

**Thyroid function in premature infants**

In premature infants, there is a physiological reduction in blood T4 levels, but TSH levels are not elevated in this situation. These cases need special observation to ensure that the low T4 levels rise into the normal range as the infant matures, which may take several weeks. Serum free T4 levels (by equilibrium dialysis method) are often normal. Thyroid supplementation during this period remains controversial.

**Treatment**

The American Academy of Pediatrics (AAP) recommends that infants be managed in consultation with a pediatric endocrinologist. (18) Treatment of CH is effective if done correctly. L-tyroxine (brand or generic l-thyroxine), in pill form, is crushed, mixed with water or expressed breast milk and administered once daily. Currently, there are no FDA-approved liquid formulations. The recommended starting dose is 10–15 mcg/day of body weight daily, usually 37.5 mcg/day to 50 mcg/day. AAP recommendations for follow-up serum T4 (or free T4) and TSH are as follows:

- Initiation of treatment and every 2 weeks until the serum TSH normalizes
- Every 1–2 months in the first 6 months
- Every 3–4 months from 6 months–3 years of age
- Every 6–12 months from age 3–end of growth period
- 4–6 weeks after any dose change

**Treatment goals**: Maintain serum T4 or free T4 in the upper half of the normal range (10–16 µg/dL for T4 or 1.2–2.04 ng/dL for free T4 [normal range may vary with assay]), and TSH normalized (<6 µIU/mL). Clinical evaluations can occur less frequently. As infants grow, the dose of thyroxine is increased. Periodic developmental testing should be done on all patients. If treatment is started early and thyroid levels are monitored closely, development remains normal.
Screening practice considerations

• Primary congenital hypothyroidism is the most common disorder detected by the NBS program.

• Ninety percent of hypothyroid infants are detected on the first specimen; in 10% of cases, hypothyroidism develops in the weeks after birth and is detected on a second screening test as production of thyroid hormone decreases after birth. (18–20)

• Some infants (usually pre-term) will manifest a delayed rise in TSH, and so are also detected on the routine second or third screening test. Practitioners therefore must remain alert to clinical symptoms in premature and older infants despite normal initial screening.

• False positive results may occur if the specimen is collected within the first few hours after birth, as the TSH rises in response to the extra-uterine environment.

• Topical iodine use on the infant or a mother who is breastfeeding and taking iodine supplements may cause transient hypothyroidism. In addition, nursing mothers drinking “seaweed soup”, which has a high iodine content, may also cause hypothyroidism in the neonate; this will resolve if ingestion of seaweed soup is discontinued.

<table>
<thead>
<tr>
<th>Results</th>
<th>Likely causes</th>
<th>Actions</th>
</tr>
</thead>
</table>
| T4 low/TSH elevated                  | • Hypothyroidism probable  
• False positive                                                                           | NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations. |
| T4 low/TSH slightly elevated         | • Mild hypothyroidism  
• Transient hypothyroidism seen with recovery from “hypothyroxinemia of prematurity”  
• False positive                                                                           | NWRNBS Program contacts practitioner by FAX and by mail requesting further testing. |
| T4 low/TSH normal (on two specimens unless premature) | • Thyroid binding globulin (TBG) deficiency  
• Central or secondary (hypopituitary) hypothyroidism  
• Non-thyroidal illness syndrome (“sick euthyroid syndrome”) associated with prematurity or acute illness  
• False positive                                                                           | NWRNBS Program contacts practitioner by FAX and by mail requesting further testing. |
Sickle Cell Disease and other Hemoglobinopathies

Sickle Cell Disease essentials

- **Incidence:** (USA) 1:2,000 births; 1:365 African Americans
- **Screening test:** Isoelectric focusing (IEF)
- **Confirmatory tests:** IEF and/or HPLC (high performance liquid chromatography)
- **Validity:** 100% found on 1st screen (unless transfused)
- **Treatment:** Comprehensive care, prophylactic penicillin, immunizations and empiric treatment of febrile episodes. Refer to pediatric hematologist.
- **Outcome:** Screening prevents death from sepsis in most infants. Long-term outcome depends on the severity of the hemoglobinopathy and response to treatment.

The primary goal of hemoglobinopathy screening is to detect clinically significant sickling hemoglobinopathies in the neonatal period, before symptoms occur. Newborn diagnosis of sickle cell disease, if coupled with family education and centralized comprehensive care, can markedly lower morbidity and mortality. (21)

Homozygous sickle cell disease (SCD) occurs when the recessive gene for hemoglobin S, sickle hemoglobin, is inherited from both parents. The term “clinically significant sickling syndrome” also includes conditions resulting from inheritance of one gene for hemoglobin S and inheritance of a second gene for certain other hemoglobin variants, such as beta thalassemia or hemoglobin C. These doubly heterozygous conditions tend to be less severe than SCD, although all are potentially capable of producing severe complications. The disease incidence in a population depends on the population’s ethnic composition.

Clinical features

Sickle syndromes are systemic diseases and may affect any organ. They are characterized clinically by chronic hemolysis, intermittent vaso-occlusion and marked variability. Some patients experience unremitting complications, while others lead full and productive lives. While newborns are generally asymptomatic, early manifestations in infancy or early childhood can be life-threatening and include overwhelming infection due to splenic dysfunction, splenic sequestration crisis, and aplastic crisis with profound anemia. Before newborn diagnosis and preventive care, mortality in the United States was 8–30% in the first three years of life. Other important complications include vaso-occlusive pain syndromes, osteomyelitis, acute chest syndrome, stroke, priapism, pyelonephritis, gallstones, skin ulcers, retinopathy, and decreased life expectancy.

Other significant hemoglobinopathies are less common and even more variable. Their manifestations range from very mild chronic hemolysis to severe dyserythropoiesis requiring a lifetime of transfusion support. Early detection of these less common conditions may prevent unnecessary diagnostic and therapeutic intervention.
Laboratory tests

All first NBS specimens are screened for hemoglobinopathies using isoelectric focusing (IEF). Various hemoglobin patterns occur. If an abnormality is detected, the sample is reanalyzed using high performance liquid chromatography (HPLC). If a hemoglobin abnormality is detected on the first sample, the second sample is also analyzed by IEF and HPLC. Thus, each hemoglobin abnormality is verified four times, using two different techniques on two different specimens. Solubility tests (Sickle-dex, Sickle-prep, etc.) are never appropriate in infancy and should not be used to confirm screening results.

Treatment

Infants with significant hemoglobinopathies should have a primary care provider and receive periodic evaluation in a comprehensive care setting. Therapy begins with education of caregivers and includes prophylactic penicillin, prompt evaluation and empirical treatment of any febrile illness, and immunizations including those for encapsulated bacteria. Close attention is necessary to monitor for the common problems of poor growth, recurrent pain and febrile illnesses. Organ-specific complications, sedation and general anesthesia require special attention. Other treatments, including the use of blood products and investigational therapies depend on the clinical course.

