



## PERMANENT ADMINISTRATIVE ORDER

**PH 83-2022**

CHAPTER 333

OREGON HEALTH AUTHORITY

PUBLIC HEALTH DIVISION

**FILED**

05/25/2022 10:50 AM  
ARCHIVES DIVISION  
SECRETARY OF STATE  
& LEGISLATIVE COUNSEL

FILING CAPTION: Update to Newborn Bloodspot Screening tests provided and Practitioner's Manual

EFFECTIVE DATE: 06/01/2022

AGENCY APPROVED DATE: 05/23/2022

CONTACT: NW Regional Newborn Screening Program 7202 NE Evergreen Parkway, Suite 100 Filed By:

503-693-4174

Hillsboro, OR 97124

Public Health Division

publichealth.rules@dhsosha.state.or.us

Rules Coordinator

### RULES:

333-024-1020, 333-024-1025, 333-024-1040, 333-024-1070

AMEND: 333-024-1020

NOTICE FILED DATE: 03/30/2022

RULE SUMMARY: Amend OAR 333-024-1020 – Revise Oregon Newborn Bloodspot Screening Practitioner's Manual to the 12th Edition, publishing in 2022. Key changes within include but are not limited to the following:

- Addition of section describing the Northwest Regional Newborn Bloodspot Screening Advisory Board
- Updates to "information about newborn bloodspot screening medical conditions" section to:
  - o Align descriptions with current clinical understanding and practice
  - o Add sections for SMA and X-ALD
- Various edits for clarity and consistency.

### CHANGES TO RULE:

#### 333-024-1020

Newborn Screening: Persons Responsible for Ensuring that First Specimens are Collected and Submitted

(1) The following, in order of priority, are responsible for ensuring that first specimens are collected and submitted in accordance with this rule:¶

(a) Hospitals and freestanding birthing centers, if the infant is born at the hospital or freestanding birthing center.¶

(b) A facility or practitioner responsible for the infant's medical care soon after birth.¶

(c) Parents or legal guardians of the infant when the birth is unattended by a practitioner.¶

(2) The persons described in section (1) of this rule must ensure that specimens are collected within the timeframes and in the manner described in OAR 333-024-1030 to 333-024-1040, and in accordance with the instructions provided by the Oregon State Public Health Laboratory available in the Oregon Newborn Bloodspot Screening Practitioner's Manual (Practitioner's Manual), 11<sup>th</sup> Edition; 2019~~22~~ found at [www.healthoregon.org/nbs](http://www.healthoregon.org/nbs), unless the infant is exempt pursuant to OAR 333-024-1050. ¶

(3) A person who collects and submits the first specimen from a two-part or three-part collection kit must provide the remaining specimen card(s) to the person described in OAR 333-024-1025 who has the responsibility for ensuring that the second specimen is collected and, when applicable, the third specimen.

Statutory/Other Authority: ORS 413.014, 433.285, 431A.750

Statutes/Other Implemented: ORS 433.285, 433.290, 433.295

RULE ATTACHMENTS DO NOT SHOW CHANGES. PLEASE CONTACT AGENCY REGARDING CHANGES.

