

Oregon Newborn Bloodspot Screening Practitioner's Manual



Northwest Regional Newborn Bloodspot Screening Program



PUBLIC HEALTH DIVISION
Oregon State Public Health Laboratory



Newborn Bloodspot Screening Practitioner's Manual

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Introduction

Welcome! The purpose of this manual is to provide useful information to health care providers about the Northwest Regional Newborn Bloodspot Screening (NBS) Program. This program provides services to multiple states and territories, including Oregon, New Mexico, Guam, Saipan, several Tribal nations and others. The Northwest Regional NBS Program is part of the Oregon State Public Health Laboratory (OSPHL). Specimens are received and tested by the NBS laboratory and abnormal results are referred to the follow-up team.

This manual describes the process of newborn bloodspot screening from collection through result reporting, and follow-up for babies who screen positive for a condition. It outlines the roles and responsibilities of the NBS Program, medical practitioners, and parents. It also discusses newborn bloodspot screening practice standards, common issues that can occur during screening, and links to helpful resources. We invite practitioners to contact us with any questions, concerns, or suggestions on how to improve this manual.

Contact information and additional resources are available at www.healthoregon.org/nbs. Healthcare practitioners working in the state of New Mexico can locate contact information for the New Mexico Newborn Genetic Screening Program at <https://www.nmhealth.org/about/phd/fhb/cms/nbgs/>.

The goal of NBS is to detect treatable medical conditions within the first two weeks of life.

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 500 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 bloodspot screening is so important are:

- Approximately 20 disorders can kill or severely harm an infant if untreated 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.

Newborn bloodspot screening is changing rapidly and will continue to change in the future. While states are trying to develop standard newborn bloodspot screening recommendations, variation continues from state to state, and practitioners must be aware of the newborn bloodspot screening practice that applies to their patients. Practitioners who are licensed in Oregon or treat Oregon residents must orient to the newborn bloodspot screening rules and regulations that apply.

If you are a practitioner serving outside of Oregon, other regulations may apply. Healthcare practitioners working in the state of New Mexico can locate information for the New Mexico Newborn Genetic Screening Program at <https://www.nmhealth.org/about/phd/fhb/cms/nbgs/>.

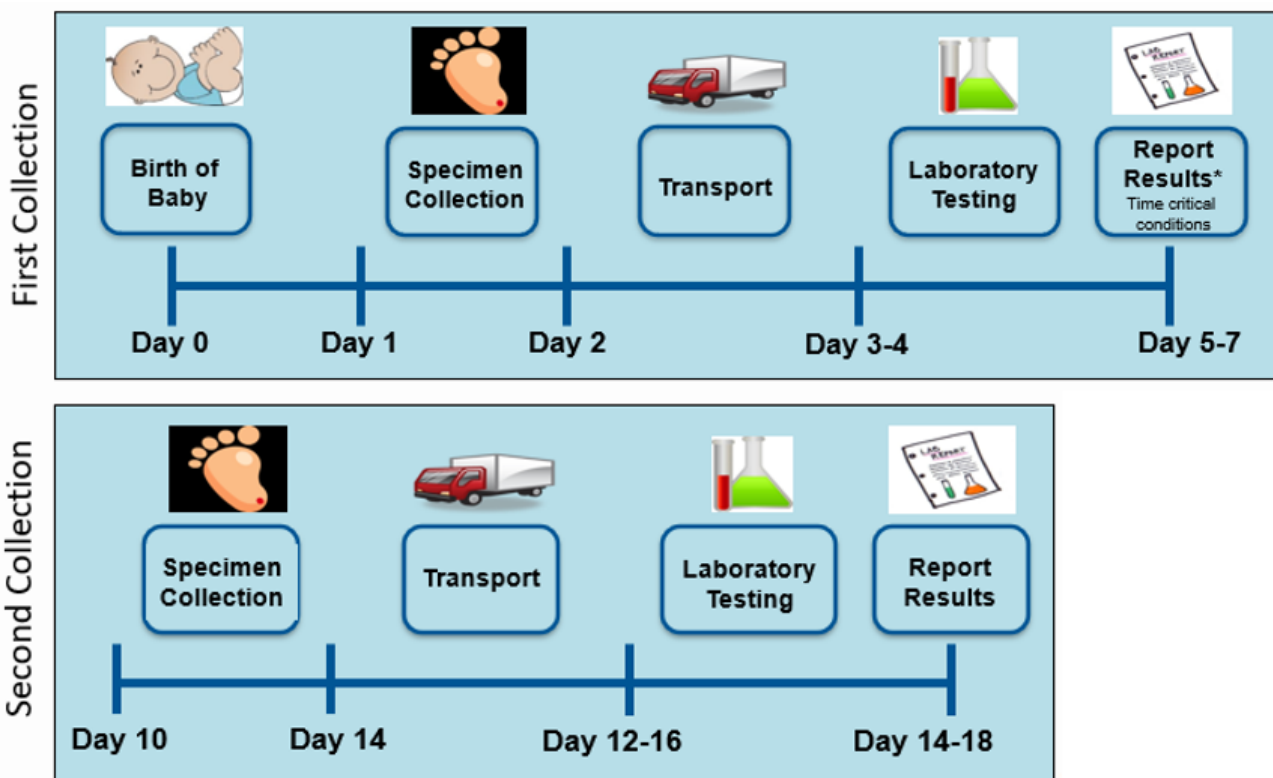
Oregon began newborn bloodspot screening for PKU (Phenylketonuria) in 1963. Since then, newborn bloodspot screening has expanded to include other metabolic conditions, cystic fibrosis, sickle cell disease, severe combined immunodeficiency (SCID), and several lysosomal diseases. The laboratory screens for the medical conditions listed in this manual. Additional related conditions may be identified and are described in the condition sections at the end of this manual.

Babies born in Oregon are screened at two different time intervals. The first specimen collected at 24-48 hours is important to identify time critical conditions that require immediate treatment, and the second specimen collected at 10-14 days will help to pick up those instances of later onset disease. Parents who choose to only have one specimen collected should be informed of the risk.

Newborn bloodspot screening is not intended to diagnose an infant's medical condition. Newborn bloodspot 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 bloodspot screening.

Effective communication is essential for newborn bloodspot screening to succeed.

Newborn bloodspot screening process



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

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



Advisory Board

The purpose of the Northwest Regional Newborn Bloodspot Screening (NWRNBS) Advisory Board (The Board) is to provide advocacy, advice, recommendations, and technical information to the program based on members' respective areas of expertise. The Board is comprised of 13 partners within the newborn bloodspot screening community that include representatives of hospitals, birth centers, families, insurance, midwifery, nursing, and pediatrics (ORS 433.299).

The Board assists the NWRNBS Program with strategic planning and the development of policies, priorities, and services related to newborn screening including the addition or removal of diseases to the screening panel. As part of the review process to add a new condition to the panel, the board evaluates the scientific evidence for screening and receives input from families and advocates, whose lives are impacted by the disease. In all recommendations made by the board, the whole newborn screening system is considered and how it can improve health outcomes for all infants and their families.

If you are interested in participating in the NWRNBS Advisory Board, please send an email to NWRegional.NBS@odhsoha.state.or.us

Additional information on the criteria and process that the advisory board uses to evaluate conditions for inclusion to the panel, can be found on the Oregon NBS website:

<https://bit.ly/NWRNBSadvisoryboard>



Definitions

“Abnormal Result” means the result of the laboratory screening meets criteria for follow-up testing and may require medical evaluation.

“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 specimen collection card (also known as the filter paper collection device) and the attached demographic form for the purposes of collection and submission of specimens for newborn bloodspot screening.

“Newborn bloodspot screening” means a test performed on infants to screen for specific medical conditions. Newborn bloodspot screening replaces the outdated terms ‘PKU test’, ‘PKU screening’ or ‘the PKU’.

“Practitioner” means: the person who takes responsibility for the delivery or health care of an infant born in Oregon. This may include the following:

- a) A physician licensed under ORS 677;
- b) A naturopathic physician licensed under ORS 685;
- c) Advanced practice registered nurse licensed under ORS 678;
- d) A chiropractic physician licensed under ORS chapter 684;
- e) A direct entry midwife licensed under ORS 687, or
- f) Any practitioner responsible for the infants’ medical care soon after birth.

“Specimen” means: a blood specimen obtained from an infant that has been applied to the filter paper of a specimen collection card.



Newborn bloodspot screening responsibilities in Oregon

This section describes the responsibilities for successful newborn bloodspot screening in Oregon. Practitioners caring for patients in other jurisdictions will need to comply with other regulations.

Newborn bloodspot 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 bloodspot screening.
- **Northwest Regional Newborn Bloodspot Screening (NWRNBS) Program:** The program consists of two entities; the laboratory and the follow-up team. The laboratory is responsible for testing, record keeping, and ensuring quality of laboratory methods and results. The follow-up team notifies providers of results, tracks abnormal and unresolved results, and provides educational resources.
- **Oregon Health & Science University (OHSU) subspecialty programs:** These partners are responsible for providing consultation services to practitioners and the NWRNBS Program.

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 also share the responsibility for ensuring their infants are tested.

Practitioners are responsible for ensuring that newborn bloodspot screening is performed.

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 OSPHL 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.

Education services

The Oregon NBS program provides training and education services to improve the quality of newborn bloodspot screening practices. These include a quality assurance surveillance program, facility site-visits, and comprehensive reviews of screening systems by the NBS public health nurse. In addition, training and education resources are available for both practitioners and parents on the Oregon NBS website.

Practitioners: <https://bit.ly/providersNBSresources>

Parents: <https://bit.ly/ParentNBSresources>

Fee exemption for Oregon births

In Oregon, no person is refused service because of the inability to pay the fee for screening (OAR 333-024-1100).

A practitioner can request a waiver of fees from the Oregon Health Authority, provided that the client is unable to pay.

Fee waivers must be received by the Oregon State Public Health Laboratory prior to birth of the baby, but no later than 30 calendar days from the day the infant was born.

Upon receipt of the fee waiver request and confirmation through Oregon Health Authority records that exemption criteria are met, the Oregon Health Authority will issue a refund check or provide or replace the kit to the payer of record.

For additional information, visit [our website](#).

Parent refusal to have the infant screened in Oregon

A parent may opt not to have their infant screened. The Oregon administrative rules provide the acceptable reasons in which parents can decline screening. (OAR 333-024-1000 and OAR 333-024-1050)

The refusal form is located on the back of the filter paper of the specimen collection card. See [“How to Fill Out the Specimen Collection Card”](#) for more information on how to fill out the specimen collection card when there is a refusal.

*For submitters outside of Oregon, reference your own state NBS program.



Medical conditions on the newborn bloodspot screening panel

Oregon newborns are screened for the following medical conditions recommended by the Northwest Regional NBS Program Advisory Board. More information on these medical conditions is available at the end of this manual and at:

- Baby's First Test: <https://babysfirsttest.org/>
- The Oregon State Public Health Laboratory: www.healthoregon.org/nbs
- The American College of Medical Genetics (ACMG) Newborn Screening ACT Sheets and Algorithms: [ACT Sheets and Algorithms](#)

Table 1: Medical conditions on the Oregon newborn bloodspot screening panel

MEDICAL CONDITION	ANALYTE(S) TESTED FOR	INCIDENCE IN NW REGION	SYMPTOMS IF NOT TREATED	COMMON MEDICAL TREATMENT
ORGANIC ACID DISORDERS				
Propionic acidemia (PA)*	C3, C3/C2	1 per 271,000	Vomiting; lethargy; acidosis possibly resulting in death	Protein-restricted diet; medical formula; carnitine therapy
Methylmalonic acid (MMA)*	C3, C3/C2	1 per 95,000	Vomiting; lethargy; acidosis possibly resulting in death	Protein-restricted diet; medical formula; carnitine therapy and hydroxocobalamin therapy
Isovaleric acidemia (IVA)	C5, C5/C2, C5/C3	1 per 148,000	Vomiting; lethargy; acidosis possibly resulting in coma, death	Protein-restricted diet; carnitine and glycine therapy
3-methylcrotonyl CoA carboxylase deficiency (3MCC)	C5OH	1 per 51,000	Most have been asymptomatic	None, except carnitine therapy if deficient

MEDICAL CONDITION	ANALYTE(S) TESTED FOR	INCIDENCE IN NW REGION	SYMPTOMS IF NOT TREATED	COMMON MEDICAL TREATMENT
3-hydroxy-3-methylglutaryl CoA lyase deficiency (HMG)	C5OH, C6DC	Rare, less than 1 per 300,000	Hypoglycemia; acidosis possibly resulting in death	Protein restriction
Holocarboxylase Synthase Deficiency	C3, C5OH	Rare, less than 1 per 300,000	Hypotonia; seizures; skin rash; alopecia; lactic acidosis; brain damage	Biotin therapy
Beta-ketothiolase deficiency (BKT)	C5:1, C5OH	Rare, less than 1 per 1 million	Severe bouts of acidosis possibly resulting in intellectual and developmental disability or death	IV support during episodes; bicarbonate supplement
2-methyl-3-hydroxybutyryl CoA dehydrogenase deficiency (2M3HBA)	C5:1, C5OH	Rare, less than 1 per 1 million	Loss of the developmental milestones and motor skills. Developmental delays.	Protein restriction
Glutaric acidemia, type 1 (GA-1)	C5DC, C5DC/C8, C5DC/C16	1 per 85,000	Often asymptomatic in newborn; sudden metabolic crisis damages basal ganglia	IV support during intercurrent illness; protein restriction; carnitine therapy
Malonic acidemia (MAL)	C3DC, C3DC/C10	Rare, less than 1 per 300,000	Intellectual disability	Carnitine therapy; MCT oil therapy; long chain fat restriction; avoidance of fasting
Isobutyryl-CoA dehydrogenase deficiency (IBD)	C4, C4/C2 C4/C3	Rare, less than 1 per 300,000	None to severe cardiomyopathy	Carnitine therapy; protein restriction; avoid fasting
2-methylbutyryl CoA dehydrogenase deficiency (2MBC)	C5, C5/C2 C5/C3	1 per 181,000 (Hmong have higher incidence)	Hypoglycemia; intellectual and developmental disability; Hmong infants are often asymptomatic	None or avoid fasting
3-methylglutaconyl CoA hydratase deficiency (3MGH)	C5OH	Rare, less than 1 per 1.3 million	Hypoglycemia; acidosis; may be asymptomatic	Protein restriction; avoid fasting
FATTY ACID OXIDATION DISORDERS				
Carnitine uptake deficiency (CUD)	C0	1 per 116,000	Hypoglycemia; cardiomyopathy	Carnitine therapy

MEDICAL CONDITION	ANALYTE(S) TESTED FOR	INCIDENCE IN NW REGION	SYMPTOMS IF NOT TREATED	COMMON MEDICAL TREATMENT
Medium chain acyl-CoA dehydrogenase deficiency (MCAD)*	C6, C8, C10:1, C8/C10	1 per 19,000	Hypoglycemia possibly resulting in coma, death; may be asymptomatic	Avoid fasting; carnitine therapy if deficient
Very long chain acyl-CoA dehydrogenase deficiency (VLCAD)*	C14, C14:1, C14:1/C16	1 per 62,500	Hypoglycemia with or without cardiomyopathy; muscle fatigue	Avoid fasting; low fat diet with MCT oil supplement; carnitine therapy
Long chain 3 hydroxyacyl-CoA dehydrogenase deficiency (LCHAD)*	C16OH	1 per 541,000	Hepatic dysfunction; hypoglycemia; failure to thrive	Long chain fatty acid restriction; medium chain triglycerides (MCT) oil supplement; carnitine therapy; avoid fasting
Trifunctional protein deficiency (TFP)	C16OH	Very rare. Incidence unknown	Feeding difficulties; lethargy; hypoglycemia; low muscle tone; liver problems	Long chain fatty acid restriction; medium chain triglycerides (MCT) oil supplement; carnitine therapy; avoid fasting
Short chain acyl-CoA dehydrogenase deficiency (SCAD)	C4, C4/C2 C4/C3	1 per 81,000	Most asymptomatic; hypotonia, intellectual and developmental disability	None
Glutaric acidemia type II, also known as Multiple acyl-CoA dehydrogenase deficiency (MADD)	C4, C5, C6, C8, C10, C12,	1 per 541,000	Multiple congenital abnormalities; acidosis; hypoglycemia	Low fat diet; avoid fasting
Carnitine palmitoyl transferase deficiency, type I (CPT-I)	C0, C0/ (C16+C18)	1 per 812,000	Hypoketotic hypoglycemia, brought on by fasting or intercurrent illness; Average age at presentation: birth to 18 months	Avoid fasting and long chain fatty acids; MCT oil supplement
Carnitine palmitoyl transferase deficiency, type II (CPT-II)*	C16 (C16+C18:1)/C2	1 per 400,000	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	Avoid fasting and severe exercise; MCT oil supplement