Carrier detection makes SCD screening different

Sickle cell disease screening regularly identifies carriers (heterozygotes) as well as those affected by a given disease. In fact, many more carriers than disease states are identified for all hemoglobinopathies. If both parents are carriers of an autosomal recessive genetic trait, the risk of any infant of that couple being homozygous, and therefore having the disease, is 1:4.

Screening practice considerations

- Newborn screening for hemoglobinopathies is not done on the second specimen unless an abnormality has been identified on the first specimen. It is crucial to use the first kit for the first test; the cards are not interchangeable.

- Transfusion of red blood cells before collecting the newborn screening specimen will invalidate the hemoglobinopathy test. Always obtain a specimen before any transfusion regardless of the infant’s age.

- Some hemoglobinopathies, particularly some thalassemias, are not reliably detected by newborn screening and a normal screening result does not rule out the possibility that a patient has a hemoglobinopathy. Further testing or consultation should be sought if indicated by clinical suspicion.
<table>
<thead>
<tr>
<th>Results</th>
<th>Likely causes</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS (absence of A)</td>
<td>• Sickle cell disease; or&lt;br&gt;• Sickle beta thalassemia</td>
<td>NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.</td>
</tr>
<tr>
<td>FSC (absence of A)</td>
<td>• Sickle hemoglobin SC disease</td>
<td>NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.</td>
</tr>
<tr>
<td>FC (absence of A)</td>
<td>• Homozygous C disease</td>
<td>NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.</td>
</tr>
<tr>
<td>FE (absence of A)</td>
<td>• Homozygous hemoglobin E; or&lt;br&gt;• Hemoglobin E-beta thalassemia</td>
<td>NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.</td>
</tr>
<tr>
<td>FAS</td>
<td>• Sickle cell carrier; or&lt;br&gt;• Sickle beta thalassemia; or&lt;br&gt;• Sickle cell disease in transfused infant</td>
<td>NWRNBS Program will report by letter regarding test results and any other recommendations.</td>
</tr>
<tr>
<td>FAC</td>
<td>• Hemoglobin C carrier&lt;br&gt;• Homozygous C disease in a transfused infant</td>
<td>NWRNBS Program will report by letter regarding test results and any other recommendations.</td>
</tr>
<tr>
<td>FA + slow band</td>
<td>• Possible carrier for hemoglobin E, O, D or G</td>
<td>NWRNBS Program will report by letter regarding test results and any other recommendations.</td>
</tr>
<tr>
<td>FA + fast band</td>
<td>• Possible alpha thalassemia&lt;br&gt;• Bart’s hemoglobin is a marker for alpha thalassemia</td>
<td>NWRNBS Program will report by letter regarding test results and any other recommendations.</td>
</tr>
<tr>
<td>F only</td>
<td>• Premature infant; or&lt;br&gt;• Beta thalassemia major</td>
<td>NWRNBS Program will report by letter regarding test results and any other recommendations.</td>
</tr>
<tr>
<td>Predominance of A</td>
<td>• Transfused infant; or&lt;br&gt;• Patient outside of neonatal age range</td>
<td>NWRNBS Program will report by letter regarding test results and any other recommendations.</td>
</tr>
</tbody>
</table>
Amino Acid Conditions

Hypermethioninemia

*Homocystinuria (cystathionine beta-synthase deficiency)*

**Homocystinuria essentials**

- **Incidence:** 1:100,000
- **Screening test:** Methionine by MS/MS
- **Confirmatory tests:** Quantitative methionine, homocystine in blood and urine
- **Validity:** 20% 1st screen; 80% 2nd screen
- **Treatment:** Pyridoxine if responsive; if not responsive, low protein diet with cysteine and betaine supplements
- **Outcome:** Excellent if treated early and adherence is good

The most common form of genetic homocystinuria is cystathionine beta-synthase deficiency (CBS). CBS is required for conversion of methionine to cysteine and deficiency results in the accumulation of homocystine, methionine and cysteine-homocystine disulfides in the blood and urine. Unfortunately, methionine rises slowly in affected infants and may not be detectable on specimens obtained in the first few days after birth. Homocystinuria is inherited as an autosomal recessive trait.

**Clinical features (22,23)**

Untreated patients appear normal at birth, but by the first or second year intellectual and developmental disability may be apparent, most will develop dislocation of the lenses and a marfanoid body habitus, osteoporosis, and ultimately thrombo-embolism may develop which can result in stroke and serious, permanent disabilities or death.

**Methionine adenosyltransferase (MAT) deficiency**

A number of infants in the United States, identified through newborn screening with persistently elevated methionine, have been shown to have MAT deficiency. All but one patient has been asymptomatic, with normal growth and development.

**Laboratory test**

Elevation of methionine is detected by MS/MS

*Not all forms of hypermethioninemia or even all cases of CBS deficiency will be detected by MS/MS.*
Treatment

Some patients will respond to pyridoxine in large doses (250–1,200 mg/day). For patients unresponsive or partially responsive to pyridoxine, a protein-restricted diet supplemented with cysteine and betaine is usually effective. The outcome for treated patients is dependent on the age at diagnosis, adherence with therapy and severity of defect. For those with good compliance, outcome is normal.

Screening practice considerations

- Methionine rises slowly in affected infants, so that the first screening specimen may be normal; 80% of the homocystinuria patients detected in the NWRNBS Program have been found on routine second tests.
- Methionine may be elevated secondary to liver disease, prematurity or parenteral nutrition.

<table>
<thead>
<tr>
<th>Results</th>
<th>Likely causes</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine slightly elevated</td>
<td>• Homocystinuria/MAT deficiency possible</td>
<td>NWRNBS Program requests repeat filter paper specimen by mail.</td>
</tr>
<tr>
<td></td>
<td>• Tyrosinemia, Type I, galactosemia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Liver disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Parenteral nutrition</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• High protein diet</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• False positive</td>
<td></td>
</tr>
<tr>
<td>Methionine elevated</td>
<td>• Homocystinuria/MAT deficiency probable</td>
<td>NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations</td>
</tr>
<tr>
<td></td>
<td>• Tyrosinemia, Type I</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Liver disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Parenteral nutrition</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• High protein diet</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• False positive</td>
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</tr>
</tbody>
</table>

Phenylketonuria (PKU) and Hyperphenylalaninemia

Hyperphenylalaninemia essentials

- **Incidence:** 1:16,300 births
- **Screening test:** Phenylalanine elevated by MS/MS; phenylalanine/tyrosine ratio elevated
- **Confirmatory tests:** Quantitative amino acids; biopterins in blood and urine
- **Validity:** >99% on 1st screen
- **Treatment:** Low phenylalanine diet; biopterin supplementation
- **Outcome:** Normal if treated early and adherence is good
Detection of elevated phenylalanine levels requires urgent follow-up. The disorder is due to a recessively inherited enzyme defect in which the body cannot use the amino acid phenylalanine properly. All other metabolic processes are intact, but phenylalanine, which comes from all dietary protein, accumulates in the blood to toxic levels. All forms of hyperphenylalaninemia from mild to severe and including biopterin defects are inherited as autosomal recessive disorders.