# Oregon Newborn Bloodspot Screening Practitioner's Manual



Northwest Regional Newborn Bloodspot Screening Program

# Newborn Bloodspot Screening Practitioner's Manual

## **Oregon Health Authority**

### **Public Health Division**

John L. Fontana, PhD, (HCLD) ABB

Patrice Held, PhD, FACMG

Sheri Hearn, MPH

Sarah Humphrey King, CHES

Sara Etienne, BS

Kristi Murphy, MS GC

## **Oregon Health & Science University**

Kara Connelly, MD

Erika Finanger, MD

Cary Harding, MD

Shyam Joshi, MD

Kim Kripps MD

Stephen L. LaFranchi, MD

Mike Powers, MD

Sarah Viall, PPCNP-BC

Leah Wessenberg, FNP

Trisha Wong, MD

Melinda Wu, MD

Amy Yang, MD

Tyler Yates, MD

12th Edition, 2022

# Table of Contents

» Table of Contents .....	3	» Tips to avoid rejected specimens .....	34
» Introduction .....	5	» Information about newborn bloodspot screening medical conditions .....	36
» Advisory Board .....	7	» Cystic Fibrosis (CF) .....	36
» Definitions .....	8	» Congenital Adrenal Hyperplasia (CAH) .....	39
» Newborn bloodspot screening responsibilities in Oregon .....	9	» Primary Congenital Hypothyroidism (CH) .....	41
» Education services .....	10	» Sickle Cell Disease and other Hemoglobinopathies .....	44
» Fee exemption for Oregon births .....	10	» Amino Acid Conditions .....	46
» Parent refusal to have the infant screened in Oregon .....	10	» Fatty Acid Oxidation (FAO) Conditions .....	53
» Medical conditions on the newborn bloodspot screening panel .....	11	» Organic Acid Conditions (OA) .....	55
» Newborn bloodspot screening kits .....	17	» Urea Cycle Conditions (UCD) .....	58
» Timing for specimen collection .....	18	» Galactosemia .....	62
» Patient demographic information .....	21	» Biotinidase Deficiency .....	64
» Heel-stick specimen collection instructions .....	23	» Severe Combined Immunodeficiency (SCID) .....	65
» Specimen transport .....	26	» Lysosomal Storage Disorders (LSDs) .....	67
» Reporting of results .....	28	» Fabry Disease .....	67
» Situations that may impact newborn bloodspot screening results .....	30	» Pompe Disease .....	71
» Requesting newborn bloodspot screening records .....	32	» Mucopolysaccharidosis Type I (MPS I) .....	74
» Use, release, and retention of residual bloodspot specimens .....	33	» Gaucher Disease .....	76
		» Spinal Muscular Atrophy (SMA) .....	79
		» X-Linked Adrenoleukodystrophy (X-ALD) .....	82
		» References .....	86



### **Acknowledgment**

We are indebted to the newborn bloodspot screening state coordinators, medical consultants, and practitioners for their assistance and advice.

### **Recommended citation**

NW Regional Newborn Bloodspot Screening Program.  
Newborn Bloodspot Screening Practitioner's Manual, 12th Edition; 2022.



# 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 OSPHL and abnormal results are referred to the NBS Follow-up Team.

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

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

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

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 as of 2018, some lysosomal storage disorders. The OSPHL 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.

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

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

**Newborn 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.**



# 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 for the review and creation of legislative reports based on members' respective areas of expertise. The Board assists NWRNBS with strategic planning and the development of policies, priorities and services related to newborn bloodspot screening. The Board's role also includes reviewing conditions to be recommended for the addition or removal from the test panel of diseases. In all activities, The Board considers the newborn screening system as a whole, to improve health outcomes for all infants and their families. The Board is comprised of 13 partners within the newborn bloodspot screening community. The members include representatives of hospitals, birth centers, families, insurance, midwifery, nursing, pediatrics and other perspectives.

If you are interested in participating with the NWRNBS Advisory Board, please send an email to [nbs.advisoryboard@dhsosha.state.or.us](mailto:nbs.advisoryboard@dhsosha.state.or.us).



# 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 filter paper collection device, attached demographic form and other items provided by the Oregon State Public Health Laboratory for the the purposes of collection and submission of specimens for newborn bloodspot screening.

**“Practitioner”** means: the person who takes responsibility for the delivery or health care of an infant born in Oregon and is one of the following:

- a) A physician licensed under ORS 677;
- b) A naturopathic physician licensed under ORS 685;
- c) Advanced practice registered nurse licensed under ORS 678;
- d) A chiropractic physician licensed under ORS chapter 684; or
- e) A direct entry midwife licensed under ORS 687.