MEDICAL CONDITION	ANALYTE(S) TESTED FOR	INCIDENCE IN NW REGION	SYMPTOMS IF NOT TREATED	COMMON MEDICAL TREATMENT
Carnitine acylcarnitine translocase deficiency (CACT)	C16, (C16+C18:1)/C2	Very rare. Incidence unknown.	Fatigue; irritability; poor appetite; fever; diarrhea; vomiting; hypoglycemia; seizure; hypotonia	Avoid fasting and severe exercise; MCT oil supplement; L- carnitine supplement
AMINO ACID DISORDERS				
Argininosuccinate lyase deficiency (Argininosuccinic aciduria; ASA)*	ASA, ASA/Citrulline	1 per 125,000	Hyperammonemia; intellectual and developmental disability; seizure; death	Protein-restricted diet; medical formula; medication
Citrullinemia, type I (CIT)*	Citrulline	1 per 325,000	Hyperammonemia; intellectual and developmental disability; seizure; death	Protein-restricted diet; medical formula; medication
Maple syrup urine disorder (MSUD)*	Leucine, Leu/Alanine	1 per 271,000	Vomiting; lethargy; acidosis possibly resulting in death	Protein-restricted diet; and medical formula
Homocystinuria (HCY)	Methionine	1 per 203,000	Intellectual and developmental disability; dislocation of lenses; marfanoid body habitus; strokes	Pyridoxine; protein- restricted diet; medical formula; Foltanx
Phenylketonuria (PKU)	Phenylalanine, Phenylalanine/Tyr osine	1 per 28,500	Profound intellectual and developmental disability; seizures	Protein-restricted diet; medical formula; Kuvan if responsive
Tyrosinemia, type I	Succinylacetone	1 per 812,000	Vomiting; lethargy; liver disease; coagulopathy; renal tubular acidosis	Protein-restricted diet; medical formula; medication
Tyrosinemia, type II and type III	Tyrosine	1 per 652,000	Corneal thickening; developmental delay; hyperkeratosis of palms and soles	Protein-restricted diet; medical formula; medication
Arginase deficiency (ARG)	Arginine	1 per 1.6 million	Irritability; developmental delay; spastic tetraplegia	Protein-restricted diet; medical formula; medication
ENDOCRINE DISORDERS				
Primary congenital hypothyroidism	TSH	1 per 2,300	Intellectual and developmental disability; other brain damage; growth delay	Thyroid hormone

MEDICAL CONDITION	ANALYTE(S) TESTED FOR	INCIDENCE IN NW REGION	SYMPTOMS IF NOT TREATED	COMMON MEDICAL TREATMENT
Congenital adrenal hyperplasia (CAH)*	17-OH-progesterone	1 per 12,700	Addisonian crisis/salt wasting in 3/4 infants; dehydration; shock; hyperkalemia; virilization of females	Glucocorticoid and/or mineralocorticoid therapy
PULMONARY DISORDERS				
Cystic fibrosis (CF)	Immunoreactive Trypsinogen (IRT) 2nd tier DNA analysis	1 per 6,500	Lung disease; growth failure	Pulmonary therapy; prevent infection; replace digestive enzymes
OTHER METABOLIC DISORDERS				
Biotinidase deficiency	Biotinidase	1 per 60,000	Intellectual and developmental disability; seizures; skin rash; alopecia; hearing loss; death	Biotin therapy
Classic galactosemia (GALT)*	Galactosemia enzyme (GALT) 2nd tier total galactose	1 per 95,000	Neurodevelopmental impairment; liver disease; cataracts; Gram-negative sepsis in newborns	Galactose-restricted diet
HEMOGLOBIN DISORDERS				
Sickle cell disease	Hemoglobin patterns	1 per 10,000 (1 per 365 in Black or African Americans)	In sickle cell disease: death by sepsis or splenic sequestration anemia; sickling crisis	Penicillin and comprehensive care
IMMUNOLOGY DISORDERS				
Severe combined immunodeficiency (SCID)	T-cell receptor excision circles (TRECs)	1 per 50,000 to 1 per 100,000	Severe respiratory infection; poor growth; rashes appear like eczema; chronic diarrhea; recurrent oral thrush	Bone marrow transplant
LYSOSOMAL DISORDERS				
Pompe* (glycogen storage disease Type II)	Alpha-glucosidase (GAA)	1 per 28,000	Generalized muscle weakness; respiratory failure; cardiomegaly; enlarged liver; hearing loss	Enzyme replacement therapy

MEDICAL CONDITION	ANALYTE(S) TESTED FOR	INCIDENCE IN NW REGION	SYMPTOMS IF NOT TREATED	COMMON MEDICAL TREATMENT
Mucopolysaccharidosis Type I (MPS I)*	Alpha-L-iduronidase (IDUA)	Between 1 per 87,000 and 1 per 185,000	Skeletal abnormalities; cognitive impairment; heart disease; cloudy corneas; deafness	Bone marrow transplant; enzyme replacement therapy
Fabry	Alpha-galactosidase (GLA)	Between 1 per 1,500 and 1 per 13,000	Renal failure; Hypertrophic cardiomyopathy; Pain in hands and feet; poor sweating; irritable bowels; proteinuria; hearing loss	Enzyme replacement therapy
Gaucher*	Beta-glucocerebrosidase (GBA)	1 per 57,000	Enlarged spleen and liver; low platelets; anemia; bone disease; Type III have eye tracking issues as well	Enzyme replacement therapy
Infantile Krabbe Disease (IKD)	Galactocerebrosidase (GALC)	1:100,000 to 250,000	Extreme irritability; feeding difficulties; failure to thrive; spasticity	Bone marrow transplant
OTHER CONDITIONS				
Spinal muscular atrophy (SMA)2	Exon 7 of the SMN1 gene	1:11,000	Age of onset and severity vary depending on type; some level of muscle weakness and atrophy can be expected	Disease modifying treatment; gene therapy
X-linked adrenoleukodystrophy (X-ALD)	C26:0 Lysophosphatidylcholine (C26:0-LPC)	1:4,845	Progressive damage in tissues and organs, particularly in the adrenal glands, brain and spinal cord	Cortisol replacement and/or hematopoietic stem cell transplant (HSCT)

* Infants may have severe neonatal presentation.

Newborn bloodspot screening may identify other related medical conditions that are not listed above. Information regarding these related conditions can be found in the relevant condition sections below. It is within the discretion of an infant's health care provider and parents or legal guardians to determine what, if any, medical follow-up is needed in these circumstances.



Newborn bloodspot screening kits

Oregon practitioners must order newborn bloodspot screening kits 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.

New Mexico practitioners can find information about ordering kits at www.nmhealth.org/about/phd/fhb/cms/nbgs/. 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.

Figure 1: Specimen barcode and kit number:



Double Kits

Double kits are used for most births. Each specimen in the kit has a barcode and kit number that allow the second specimen to be matched easily by the screening lab to the data from the first specimen. This matching system helps to link the data from newborn bloodspot 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 infants who meet specific criteria for a third specimen collection. See [page 22](#) for more information.

Each specimen in the kit has a barcode and a kit number that allow the second specimen and third specimen to be matched easily by the screening lab to the data from the first specimen. This matching system helps to ensure that newborn bloodspot 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 first 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.



Timing for specimen collection

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.

**NBS Follow-up Team
503-693-4174**

Newborn bloodspot screening must be collected as described below. If an infant presents for medical care outside of the timelines established below, collect and submit the bloodspot specimen as soon as possible up to six months of age (OAR 333-024-1030).

Routine births

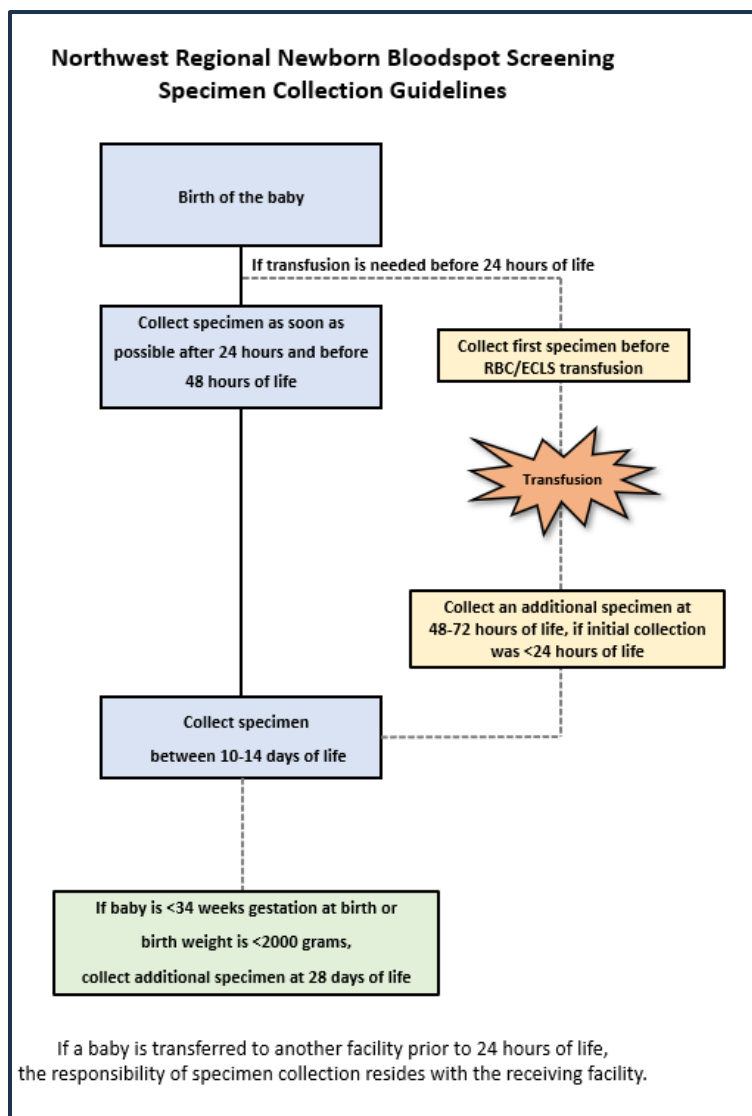
For routine births use a newborn bloodspot screening double kit. The first specimen must be collected as soon as possible after 24 hours of life but before 48 hours of life and a second specimen must be collected between 10 and 14 days of life as shown in the Specimen Collection Guidelines chart on the next page.

After the first specimen is collected, the second specimen in the double kit is routed to the provider who will collect the second specimen. Many hospitals choose to send the second part of the kit with the parent to give to the follow up provider. It is also acceptable for the hospital to send the second specimen collection card directly to the PCP clinic where the baby will receive care. This practice works the best in rural areas and in cases where the hospital and PCP clinic are co-located.

If the primary care provider does not receive a second specimen collection card to perform a collection between 10 and 14 days, a single kit should be used to collect a specimen.

Special Specimen Collection Circumstances

NBS Specimen Collection Guidelines:



RBC/ECLS transfusion:

If an infant is going to have an RBC/ECLS transfusion prior to 24 hours of life, collect the first specimen prior to the transfusion. Collect an additional specimen at 48-72 hours of life, if the initial collection was prior to 24 hours of life. Check the 'transfusion' box on the specimen collection card and indicate the date/time of the last RBC transfusion. Another specimen will need to be collected at 10-14 days of life.

Infants who require an additional specimen collection:

An infant should have an additional specimen collection at 28 days of life if they meet either of the following criteria:

- Infant was born at less than 34 weeks gestation; OR
- Birth weight was less than 2000 grams.

If the infant meets either of the above criteria, use a 3-part specimen collection kit for specimen collection. If a double kit is used, a single kit should be used for the collection at 28 days.

Transfer to a different medical facility prior to first specimen collection

If an infant is transferred to a different medical facility in Oregon **prior** to first specimen collection at 24 hours of life, the receiving facility is responsible for collecting the NBS specimen using the recommended guidelines. The facility that delivered the baby should still complete the demographic information on the first specimen collection card and check the 'transferred' box on the 'blood not submitted' section. The cards for that infant should be sent to the OSPHL. **Do not send specimen collection cards to the receiving facility.**

Transfer to a different medical facility after first specimen collection

For infants that are transferred to another facility **after** the first specimen has been collected, the receiving facility is responsible for collecting any additional screenings. The transferring facility should complete the demographic information on the remaining specimen collection cards (second or third card), check the 'transferred' box on the 'blood not submitted' section, and send the remaining specimen collection cards to the OSPHL. **Do not send specimen collection cards to the receiving facility.**

Out of state transfers:

If an infant is transferred out of state **prior** to the first specimen collection, the receiving facility is responsible for collecting the NBS specimen following their state NBS collection guidelines. The transferring facility should complete the demographic information on the first specimen collection card, check the 'transferred' box on the 'blood not submitted' section and send all the infant's specimen collection cards to the OSPHL. **Do not send specimen collection cards to the receiving facility.**

If an infant is transferred out of state **after** the first specimen has been collected, the receiving facility is responsible for collecting additional NBS specimens following their state NBS collection guidelines. The transferring facility should complete the demographic information on the second specimen card, check the 'transferred' box on the 'blood not submitted' section and send the specimen collection card to the OSPHL. **Do not send specimen collection cards to the receiving facility.**



Early discharge

A first specimen should be collected as soon as possible after 24 hours of life but before 48 hours. If a family is requesting an early discharge, they should be counseled on the risks of inaccurate results that increase when a specimen is collected prior to 24 hours of life. If a family decides to leave prior to 24 hours, collect the first specimen before they leave your care. Some infants may not return for routine postnatal care.

Older infants

The Oregon State Public Health Laboratory has established procedures for testing specimens from newborns and infants up to 6 months of age. A specimen must be collected

and received at the state lab for testing before the baby is 6 months of age. The Oregon State Public Health Laboratory cannot perform newborn bloodspot screening testing for infants older than 6 months of age.



Completing the specimen collection card

A specimen collection card must be completed for every infant, even if blood will not be collected. The specimen collection card must be complete, accurate, and legible for the proper identification of the infant and interpretation of results.

- Do not use acronyms or abbreviations when completing the specimen collection card.
- Incomplete demographic information may result in a delay in specimen testing.
- The state lab staff do not have access to any type of electronic health record system.
- All the fields on the specimen collection card must be completed.
- Use a medium ballpoint pen with black ink to ensure that the information goes through the multiple layers of the card.
- Do not use cursive handwriting.

Only fill out the specimen card for the specimen you will be collecting. If you are a birth facility submitter, do not fill out the information on the second specimen card that is sent home with parents.

Specimen collection card care and storage

Unused specimen collection cards should always be stored on their edge, in a filing cabinet or accordion folder to decrease the risk of compressing or damaging the filter paper. Specimen collection cards should never be folded prior to, during, or after collection. **Do not tape, paper clip or staple anything to the specimen collection card.**

The specimen collection card supply should be reviewed quarterly to ensure cards will not expire in the next 3 months. Cards that are close to their expiration date can be exchanged for new cards. Contact the OSPHL at 503-693-4100 and ask for NBS Kit Orders.