**Clinical features**

Infants with PKU seem to be normal for many months; however, without treatment, severe intellectual and developmental disability, seizures, eczema and other problems usually develop. In older untreated patients, the skin and hair may be fair, the eyes may be blue and a mousey odor of the skin or urine is common. Untreated blood phenylalanine level is often over 1,200 µM/L in infants with severe PKU. Overall, PKU occurs in about 1 in 10,000–15,000 Caucasian and Hispanic births and is less common in other races. Although severe mental deficiency usually occurs in untreated cases, occasional asymptomatic adults are found with normal or near normal intelligence, despite high phenylalanine levels.

Plasma phenylalanine is not detectably elevated in cord blood. It starts rising within 24 hours after birth and often reaches 1,200 µM/L or more within a few days. The screening test is abnormal within 24 hours of birth.

The phenylalanine/tyrosine ratio is uniformly abnormal in true cases and can be used to differentiate false positive cases.

**Variant forms of PKU (hyperphenylalaninemia)**

Several intermediate forms of hyperphenylalaninemia occur in which the plasma phenylalanine levels are lower than in classic PKU. In these cases, intellectual and developmental disability is variable and in the milder variants is completely absent. In infancy, these patients can mimic severe PKU, and for adult women the risk of maternal PKU syndrome increases in proportion to the plasma phenylalanine.

Some forms of hyperphenylalaninemia are caused by defects of the cofactor biopterin metabolism and blood phenylalanine levels are variable. These patients have progressive neurological damage with seizures and steady deterioration that becomes noticeable sometime between 6 and 20 months of age despite early treatment with a low phenylalanine diet. Definitive tests can differentiate these variant forms of PKU. In view of the severity of this group of diseases, all infants with persistently abnormal levels of phenylalanine must have testing by special blood and urine tests for biopterin abnormalities.
Maternal PKU and hyperphenylalaninemia

Women with significant hyperphenylalaninemia have an increased risk of miscarriage and their offspring (who usually do not have PKU) may have intra-uterine growth retardation that persists postnataally. More than 90% of infants of untreated mothers with classical PKU have microcephaly, intellectual and developmental disability and/or congenital heart defects. They have a transient elevation of phenylalanine (240–1,200 µM/L) that falls to normal within 24 hours. A phenylalanine restricted diet begun before conception and during pregnancy can often prevent damage to the fetus. Most childbearing women today, if born in the United States, should have been screened as infants, so the chances of undiagnosed hyperphenylalaninemas are remote but still present.

Laboratory tests

PKU and hyperphenylalaninemia are detected using tandem mass spectrometry (MS/MS); the normal phenylalanine level is elevated and the phenylalanine/tyrosine ratio is elevated.

Treatment (24–27)

With proper treatment, intellectual and developmental disability is totally preventable. Treatment should be started as soon after birth as possible (preferably in the first week) in any infant recommended for treatment by the consultants and should be continued indefinitely. Frequent monitoring is required, especially in the first few weeks, because variant forms of hyperphenylalaninemia may be indistinguishable from classic PKU and improper nutritional therapy can be fatal.

If treatment is not started for some weeks, the results are more variable and the IQ tends to be lower. Patients whose treatment begins after 6 months are likely to remain intellectually disabled. Older patients usually show little change in IQ with treatment, but a low phenylalanine diet may help to control behavior problems.

Screening practice considerations

• Detection may depend on the amount of protein ingested or endogenously produced by the infant, but most affected infants (90%) have abnormal results even in the first 24 hours of life regardless of intake. Those with milder forms of hyperphenylalaninemia require longer periods of feeding or catabolism to develop abnormal results.
• Contamination of the filter paper with food or liquids containing Aspartame may cause false positive results or an inadequate specimen.

<table>
<thead>
<tr>
<th>Results</th>
<th>Likely causes</th>
<th>Actions</th>
</tr>
</thead>
</table>
| Phenylalanine elevated; Phe/Tyr elevated | • PKU possible  
• Variants forms of PKU  
• Mother has PKU  
• False positive  
• Transient hyperphenylalaninemia | NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations. |
Tyrosinemia, Type I, II and transient *

**Tyrosinemia essentials**

- **Incidence:** 1:652,000 (types I & II) (1: 1,000 transient)
- **Screening test:** Tyrosine by MS/MS; succinylacetone
- **Confirmatory tests:** Succinylacetone, blood amino acids, enzyme and mutation analysis
- **Validity:** >99% on either screening test for tyrosinemia type I
- **Treatment:** Low protein phe/tyr diet, medications and possible liver transplant in type I; low phe/tyr diet in type II. Transient tyrosinemia resolves within a month or two of birth or Vitamin C supplements for a few days will shorten the time.
- **Outcome:** Type I: 2-(nitro-4-trifluoromethylbenzoyl)-1-3-cyclohexanedione (NTBC) stops progression of disease and allows normal growth and development. The long-term risk of liver adenomas is still unknown, prompting some families to opt for liver transplant. Type II and transient: Normal outcome Elevated tyrosine may result from an inherited defect of tyrosine catabolism or, as in transient tyrosinemia, delayed maturation of liver enzymes or liver disease.

**Transient Tyrosinemia (28)**

Transient Tyrosinemia of the newborn is common (1:1,000) and more common among Inuit and Eskimo populations in Alaska. Transient tyrosinemia is thought to arise from delayed maturation of the liver enzyme, 4-hydroxyphenylpyruvic acid dehydrogenase (4HPPD), coupled with increased protein intake and/or occult ascorbic acid deficiency. Tyrosine levels may be quite high (>480 µM/L) peaking at 14 days of life and resolved by 1 month. Premature infants or those on parenteral nutrition may have prolonged hypertyrosinemia.

**Clinical features**

Transient Tyrosinemia of the newborn may present with lethargy or decreased motor activity, but is usually a biochemical abnormality found in an otherwise normal newborn. Transient tyrosinemia is not associated with long-term sequelae, although this has not been systematically studied.

**Treatment**

Transient Tyrosinemia, while probably benign, may in some cases be treated with protein restriction to 2g/kg/day and administration of ascorbic acid (50–200 mg/day orally for 5–7 days) to infants found to have transient tyrosine (after types I & II are excluded). If the infant is breastfeeding, ascorbic acid alone may be crushed, dissolved in water and administered orally. Ascorbic acid, a co-factor for 4HPPD, helps to increase the enzyme's activity which will resolve the hypertyrosinemia more quickly if there are concerns about the infant's status.