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

**“Specimen”** means: a blood specimen obtained from an infant by means of capillary puncture or skin puncture (heel stick) that has spotted onto the newborn bloodspot screening kit and allowed to air dry.



# Newborn bloodspot screening responsibilities in Oregon

**This section describes the responsibilities for successful newborn bloodspot screening in Oregon. Practitioner's 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.
- **Oregon State Public Health Laboratory (OSPHL) and NBS Follow-up Team:** The laboratory is responsible for testing, record keeping, ensuring quality of laboratory methods, notifying providers of results, tracking abnormal and unresolved results, and providing educational materials.
- **Oregon Health & Science University (OHSU) subspecialty programs:** These partners are responsible for providing consultation services to practitioners and the OSPHL.

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

**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 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 Education Coordinator. In addition, education resources are made available to practitioners and parents at [www.bitly.com/nbs-resource](http://www.bitly.com/nbs-resource).

## Fee exemption for Oregon births

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

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

## Parent refusal to have the infant screened in Oregon

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

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

**NBS Follow-up Team**

**Fax: 503-693-5601**



# Medical conditions on the newborn bloodspot screening panel

Oregon newborns are screened for the following medical conditions recommended by the Advisory Committee on Heritable Disorders in Newborns and Children and the Northwest Regional Newborn Bloodspot Screening (NWRNBS) Program Advisory Board. More information on these medical conditions is available at the end of this manual and at:

- Baby's First Test: <http://babysfirsttest.org/>
- The Oregon State Public Health Laboratory: [www.healthoregon.org/nbs](http://www.healthoregon.org/nbs)
- The American College of Medical Genetics (ACMG): [ACT Sheets and Algorithms](#).
- Western States Regional Genetics Network: [www.newbornscreening.info](http://www.newbornscreening.info)

**Newborn bloodspot screening is not diagnostic. Both false negative and false positive results may occur. Confirmatory testing is required for diagnosis.**

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

Medical Condition	Analyte(s) tested for	Incidence in NW region	Symptoms <i>if not treated</i>	Common Medical Treatment
<b>Organic Acid Disorders</b>				
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	1 per 148,000	Vomiting; lethargy; acidosis possibly resulting in coma, death	Protein-restricted diet; carnitine and glycine therapy

Medical Condition	Analyte(s) tested for	Incidence in NW region	Symptoms <i>if not treated</i>	Common Medical Treatment
3-methylcrotonyl CoA carboxylase deficiency (3MCC)	C5OH	1 per 51,000	Most have been asymptomatic	None, except carnitine therapy if deficient
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
Multiple carboxylase deficiency (MCD)	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	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	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	Rare, less than 1 per 300,000	None to severe cardiomyopathy	Carnitine therapy; protein restriction; avoid fasting
2-methylbutyryl CoA dehydrogenase deficiency (2MBC)	C5	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

Medical Condition	Analyte(s) tested for	Incidence in NW region	Symptoms <i>if not treated</i>	Common Medical Treatment
<b>Fatty Acid Oxidation Disorders</b>				
Carnitine uptake deficiency (CUD)	C0	1 per 116,000	Hypoglycemia; cardiomyopathy	Carnitine therapy
Medium chain acyl-CoA dehydrogenase deficiency (MCAD)*	C6, C8, C10, 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, 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)*	C14:1, C16, C16OH, C18, C18OH	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)	C14:1, C16, C16OH, C18, C18OH	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	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, C14, C16, C18:1	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

Medical Condition	Analyte(s) tested for	Incidence in NW region	Symptoms <i>if not treated</i>	Common Medical Treatment
Carnitine palmitoyl transferase deficiency, type II (CPT-II)*	C16, C18, C18:1	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
Carnitine acylcarnitine translocase deficiency (CACT)	C16, C18, C18:1	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
<b>Amino Acid Disorders</b>				
Argininosuccinate lyase deficiency (Arginosuccinic aciduria; 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	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	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