Rotate the supply of specimen collection cards when new cards arrive so the cards that expire the soonest are used first.

The filter paper portion of the specimen collection card should never be handled or touched to avoid contamination.

How to fill out the specimen collection card:

1. Ensure you are using the correct specimen collection card.

The first specimen must be collected using the first specimen collection card, the second specimen collected using the second specimen card etc.

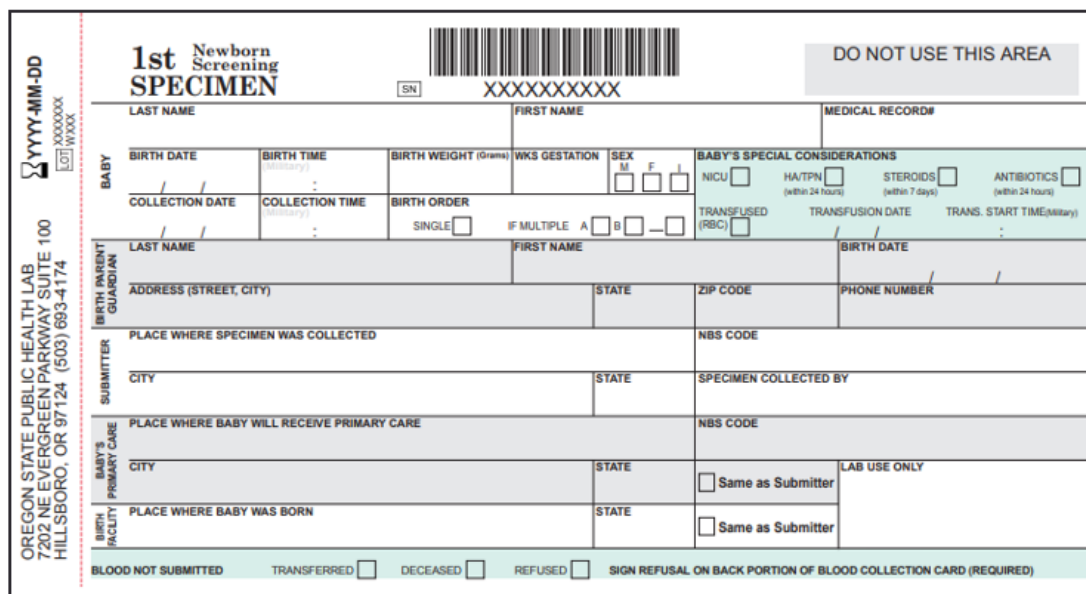
If the specimen collection cards are not used in the correct order, the infant's results may not link correctly within the laboratory information system. This could delay screening for hemoglobinopathies, cystic fibrosis, SMA, LDs, and SCID, which are routinely performed only on the first specimen.

Be sure to use the correct part of the kit for your collection.

2. Check the expiration date on the specimen collection card.

The expiration date can be found along the margin of the card, on the back of the protective cover and on the filter paper. A specimen will be reported as unsatisfactory if it expires before testing can be performed. If you have expired cards in your inventory, reach out to the OSPHL for exchange information.

Figure 2: Newborn Bloodspot Screening Specimen Collection Card Demographics Section



The form is titled "1st Newborn Screening SPECIMEN" and includes a barcode. It is divided into several sections for data entry:

- BABY:** LAST NAME, FIRST NAME, MEDICAL RECORD#, BIRTH DATE, BIRTH TIME, BIRTH WEIGHT (Grams), WKS GESTATION, SEX (M/F), NICU, HA/TPN, STEROIDS, ANTIBIOTICS, TRANSFUSED (RBC), TRANSFUSION DATE, TRANS. START TIME.
- BIRTH PARENT GUARDIAN:** LAST NAME, FIRST NAME, BIRTH DATE, ADDRESS (STREET, CITY), STATE, ZIP CODE, PHONE NUMBER.
- SUBMITTER:** PLACE WHERE SPECIMEN WAS COLLECTED, CITY, STATE, NBS CODE, SPECIMEN COLLECTED BY.
- BABY'S PRIMARY CARE:** PLACE WHERE BABY WILL RECEIVE PRIMARY CARE, CITY, STATE, NBS CODE, LAB USE ONLY (Same as Submitter).
- BIRTH FACILITY:** PLACE WHERE BABY WAS BORN, STATE, LAB USE ONLY (Same as Submitter).

At the bottom, there are checkboxes for "BLOOD NOT SUBMITTED", "TRANSFERRED", "DECEASED", "REFUSED", and a note to "SIGN REFUSAL ON BACK PORTION OF BLOOD COLLECTION CARD (REQUIRED)".

3. Fill in each section of the card

- **Baby's information:** This information is used to identify the baby and provide the lab with information about the birth and specimen collection. Enter all the requested information.

- The name of the infant should match the birth certificate whenever possible.
 - Time should be entered using military time.
 - Weight should be entered in grams.
 - It is acceptable to apply a patient label to the back of demographics section of the specimen collection card, but please remember to complete all the requested information.
- **Baby's special considerations:** Review the baby's special considerations and check the appropriate boxes if baby has received steroids within the past seven days, antibiotics within the past 24 hours, TPN at time of specimen collection including formula with added amino acids, or an RBC transfusion, including RBCs given in utero.
- a. **Birth parent/guardian's information:** This information is used to locate and contact the family, if needed.
 - Enter all the information for one of the parents or the guardian who will be caring for baby.
 - For adoption/surrogacy, enter one of the adoptive parent's information.
 - For a baby who is in foster care, enter the foster care parent's information or the information of the case worker if the foster placement has not been determined.
 - **Make sure to include the phone number.**
 - b. **Submitter's information:** This information is used for data tracking and to contact the submitter if there are questions about the specimen collection.
 - Enter the information where the specimen was collected.
 - For a hospital birth, enter the birth facility name and the unit if more than one unit at the facility collects newborn screens (ie MBU, NICU, Pediatric unit.)
 - For a community birth provider, enter the clinic practice business name or the full name of the community birth provider if they are not affiliated with a clinical practice.
 - For a primary care provider clinic enter the full name of the clinic.
 - Enter the NBS code for the submitter. A searchable database of NBS codes can be found on our website at <https://bit.ly/NBSCodes>.
 - Specify the clinic location if there are multiple locations.
 - Enter the city and state information.
 - c. **Baby's primary care:** This information will be used to identify the baby's primary care provider clinic. The primary care provider will receive and act upon the screening results as needed.

- Enter the full name of the clinic where baby will receive primary care. This could be the NICU if the baby will be having an extended stay.
 - Enter the NBS code for the baby's primary care clinic.
 - Enter the city and state where the facility is located.
 - If the baby's primary care clinic is the same as the submitter information, check the 'same as submitter' box in this section. If this box is checked it is not necessary to fill in the rest of the 'baby's primary care section'.
- d. **Birth facility:** This information is used to identify the facility where baby was born.
- For a hospital, enter the hospital name.
 - For a community birth provider, enter your full name or your clinic name.
 - Enter the state where the facility is located.
 - If the place where baby was born is the same as the submitter information, check the 'same as submitter' box. If this box is checked it is not necessary to fill in the rest of the 'birth facility' section.
- e. **Blood not submitted:** The NBS program requires a specimen collection card be completed for every baby. To ensure that every baby has the opportunity to be screened, the collector must complete the specimen collection card, even if blood will not be collected. The NBS program will replace double kits submitted by birth facilities and community birth providers. There are 3 possible reasons that blood is not collected for the baby: baby is transferred prior to specimen collection, baby expires prior to specimen collection, or parent/guardian refuses the specimen collection.
- Baby is transferred: (see [pp22-23](#) for more specific information).
 - Baby expires: Complete the information on the first specimen collection card. Check the 'deceased' box. Send all the specimen collection cards for that baby back to the OSPHL. In the case of a fetal demise, it is not necessary to complete a specimen collection card.
 - Parent or guardian refusal: If the parent or guardian refuses to have the NBS specimen collected, additional education should be provided about the benefits of newborn bloodspot screening.
 - After additional education if parent or guardian declines, they must review, complete and sign the refusal form, located on the back of the filter paper of the specimen collection card. If the parent or guardian refuses to sign the refusal form, indicate that on the card. The exemption form is available in other languages on the Oregon NBS website: <https://bit.ly/providersNBSresources>.
 - Complete the information on the first specimen collection card. Send the first specimen card to the OSPHL. Send the second specimen card home with the family, if they are willing. Sometimes a family will accept screening when they meet with the PCP.



Heel stick specimen collection instructions

The preferred newborn bloodspot screening (NBS) specimen is capillary blood obtained from a heel stick. A training detailing proper collection and helpful resources can be found at <https://bit.ly/providersNBSresources>.

Prior to specimen collection:

There are steps you can take to improve blood flow and/or reduce pain for the infant prior to specimen collection:

- **Dress baby warmly**
 - Dress baby in warm clothing and then swaddle baby in a blanket before and during the specimen collection.
- **Position baby upright**
 - Position baby so the foot will be lower than the level of the heart prior to and during specimen collection. A parent or caregiver can hold the baby upright during the specimen collection. If the baby will be placed in an isolette or on an exam table during specimen collection, angle the surface so the infant's heart is above the foot. Allowing the leg and foot to dangle can also be helpful to promote blood flow.
- **Leg Massage**
 - Gently massage the infant's leg from the thigh towards the toes, promoting blood flow to the foot.
- **Breastfeeding or non-nutritive sucking**
 - The birth parent can breastfeed during specimen collection to reduce pain. If a pacifier has already been introduced, non-nutritive sucking can also aid in pain reduction.
- **Oral glucose: sucrose solution**
 - Follow your facility protocol for giving the infant an oral glucose or sucrose solution prior to specimen collection to reduce pain.

Do not touch any part of the filter paper circles before, during or after collection. Multiple agents can contaminate the filter paper.

Do not compress the filter paper before, during, or after collection.

- Anesthetic topical creams are not recommended due to possible interference with the screening.

It is important to let parents know ahead of the procedure that it may take more than one heel stick to collect the specimen. Typically, the specimen can be collected in one heel stick, but occasionally an additional heel stick is needed to collect enough blood.

Puncture device

Use a puncture device that is specific to infant heel stick blood collection. Puncture devices are available in two sizes for infants.

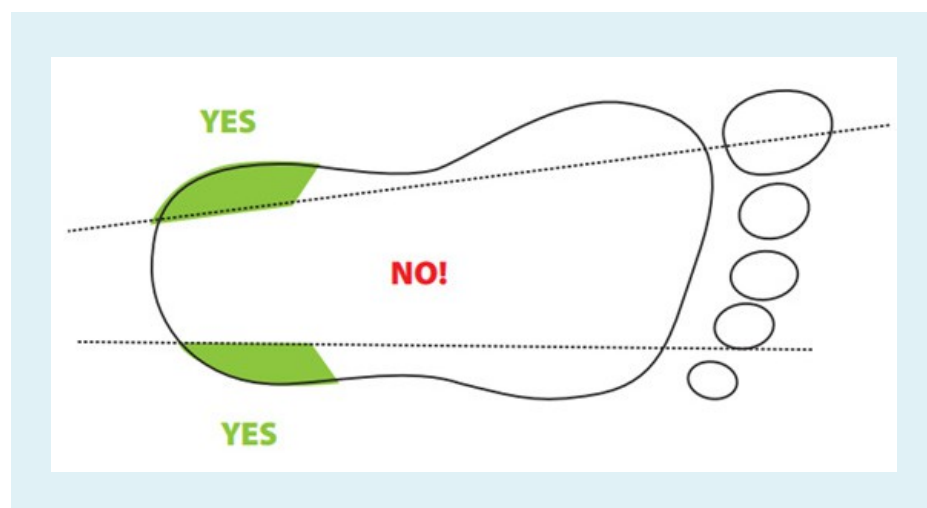
For the average weight newborn, the puncture device should not exceed a puncture depth of 2.0 mm or a length of 2.0 mm.

For babies born at less than 37 weeks gestation, or who have a birth weight of less than 2500 grams, do not exceed a puncture depth of 0.85 mm or a length of 1.75mm.

How to collect the specimen

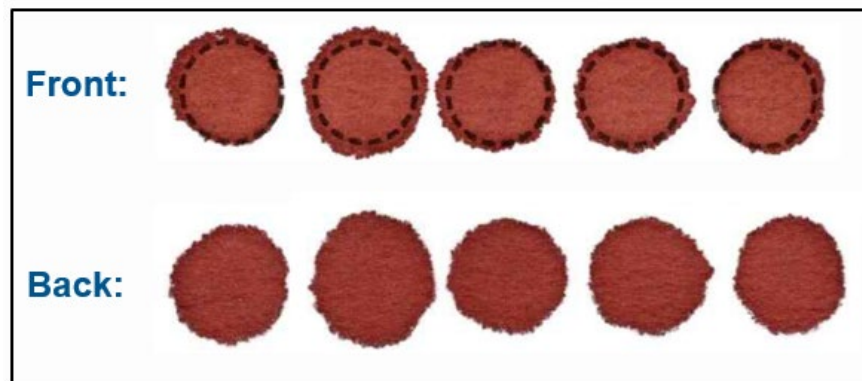
1. Check the expiration date on the specimen collection card to ensure it will not expire prior to being tested at the OSPHL.
2. Complete the demographics section of the card prior to specimen collection. Remove the blue carbon copy of the card. This copy is intended to be kept for your records.
3. Select a heel stick site on the infant's heel. Either side of the base of the heel is an appropriate site for the heel stick (see figure 3).

Figure 3 Recommendation for heel puncture site in newborns

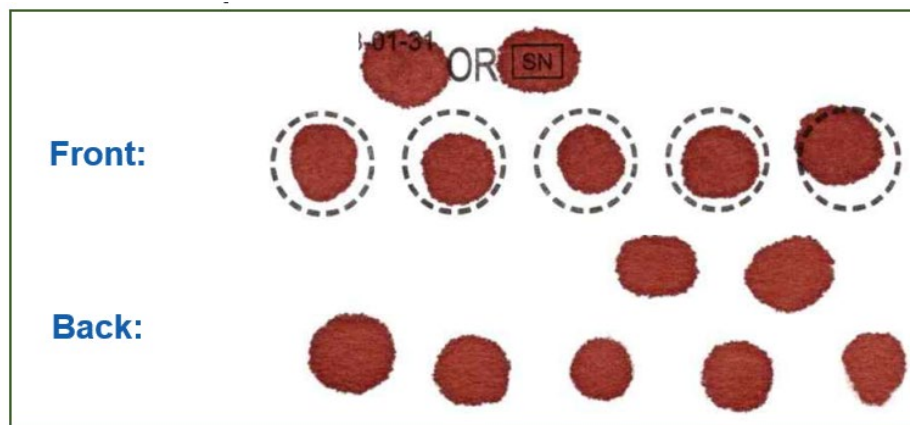


4. Use a commercial heel warmer on the heel stick site to improve blood flow.
5. Cleanse the heel stick site with alcohol and let air dry. Do not use betadine, iodine, lotion, or essential oils on the infant's foot prior to collecting the specimen.
6. Hold the foot firmly and position the puncture device against the heel stick site. If the puncture device has instructions on how to position the device against the heel, follow the instructions. If there are no instructions, the puncture should be across (perpendicular to) the heel prints, not parallel to them. The ridges of the heel prints will hold a drop of blood in place, so a larger drop can collect.
7. Apply gentle pressure to the heel stick site with the puncture device. Puncture the heel with the puncture device in one smooth motion. **Do not 'double stick' the puncture site with another puncture at the same site.**
8. Wipe away the first drop of blood with a clean or sterile gauze pad to remove tissue fluids.
9. Apply gentle intermittent pressure around the heel to allow **one large drop of blood** to collect. Do not milk or squeeze the heel right at the puncture site.
10. Allow the drop of blood to touch or drop onto the center of the first circle on the filter paper. **Only one drop of blood can be applied to each circle on the filter paper.** You may fill the circles from the front or back of the specimen collection card, but you may only fill from one side. Do not fill both sides of the specimen collection card.
11. Allow the drop of blood to soak through the filter paper. Do not touch the heel to the filter paper or swipe the blood across the filter paper.
12. Repeat the same procedure, following steps 6 through 10 for each of the remaining circles on the filter paper. Apply one drop of blood in each circle.
13. When the circles have been filled, with one drop of blood in each circle, review your work while the baby is still with you. Each circle on the filter paper should be filled with one drop of blood. The blood should saturate evenly through the card. Turn the card over to observe for even saturation through the card.