* Not all cases of tyrosinemia will be detected by newborn screening.
**Hepatorenal Tyrosinemia, Type I (29)**

Tyrosinemia, Type I or fumarylacetoacetate hydrolase (FAH) deficiency occurs in 1:100,000 births. Hepatorenal tyrosinemia is inherited as an autosomal recessive trait.

**Clinical features**

Tyrosinemia, Type I causes severe liver and renal disease and peripheral nerve damage. Presentation in infancy includes vomiting, lethargy, diarrhea and failure to thrive. Liver disease with hepatomegaly, hypoproteinemia, hyperbilirubinemia, hypoglycemia and coagulopathy may be present. In untreated infants, renal proximal tubular dysfunction results in aminoaciduria, hyperphosphaturia and hypophosphotemic rickets. Untreated, death in infancy or childhood from acute liver failure, neurological crises, or hepatocellular carcinoma is usual.

**Treatment**

Therapy with oral NTBC blocks the formation of the toxic metabolites. NTBC is effective in preventing or halting liver and renal damage and averting acute neurological crises. Long-term ability of NTBC to prevent the development of hepatic carcinoma is yet unknown. The ultimate treatment, liver transplantation, has been successful in many cases. Adjunct therapy with dietary restriction of tyrosine as well as symptomatic treatment of clotting defects, rickets and proximal tubular losses may also be needed.

**Occulocutaneous Tyrosinemia**

Tyrosinemia, Type II is caused by a deficiency of the enzyme tyrosine aminotransferase (TAT) and is inherited as an autosomal recessive trait. TAT deficiency is rare, with about 100 cases described worldwide, although more infants may be identified as MS/MS screening continues to be implemented. (30)

**Clinical features (29, 30)**

TAT is manifested primarily in the eyes, the skin and the central nervous system. In the eyes, tyrosine crystals accumulate resulting in painful corneal erosions. Equally painful hyperkeratotic plaques develop on the plantar surfaces of hands, feet and digits. Symptoms usually develop in the first year of life, but have been present on the first day of life or not occur until adulthood. A variable degree of intellectual and developmental disability is present in about 50% of cases.
**Treatment**

A diet restricting phenylalanine and tyrosine is effective in clearing and/or preventing ulcerations.

**Laboratory tests**

Tyrosinemia is detected using succinylacetone by MS/MS; the cutoff tyrosine level is \( \leq 480 \, \mu M/L \). There is considerable overlap in tyrosine levels between normal infants, those with transient tyrosinemia and affected infants, making the tyrosine level itself not very specific.

Clinical correlation, blood amino acids and urine succinylacetone are necessary to differentiate these cases.

**Screening practice considerations**

- Tyrosine may be slow to rise in affected infants, making it more likely to be found on routine second testing. Practitioners must remain alert to the possibility of tyrosinemia in any infant with liver disease, corneal or keratotic lesions.

<table>
<thead>
<tr>
<th>Results</th>
<th>Likely causes</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine elevated</td>
<td>• Transient tyrosinemia</td>
<td>NWRNBS Program requests repeat filter paper in 2-3 months by mail.</td>
</tr>
<tr>
<td>Succinoylacetone elevated 1.0</td>
<td>• Tyrosinemia possible</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Liver disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Parenteral nutrition</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• False positive</td>
<td></td>
</tr>
<tr>
<td>Tyrosine slightly elevated</td>
<td>• Transient tyrosinemia</td>
<td>NWRNBS Program requests repeat filter paper by mail.</td>
</tr>
<tr>
<td>Succinoylacetone decreased</td>
<td>• Tyrosinemia possible</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Liver disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Parenteral nutrition</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• False positive</td>
<td></td>
</tr>
<tr>
<td>Succinoylacetone increased.</td>
<td>• Tyrosinemia type I</td>
<td>NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.</td>
</tr>
<tr>
<td>Tyrosine normal.</td>
<td>• False positive</td>
<td></td>
</tr>
</tbody>
</table>
Fatty Acid Oxidation (FAO) Conditions

FAO condition essentials

- **Neonatal emergency:** Approximately 10% of infants with FAO disorders will die in the first few days after birth, generally before screening results are known.
- **Incidence:** 1:6,000 births; MCAD is the most common, approximately 1: 15,000 births; LCHAD is 1: 50,000 and VLCAD, 1: 31,000
- **Screening test:** Acylcarnitines by MS/MS
- **Confirmatory tests:** Acylcarnitine profiles, enzyme assay and/or mutation analysis
- **Validity:** 90% on the 1st screen, 10% on the 2nd screen
- **Treatment:** Avoid fasting, IV glucose support during intercurrent illness
- **Outcome:** Variable depending on the FAO. MCAD patients do well if caught early and episodes are prevented.

Mitochondrial beta-oxidation of fatty acids is crucially important in the body’s ability to produce energy during fasting. In infants, a “fasting” state can be produced in as little as four hours. Fatty acids must be transported into the cytoplasm and then into the mitochondria for oxidation; carnitine is required for these transport steps. Once in the mitochondria, fatty acid chains 4-18 carbons in length must be oxidized, two carbons at a time, each reaction using a chain-specific enzyme, before ketogenesis can occur. Over 20 individual steps occur in beta-oxidation some with multiple enzyme complexes. An enzyme block anywhere in this process or a carnitine deficiency will result in hypoketotic hypoglycemia and tissue damage related to the toxic accumulation of unoxidized fatty acids.

**Fatty Acid Oxidation conditions***

- Carnitine transport defect (enzyme unknown) (CUD)
- Carnitine/acylcarnitine translocase (CT) deficiency
- Carnitine palmitoyl transferase I (CPT I) deficiency
- Carnitine palmitoyl transferase II (CPT II) deficiency
- Very long chain acyl-CoA dehydrogenase (VLCAD) deficiency
- Long chain L-3 hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency
- Medium chain acyl-CoA dehydrogenase (MCAD) deficiency
- Short chain acyl-CoA dehydrogenase (SCAD) deficiency
- Multiple acyl-CoA dehydrogenase deficiency (MADD aka glutaric acidemia II [GA II])
- Trifunctional protein (TFP) deficiency

MCAD is the most common, but NBS has identified infants with all the FAO disorders. All are inherited as autosomal recessive traits.

* These are not all the FAO conditions, only the ones thought to be detectable with MS/MS. At this time the sensitivity and specificity of MS/MS to detect all affected infants is unknown.
Clinical features (32, 33)

FAO disorders have overlapping symptoms and organ involvement, which are classified into three major categories as described below.