Medical Condition	Analyte(s) tested for	Incidence in NW region	Symptoms <i>if not treated</i>	Common Medical Treatment
<b>Endocrine Disorders</b>				
Primary congenital hypothyroidism	Thyroid hormone T4 and Second tier TSH	1 per 2,300	Intellectual and developmental disability; other brain damage; growth delay	Thyroid hormone
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
<b>Pulmonary Disorders</b>				
Cystic fibrosis (CF)	Immunoreactive Trypsinogen (IRT)	1 per 6,500	Lung disease; growth failure	Pulmonary therapy; prevent infection; replace digestive enzymes
<b>Other Metabolic Disorders</b>				
Biotinidase deficiency	Biotinidase	1 per 1.05 million	Intellectual and developmental disability; seizures; skin rash; alopecia; hearing loss; death	Biotin therapy
Classic galactosemia (GALT)*	Galactosemia enzyme (GALT)	1 per 95,000	Neurodevelopmental impairment; liver disease; cataracts; Gram-negative sepsis in newborns	Galactose-restricted diet
<b>Hemoglobin Disorders</b>				
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
<b>Immunology Disorders</b>				
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

Medical Condition	Analyte(s) tested for	Incidence in NW region	Symptoms <i>if not treated</i>	Common Medical Treatment
<b>Lysosomal Storage Disorders</b>				
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
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
<b>Other Conditions</b>				
Spinal muscular atrophy (SMA) <sup>2†</sup>	Exon 7 of the <i>SMN1</i> 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) <sup>‡</sup>	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.

<sup>†</sup> Screening for this condition is anticipated to begin June 1, 2022.

<sup>‡</sup> Screening for this condition is anticipated to begin on or before January 1, 2023.

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](http://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 here: [www.nmhealth.org/about/phd/fhb/cms/nbgs/](http://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.

**Newborn bloodspot screening kits are pre-coded for the facility or practitioner that ordered the kit and should not be loaned to, or borrowed from, other facilities.**

**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 2nd specimen to be matched easily by the screening lab to the data from the 1st specimen. This matching system helps to link the data from newborn 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 in neonatal intensive care units (NICU). Each specimen in the kit has a barcode and a kit number that allow the 2nd specimen and 3rd specimen to be matched easily by the screening lab to the data from the 1st specimen. This matching system helps to ensure that newborn 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 1st specimen should be written on the single kit to help with matching the data for the infant. These kits will also be used when the OSPHL requests a repeat specimen.



# 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 time lines established below, collect and submit the bloodspot as soon as possible up to six months of age.

## 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 age but before 48 hours of age and a second specimen must be collected between 10 and 14 days of age as shown in [Table 2](#).

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

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

## Infants admitted to the NICU

For babies that require admission to a neonatal intensive care unit, collect the first specimen **as soon as possible after 24 hours of age but before 36 hours of age**. If the infant is being transfused, collect the specimen prior to transfusion regardless of the age of the infant. If an infant is transfused prior to 24 hours of age the second specimen must be collected at 48-72 hours of age. If the infant is **not** transfused prior to 24 hours of age the second specimen must be collected between 10 and 14 days of age. A third specimen must be collected at approximately 1 month, but no sooner than 28 days after birth.

For infants that are discharged or transferred after the first specimen (or second specimen) is collected, the remaining collection cards from the triple kit must be routed to the provider who will collect these specimens.

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

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

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

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

## Early discharge

If a family is requesting an early discharge, collect the 1st specimen before they leave your care. Some infants may not return for routine postnatal care. Please be certain to check the 'early discharge' box on the demographic portion of the card, to alert the lab that this is the reason for the early collection.

## Baby expires

In many cases, blood spot specimens from an infant who expired are a valuable resource for the family.

We recommend that you collect a newborn bloodspot screening specimen either at the typical screening interval, or sooner if needed and in consideration for the wishes of the baby's guardians.