14. If the specimen looks similar to the example below, the specimen collection is complete.



If the circles are not completely filled, or the saturation through the card is uneven, you can add 2-3 extra drops of blood outside of the circles on the filter paper (see image below). These additional drops of blood will provide more blood for testing. **Note: drops of blood cannot touch, overlap, or layer on top of each other.**

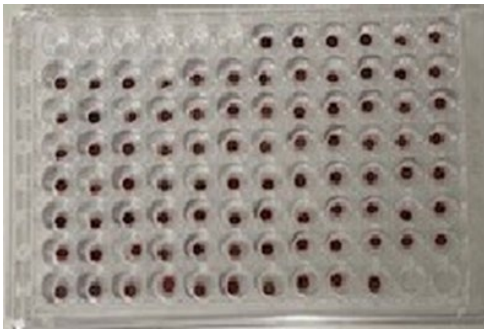


15. If blood flow stops and you have not finished filling each circle, repeat the specimen collection procedure with a new puncture device and choose a different heel stick site. You will need repeat all the steps in the specimen collection procedure.
16. When the specimen collection is complete, apply gentle, direct pressure to the puncture site using a clean or sterile gauze pad until bleeding has stopped, and provide additional care per your facility policy.
17. Dry the specimen in a flat (horizontal) position for 3-4 hours until completely dry. The filter paper should not touch any surface during the drying process. Do not expose the specimen to sunlight, heat, or fans. Do not hang the specimen to dry. Do not place the specimen in a plastic bag, leave in a hot

mailbox, or in a hot car. These practices can destroy some of the proteins and enzymes that are required for accurate screening.

18. Apply the protective cover over the filter paper after the specimen is completely dry.
19. Ensure that the specimen is completely dry before sending it to the state lab.

The importance of even saturation



When the lab staff are punching out the discs of blood for testing, each disc of blood must contain the same amount (volume) of blood. This is important to get valid, reliable results. Specimens with layered or overlapping blood drops will have an inconsistent amount of blood. This can lead to inaccurate results (false negative or false positive results).

The amount of blood needed for screening

For a first specimen, enough blood is needed for a minimum of 10 punches, plus more if repeat or additional screening is needed.

For a second specimen, enough blood is needed for a minimum of 7 punches, plus more if repeat or additional screening is needed.

Each punch is 3.2 mm in diameter. 3-4 punches can typically be taken from a full circle (bloodspot).

Repeat or additional testing that will require additional punches can affect any specimen. Babies who are pre-term, low birth weight, or ill are more likely to require additional punches from their specimen.



Venous blood collection

Capillary blood obtained from a heel stick is the preferred collection method for newborn bloodspot screening. In the rare circumstance that a heel stick is contraindicated, a specimen may be obtained from a peripheral or central line. Umbilical cord blood is not an acceptable source for specimen collection.

When selecting the appropriate site for venous blood collection consider the following:

- Blood should be collected from an extremity that has not been used to give IV fluids (including blood).
- The blood should not be collected into vacuum tubes that contain heparin or other anticoagulation agents.
- Transfer devices increase the risk of an unsatisfactory specimen. Ensure that only one drop of blood is applied to each circle on the filter paper and the transfer device does not touch the filter paper.

Unsatisfactory specimens

An unsatisfactory specimen is a specimen that the lab is unable to use for testing. The specimen will need to be recollected, which will cause a delay in screening results, confirmatory testing, and treatment for affected infants. This can negatively impact patient outcomes.

A specimen may be determined to be unsatisfactory for multiple reasons.

Uneven saturation:

Uneven saturation can be caused by several reasons.

a. **Multiple blood drops are applied to the same circle.**

This is the most common type of unsatisfactory specimen the OSPHL receives. When more than one blood drop is applied to each circle, the drops connect and layer on top of each other. The blood drops do not saturate evenly through the card, creating areas where there is too much blood volume and areas where there is not enough blood volume for testing. **Only one large drop of blood should be applied to each circle on the filter paper.**

- **Blood is applied to the filter paper with a transfer device such as a syringe or capillary tube.**

When a transfer device is used to collect the blood, it is difficult to apply only one blood drop to each circle. Applying more than one drop of blood to each circle will cause uneven saturation. Using a transfer device increases the risk that the blood will clot or separate from the serum, which can also cause uneven saturation. The filter paper can be scratched or ripped if the transfer device touches the filter paper during blood application. Blood should always be applied directly from the heel to the filter paper.

b. Hanging the specimen collection card to dry

If the filter paper is hung vertically or at an angle during the drying process, the red blood cells will move down the card instead of saturating through the card during drying. Specimen collection cards should always be laid flat (horizontal) to dry for 3-4 hours, until completely dry.

c. Serum separation

The blood can separate from the serum when a transfer device is used. It can also be caused by squeezing or milking right at the puncture site, which pushes tissue fluid into the specimen.

Damage to the filter paper

Although rare, if the filter paper is damaged prior to specimen collection, this can affect its ability to absorb and saturate blood evenly through the card. **Unused** specimen collection cards should always be stored on their edge and not laid flat to avoid compression damage.

Insufficient blood

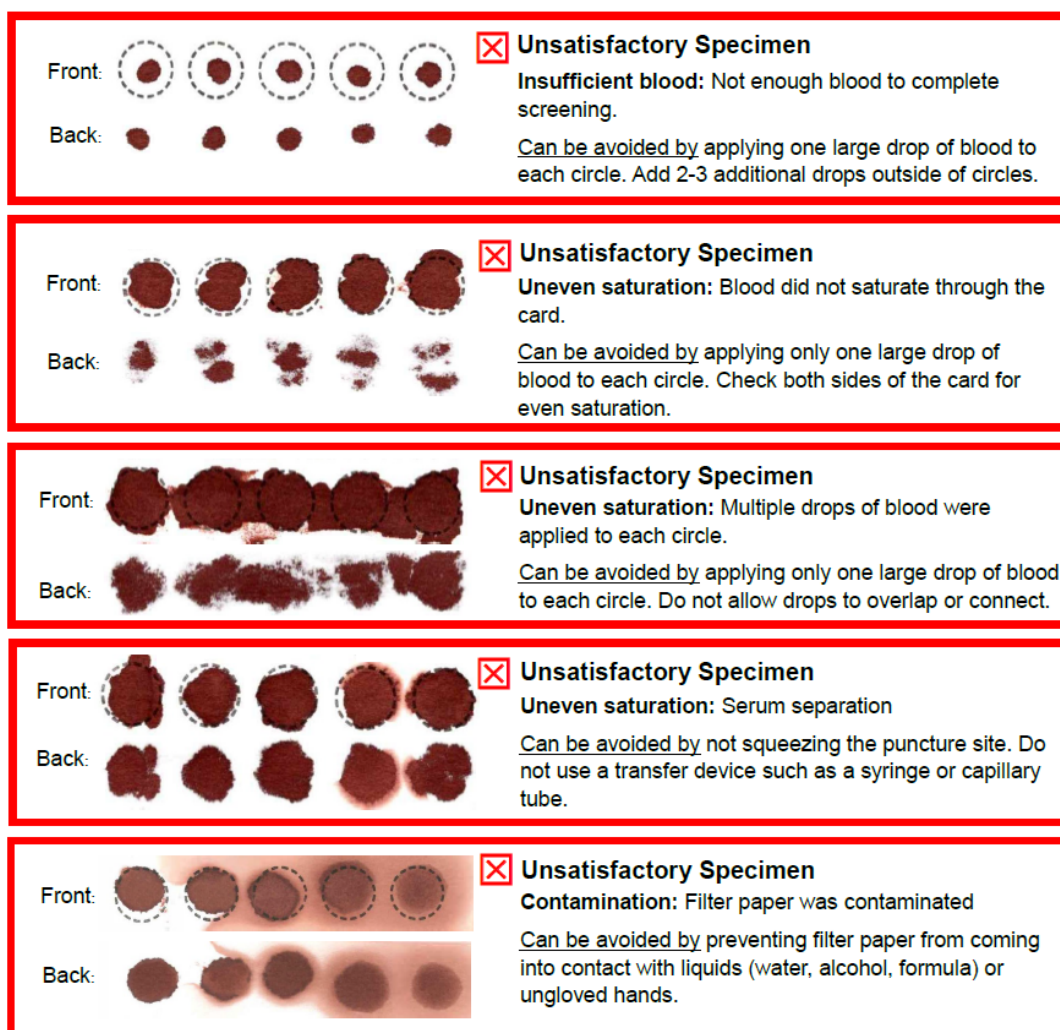
There needs to be enough blood to perform all the testing for each specimen. When the blood drops are small and/or do not saturate evenly through the card, the specimen will be determined to be unsatisfactory. A large drop of blood should be applied to each circle on the filter paper. 2-3 additional drops of blood can be applied outside of the circles on the filter paper if needed.

Contamination

Contamination of the specimen can be caused by numerous factors.

- a. Not letting the alcohol dry completely prior to puncturing the heel.
- b. Not wiping away the first drop of blood after the heel stick
- c. Specimen may become contaminated with fluid (formula, water) or ungloved hands.

Figure 4: Unsatisfactory specimen examples:



Specimen too old to test

A specimen that is received more than 14 days from the date of collection is too old to test and will be determined to be unsatisfactory. A specimen should be sent to the state lab as soon as possible after it is completely dry and no later than 24 hours after collection.

Expired kit

A specimen that is collected on a specimen collection card that is expired or will expire prior to testing will be determined to be unsatisfactory. Always check the expiration date of each specimen collection card prior to collection.

Specimen recollection

When a specimen is determined to be unsatisfactory, it will need to be recollected as quickly as possible (OAR 333-024-1060).

If a first specimen is determined by the OSPHL to be unsatisfactory, use the second specimen card to recollect the specimen. The infant should be scheduled as soon as possible to have the specimen recollected. However, if the specimen is not collected until after 9 days of life for the infant, and the results are normal, this recollection will also count as the second specimen and no further specimens will need to be collected.

If a second specimen is determined by the OSPHL to be unsatisfactory, a single specimen collection card should be used for the recollection. If you are not sure if a specific infant needs an additional NBS specimen collected, contact the NBS follow up team.



Specimen tracking within your facility

It is vitally important that every newborn bloodspot specimen collected from your facility reaches the state lab for screening. Every specimen should be monitored and tracked from time of collection through shipping to the OSPHL and receiving the screening results.

Birth hospitals should implement the following:

- Daily birth census comparison to NBS specimens collected, to ensure every infant has had the opportunity to be screened and a specimen collection card has been filled out for every infant.
- Chain of custody documentation for every specimen collected.
 - If a specimen is being sent to a different unit within the facility, a packing list or manifest should be sent to ensure all specimens have been received. This can reduce the chance of a specimen going missing. There have been occasions where a specimen has been lost within the birth facility because the specimen was not tracked between units. For example, if NBS specimens are collected on the mother/baby unit and sent to the hospital lab for shipping to the OSPHL, the mother/baby unit should provide a manifest to go with the specimens. The hospital lab staff should check to make sure all specimens listed on the manifest were received from the unit before specimen packaging to send to the OSPHL. Any discrepancy should be reported to the mother/baby unit immediately.



Specimen transport

It is critically important that the Oregon State Public Health Laboratory (OSPHL) receive newborn bloodspot screening specimens as soon as possible after collection and drying. Many of the conditions on the newborn bloodspot screening panel can cause serious injury or death in the first weeks of life. Early diagnosis and treatment for these medical conditions must occur rapidly to improve patient outcomes.

Delays in transporting specimens may result in a specimen being rejected for testing.

Specimens should be sent to the OSPHL as soon as they are completely dry (3-4 hours after collection) and no later than 24 hours after collection. Specimens that arrive prior to 10:30 am will begin screening that same day. Specimens that arrive after 10:30am will begin screening the following day.

1. Prior to shipping, every specimen collection card should be reviewed to ensure it is complete, accurate and legible.
2. Place the dry specimen(s) into an envelope. Tyvek envelopes are acceptable and preferred to prevent water damage to specimens during transit. Do not ship specimens in plastic bags or containers. Multiple specimens can be placed in the same envelope. Keep in mind this may affect shipping costs.
3. When sending multiple specimens, a packing list or manifest should be included. This will be used by the state lab staff to match specimens received. Discrepancies will be reported to the submitter.
4. Send the specimens to the OSPHL as soon as possible after drying and no later than 24 hours after collection.
5. Specimens should be received by the OSPHL within 48 hours of collection. All specimens must be sent by express mail, courier or another timely delivery service such as FedEx or UPS.

6. Do not expose specimens to the weather elements such as a hot car or placing it in a mailbox for long periods where it can be exposed to heat, cold or rain. Hot and cold temperatures can affect results.
7. Send the specimens to:

Oregon State Public Health Laboratory
Newborn Bloodspot Screening Program
7202 NE Evergreen Parkway, Suite 100
Hillsboro, OR 97124
8. Keep a tracking log of all NBS specimens sent to the OSPHL. Include the kit number, infant's name, DOB, date/time specimen left your facility and shipping tracking number if applicable.
9. Review each screening result and match to the tracking log. Any discrepancy or missing specimen results should be reported to the OSPHL immediately.

Batching specimens to reduce facility shipping costs leads to unnecessary and potentially deadly delays in newborn bloodspot screening.

Prompt specimen transit is essential to identify infants who may be impacted by a screening condition in the first few days of life. Use of a courier service or expedited shipping is strongly recommended. 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 quickly. Delays within a facility may be attributed to inefficient internal processes, slow courier services, simple forgetfulness, or, most dangerously, batching specimens.

The OSPHL is open Monday through Friday from 7am through 5pm. On Saturdays the lab is open to receive specimens from 9 am until noon. The OSPHL is closed on Sundays and all state observed holidays.



Reporting of results

Results are available online

As of July 1, 2024, normal newborn bloodspot screening results are no longer mailed to submitters. Newborn bloodspot screening result reports can be accessed online through the OSPHL reporting website, Secure Remote Viewer (SRV), as soon as they are available. You can find instructions for the SRV and the SRV access request [form](#) on the NBS website <https://bit.ly/SRVportal>. If you have questions, contact the NBS Follow-up Team at 503-693-4174.

Confirmatory testing may be requested following an abnormal screening result.

The confirmatory test results must be reported to the NBS Follow-up Team by:

Calling **503-693-4174** or

Faxing the results to
503-693-5601

Results reporting

Newborn bloodspot screening results are available in SRV to the “Submitter” and the “PCP/Clinic”, as identified on the specimen kit, after being released by the OSPHL.

Providers and care teams will be contacted directly for infants with abnormal results that require confirmation 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: <https://bit.ly/providersNBSresources>

Newborn bloodspot 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.



What should I do if I can't locate screening results in SRV?

There are four different search methods that can be used to locate NBS screening results in SRV. Please review the [SRV instruction sheet](#) for additional information.

If you are still unable to locate an infant's screening results within one week following collection and shipping the specimen, contact our NBS follow up team.

The practitioner must communicate abnormal results to the parent or guardian of the infant.



Situations that may impact newborn bloodspot screening results

The guidance below is to provide a summary of common factors that may affect newborn bloodspot screening results. Other factors may be discussed with clinicians following result availability.

Preterm, low birth weight, or sick infants

Newborn bloodspot screening for preterm, 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. See additional information contained within each disease summary below.

Parenteral nutrition and carnitine therapy

Specimens should not be taken from the line used to deliver total parenteral nutrition (TPN) and carnitine. Parenteral nutrition and carnitine can impact the concentration of amino acids and acylcarnitines.