**Hepatic (34, 35):** No typical age of presentation, which may be on the first day of life through adulthood. As of 2010, three infants in our region with MCAD presented with sudden cardio/pulmonary arrest before screening results were known; one infant survived. Precipitating factors are fasting and/or stress associated with intercurrent illness. Patients present with “Reyes-like” symptoms including vomiting, lethargy, hypoketotic hypoglycemia, mild hyperammonemia, hyperuricemia, hypocarnitinemia and abnormal liver function tests. Liver biopsy often shows steatosis. Hepatic presentation is common in MCAD, VLCAD, LCHAD, neonatal CPT I & II and mild CT deficiency. Patients with LCHAD may develop retinal pigmentary changes and progressive visual loss in childhood despite early diagnosis and treatment.

**Cardiac:** Cardiac abnormalities include hypertrophic or dilated cardiomyopathy. Pericardial effusion or cardiac failure can lead to death in these patients. FAO disorders with cardiac involvement include carnitine transporter defects, LCHAD, TFP deficiency, neonatal CPT II and VLCAD.

**Muscular:** There is usually moderate to severe hypotonia with recurrent rhabdomyolysis. Creatinine kinase may be greatly elevated. In infants and children seizures and/or developmental delay may also be present. Rhabdomyolysis is common in the adult form of CPT II, LCHAD, TFP deficiency and VLCAD.

A mother carrying an affected LCHAD fetus is prone to developing a life-threatening acute fatty liver during pregnancy or HELLP syndrome (hemolysis, elevated liver enzymes, low platelets). The reasons for this are not yet understood, but FAO disorders should be considered in infants whose mothers have a history of these pregnancy complications. (36)

**Treatment**

Even with screening, some infants with FAO disorders may die before laboratory results are available. Treatment for MCAD and some other FAOs is extraordinarily simple once the diagnosis is suspected. Avoidance of fasting, particularly as infants and young children, is the primary treatment. Carnitine supplementation (100mg/kg/day) is used to provide a pathway for removal of toxic intermediate metabolites in some FAOs. With appropriate treatment hepatic, cardiac and muscular complications can be reduced or eliminated. Patients with these disorders may require IV support for fluid and calories during intercurrent infections or illnesses. With pre-symptomatic diagnosis and appropriate therapy, outcome can be normal for infants with MCAD. (36, 37) Outcomes for the other disorders are still being evaluated.
Screening practice considerations

• Neonatal forms of FAO disorders can present in the first few days of life.
• Practitioners must remain alert to the possibility of FAO disorders in any neonate, infant or child with hypoketotic hypoglycemia or “Reyes-like” episodes or mother’s with HELLP syndrome or fatty liver of pregnancy.
• Infants affected with an FAO who are well fed may have normal screening results, masking the presence of the disorder.
• Practitioners caring for Alaska or Canadian Native infants should ensure infants are tested twice, once between 24–48 hours of age and the second about 2 weeks of age as there is a higher incidence of CPT 1 in these infants.

Organic Acid Conditions (OA)

OA condition essentials

• Neonatal emergency: Infants with severe forms of organic acidemias will be symptomatic within a few days of birth and may die or suffer brain damage if not diagnosed and treated promptly.
• Incidence: 1:20,000 births
• Screening test: MS/MS detection of leucine and acylcarnitines. Approximately 15 OAs can be detected through NBS.
• Confirmatory tests: Quantitative amino acids, acylcarnitines, organic acids, enzyme assay and/or mutation analysis
• Validity: >99% detected on first screen
• Treatment: Specific amino acid dietary restrictions and medications
• Outcome: Variable, from poor to excellent, depending on neonatal course, disease severity, compliance with treatment and other environmental factors Organic acidemias (OA) result from enzyme deficiencies involved in the catabolism of multiple amino acids and other metabolites. Maple syrup urine disease is detected by an elevation of the amino acid leucine and an abnormal leucine/alanine ratio. All the other OAs are detected through elevations in acylcarnitines. All have autosomal recessive inheritance and have a collective incidence of 1:20,000. Screening for OAs is done for all infants in our region.

The following OAs are screened for by MS/MS:

• Beta-ketothiolase deficiency
• Glutaric acidemia, type I (glutaryl-CoA dehydrogenase deficiency)
• Isobutyryl CoA dehydrogenase deficiency
• Isovaleric acidemia, (isovaleryl-CoA dehydrogenase deficiency)
• Malonic aciduria
• Maple syrup urine disease (branched chain alpha-ketoacid dehydrogenase deficiency)
• Methylmalonic acidemias, methylmalonyl CoA mutase deficiency and defects of B-12 metabolism
• Propionic acidemia
• 3-Hydroxy-3-methylglutaryl (HMG) CoA lyase deficiency
• 2-Methyl-3-hydroxybutyryl CoA dehydrogenase deficiency
• 2-Methylbutyryl CoA dehydrogenase deficiency (mitochondrial acetoacetyl-CoA thiolase deficiency)
• 3-Methylcrotonyl CoA carboxylase (3MCC) deficiency
• 3-Methylglutaconyl CoA hydratase deficiency (3-methyl-glutaconic aciduria, type I)
• Multiple carboxylase deficiency

Clinical features (38–40)

Neonatal onset: Most of these disorders have severe forms that present in the first week of life and constitute a neonatal emergency. Infants are generally well at birth, but develop poor feeding, irritability, lethargy, vomiting, and severe metabolic ketoacidosis, with or without hypoglycemia, in the first few days of life; this progresses to coma and death in the first month if treatment is not instituted. In methylmalonic and propionic acidemias, ammonia may also be elevated. Isovaleric acidemia is associated with the odor of “sweaty socks.” Maple syrup urine disease has a characteristic “burnt sugar” or “maple syrup” odor which can be noticed in the urine, sweat and ear cerumen of the affected infant as early as the fifth day of life. Isobutyryl CoA dehydrogenase deficiency is associated with a dilated cardiomyopathy. Even with prompt treatment, many infants with neonatal forms of organic acidemias sustain psychomotor damage and may have significant long-term morbidity. These infants may be ill before the results of the screening tests are known. Contact the metabolic consultants urgently if an OA is suspected.

Late onset: Milder variants may present with an acute decompensation brought on by an intercurrent illness similar to those described above, or with failure to thrive, hypotonia, intellectual and developmental disability or seizures and a history of bouts of vomiting, protein intolerance, acidosis and/or hypoglycemia. While these patients typically have “milder” disease, the neurological damage may be just as severe as those presenting earlier. Newborn screening may be very beneficial to these infants as the initial crisis may be prevented.