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

- **Calling 503-693-4174 or**
- **Faxing the infant's information to 503-693-5601**

# Older infants

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

Table 2: Age of infant at specimen collection

	Collection Kit	First specimen	Second specimen	Third specimen
Routine Birth	Double Kit	As soon as possible after 24 hours of age but before 48 hours of life	10–14 days of age	Not Collected
NICU infants transfused prior to 24 hours of age	Triple Kit	Prior to transfusion	48–72 hours after birth	~ 1 month, no sooner than 28 days
NICU infants <u>not</u> transfused prior to 24 hours of age	Triple Kit	As soon as possible after 24 hours of age but before 36 hours of age and prior to transfusion	10–14 days of age	~ 1 month, no sooner than 28 days





# Patient demographic information

## **Incomplete demographic information may result in your specimen not being tested.**

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

Accurate and complete patient, provider, and specimen collection information must be provided on every collection card to allow for rapid follow-up if results are abnormal. This information is required by Clinical Laboratory Improvement Amendments of 1988 (CLIA) and must be legible.

The person performing the collection must:

1. Verify that the collection kit will not expire before all parts of the kit can be tested by the laboratory. If a double kit will expire within 1 month of the collection, please use a different kit. The expiration date is on the spine and the back of the kit as well as on the top of the filter paper portion.
2. Identify the infant and match with the correct screening kit. Make sure to select the correct kit part (1st, 2nd or 3rd) depending on the specimen being collected.

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

**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.**

3. **ALL** demographic fields must be filled in before collecting the specimen (see figure 2).
  - a. If the birth mother will not be maintaining custody of the infant, provide the name, address and phone number for the infant's guardian in the "Mother" fields. This information may be used to locate the infant for follow-up.
  - b. Labels may be used to provide demographic information. They must be included on all layers of the screening kit. They must not cover demographic information fields that will be hand-written.

**Provide the name and contact information for the provider who is responsible for the infant's medical care and treatment *after discharge* in the "Send report to PCP / Clinic" field. This practitioner will be sent screening results and follow-up information. Do not use the resident, attending, or on-call provider!**

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


RETURN TO: OREGON STATE PUBLIC HEALTH LAB  
 PO BOX 275, PORTLAND, OR 97207-0275 (503)693-4174

7070717  
W161

LOT

**1st Newborn Screening**

**SPECIMEN OR** SN



\* 1 1 7 5 0 6 8 4 0 5 \*

DO NOT WRITE  
IN THIS SPACE

Baby's Last Name		Baby's First Name		Send Report to PCP/Clinic:		CODE	
( ) Single Birth, or ( ) Multi-Birth A B C D E F Circle One				Address/Telephone Number			
Sex: M F		ID Chart #		Birth Date ( / / )		Time of Day (circle one) : am pm	
Food Source Last 24 Hours:		Soy Formula, [5] NPO		Check all that apply		Birth Wt. Lbs. oz	
[4] Breast, [3] Lactose Formula, [6] Other		[0] Other		Specimen Date ( / / )		Time of Day (circle one) : am pm	
Other Factors:		Transfusion		Present Wt. Lbs. oz			
<input type="checkbox"/> Early discharge <input type="checkbox"/> TPN		Last RBC Transfusion Date / / OR <input type="checkbox"/> None		Baby's Race: [1] White, [2] Black, [3] Amer. Ind./Native, [4] Unknown/Other, [0] Asian / Pacific Islander		Alaskan Native Hispanic? No Yes	
Hosp. or Hosp. CODE/Submitter ID and Address				Mother's Last Name		First Name	
				Mother's Address-Number & Street		Mother's Birth Date	
				City		State Zip Code	
Specimen taken by:				Telephone Number: Specimen reference #			



# Heel-stick specimen collection instructions\*

Each facility or medical provider must establish a procedure for staff performing newborn bloodspot screening specimen collections. Resources are available from the Clinical Laboratory Standards Institute that can help with creating or updating your procedure.