Report that the baby was receiving TPN or carnitine at the time of collection on the specimen collection card.

Red blood cell transfusions

Infants should have a specimen collected prior to a red blood cell 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 60 days for an affected infant to accumulate abnormal analyte values after a transfusion, significantly delaying diagnosis and treatment. The screening laboratory will request collection of another specimen 60 days after the last transfusion.

Report that a transfusion occurred on the specimen collection card.

A transfusion of fresh frozen plasma, cryo, or platelets does not need to be reported.

Pivalic acid antibiotic therapy

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

Maternal conditions may affect newborn bloodspot 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
- Mothers with Cystic Fibrosis, who are receiving modulators





Requesting newborn bloodspot screening results

- If the child is younger than 6 years, practitioners may request the newborn bloodspot screening records by faxing the child's full name, date of birth, kit number, and parent or guardian's name (at the time of the child's birth) and date of birth on your facility letterhead to 503-693-5601.
- **Records are not available** for patients older than 6 years of age. Newborn bloodspot screening results are kept for 6 years and then are securely destroyed.
- 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 is provided by Baby's First Test at www.babysfirsttest.org.
- Parents or legal guardians may request the infant's newborn bloodspot screening records by completing this [form](#). Health care providers are able to access results in the SRV.





Use, release, and retention of residual bloodspot specimens

(OAR 333-024-1090)

After newborn bloodspot 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.
- Routine program evaluation and quality improvement.
 - This includes, but is not limited to:
 - Evaluation of reagents, kits, and instrument performance;
 - Demonstration of competency for testing personnel and for health care providers collecting the specimen;
 - Validation and verification of new equipment and screening methods.

The residual bloodspot specimens will be de-identified prior to use. No patient identifiers will remain.

The OSPHL does not release for research or sell residual bloodspot specimen cards.

The residual bloodspot specimen cards can be requested by:

- Parent or legal guardian(s) of the infant, following the procedure detailed on the Oregon NBS website, www.healthoregon.org/nbs
- A court order

Residual specimens are retained by the OSPHL for 12 months. Specimens will be destroyed during the month after the retention time is met, using a method that protects patient confidentiality and privacy.



Information about newborn bloodspot screening medical conditions

The information in these pages was provided by medical specialists contracted by the Northwest Regional Newborn Bloodspot Screening Program, and at the time of publication was considered the most current standard of care.

Cystic Fibrosis (CF)

Screening Process

- **First-tier test:** The first-tier immunoassay measures immunoreactive trypsinogen (IRT). Trypsinogen is the enzyme produced in the pancreas that is transiently elevated in the blood of most CF infants at birth.ⁱ
- **Second-tier test:** For specimens with an elevated IRT on one (if sufficiently high) or both screening specimens, second-tier DNA screening for 62 common variants is performed. See table below.
- **False positives / False negatives:** IRT may be falsely elevated in premature, stressed, or sick infants. Alternatively, IRT can be falsely low in infants with CF who are born with meconium ileus. It is estimated that a small portion (less than 10%) of babies with CF will not have an elevated IRT on the screening test.
 - 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/or DNA mutation analysis.
 - CF infants with meconium ileus or who are pancreatic sufficient may not have an elevated IRT. If meconium ileus is present, then

diagnostic testing for CF should be performed, in addition to routine newborn screening.

- 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.
 - Infants born to mothers with CF receiving modulator therapy 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.
- **Confirmatory testing:** Sweat chloride testing and 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.ii iii 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.

Disease Information

- **Incidence:** 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.

- **Causes:** Cystic fibrosis (CF) is a recessively inherited defect of the cystic fibrosis transmembrane conductance regulator (CFTR) protein.
- **Clinical features if untreated:** 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.
- **Treatment:** Comprehensive, multidisciplinary care, pancreatic enzyme replacement, soluble vitamin replacement, high-calorie/high-fat diet, airway clearance regimen, and new specific targeted therapies based on genotype.

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 is 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.
- **Outcome:** Early diagnosis improves pulmonary function and nutrition outcomes. With new treatments and ongoing comprehensive care, persons with Cystic Fibrosis can live a long and fulfilling life.

CF DNA Variant Panel Used for Second-Tier Screening

Legacy Variant Name	HGVS (cDNA)	Legacy Variant Name	HGVS (cDNA)
1717-1G>A*	c.1585-1G>A	W1282X*	c.3846G>A
1898+1G>A*	c.1766+1G>A	2183AA>G	c.2051_2052delAAinsG
2184delA*	c.2052del	1078delT	c.948del
2184insA	c.2052dup	1154insTC	c.1019_1020dup
2789+5G>A*	c.2657+5G>A	1548delG	c.1418del
3120+1G>A*	c.2988+1G>A	1677delTA	c.1545_1546del
3272-26A>G	c.3140-26A>G	1811+1.6kbA>G	c.1680-886A>G
3659delC*	c.3528del	1898+5G>T	c.1766+5G>T
3849+10kbC>T*	c.3718-2477C>T	2789+2insA	c.2657+2_2657+3insA
621+1G>T*	c.489+1G>T	3876delA	c.3744del
711+1G>T*	c.579+1G>T	3905insT	c.3773dup

Legacy Variant Name	HGVS (cDNA)	Legacy Variant Name	HGVS (cDNA)
A455E*	c.1364C>A	394delTT	c.262_263del
D1152H	c.3454G>C	A559T	c.1675G>A
F508del*	c.1521_1523del	E60X	c.178G>T
G542X*	c.1624G>T	F311del	c.935_937del, c.933_935del
G551D*	c.1652G>A	G970D	c.2909G>A
G622D	c.1865G>A	I618T	c.1853T>C
G85E*	c.254G>A	M1101K	c.3302T>A
I507del*	c.1519_1521del	P67L	c.200C>T
L206W	c.617T>G	Q493X	c.1477C>T
N1303K*	c.3909C>G	Q890X	c.2668C>T
P5L	c.14C>T	R1066C	c.3196C>T
PolyT	c.1210-12T[5_9]	R1070W	c.3208C>T
PolyTG	c.1210-34TG[10_12]	R1158X	c.3472C>T
R1070Q	c.3209G>A	R117C	c.349C>T
R1162X*	c.3484C>T	R352Q	c.1055G>A
R117H*	c.350G>A	R75X	c.223C>T
R334W*	c.1000C>T	S549N	c.1646G>A
R347H	c.1040G>A	V456A	c.1367T>C
R347P*	c.1040G>C	V520F	c.1558G>T
R553X*	c.1657C>T	Y1092X	c.3276C>A, c.3276C>G
R560T*	c.1679G>C	Y122X	c.366T>A
S945L	c.2834C>T		

Congenital Adrenal Hyperplasia (CAH)

Screening Process

- **Neonatal emergency:** 3/4 will develop salt wasting crisis, which can be fatal, in the first week to month of life. This disorder may be quickly life threatening and is a neonatal emergency. In both sexes, salt wasting and shock may develop rapidly within 7–28 days of birth.
- **First-tier test:** 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/ False negatives:** Occur more frequently in premature, low birth weight or sick infants.
 - 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 30% of infants will be detected only on a second screen.^{iv v vi}
- **Confirmatory testing:** 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.

Disease Information

- **Incidence:** 1:12,700 newborns
- **Causes:** CAH is a group of inherited disorders affecting hormone production within the adrenal gland. In the case of CAH, 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 if untreated:** If not found and treated early, it can be fatal.
 - 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.^{vii}
 - 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.^{viii}

- **Treatment:** Infants should be treated with hydrocortisone and mineralocorticoids in consultation with a pediatric endocrinologist.
- **Outcome:** Early detection and treatment can be lifesaving. Chromosome analysis in infants with ambiguous genitalia will prevent gender misassignment.^{ix} Ultimate outcome depends on severity of the defect, days to treatment, and adherence. Refer to pediatric endocrinologist.

Primary Congenital Hypothyroidism (CH)

Screening Process

- **First-tier test:** Screening is based on an immunoassay for thyroid stimulating hormone (TSH). Affected infants have high levels of TSH. Infants with milder disorders have intermediate levels.
- **False positives / False negatives:**
 - False positives occur more frequently in early collection (prior to 24 hours of life); premature or low birth weight babies (<34 weeks gestation or <2000g) or sick infants.
 - About 10% of infants will manifest a delayed rise in TSH making it more likely to be detected on the routine second or third screen, and increasing the risk for a false negative result.^{x xi}
- **Confirmatory testing:** Confirmation is by measurement of serum TSH and free T4. 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.

Disease Information

- **Incidence:** 1:2,000 newborns
- **Causes:** 85% of cases are caused by thyroid dysgenesis. Hereditary inborn errors of thyroid hormone biosynthesis are the cause of the remaining 15%.

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.

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.

- **Clinical features if untreated:** If not found and treated early (3-6 weeks of life), deficiency of thyroid hormone in an infant may result in intellectual and developmental disability and other signs of brain damage.
 - Infants may be asymptomatic before 3 months of age, by which time some brain damage has usually occurred. 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 fontanelles, 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.
- **Treatment:** The American Academy of Pediatrics (AAP) recommends that infants be managed in consultation with a pediatric endocrinologist.^{xii} Treatment of CH is L-thyroxine (brand or generic l-thyroxine), in pill form, is crushed, mixed with water or expressed breast milk and administered once daily.
- **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.^{xiii}

Sickle Cell Disease and other Hemoglobinopathies

Screening Process

- **First-tier test:** Capillary electrophoresis (CE) to look at hemoglobin banding pattern.
- **Second-tier test:** Isoelectric focusing (IEF) to separate the different forms of hemoglobin by another separation technique.

- **False positives / False negatives:** Transfusion of red blood cells before collecting the newborn bloodspot screening specimen will invalidate the hemoglobinopathy test (risk for false negative). Always obtain a specimen before any transfusion regardless of the infants' age.

Some hemoglobinopathies, particularly some thalassemias, are not reliably detected by newborn bloodspot 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.

Sickle cell disease screening 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.

- **Confirmatory testing:** If a hemoglobin abnormality is detected on the first sample, the second sample is also analyzed by CE and IEF. 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.

Disease Information

- **Incidence:** 1:2,000 births; 1:365 African Americans
- **Causes:** Homozygous sickle cell disease (SCD) occurs when the recessive gene for hemoglobin S, sickle hemoglobin, is inherited from both parents.
- **Clinical features if untreated:** 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.

- **Treatment:** Infants with significant hemoglobinopathies should have a primary care provider and receive periodic evaluation by a pediatric hematologist with expertise in hemoglobinopathies. 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.
- **Outcome:** Newborn diagnosis of sickle cell disease, if coupled with family education and centralized comprehensive care, can markedly lower morbidity and prevent death from sepsis.^{xiv} Long-term outcome depends on the severity of the hemoglobinopathy and response to treatment.

Amino Acid Conditions

Homocystinuria

*Homocystinuria (cystathionine beta-synthase deficiency)**

Screening Process

- **First-tier test:** Analysis of methionine by tandem mass spectrometry (MS/MS).
- **False positives / False negatives:** Methionine elevations can be secondary to liver disease, prematurity, or parenteral nutrition, leading to a false positive newborn screen for homocystinuria.

Patients with methionine adenosyltransferase (MAT) deficiency also have persistently elevated methionine, but generally are asymptomatic, with normal growth and development. These patients would also be considered a false positive for homocystinuria.

In patients with homocystinuria, methionine may rise slowly and may not be detectable on specimens obtained in the first few days after birth, leading to a risk for false negatives.

- **Confirmatory testing:** Quantitative plasma amino acids to measure methionine, Total homocysteine in blood.

Disease Information

- **Incidence:** 1:100,000 newborns
- **Causes:** Cystathionine beta-synthase deficiency (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. Homocystinuria is inherited as an autosomal recessive trait.
- **Clinical features if untreated:** 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.
- **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.
- **Outcome:** 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.

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

Phenylketonuria

Screening Process

- **First-tier test:** Analysis of phenylalanine and tyrosine by tandem mass spectrometry (MS/MS).
- **False positives / False negatives:** Elevated phenylalanine levels can be secondary to liver disease, prematurity, or parenteral nutrition, leading to a false positive

newborn screen. Contamination of the filter paper with food or liquids containing aspartame may also cause false positive results.

Detection of phenylketonuria may depend on the amount of protein ingested or endogenously produced by the infant, but most affected infants have abnormal results even in the first 24 hours of life regardless of intake. Those with milder forms may require longer periods of feeding or catabolism to elevated phenylalanine levels.

- **Confirmatory testing:** Quantitative plasma amino acids, Biopterins in blood and urine

Disease Information

- **Incidence:** 1: 16,300 newborns
- **Causes:** Phenylketonuria 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. Phenylalanine accumulates in the blood to toxic levels.
- **Clinical features if untreated:** Infants with phenylketonuria may appear unaffected at birth; however, without treatment, severe intellectual and developmental disability, seizures, eczema and other problems will develop. In older untreated patients, the skin and hair may be fair, and a mousey odor of the skin or urine is common. Although severe intellectual deficiency usually occurs in untreated cases, occasional asymptomatic adults are found with normal or near normal intelligence, despite high phenylalanine levels. Untreated blood phenylalanine level is often over 1,200 $\mu\text{M/L}$ in infants with severe phenylketonuria.
 - **Hyperphenylalaninemia:** Intermediate/mild forms of hyperphenylalaninemia can 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.
 - **Biopterin defect:** 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 by two years of age despite early treatment with a low phenylalanine diet.

- **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 postnatally. Infants of untreated mothers with classical PKU have microcephaly, intellectual and developmental disability and/or congenital heart defects. A phenylalanine restricted diet begun before conception and during pregnancy can often prevent damage to the fetus.
- **Treatment:** A low protein diet, devoid of phenylalanine, should be started as soon after birth as possible (preferably in the first week). Frequent monitoring of phenylalanine levels 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.
- **Outcome:** The outcome for treated patients is dependent on the age at diagnosis, adherence with treatment. For those with good compliance, outcome is normal. Patients whose treatment begins after 6 months are likely to remain intellectually disabled.

Tyrosinemia type I, II

Screening Process

- **First-tier test:** Analysis of tyrosine and succinylacetone by tandem mass spectrometry (MS/MS).
- **False positives / False negatives:** Elevated tyrosine can be secondary to liver disease, prematurity, or parenteral nutrition, leading to a false positive newborn screen.

Tyrosine may rise slowly in affected newborns and may not be detectable on specimens obtained in the first few days after birth, leading to a risk for false negatives.

- **Confirmatory testing:** Quantitative plasma amino acids, succinylacetone in urine, enzyme activity, and mutation analysis

Disease Information

- **Incidence:** 1:100,000 newborns for tyrosinemia type I. Less than 100 cases of tyrosinemia type II have been described worldwide.
- **Causes:**
 - **Tyrosinemia Type I** is due to a recessively inherited enzyme defect, fumarylacetoacetate hydrolase, in which the body cannot use the amino acid tyrosine properly.
 - **Tyrosinemia Type II** is caused by a deficiency of the enzyme tyrosine aminotransferase (TAT) and is inherited as an autosomal recessive trait.
- **Clinical features if untreated:**
 - **Tyrosinemia type I:** If untreated, 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, hyper phosphaturia and hypophosphotemic rickets. Untreated, death in infancy or childhood from acute liver failure, neurological crises or hepatocellular carcinoma is usual.
 - **Tyrosinemia type II:** If untreated, Tyrosinemia type II 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.
 - **Transient Tyrosinemia:** Transient tyrosinemia is a biochemical abnormality found in an otherwise normal newborn. It is not associated with long-term sequelae, although this has not been systematically studied.
- **Treatment:**
 - **Tyrosinemia type I:** The medication, (2-(nitro-4-trifluoromethylbenzoyl)-1-3-cyclohexanedione (NTBC)), blocks the formation of the toxic metabolites and 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.