Asymptomatic cases: There are numerous reports of cases of isolated 3-methylcrotonyl-CoA carboxylase deficiency who have remained asymptomatic despite biochemical and/or enzymatic confirmation of the condition. The etiology of these variant presentations is not yet understood. Mild forms of methylmalonic acidemia have been found.
Glutaric Acidemia, type I: Glutaric Acidemia, Type I or GA I is an organic acidemia with clinical features unlike those described above. (40–42) In this disease, there is an accumulation of glutaric acid and 3-hydroxy glutaric acid, which are believed to be toxic to cells, particularly in the central nervous system. The classic presentation is macrocephaly at or shortly after birth. Infants have a period of apparently normal development but may have soft neurological signs, like jitteriness, irritability and truncal hypotonia. Generally, between 6 and 18 months of age, patients will experience an acute encephalopathic episode resulting in damage to the basal ganglia and atrophy of the caudate and putamen. This occurs over the course of a few hours to a day and is irreversible and untreatable. Severe dystonia, dyskinesia and other neurological findings result, either in a static or slowly progressive form. These children are often misdiagnosed as having extra pyramidal cerebral palsy. Approximately 25% of GA I patients will present with motor delay, hypotonia, dystonia and dyskinesia that develop gradually during the first few years of life, without any apparent acute crisis. Intellect is relatively intact. Infants with GA I are prone to acute subdural and retinal hemorrhages after minor head trauma. This can be misdiagnosed as child abuse. Finally, 5% of all Amish patients have been completely asymptomatic without any crises and normal development. Neurological crises and symptoms rarely occur after 5 years of age.

Laboratory tests

All these disorders are detected using MS/MS. Leucine can be elevated in infants receiving parenteral nutrition, usually along with other amino acid elevations. In a normal newborn, however, elevations of these compounds are unusual and require rapid follow-up. There is evidence that not all affected infants will be found by NBS. (42)

Treatment

Any infant in whom a neonatal onset organic acidemia is suspected should be treated as a neonatal emergency. Infants with these disorders should in most, if not all, cases be transferred to a major medical center with a metabolic specialist as quickly as possible. The diagnosis, investigations and management are very complicated. Death or permanent neurological deficits can occur rapidly in untreated cases. Infants who are asymptomatic at the time that abnormal screening results are reported may be handled less urgently, depending on the clinical status and individual circumstances. Treatments, which must be continued for life, consist of strict dietary amino acid restrictions and medications.

Infants with GA I, in addition to diet and medications, must have aggressive supportive care during intercurrent illness throughout the first 5–6 years of life. This generally entails hospitalization, IV fluid and calories during all febrile or flu like illnesses.

For individuals with MSUD, isovaleric acidemia and one or two other organic acidemias, prospective and early identification through newborn screening will be life-saving and outcomes are expected to be good. Eighty percent of infants with GA1,
treated pre-symptomatically, have avoided striatal necrosis. For other less common conditions, the outcome is still being evaluated.

Screening practice considerations
• Affected infants must be detected early if major problems are to be prevented.
• Practitioners must remain alert to the possibility of these diseases in any infant with lethargy, acidosis or coma.

Urea Cycle Conditions (UCD)

Urea Cycle essentials
• Neonatal emergency: Infants with severe hyperammonemia may die in the first week to 10 days if not diagnosed and treated.
• Incidence: 1:60,000 births (all 3 disorders)
• Screening test: Citrulline, argininosuccinic acid and arginine by MS/MS
• Confirmatory tests: Quantitative amino acids, urine organic acids and enzyme assay in red blood cells or hepatocytes
• Validity: >99% of citrullinemia and ASA on first test. The only arginase deficient infant diagnosed in Oregon was found on the second screen.
• Treatment: Neonatal rescue from hyperammonemic coma is complicated and should be done under the guidance of an experienced metabolic physician. Day-to-day hyperammonemia is controlled with a low protein diet, medications and amino acid supplements. Complete or partial liver transplant eliminates the need for diet and may improve development.
• Outcome: For those with citrullinemia and ASA who survive a neonatal coma, the outcome is usually fair to poor. Brain damage is common and the risk of hyperammonemia continues throughout life. Complications from arginase deficiency should be preventable with early and continuous treatment.

The urea cycle is the metabolic pathway responsible for the detoxification of ammonia and for the synthesis of arginine and urea. There are six enzymes in the urea cycle, each of which if missing, will result in hyperammonemia and one of the six disorders of the urea cycle. Each of these enzyme deficiencies has genetic and clinical variability from mild to lethal. Only three UCDs can be detected by newborn screening:
• Arginase deficiency
• Argininosuccinic aciduria (ASA)
• Citrullinemia, type I and II

They are inherited as autosomal recessive traits.
Arginase deficiency (43)

Clinical features

Arginase deficiency is associated with irritability, inconsolable crying, anorexia, vomiting and developmental delay in infancy. This progresses to spastic tetraplegia with lower limbs more severely affected than the upper, psychomotor retardation, hyperactivity and growth failure. Hyperammonemia may result in encephalopathy, but is often milder than that seen in other urea cycle defects. A severe neonatal form presents with cholestatic jaundice, liver failure and death.

Citrullinemia, Type I (CTLN1) and Argininosuccinic Aciduria (ASA) (44,45)

Clinical features-neonatal onset

Infants appear normal at birth and for the first 24 hours. Usually between 24–72 hours symptoms of hyperammonemia will appear as lethargy, vomiting, hypothermia, hyperventilation progressing to coma, cerebral edema and death without intervention. Unfortunately, a misdiagnosis of sepsis is made in 50% of the cases, wasting precious time. In addition to ammonia, both glutamate and glutamine are usually elevated. Specific elevations in citrulline, argininosuccinic acid, arginine and orotic acid are helpful in determining the exact type of urea cycle defect.

Clinical features-late onset

Late onset forms of urea cycle disorders most often present as non-specific developmental delay, seizures or other neurological symptoms which are associated with a history of repeated bouts of lethargy, vomiting, irritability or headaches. Food refusal and failure to thrive are not uncommon.

Asymptomatic cases

Newborn screening has detected several infants with very mild citrullinemia, who do not require any treatment when healthy, but may be at risk of decompensation under stress, infection or high protein intake.

Citrin Deficiency (Citrullinemia, Type II and Neonatal Intrahepatic Cholestasis [NICCD]) (46)

Citrin is a mitochondrial membrane aspartate-glutamate carrier that acts to transfer cytosolic NADH into the mitochondria. There are two distinct disorders associated with citrin deficiency. It is unknown how well NBS tests will identify these patients.
Clinical features-neonatal onset

Neonatal intrahepatic cholestasis due to citrin deficiency (NICCD) has been found in over 200 Japanese and Asian infants and a handful of non-Asian infants, usually between 1–5 months of age. Liver disease may be accompanied by jaundice and fatty infiltrates. While liver failure may necessitate transplant in infancy, the liver disease generally resolves by a year of age for most patients. At least one of these infants has progressed to citrullinemia type II at the age of 16 years.

Clinical features-late onset

Patients with citrullinemia type II (CTLN2) present in childhood or adulthood (11–64 years of age). Symptoms may be acute or develop slowly. These include enuresis, delayed menarche, insomnia, night sweats and terrors, recurrent vomiting, diarrhea, tremors, confusion, lethargy, delusions and episodes of coma. Citrulline and ammonia are elevated. Within a few years of the diagnosis, episodes of pancreatitis, hyperlipidemia and death from cerebral edema generally occur. Hepatocellular carcinoma has been reported in a few cases.