**Contact the OSPHL  
at 503-693-4174  
for assistance with  
specimen collection.**

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

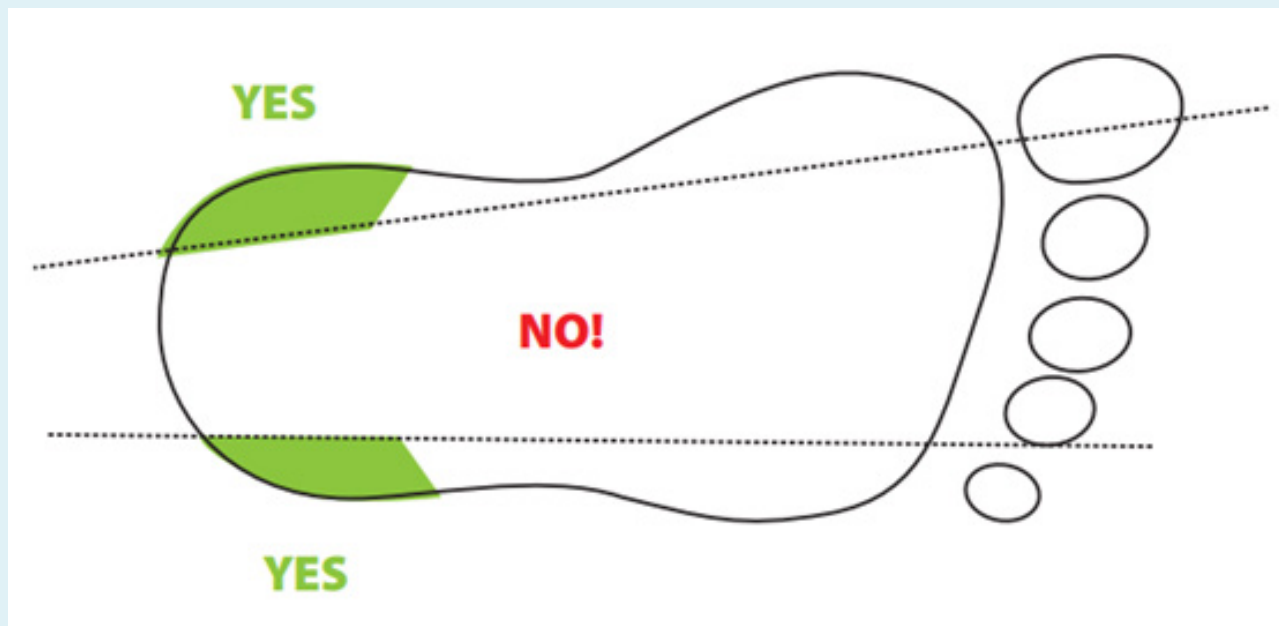
A training detailing proper collection and helpful resources can be found at [www.bitly.com/nbs-resource](http://www.bitly.com/nbs-resource). Tips to avoid rejected specimens are provided later in this manual.

1. Use a scalpel bladed lancet manufactured specifically for heel stick collection from an infant. Do not use a lancet longer than 2.0 mm. Do not use capillary tubes or other collection devices.
2. Select a lance site on the infant's heel (see Figure 3). Cleanse lance site with alcohol and air dry. Do not use betadine, iodine, lotion, or essential oils on the baby prior to collecting the specimen.
3. Perform lancing on the most medial or most lateral portion of the plantar surface of the heel.

---

\* These recommendations conform to CLSI publication NBS01-A6.