- **Tyrosinemia type II:** A diet restricting phenylalanine and tyrosine is effective in clearing and/or preventing ulcerations.
- **Outcome:**
 - **Tyrosinemia type I:** Medications (2-(nitro-4-trifluoromethylbenzoyl)-1-3-cyclohexanedione (NTBC)) stops progression of the disease and allows for normal growth and development. The long-term risk of liver adenomas is still unknown, prompting some families to opt for liver transplant.
 - **Tyrosinemia type II:** For those with good compliance, outcome is normal.

Fatty Acid Oxidation (FAO) Conditions

Screening Process

- **Neonatal emergency:** The neonatal forms of FAO disorders can present in the first few days of life and can quickly become life threatening. Approximately 10% of infants with FAO disorders die in the first few days after birth, sometimes before screening results are known.
- **First-tier test:** Acylcarnitine analysis by tandem mass spectrometry
- **Fatty Acid Oxidation Disorders on the Screening Panel:**
 - Medium chain acyl-CoA dehydrogenase (MCAD) deficiency
 - Very long chain acyl-CoA dehydrogenase (VLCAD) deficiency
 - Long chain L-3 hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency
 - Trifunctional protein (TFP) deficiency
 - Short chain acyl-CoA dehydrogenase (SCAD) deficiency
 - Multiple acyl-CoA dehydrogenase deficiency (MADD aka glutaric acidemia II (GA II))
 - Carnitine uptake defect (CUD)
 - Carnitine/acylcarnitine translocase (CACT) deficiency
 - Carnitine palmitoyl transferase I (CPT I) deficiency

- Carnitine palmitoyl transferase II (CPT II) deficiency
- **False positives / False negatives:** Infants affected with an FAO who are well fed may have normal screening results, masking the presence of the disorder.
- **Confirmatory testing:** Acylcarnitine profile in plasma, enzyme assay and/or mutation analysis

Disease Information

- **Incidence:** 1:6,000 births; MCAD deficiency is the most common, approximately 1:15,000 births; LCHAD deficiency is 1: 50,000, and VLCAD deficiency is 1: 31,000. All are inherited as autosomal recessive traits.
- **Causes:** Mitochondrial beta-oxidation of fatty acids is critically important to the body's ability to produce energy during fasting. Infants initiate "fasting" in as little as four hours after a feeding. 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 are 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.
- **Clinical features if untreated:** FAO disorders have overlapping symptoms and organ involvement, which are classified into three major categories as described below. There is no typical age of presentation as affected individuals can present on the first day of life through adulthood.
 - **Hepatic:**^{xv xvi} Precipitating factors for FAO disorders are fasting and/or stress associated with intercurrent illness. Patients present with "Reyes-like" symptoms including vomiting, lethargy, hypoketotic hypoglycemia, mild hyperammonemia, hyperuricemia, low carnitine and abnormal liver function tests. Liver biopsy often shows steatosis.

Hepatic presentation is common in MCAD deficiency, VLCAD deficiency, LCHAD deficiency, neonatal CPT I & II and CACT deficiency.

- **Cardiac:** Cardiac abnormalities include hypertrophic or dilated cardiomyopathy. Pericardial effusion or cardiac failure can lead to death in these patients. Infants with MCAD deficiency can present with sudden cardio/pulmonary arrest before screening results are known.

FAO disorders with cardiac involvement include carnitine transport defects, MCAD deficiency, VLCAD deficiency, LCHAD deficiency and TFP deficiency.

- **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 deficiency, TFP deficiency and VLCAD deficiency.

- **Additional considerations for LCHAD deficiency:** Patients with LCHAD deficiency may develop retinal pigmentary changes and progressive visual loss in childhood despite early diagnosis and treatment.

A mother carrying a fetus affected with LCHAD deficiency 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 LCHAD deficiency should be considered in infants whose mothers have a history of these pregnancy complications.^{xvii}

- **Treatment:** Treatment for MCAD deficiency 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. Patients with these disorders may require IV support for fluid and calories during intercurrent infections or illnesses.
- **Outcome:** The outcome is variable depending on the FAO disorder. Patients with MCAD deficiency do well if diagnosed early. Outcomes for the other disorders are still being evaluated.

Organic Acid Conditions (OA)

Screening Process

- **Neonatal emergency:** Infants with a severe form of an organic acidemia will likely be symptomatic within a few days of life. There is a high risk of brain injury and death, if not diagnosed and treated promptly.
- **First-tier test:** Analysis of leucine and acylcarnitines by tandem mass spectrometry (MS/MS).
- **Organic Acidemias on the Screening Panel:**
 - Maple syrup urine disease (branched chain alpha-ketoacid dehydrogenase deficiency)
 - Propionic acidemia
 - Methylmalonic acidemia (cobalamin disorders)
 - Methylmalonic acidemia (methylmalonyl-CoA mutase deficiency)
 - Isovaleric acidemia
 - 3-Methylcrotonyl CoA carboxylase (3MCC) deficiency
 - 3-hydroxy-3-methylglutaric acidemia (HMG)
 - Multiple carboxylase deficiency (MCD) aka Holocarboxylase synthetase deficiency
 - Beta-ketothiolase deficiency
 - 2-Methyl-3-hydroxybutyric acidemia
 - Glutaric acidemia type I
 - Malonic acidemia
 - Isobutyrylglycinuria
 - 2-Methylbutyrylglycinuria
 - 3-methyl-glutaconic aciduria
- **False positives / False negatives:** Infants receiving parenteral nutrition may have elevations of leucine and isovalerylcarnitine (C5) and yield false positive results for related organic acidemias.

There is also evidence that not all infants affected with an organic acidemia will be identified by NBS (risk for false negatives).^{xviii}
- **Confirmatory testing:** Quantitative plasma amino acids, acylcarnitine analysis in plasma, urine organic acid analysis, enzyme assay and/or mutation analysis.

Disease Information

- **Incidence:** 1:20,000 newborns
- **Causes:** Organic acidemias result from an enzyme deficiency in the catabolic pathway of amino acids and other metabolites. This causes an accumulation of abnormal (and usually toxic) organic acid metabolites and increased excretion of organic acids in urine.
- **Clinical features if untreated:**
 - **Neonatal onset:** Most organic acidemias have severe forms that presents 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. In methylmalonic and propionic acidemias, ammonia may also be elevated. This progresses to coma and death in the first month, if left untreated. Infants with a neonatal onset form of the disease may be ill before the results of the screening tests are known. Even with prompt treatment, some infants with a neonatal form of an organic acidemia may sustain psychomotor damage and may have significant long-term morbidity.
 - **Later onset:** Patients with milder forms of an organic acidemia may present with an acute decompensation brought on by a recurrent illness or may present with failure to thrive, hypotonia, intellectual and developmental disability, or seizures. Typically, there is a history of bouts of vomiting, protein intolerance, acidosis and/or hypoglycemia. Patients with mild disease may still have neurological damage just as severe as those with neonatal onset.
 - **Asymptomatic cases:** There are numerous reports of patient with 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.
 - **Glutaric Acidemia type I:** Glutaric Acidemia Type I (GAI) is an organic acidemia with clinical features unlike those described above.^{xix xx xxi} 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, if untreated, patients will experience an acute encephalopathic episode prior to two years of age resulting in damage to the basal ganglia, which is irreversible. Intellect remains relatively intact. Infants with GAI are also prone to acute subdural and retinal hemorrhages after minor head trauma. Neurological crises and symptoms rarely occur after 5 years of age.

- **Treatment:** In general, patient with organic acidemias must be compliant with specific amino acid dietary restrictions and medications for life.

Any affected infant with a neonatal onset should be treated as an emergency and transferred to a major medical center with a metabolic specialist as quickly as possible. 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.

- **Outcome:** The outcome is variable, from poor to excellent, depending on the condition, neonatal course, disease severity, compliance with treatment and other environmental factors.

Urea Cycle Conditions (UCD)

Screening Process

- **Neonatal emergency:** Infants with severe hyperammonemia may die in the first week, if not diagnosed and treated. Practitioners must remain alert to the possibility of these disorders in any newborn with lethargy or coma.
- **First-tier test:** Analysis of citrulline, argininosuccinic acid and arginine by tandem mass spectrometry (MS/MS).
- **Urea Cycle Conditions on the Screening Panel:** Only three UCDs can be detected by newborn bloodspot screening:
 - Arginase deficiency
 - Argininosuccinic aciduria (ASA)
 - Citrullinemia, type I
- **False positives / False negatives:** Citrulline elevations can be secondary to liver disease, prematurity, or parenteral nutrition, leading to a false positive newborn screen.

Patients with neonatal intrahepatic cholestasis due to citrin deficiency (Citrullinemia Type II) may also have persistently elevated citrulline. These patients would also be considered a false positive for citrullinemia type I.

In patients with arginase deficiency, arginine may rise slowly and may not be detectable on specimens obtained in the first few days after birth, leading to a risk for false negatives.

- **Confirmatory testing:** Quantitative plasma amino acids, urine organic acids, urine orotic acid, and enzyme assay in red blood cells or hepatocytes.

Disease Information

- **Incidence:** 1:60,000 births (all 3 disorders)
- **Causes:** 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. If any enzyme is deficient, the ammonia levels will rise. There is significant clinical variability depending upon the severity of the deficiency, from mild to lethal.
- **Clinical features of Citrullinemia Type I (CTLN1) and Argininosuccinic Aciduria (ASA), if untreated:**
 - **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.
 - **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:** Newborn bloodspot 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.

- **Clinical features of Arginase Deficiency, if untreated:** 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 delay, 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.
- **Treatment:** Neonatal rescue from hyperammonemic coma is complicated and should be done under the guidance of an experienced metabolic physician. 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. Complete or partial liver transplant eliminates the need for dietary therapy and may improve clinical outcomes.
- **Outcomes:** Outcomes for patients with citrullinemia type I and ASA, who are rescued from prolonged neonatal hyperammonemia, is extremely poor. Brain damage is common and the risk of hyperammonemia continues throughout life. Even patients treated prospectively from birth may be symptomatic. ^{.xxii} Those with late onset disease fare better, and presymptomatic diagnosis and treatment may allow normal development.

Complications from arginase deficiency should be preventable with early and continuous treatment.

Classical Galactosemia

Screening Process

- **Neonatal emergency:** If left untreated 50% of affected babies will die in the first 7-10 days of life.
- **First-tier test:** Galactose-1-phosphate uridyl transferase (GALT) quantitative enzyme assay.

- **Second-tier test:** Free galactose and galactose-1-phosphate (Hill test).
- **False positives / False negatives:** >99% of Classical Galactosemia cases will be found on the first specimen. This test may not differentiate milder variants, G6PD, or liver disease from classical galactosemia.
 - 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 collecting the newborn bloodspot screening specimen will invalidate the GALT assay. Obtain a specimen before transfusion.
 - Galactose metabolites (Hill test) may be normal in infants being fed a soy-based formula.
- **Confirmatory testing:** Enzyme assay for GALT activity and quantification of galactose-1-phosphate.

Disease Information

- **Incidence:** 1:60,000 births
- **Causes:** Autosomal recessive disorder. Severely diminished or missing galactose-1-phosphate uridyl transferase enzyme leading to toxic levels of galactose-1-phosphate in tissues.
- **Clinical features if untreated:** If not found and treated early, it can be fatal. 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 disabilities will develop.
- **Treatment:** Galactosemia is treated by dietary galactose restriction, accomplished in the infant period by using soy-based or partially hydrolyzed infant formulas. The diet must be followed closely, for life.
- **Outcome:** Even with early treatment, somewhat diminished IQ will present, verbal and motor dyspraxia in 60% of patient, ovarian failure in 80% of females and post-natal growth delay during childhood.

Biotinidase Deficiency

Screening Process

- **First-tier test:** Biotinidase qualitative colorimetric enzyme assay. Detection of enzyme activity is by a qualitative colorimetric assay. In the presence of the enzyme a color change occurs.
- **False positives / False negatives:** 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 collecting the newborn bloodspot screening specimen will invalidate the biotinidase assay. Obtain a specimen before transfusion.
- **Confirmatory testing:** Quantitative biotinidase enzyme assay.

Disease Information

- **Incidence:** 1:60,000 births
- **Causes:** This recessively inherited disorder affects the cells' ability to recycle the vitamin-cofactor biotin, which impairs the function of mitochondrial carboxylases.
- **Clinical features if untreated:** 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.
- **Treatment:** 5-10 mg biotin/day. 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.
 - Infants with partial deficiency (5–10%) have been identified through newborn bloodspot 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.

- **Outcome:** Excellent if compliant with biotin therapy

Severe Combined Immunodeficiency (SCID)

Screening Process

- **First-tier test:** The screening test uses polymerase chain reaction (PCR) to evaluate the number of T cell receptor excision circles (TRECs) in 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.
- **False positives:** TRECs may also be low in infants with non-SCID-related causes of T-cell lymphopenia, who will also require evaluation and management.
- **Confirmatory testing:** CBC with differential and flow cytometry to determine the extent of the cell lymphopenia.

Disease Information

- **Incidence:** 1:50,000-1:100,000 births
- **Causes:** The term severe combined immunodeficiency is a group of inherited disorders 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 if untreated:** Infants may be symptomatic at birth, though most are completely healthy. 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 infection 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. 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. However, in patients with SCID, these viruses and bacteria may cause severe, life-threatening infection.

- **Treatment:** Infants may receive bone marrow transplant, gene therapy or enzyme replacement depending on the exact mutation causing their particular form of SCID.
- **Outcome:** Excellent if treatment is received in a timely manner.

Lysosomal Disorders (LDs)

Fabry Disease

Screening Process

- **First-tier test:** Tandem mass spectrometry (MS/MS) to detect Alpha-galactosidase A (GLA) enzyme activity.
 - GLA enzyme is measured in one valid specimen only. If GLA enzyme activity is normal on the first screen, it is not repeated on the second or other subsequent specimens.
- **Second-tier test:** *GLA* sequencing and copy number variant analysis.
- **Confirmatory testing:** GLA enzyme activity in plasma and leukocytes, DNA analysis of family members may also be warranted.

Disease Information

- **Incidence:** Estimates range from 1 in 3,000 infants detected by newborn bloodspot screening to 1 in 10,000 males diagnosed after development of symptoms.
- **Causes:** Fabry disease is inherited in an X-linked manner. Mutations in *GLA* result in reduced formation of alpha-galactosidase A, the lysosomal enzyme responsible for processing of sphingolipids. This leads to accumulation of globotriaosylceramide (GL-3) and progressive damage in tissues and organs throughout the body, particularly in the endothelium of small vessels, heart valves and muscle and renal podocytes.
- **Clinical features if untreated:** In the classic form, typically affecting males, the symptoms start in childhood to adolescence and feature neuropathic pain in

the hands/feet (aka acroparesthesia), skin lesions (angiokeratomas), decreased sweating (typically hypohidrosis), corneal opacities and proteinuria. Without treatment, this progresses to end-stage renal disease (ESRD), hypertrophic cardiomyopathy, cardiac arrhythmia, and/or heart valve disease, as well as stroke in some patients, in the third to fifth decade of life.

- Atypical forms of Fabry disease also occur and may present with more isolated signs or symptoms. These forms can include 1) a cardiac variant seen in later decades of life with left ventricular cardiomyopathy, arrhythmia and proteinuria but not associated with ESRD; 2) a renal variant with ESRD but absent acroparesthesias; or 3) cerebrovascular disease presenting with stroke or transient ischemic attack (TIA).
- While Fabry disease is a disorder primarily affecting males, female heterozygotes can be detected by screening. Female carriers have varying presentations due to random X-chromosome inactivation with the most severely impacted females expressing the X chromosome with the pathogenic *GLA* variant in the affected organs. Some female carriers will remain asymptomatic while others can display a classic disease presentation and may benefit from early intervention.
- In affected males, the infant's mother is an obligate heterozygote and should also be evaluated for symptoms of the disease. Rarely, de novo pathogenic variants arise spontaneously.
- **Treatment:** Enzyme replacement administered via infusion and in some cases oral chaperone therapy.
 - Individuals identified by newborn bloodspot screening are not expected to require or benefit from treatment in infancy or early childhood. However, baseline laboratory evaluations and regular monitoring will be conducted starting with the confirmation of diagnosis.
 - Primary available treatment is enzyme replacement therapy (ERT) typically administered by IV infusion every two weeks. Because infusions come with their own significant medical burden, this treatment is reserved for individuals with signs or symptoms of disease progression. Oral chaperone therapy is also available for a subset of affected adult individuals, but only certain genetic variants are amenable to this therapy.