Laboratory tests

Elevations of citrulline and arginine are detected by MS/MS. The laboratory cutoff for citrulline is ≤70 µM/L; for arginine, ≤110 µM/L; argininosuccinic acid, ≤1.50 µM/L. Transient elevations of plasma arginine and citrulline in the newborn are unusual unless the infant is premature and/or receiving parenteral nutrition.

Infants with NICCD may or may not have citrulline elevations. Approximately half of the Japanese patients came to attention with elevated galactose, methionine and/or phenylalanine on NBS before the advent of MS/MS. Approximately 10% of NICCD patients had normal citrulline.

Treatment (Citrullinemia, Type I & ASA)

All patients with a neonatal presentation represent medical emergencies and outcomes may be variable. Patients with neonatal onset disease will typically require aggressive treatment with hemodialysis. All patients, both late onset and those rescued from neonatal hyperammonemia, will require treatment with low protein diets and medications to prevent hyperammonemia and remove toxic compounds. The outcome for patients rescued from prolonged neonatal hyperammonemia is dismal. Brain damage is likely. Even patients treated prospectively from birth may not be normal. (46) Those with late onset disease fare better, and presymptomatic diagnosis and treatment may allow normal development.
**Treatment: NICCD and CTLN2**

NICCD responds well to protein restriction in infancy for most patients. Those who do not respond or who develop progressive liver failure graduate to liver transplantation.

Patients with CTLN2 receive a liver transplant, as they will proceed to death without it. Dietary restriction of protein is ineffective. Long-term outcome is unknown.

**Screening practice considerations**

- Neonatal emergency.
- Infants with neonatal onset disease may be sick or die before screening results are known.
- Practitioners must remain alert to the possibility of these disorders in any newborn with lethargy or coma.
- Arginine may rise slowly in some cases and is more likely to be found on the second screening test.
- Citrin deficiency is more common in Asian infants.

<table>
<thead>
<tr>
<th>Results</th>
<th>Likely causes</th>
<th>Actions</th>
</tr>
</thead>
</table>
| Arginine >110 μM/L | • Arginase deficiency possible  
• Transient argininemia  
• Liver disease  
• False positive | NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations. |
| ASA >1.50 μM/L | • Argininosuccinic aciduria possible  
• Liver disease  
• False positive | NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations. |
| Citrulline >70 μM/L | • Citrulinemia, argininosuccinic aciduria possible  
• Transient citrullinemia  
• Liver disease  
• False positive | NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations. |
| Citrulline >120 μM/L on second specimen | • Mild citrulinemia, argininosuccinic aciduria possible  
• Transient citrullinemia  
• Liver disease  
• False positive | NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations |
Galactosemia essentials

- **Neonatal emergency:** 50% will die in the first 7-10 days usually from gram-negative sepsis. Acute liver disease can produce a coagulopathy and vitreous hemorrhage.
- **Incidence:** 1:60,000
- **Screening test:** GALT test (quantitative enzyme assay); Second tier: Hill test (free galactose and galactose-1-phosphate) is done on every infant with abnormal GALT test.
- **Confirmatory tests:** Enzyme assay GALT, RBC gal-1-phosphate
- **Validity:** >99% found on 1st specimen, unless transfused
- **Treatment:** Lactose restricted diet
- **Outcome:** Somewhat diminished IQs as a group, verbal and motor dyspraxia in 60%, ovarian failure in 80% of females and post-natal growth delay during childhood

Dietary galactose is most commonly ingested as lactose, the principal carbohydrate of human milk and most non-soy commercial infant formulas, which is hydrolyzed to glucose and galactose in the intestine. After absorption, galactose is metabolized by several enzymes including galactokinase and galactose-1-phosphate uridyl transferase (GALT). When deficient, the latter causes galactosemia. Galactosemia is an autosomal recessively inherited condition.

Clinical features (47)

Detection of galactosemia requires urgent follow-up and is considered a neonatal emergency. The early clinical features of severe untreated galactosemia include neonatal hypoglycemia, liver damage, jaundice, weight loss, lethargy and sepsis. Vitreous hemorrhage from coagulopathy has been reported in some infants. Death may result from gram-negative sepsis within 1–2 weeks of birth. If the infant remains untreated and survives the neonatal period, cataracts, cirrhosis, renal Fanconi syndrome and intellectual and developmental disability are usual.

Several genetic variants with less severe reduction in the enzyme activity occur (e.g., the Duarte variant). The screening test is not designed to detect variant galactosemia and is not completely sensitive for this purpose. Most of these cases are asymptomatic and are detected on newborn screening because of abnormalities in GALT.

Laboratory tests

Two screening tests are used to detect galactosemia in a two-tiered sequence:

- **GALT activity:** The enzyme test depends upon fluorescence produced by the normal galactose enzyme cascade in red blood cells. A temporarily abnormal
result (diminished or absent fluorescent activity) is found in some infants. The test may be persistently abnormal if the enzyme activity is <50% of normal. It does not differentiate milder variants from severe defects or G6PD.

- **Galactose:** Slight elevations can occur in normal neonates, but galactose metabolites are greatly elevated in infants with galactosemia if they are receiving a lactose-containing formula or breast milk. Liver disease may also cause an elevation of galactose metabolites. All infants with an abnormal GALT or who have been transfused will be screened for galactose.

**Treatment**

Galactosemia is treated by dietary galactose restriction. The diet must be followed for life and requires close supervision. Even with early diagnosis and strict dietary restrictions children with galactosemia are at risk for speech disorders, tremors, growth and developmental delays and in females, ovarian failure.

**Screening practice considerations**

- This disorder kills quickly. Transport all specimens 4–12 hours after collection and no later than 24 hours after collection.
- The GALT test should be abnormal in virtually all severe classic galactosemic infants even if the specimen is obtained before lactose is ingested, unless the infant has been transfused. Obtain a specimen before any transfusion.
- The GALT enzyme is prone to degradation if the sample is delayed in the mail or exposed to excess temperature or humidity. This produces a false positive GALT result.
- Galactose accumulation depends on lactose ingestion so that blood galactose metabolites may be normal in infants being fed a soy-based formula.