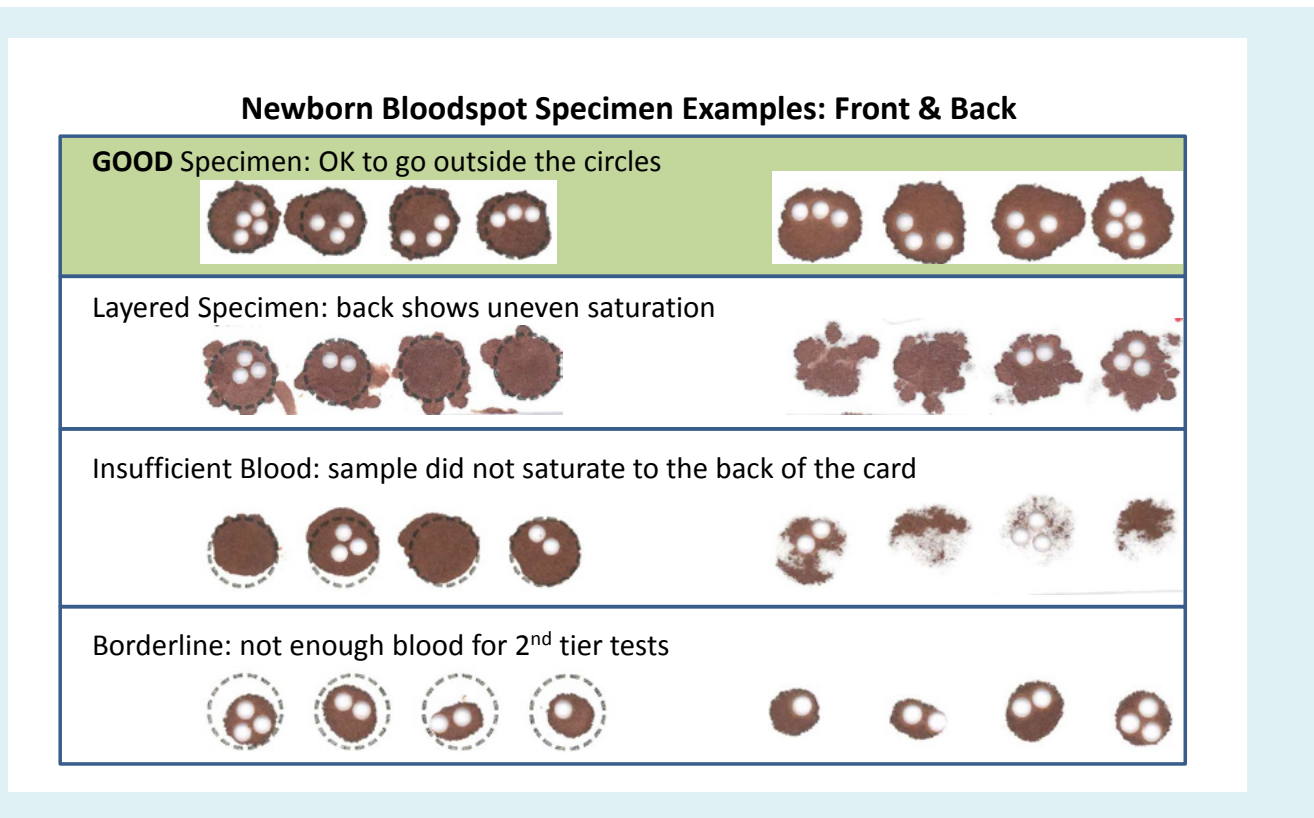
Figure 3: Recommendation for heel puncture site in newborns



4. Lance the heel with the sterile scalpel bladed lancet. Wipe away the first drop of blood to remove tissue fluids. Do not “milk” or squeeze the heel.
5. Allow a single drop of blood to collect on the heel that is large enough to fill a collection circle. Do not layer multiple spots of blood on top of one another.
6. Touch the filter paper gently to the drop of blood. Only apply blood to one side of the filter paper (it doesn’t matter which side is used).
7. Allow the blood to soak through the filter paper so that the blood spot looks similar on both the front and back of the collection kit.