- **Outcome:** Outcomes are improved with frequent monitoring and intervention to halt and prevent further progression of disease.

Pompe Disease

Screening Process

- **First-tier test:** Tandem mass spectrometry (MS/MS) to detect acid alpha-glucosidase (GAA) enzyme activity.
 - GAA enzyme is measured in one valid specimen only. If GAA enzyme activity is normal on the first screen, it is not repeated on the second or other subsequent specimens.
- **Second-tier test:** GAA gene sequencing and copy number variant analysis
- **False positives:** Can occur in heterozygous carriers and in presence of pseudodeficiency variants (present in <1% of European Caucasians, 3.9% in some East Asian populations.)
- **Confirmatory testing:** Diagnosis of Pompe disease is established by presence of biallelic pathogenic variants in GAA AND reduced GAA on diagnostic enzyme testing consistent with disease. If IOPD is suspected, urgent echocardiography and CK are recommended along with possible evaluation of AST, ALT and urine glucotetrasaccharide (Hex4) to confirm. In LOPD, these studies may be normal at the time of diagnosis in a newborn.

DNA analysis may assist in distinguishing between Infantile-onset Pompe Disease (IOPD) and Late-onset Pompe Disease (LOPD) in newborns identified by screening. Biallelic IOPD-associated or null variants are expected to cause IOPD. The most common LOPD-associated variant is c.-32-13T>G which is associated with as much as 90% of LOPD. The presence of at least one copy of c.-32-13T>G predicts LOPD.

Disease Information

- **Incidence:** Estimates range from 1 in 28,000 – 40,000 births
- **Causes:** Pompe disease is inherited in an autosomal recessive manner resulting in insufficient GAA enzyme

- **Clinical features if untreated:** Mutations in GAA result in reduced formation of acid alpha-glucosidase (GAA), the lysosomal enzyme responsible for processing glycogen in the lysosome. This leads to accumulation and progressive damage in tissues and organs throughout the body, particularly in the heart, skeletal and smooth muscle of the nervous system.

Pompe disease is classified based on age of onset, severity and organ involvement into categories of Infantile-onset (IOPD) and Late-onset (LOPD) disease. IOPD manifests before 12 months of age (possibly beginning in utero) and features hypertrophic cardiomyopathy, hypotonia, muscle weakness and eventually respiratory failure. Without intervention, affected individuals often experience a shortened lifespan of under two years. LOPD generally occurs later than 12 months, though earlier presentations have been described, but does not feature cardiomyopathy in infancy or childhood. Without treatment, these individuals have progressive proximal muscle weakness and respiratory insufficiency. The distinguishing feature between IOPD and LOPD in the newborn period is an abnormal echocardiogram and elevated urine Hex4.

- **Treatment:** Currently available treatment is enzyme replacement therapy (ERT) initiated prior to the development of tissue and organ damage to halt or slow progression.

Reversal of muscle fibrosis is not achieved by this therapy. ERT is administered by IV infusion every two weeks. Because infusions come with their own significant medical burden, this treatment is reserved for individuals with IOPD or those with LOPD with signs or symptoms of disease. As of this time, there are no oral therapies available. Additional supportive management is also provided for individuals with respiratory insufficiency, feeding difficulty, hearing loss and motor impairments.

Individuals with LOPD identified by newborn bloodspot screening may not require or benefit from treatment in infancy or childhood. However, baseline laboratory evaluations and regular monitoring will be conducted starting with the confirmation of diagnosis.

- **Outcome:** Significant improvements are expected in cardiac and respiratory function and prolonged lifespan in IOPD with early treatment. Improvement in muscle functions is expected in LOPD.

Common variants identified through sequencing and copy number analysis:

GAA Pathogenic Variant	Associated with (IOPD or LOPD)	Commonly Affected Populations
c.525delT	IOPD	Dutch
c.2482_2646del165	IOPD	Dutch
c.1935C>A	IOPD	Taiwanese/Chinese
c.2560C>T	IOPD	African
c.-32-13T>G	LOPD	European descent

Mucopolysaccharidosis Type I (MPS I)

Screening Process

- **First-tier test:** Tandem mass spectrometry (MS/MS) to detect Alpha-L-iduronidase (IDUA) enzyme activity.
 - IDUA enzyme is measured in one valid specimen only. If IDUA enzyme activity is normal on the first screen, it is not repeated on the second or other subsequent specimens.
- **Second-tier test:** *IDUA* gene sequencing and copy number variant analysis
- **False positives:** Can occur in heterozygous carriers and in the presence of pseudodeficiency variants (particularly common in individuals of African ancestry).
- **Confirmatory testing:** Alpha-L-iduronidase (IDUA) enzyme, glycosaminoglycans (GAGs) (aka mucopolysaccharides or MPS) in blood and/or urine.

Diagnosis of MPS I is established by presence of biallelic pathogenic variants in *IDUA* along with reduced IDUA and elevated GAGs on diagnostic testing.

DNA analysis may assist in determining severe versus attenuated disease in newborns identified by screening. Biallelic severe disease-associated variants are expected to cause severe disease.

In cases where more than one disease-associated variant is detected by DNA analysis, parental testing may be needed to clarify risk for disease. If the

variants were inherited from both parents (in trans-) the child is likely affected. However, if the variants were both inherited from only one parent (in cis-) the individual is an unaffected carrier. Certain genetic variants are often found to be inherited in cis- and this may be reassuring, however, diagnostic testing is always required to rule-out disease after abnormal screening.

Disease Information

- **Incidence:** Estimates range from 1 in 87,000 – 185,000 births
- **Causes:** MPS I is inherited in an autosomal recessive manner resulting in insufficient IDUA enzyme.
- **Clinical features if untreated:** Mutations in *IDUA* result in reduced formation of alpha-L-iduronidase (IDUA), the lysosomal enzyme responsible for processing certain glycosaminoglycans (GAGs). This leads to accumulation and progressive damage in tissues and organs throughout the body including the brain.

MPS I is classified based on age of onset and severity into categories of severe (formerly “Hurler”) and attenuated (formerly “Hurler-Scheie” or “Scheie”) disease.

Without early intervention severe disease is typically apparent in the first year of life and characterized by multi-system involvement and rapid progression. Primary features of this form include coarse facial features, cardiac involvement, hernias, progressive developmental delay, and a shortened lifespan.

Attenuated disease can be widely variable in presentation, usually apparent between early childhood and adolescence with less progressive symptoms. These individuals typically have less obvious facial coarseness as well as organomegaly, skeletal and joint manifestations, valvular heart disease and progressive pulmonary disease but possibly with normal intellect and lifespan.

- **Treatment:** Treatment via hematopoietic stem cell transplantation (HSCT) is standard of care in severe MPS I. Due to the morbidity and mortality associated with transplant this is not currently used in attenuated forms of the disease. HSCT is expected to show significant improvements in survival, growth, facial coarseness, organomegaly, hearing, cardiac and respiratory symptoms. Limited improvements are seen in skeletal manifestations, corneal clouding, and cognitive decline.

Enzyme replacement therapy (ERT) may be used in attenuated disease and in severe disease post-HSCT and is expected to improve organomegaly, growth, joint mobility and respiratory symptoms. Reversal of fibrosis or tissue degeneration is not achieved by this therapy. Because ERT is administered by IV infusion every two weeks and infusions come with their own significant medical burden, this treatment is also reserved for individuals with signs or symptoms of disease progression.

Individuals with attenuated MPS I identified by newborn bloodspot screening may not require or benefit from treatment in infancy or childhood. However, baseline laboratory evaluations and regular monitoring will be conducted starting with the confirmation of diagnosis.

- **Outcome:** In severe disease with early HSCT, or attenuated disease with early ERT, significant improvements expected in lifespan and overall disease burden.

Gaucher Disease

Screening Process

- **First-tier test:** Tandem mass spectrometry (MS/MS) to detect acid beta glucocerebrosidase (GCase) enzyme
 - GCase enzyme is measured in one valid specimen only. If GCase enzyme activity is normal on the first specimen, it is not repeated on the second or other subsequent specimens.
- **Second-tier test:** Liquid chromatography tandem mass spectrometry (LC MS/MS) to measure glucopsychosine concentration in the dried blood spot.
- **False positives:** Published false positive rate is 0.07% for the NeoLSD kit (PerkinElmer) used to measure GCase enzyme activity. The addition of second-tier measurement of glucopsychosine provides additional specificity, reducing false positives. Unaffected, heterozygous carriers may also be identified.
- **Confirmatory testing:** Diagnosis of Gaucher disease is established by presence of biallelic pathogenic variants in *GBA* along with reduced GCase enzyme activity and chitotriosidase activity consistent with disease.

Disease Information

- **Incidence:** In the U.S, estimated at 1 in 40,000 births. In the Ashkenazi Jewish population, prevalence is 1:855 individuals.
- **Causes:** Gaucher disease is inherited in an autosomal recessive manner resulting in insufficient GCase activity.
- **Clinical features if untreated:** Mutations in *GBA* result in reduced formation of acid beta-glucocerebrosidase (GCase), the lysosomal enzyme responsible for processing glucosylceramide (GL-1). This leads to accumulation and progressive damage in tissues and organs throughout the body, particularly the bones, liver and spleen.

Gaucher disease is classified based on the absence (Type 1) or presence (Types 2 or 3) of central nervous system (CNS) involvement. Type 1 Gaucher is the most common form and features hepatosplenomegaly, pancytopenia and bone marrow infiltration resulting in osteopenia, bone pain, fractures or osteonecrosis. Historically, these individuals were diagnosed in childhood through adulthood. Type 2, or acute, Gaucher disease is seen in children before the age of two years and characterized by hypotonia, failure to thrive, organomegaly, rapid progression and a shortened lifespan. Type 3, or subacute/chronic disease may also have symptoms apparent before age two and often present with oculomotor involvement, growth failure and organomegaly. However, a much slower progression is expected, with these individuals generally living to adulthood.

- **Treatment:** Individuals with Type 1 Gaucher disease identified by newborn bloodspot screening may not require or benefit from treatment in infancy or early childhood. However, baseline laboratory evaluations and regular monitoring will be conducted starting with the confirmation of diagnosis.
 - Primary available treatment is enzyme replacement therapy (ERT) administered by IV infusion every two weeks. Because infusions come with their own significant medical burden, this treatment is reserved for individuals with signs or symptoms of disease progression or in those with DNA variants or family history consistent with severe disease.
 - Oral substrate reducing therapy (SRT) is also available as second-line or for adult individuals who cannot tolerate ERT.
- **Outcome in early diagnosis:** Clinical improvements are expected in Types 1 and 3 receiving early treatment. Treatment of Type 2 does not result in significant change in outcomes.

Gaucher disease variants, disease types and affected population

GBA Pathogenic Variants	Gaucher disease type expected	Affected individuals
p.Asn409Ser homozygotes	Type 1	29%
p.Asn409Ser + another variant	Type 1	20%
p.Asn409Ser + p.Leu483Pro	Type 1; childhood onset	16%
p.Asn409Ser + c.84dupG	Type 1; childhood onset	12%
p.Leu483Pro homozygotes	Types 2 or 3; severe neuronopathic	6%
p.Asn409Ser + c.115+1G>A	Type 1; childhood onset	3%

Infantile Krabbe Disease (IKD)

Screening Process

- **First-tier test:** Tandem mass spectrometry (MS/MS) to detect acid galactocerebrosidase (GALC) enzyme
 - GALC enzyme is measured in one valid specimen only. If GALC enzyme activity is normal on the first specimen, it is not repeated on the second or other subsequent specimens.
- **Second-tier test:** Liquid chromatography tandem mass spectrometry (LC MS/MS) to measure psychosine concentration in the dried blood spot.
- **False positives / False negatives:** The use of second-tier psychosine testing with a cutoff of $\geq 10\text{nM}$ allows for high sensitivity and specificity for early Infantile Krabbe Disease.
 - This screening algorithm will also identify newborns at an increased risk for late onset forms or atypical forms of Krabbe Disease.
- **Confirmatory testing:** Diagnosis of IKD is established by measuring GALC activity in leukocytes, serum psychosine levels, and molecular analysis of *GALC* gene.

Disease Information

- **Incidence:** 1:100,000 to 250,000 of early IKD
- **Causes:** IKD is inherited in an autosomal recessive manner. Variants in the *GALC* gene result in functional deficiency of the

lysosomal enzyme, galactocerebrosidase (GALC), which leads to increased psychosine levels. Psychosine is toxic at high concentrations.

- **Clinical features if untreated:** Babies born with IKD, the most severe form of Krabbe disease and primary target of timely newborn screening, develop normally for the first few months and then, somewhere between two and six months of life, develop symptoms of disease. Initial signs and symptoms of IKD include extreme irritability, feeding difficulties, failure to thrive and spasticity. Affected babies rarely reach milestones beyond some head control and smiling, and they lose these abilities rapidly as the disease progresses.
 - The table below summarizes symptoms and disease progression without treatment for early IKD and other later-onset forms of Krabbe disease.
- **Treatment:** Hematopoietic stem cell transplantation (HSCT) is the recommended treatment for newborns confirmed to have IKD. For patients with IKD, transplant should occur by four to six weeks of life, as patients with earlier transplant may demonstrate better outcomes. There are risks for morbidity and mortality associated with HSCT including risk of infections, graft-vs-host disease and rejection of the donor cells.
- **Outcome:** HSCT is the only currently available treatment for IKD. HSCT prior to onset of symptoms improves clinical outcomes. Current data indicates improved survival as well as developmental and neurologic benefits from transplantation, although individuals may still experience ongoing developmental delays and motor disabilities.

	Disease Classification	Symptoms	Disease Progression Without Treatment
Early Onset	Early Infantile (0-6 months)	Crying/Irritability, feeding difficulties, poor head control, fisted hands, developmental delay	Progressive neurologic deterioration, seizures, psychomotor regression, loss of vision, hearing and voluntary movement

	Late Infantile (7-36 months)	Similar to early infantile	Similar to early infantile but slower progression
Late Onset	Juvenile onset (3-15 years)	Vision problems, muscle weakness, gait changes, loss of developmental milestones	Variable progression with older patients generally experiencing slower progression
	Adult Onset (greater than 15 years)	Gait disturbance, weakness, lower limb hypoesthesia, cognitive regression, spastic paraparesis	Variable progression with older patients generally experiencing slower progression

Spinal Muscular Atrophy (SMA)

Screening Process

- **First-tier test:** Polymerase chain reaction to detect deletion of exon 7 of the *SMN1*
- **False negatives:** 5%. Detection of deletions in *SMN1* account for only 95% of cases of SMA.
- **Confirmatory testing:** Sequencing to identify deletions/mutations in the *SMN1* gene and copy number variants in the *SMN2* gene.

Disease Information

- **Incidence:** 1:11,000 births
- **Causes:** There are many types of spinal muscular atrophy that are caused by changes in the same genes. Less common forms of SMA are caused by mutations in other genes including the *VAPB* gene located on chromosome 20, the *DYNC1H1* gene on chromosome 14, the *BICD2* gene on chromosome 9, and the *UBA1* and *BICD2* gene on the X chromosome. The types differ in age of onset and severity of muscle weakness; however, there is overlap between the types. Newborn bloodspot screening will only detect homozygous deletions in *SMN1*.

- **Clinical features if untreated:** There is a wide range of impairment seen in SMA caused by defects in the SMN1 gene, from onset before birth affecting breathing and feeding to mild weakness in adults. Accordingly, SMA can be classified into four types, based on highest motor milestone achieved.
- **Treatment:** Disease modifying treatment is available and outcomes are significantly better with earlier treatment. There are currently three disease modifying therapies available, including gene therapy.