<table>
<thead>
<tr>
<th>Results</th>
<th>Likely causes</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GALT test</strong></td>
<td><strong>Galactose metabolites</strong></td>
<td></td>
</tr>
</tbody>
</table>
| <3.5 u/dL | ≥20 mg/dL | • Severe galactosemia  
• Variant galactosemia  
• False positive | NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations. |
| <3.5 u/dL | <20 mg/dL | • Severe galactosemia with little lactose intake  
• Variant galactosemia  
• Other enzyme defects in red blood cells  
• Improperly handled sample (heat damage or transit delay) | Contact by mail if infant is ≥48 hrs old; contact by fax if <48 hrs old or if not on lactose. |
Biotinidase Deficiency

Biotinidase deficiency essentials

- **Incidence:** 1:60,000 births
- **Screening test:** Biotinidase qualitative colorimetric enzyme assay
- **Confirmatory tests:** Quantitative biotinidase enzyme assay
- **Validity:** 100% found on 1st screen
- **Treatment:** 5-10 mg biotin/day
- **Outcome:** Excellent if compliant with biotin therapy

This recessively inherited disorder affects the cells’ ability to recycle the vitamin-cofactor biotin, which impairs the function of mitochondrial carboxylases. Screening is done for all infants in the region.

Clinical features (48,49)

Infants with profound biotinidase deficiency are normal at birth, but develop one or more of the following symptoms after the first weeks or months of life: hypotonia, ataxia, seizures, developmental delay, alopecia, seborrheic dermatitis, hearing loss and optic nerve atrophy. Metabolic acidosis can result in coma and death.

Infants with partial deficiency (5–10%) have been identified through newborn screening and family studies. They may remain asymptomatic with no treatment or exhibit milder symptoms than infants with profound deficiency. A reduced dose of biotin is recommended for these infants as the consequences of possible complications are too great.

Laboratory tests

Detection of enzyme activity is by a qualitative colorimetric assay. In the presence of the enzyme a color change occurs.

Treatment

Daily biotin supplements clear the skin rash and alopecia and improve the neurological status in patients not diagnosed by screening. With early diagnosis and treatment made possible by screening, all symptoms can be prevented.

Screening practice considerations

- The enzyme is prone to damage if the sample is delayed in the mail or exposed to high temperatures or excess humidity.
- Transfusion of red cells before drawing the newborn screening specimen will invalidate the biotinidase assay. Obtain a specimen before transfusion.
### Results

<table>
<thead>
<tr>
<th>Results</th>
<th>Likely causes</th>
<th>Actions</th>
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</thead>
<tbody>
<tr>
<td>Color change does not occur</td>
<td>• Biotinidase deficiency possible</td>
<td>NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.</td>
</tr>
<tr>
<td></td>
<td>• False positive</td>
<td></td>
</tr>
</tbody>
</table>

### Severe Combined Immunodeficiency (SCID)

#### SCID essentials

- **Incidence:** 1:50,000-1:100,000 births
- **Screening test:** Polymerase chain reaction to detect T-cell Receptor Excision Circles (TRECs)
- **Confirmatory tests:** CBC, lymphocyte subset flow cytometry
- **Validity:**
- **Treatment:** Bone marrow transplant, gene therapy or enzyme replacement
- **Outcome:** Good if treated within first 3 months of life

SCID is an inherited disorder that results in severe deficiency of T lymphocytes. Depending on the genetic mutation, B lymphocytes and Natural Killer cells may also be deficient.

#### Clinical features (12)

Infants may be symptomatic at birth, though most are completely healthy at birth. Symptoms of untreated SCID include recurrent infections, failure to thrive, diarrhea and thrush. The average age of diagnosis is approximately 3-6 months of age in those not screened. This usually results in the onset of one or more serious infections within the first few months of life. These infections are typically serious, and may be life threatening and may include pneumonia, meningitis, or bloodstream infections. Children affected by SCID can also become ill from live viruses present in some vaccines. These vaccines (such as chickenpox, measles, rotavirus, and oral polio) contain viruses and bacteria that are weakened and don’t harm children with a healthy immune system. In patients with SCID however, these viruses and bacteria may cause severe, life-threatening infections.

#### Causes of SCID

The term severe combined immunodeficiency is a group of disorders. All forms of SCID are inherited with the most common an x-linked dominant disorder that affects only males. Other forms of SCID are autosomal recessive.
**Laboratory tests**

Screening is based on evaluating the number of T cell receptor excision circles (TRECs) in the dried blood spots. TRECS are a piece of DNA produced during the formation of t-cells in the thymus. Although this testing is DNA based, TREC analysis is not a test of gene mutations. TRECs may be low in infants with non-SCID-related causes of T-cell lymphopenia, who will also require evaluation and management.

**Confirmation**

Confirmation is by measuring CBC with differential and flow cytometry to determine the extent of the cell lymphopenia.

**Treatment**

Infants may receive bone marrow transplant, gene therapy or enzyme replacement depending on the exact mutation causing their particular form of SCID.

**Lysosomal Storage Disorders (LSDs)**

More information is available at [www.healthoregon.org/nbs](http://www.healthoregon.org/nbs).

**What are LSDs?**

LSDs are a group of over 40 genetic disorders that result in enzyme deficiencies within the lysosomes of the body’s cells, causing the build-up and storage of certain compounds which results in irreversible damage to the muscles, nerves, and organs in the body over time. Treatments are available for these disorders which are most effective if they are identified early.

**Which LSD’s are being tested:**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Enzyme</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabry Disease</td>
<td>Alpha Galactosidase A</td>
<td>GLA</td>
</tr>
<tr>
<td>Gaucher Disease</td>
<td>Acid Beta-Glucosidase</td>
<td>GBA</td>
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<tr>
<td>Mucopolysaccharidosis Type 1 (MPS-1)</td>
<td>Alpha-L-Iduronidase</td>
<td>IDUA</td>
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<td>Pompe Disease</td>
<td>Acid Alpha-Glucosidase</td>
<td>GAA</td>
</tr>
</tbody>
</table>
**How are LSDs diagnosed?**

Newborn screening for LSDs is done by measuring enzyme activity from newborn blood spots. Second tier DNA-based testing is done when indicated by initial results prior to reporting the final result. Diagnosis following an abnormal newborn screen requires further enzyme or DNA-based testing and should be done by a specialist with experience in the diagnosis and treatment of LSDs. Consult with a specialist immediately.

**Resources**

http://www.babysfirsttest.org

https://www.newbornscreening.info

https://www.acmg.net/


3. National Newborn Screening and Genetics Resource Center, Austin TX website: genes-r-us.uthscsa.edu


31. Shekhawat PS, Matern D, Strauss AW. Fetal fatty acid oxidation disorders, their effect on maternal health and neonatal outcome: impact of expanded newborn screening on their diagnosis and management. Pediatr Res. 2005 May;57(5 Pt 2):78R-86R.


Northwest Regional Newborn Screening Program

A collaborative project involving:

Oregon Health Authority
Oregon Health & Science University
Idaho Department of Health and Welfare
New Mexico Newborn Screening Program

You can get this document in other languages, large print, braille or a format you prefer. Contact the Newborn Screening Program at 503-693-4174 or NWRegional.NBS@state.or.us. We accept all relay calls or you can dial 711.