Proactive supportive treatment by a multidisciplinary team is essential to reduce symptom severity, particularly in the most severe cases of SMA. Nusinersen (SPINRAZA™) became the first FDA-approved drug therapy for children and adults affected by SMA with approval in December 2016. Onasemnogene abeparvovec xioi (Zolgensma™) is an FDA approved gene therapy that replaces the missing or non- functional SMN1 gene. This therapy is approved for patients with SMA under the age of 2 years. Risdiplam (Evrysdi) is a daily, enteral (or “oral” if the target audience is no healthcare providers) medication approved for all ages which improves and maintains motor function in people affected by SMA.

- **Outcome:** Can vary depending on type of SMA.

Type	Other Name	Life Span	Motor	Clinical features
SMA type 0 0 copies of SMN2	Prenatal	A few weeks, <6 months	None achieved	Reduced movement of the fetus that is first seen between 30 and 36 weeks of the pregnancy. After birth, these newborns have little movement and have difficulties with swallowing and breathing
SMA type I 1-2 copies of SMN2	Werdnig Hoffmann disease or infantile onset SMA	Median survival: 8-10 months	Some head control, sit with support only	Onset before 6 months of age. The most severely affected infants (SMA type 0 or IA) have reduced movements even in utero and are born with contractures and breathing difficulties, with death typically occurring in the first year of life without treatment. Symptoms hypotonia (reduced muscle tone), diminished limb movements, lack of tendon reflexes, fasciculations, swallowing and feeding difficulties, and impaired breathing. These children also develop scoliosis (curvature

Type	Other Name	Life Span	Motor	Clinical features
				of the spine) or other skeletal abnormalities as they get older.
SMA type II 3 copies of SMN2	Intermediate SMA	75% alive at age 25 years		Onset usually between 6 and 18 months of age although some can present earlier. They are able to sit without support but are unable to stand or walk unaided, and some may lose the ability to stay seated independently over time without treatment. They may have respiratory difficulties including hypoventilation in sleep. The progression of disease is variable without treatment. Life expectancy is reduced but most individuals live into adolescence or young adulthood. With disease modifying treatment and proactive clinical care, children with SMA type II have improved motor outcomes
SMA type III 3-4 copies of SMN2	Kugelberg-Welander disease	Normal		Onset typically after 18 months of age and do achieve independent ambulation. They first show difficulty walking and running, climbing steps, or rising from a chair. The proximal leg muscles are most often affected first, with a tremor seen in the hands. Complications include scoliosis and joint contractures—chronic shortening of muscles or tendons around joints—caused by abnormal muscle tone and weakness, which prevents the joints from moving freely. Individuals with SMA type III may be prone to respiratory infections, but with care most have a normal lifespan. Disease modifying treatment can improve outcomes.
SMA type IV 4-6 copies of SMN2		Normal	Normal	Onset after 21 years of age, with mild to moderate proximal muscle weakness and other symptoms.

SMN2 Copy Number	SMA Clinical Phenotype 1		
	SMA I 2	SMA II 2	SMA III/IV 3
1	96%	4%	0%
2	79%	16%	5%
3	15%	54%	31%
>=4 4	1%	11%	88%

Table adapted from Calucho et al [2018]

1. Clinical phenotype with supportive care only
2. With supportive care only, the maximum motor function achieved is sitting.
3. With supportive care only, ambulation is achieved but may not be maintained
4. Prior et al [2004]^{xxiii} reported three asymptomatic, unrelated individuals homozygous for an *SMN1* deletion who had five copies of *SMN2*, demonstrating that expression levels consistent with five copies of *SMN2* may compensate for the lack of *SMN1* expression.

X-Linked Adrenoleukodystrophy (X-ALD)

Screening Process

- **First-tier test:** Tandem mass spectrometry (MS/MS) to detect C26:0 lysophosphatidylcholine (C26:0-LPC).
- **Second-tier test:** Liquid Chromatography Tandem mass spectrometry (LC MS/MS) to detect C26:0 lysophosphatidylcholine (C26:0-LPC).
- **False positives:** Increased levels of C26:0-LPC are seen in other disorders which may not have treatment currently available. These disorders include peroxisomal disorders such as the Zellweger spectrum disorders, Aicardi Goutières Syndrome, and several others.
 - Unlike in most autosomal recessive disorders, given the X-linked nature of this condition, there may be significant clinical implications for family members of infants who are confirmed to have X-ALD.

- Screening may identify female X-ALD disease heterozygotes as discussed above, but not all female heterozygotes will be detected on newborn bloodspot screening.
- **Confirmatory testing:** Confirmation of the diagnosis after newborn bloodspot screening is made by measurement of VLCFAs in serum and DNA testing.
 - For females, measurement of VLCFAs alone may not be reliable in ruling out or confirming the disorder so DNA is also recommended.
 - Biochemical and molecular testing cannot predict clinical outcomes, so careful monitoring of adrenocortical function and brain imaging are required throughout life for males.
 - Whenever possible, VLCFAs should be drawn after 2-3 hours fasting in addition to avoiding peanut butter.

Disease Information

- **Incidence:** Data from other newborn bloodspot screening programs found a birth prevalence of X-ALD in screened infants of 1 in 4,845. This is more common than previously published incidences ranging from 1 in 10,000 to 1 in 17,000.
- **Causes:** X-ALD is inherited in an X-linked manner. In affected males, the infant's mother is typically a heterozygous carrier. In some cases, de novo pathogenic variants arise spontaneously.
- **Clinical features if untreated:** Mutations in *ABCD1* result in reduced formation of a protein which facilitates the transport of very long chain fatty acids (VLCFAs) into the peroxisome to be broken down. This leads to accumulation of VLCFAs and progressive damage in tissues and organs, particularly in the adrenal glands, brain and spinal cord.
 - There are three overlapping forms of disease in males:
 - Childhood cerebral: Symptom onset generally between ages four and eight years and features progressive impairment of cognition, behavior, vision, hearing and motor function resulting in significant disability within two years or less without intervention. Most children will have associated adrenal insufficiency, either as a presenting manifestation of ALD or will develop it later in childhood.

- Adrenomyeloneuropathy (AMN): Manifests after the twenties as progressive leg stiffness/weakness, sphincter abnormalities, sexual dysfunction and impaired adrenocortical function. Progression continues over decades. AMN develops in almost all affected males.
 - Adrenal insufficiency: Presents in childhood with primary adrenocortical insufficiency without neurologic symptoms, however, neurologic disability and/or AMN are typical by middle age. It is expected that most affected males will develop adrenal insufficiency.
 - Heterozygous females may develop myeloneuropathy in later decades of life. Females do not typically develop adrenal insufficiency or cerebral disease.
- **Treatment:** Diagnosis allows for monitoring and treatment. Available treatments include cortisol replacement and/or hematopoietic stem cell transplant (HSCT) depending on the type of X-ALD. As of this publication, the primary available treatments for X-ALD are:
- Corticosteroid Therapy: Many individuals with X-ALD will develop adrenal insufficiency and will not produce adequate cortisol in response to stress or illness. This can be acutely life-threatening and is treated with oral corticosteroid replacement throughout life. This treatment does not impact brain or spinal cord disease.
 - Hematopoietic Stem Cell Transplant (HSCT): In individuals who develop cerebral ALD, HSCT, also known as “bone marrow transplant,” can halt progression of disease in the brain if initiated before the cerebral disease has progressed significantly. HSCT cannot reverse advanced disease, and if performed after the disease has advanced too far, may speed up disease progression.

As with any transplant, this intervention comes with significant inherent risks and is reserved for children with confirmed cerebral ALD based on brain imaging with or without identifiable symptoms. Given that best outcomes are achieved if HSCT is performed pre-symptomatically or before the disease advances too far, males with X-ALD are screened with regular brain MRIs with the goal of detecting cerebral disease early if it occurs. HSCT is not sufficient to treat adrenal insufficiency.

- Skysona (elivaldgene autotemcel): A one-time FDA approved gene therapy for boys aged 4-17. Made specifically for each patient using the patient's own blood stem cells and adds functional copies of the *ABCD1* gene to their cells. This addition allows the child's body to make ALD protein (ALDP), which helps their body to break down very long-chain fatty acids (VLCFAs) in the brain. This process slows the progression of damage to the brain and slows the decline in neurologic function.
- **Outcome:** Affected individuals will typically not develop symptoms for years to decades. Outcomes are improved with frequent monitoring and intervention to halt progression of the cerebral form of X-ALD if occurs.

Northwest Regional Newborn Bloodspot Screening

A collaborative project involving:

Oregon Health Authority
Oregon Health & Science University
New Mexico Newborn Genetic Screening Program



PUBLIC HEALTH DIVISION
Oregon State Public Health Laboratory
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You can request this document in other languages, braille or a format you prefer. Contact the Newborn Bloodspot Screening Program at 503-693-4174 or NWRegional.NBS@state.or.us. We accept all relay calls or you can dial 711.

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References

- ⁱ Henry RL, Boulton TJ, Roddick LG. False negative results on newborn screening for cystic fibrosis. *J Paediatr Child Health*. 1990 Jun;26(3):150-1.
- ⁱⁱ Parad RB, Comeau AM. Diagnostic dilemmas resulting from the immunoreactive trypsinogen/DNA cystic fibrosis newborn screening algorithm. *J Pediatr*. 2005 Sep;147(3 Suppl): S78-82.
- ⁱⁱⁱ Speiser PW, Arlt W, Auchus RJ, Baskin LS, Conway GS, Merke DP, Meyer- Bahlburg HFL, Miller WL, Murad MH, Oberfield SE, White PC. Congenital Adrenal Hyperplasia Due to Steroid 21-Hydroxylase Deficiency: An Endocrine Society Clinical Practice Guideline *J Clin Endocrinol Metab* 2018; 103:4043-88. Mussig K, Kaltenbach S, Maser-Gluth C, Hartmann MF, Wudy SA, Horger M, et al. Late diagnosis of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Exp Clin Endocrinol Diabetes*. 2006 Apr;114(4):208-14.
- ^{iv} Votava F, Torok D, Kovacs J, Moslinger D, Baumgartner-Parzer SM, Solyom J, et al. Estimation of the false-negative rate in newborn screening for congenital adrenal hyperplasia. *Eur J Endocrinol*. 2005 Jun;152(6):869-74.
- ^v Eshragh N, Doan L, Connelly K, Denniston S, Willis S, LaFranchi SH. Outcome of newborn screening for congenital adrenal hyperplasia at two time points. *Horm Res Paediatr*; 2020; 93:128-136.
- ^{vi} American Academy of Pediatrics, Rose SR; Section on Endocrinology and Committee on Genetics, American Thyroid Association, Brown RS; Public Health Committee, Lawson Wilkins Pediatric Endocrine Society, Foley T, Kaplowitz PB, Kkaye CI, Sundararajan S, Varma SK. Clinical report. Update of newborn screening and therapy for congenital hypothyroidism. *Pediatrics*. 2006;117(6):2290-2303
- ^{vii} Therrell BL Jr, Berenbaum SA, Manter-Kapanke V, Simmank J, Korman K, Prentice L, et al. Results of screening 1.9 million Texas newborns for 21-hydroxylase-deficient congenital adrenal hyperplasia. *Pediatrics*. 1998 Apr;101(4 Pt 1):583-90
- ^{viii} Therrell BL Jr, Berenbaum SA, Manter-Kapanke V, Simmank J, Korman K, Prentice L, et al. Results of screening 1.9 million Texas newborns for 21-hydroxylase-deficient congenital adrenal hyperplasia. *Pediatrics*. 1998 Apr;101(4 Pt 1):583-90.
- ^{ix} Speiser PW, Arlt W, Auchus RJ, Baskin LS, Conway GS, Merke DP, Meyer- Bahlburg HFL, Miller WL, Murad MH, Oberfield SE, White PC. Congenital Adrenal Hyperplasia Due to Steroid 21-Hydroxylase Deficiency: An Endocrine Society Clinical Practice Guideline *J Clin Endocrinol Metab* 2018; 103:4043-88. Mussig K, Kaltenbach S, Maser-Gluth C, Hartmann MF, Wudy SA, Horger M, et al. Late diagnosis of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Exp Clin Endocrinol Diabetes*. 2006 Apr;114(4):208-14.
- ^x Maniatis AK, Taylor L, Letson GW, Bloch CA, Kappy MS, Zeitler P. Congenital hypothyroidism and the second newborn metabolic screening in Colorado, USA. *J Pediatr Endocrinol Metab*. 2006 Jan;19(1):31-8.
- ^{xi} Dick MC. Standards for the management of sickle cell disease in children. *Arch Dis Child Educ Pract Ed*. 2008 Dec;93(6):169-76. Yap S, Boers GH, Wilcken B, Wilcken DE, Brenton DP, Lee PJ, et al. Vascular outcome in

patients with homocystinuria due to cystathionine beta-synthase deficiency treated chronically: a multicenter observational study. *Arterioscler Thromb Vasc Biol.* 2001 Dec;21(12):2080-5.

xii Van Trotsenburg AS, Stoupa A, Leger J, et al. Congenital hypothyroidism: a 2020 consensus guidelines update An ENDO-EUROPEAN REFERENCE NETWORK (ERN) initiative endorsed by the European Society for Pediatric Endocrinology and the European Society for Endocrinology. *Thyroid* 2021; 31 (3):387-419

xiii Van Vliet G. Neonatal hypothyroidism: treatment and outcome. *Thyroid.* 1999 Jan;9(1):79-84.

xiv Yap S, Naughten E. Homocystinuria due to cystathionine beta-synthase deficiency in Ireland: 25 years' experience of a newborn screened and treated population with reference to clinical outcome and biochemical control. *J Inherit Metab Dis.* 1998 Oct;21(7):738-47.

xv Wilson CJ, Champion MP, Collins JE, Clayton PT, Leonard JV. Outcome of medium chain acyl-CoA dehydrogenase deficiency after diagnosis. *Arch Dis Child.* 1999 May;80(5):459-62.

xvi Holton JB, Allen JT, Green CA, Partington S, Gilbert RE, Berry PJ. Inherited metabolic diseases in the sudden infant death syndrome. *Arch Dis Child.* 1991 Nov;66(11):1315-7.

xvii Holton JB, Allen JT, Green CA, Partington S, Gilbert RE, Berry PJ. Inherited metabolic diseases in the sudden infant death syndrome. *Arch Dis Child.* 1991 Nov;66(11):1315-7.

xviii Nassogne MC, Heron B, Touati G, Rabier D, Saudubray JM. Urea cycle defects: management and outcome. *J Inherit Metab Dis.* 2005;28(3):407-14.

xix Funk CB, Prasad AN, Frosk P, Sauer S, Kolker S, Greenberg CR, et al. Neuropathological, biochemical and molecular findings in a glutaric acidemia type 1 cohort. *Brain.* 2005 Apr;128(Pt 4):711-22.

xx Gallagher RC, Cowan TM, Goodman SI, Enns GM. Glutaryl-CoA dehydrogenase deficiency and newborn screening: retrospective analysis of a low excretor provides further evidence that some cases may be missed. *Mol Genet Metab.* 2005;86(3):417-20.

xxi Scaglia F, Lee B. Clinical, biochemical, and molecular spectrum of hyperargininemia due to arginase I deficiency. *Am J Med Genet C Semin Med Genet.* 2006;142(2):113-20.

xxii Bosch AM. Classical galactosaemia revisited. *J Inherit Metab Dis.* 2006;29(4):516- 25.

xxviii Kemper AR, Brosco J, Comeau AM, Green NS, Grosse SD, Jones E, et al. Newborn screening for X-linked adrenoleukodystrophy: evidence summary and advisory committee recommendation. *Genetics in Medicine* [Internet]. 2017 Jan 1 [cited 2022 Apr 21];19(1):121–6. Available from: <http://dx.doi.org/10.1038/gim.2016.68>