Figure 4: Newborn bloodspot specimen examples



Complete, even saturation of the filter paper is essential for accurate testing. The filter paper is calibrated to absorb a specific quantity of blood. Incomplete, uneven saturation or layering of the blood alters the quantity of blood used for testing and will lead to inaccurate test results. This figure is also available at: [www.bitly.com/nbs-example](http://www.bitly.com/nbs-example).

8. Collect the blood in all five circles, repeating instructions 5 through 7. If blood flow is not sufficient, re-lance the heel. It is better to fill three circles completely than to fill four circles inadequately.
9. Air dry specimens at room temperature for between 3 and 4 hours in a **horizontal** position with the blood spots exposed. Hanging wet specimens vertically will cause heavier red cells to migrate to the dependent end of the circle resulting in uneven saturation.
10. Do not expose the specimen to excess heat or humidity at any time. Do not dry on a heater, in a microwave, with a hair dryer or in sunlight. Do not place in plastic bags, leave in a hot mailbox or in a hot car. These practices can destroy some proteins and enzymes that are required for accurate test results.
11. Ensure that the specimen is completely dry before transporting.

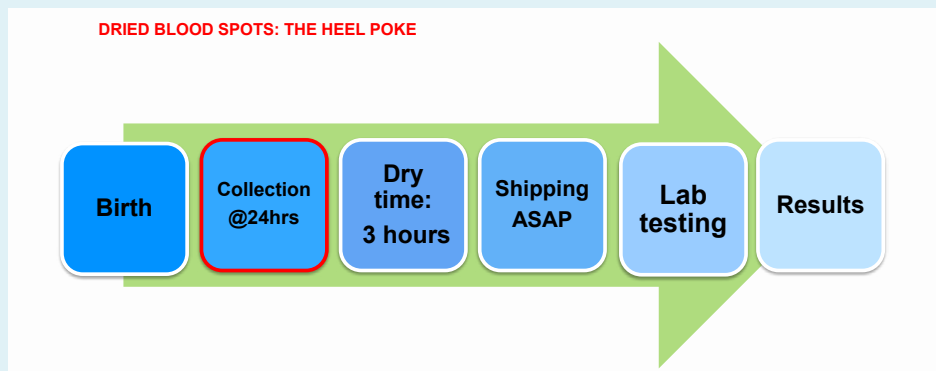


# Specimen transport

**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.**

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

**Figure 5: Newborn bloodspot screening process**



Specimens should be sent as soon as they are dried (between 3 and 4 hours) and no later than 24 hours after collection.

1. Keep a record of the specimens that are sent, including the kit numbers. A packing list or manifest should be included with the shipment.
2. Insert the dried specimen(s) into an envelope. Do not put specimens in plastic bags or containers. Do not compress the specimens.
3. Send the specimens no later than 24 hours after collection.
4. All specimens must be sent by express mail, courier or another timely delivery mechanism. Specimens should be received by the OSPHL within 48 hours of collection.

5. Send the specimens to:

Oregon State Public Health Laboratory  
Newborn Bloodspot Screening Program  
7202 NE Evergreen Parkway, Suite 100  
Hillsboro, OR 97124

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

Prompt transit is essential for identifying infants who may be impacted by a screening condition within one week of birth. 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 promptly. Delays within a facility may be from inefficient internal processes, slow courier services, simple forgetfulness, or, most dangerously, batching specimens.

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





# Reporting of results

## Results are available online

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

## Results reporting

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

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

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.

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

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

Calling 503-693-4174 or

Faxing the infant’s information to 503-693-5601



## I did not receive my newborn bloodspot screening results!

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

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

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



# 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. These include:

### Primary Congenital Hypothyroidism (CH)

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

### Lysosomal Storage Disorders (LSD)

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

## Parenteral nutrition and carnitine therapy

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 cell transfusions

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

**Report that a transfusion occurred on the specimen collection card.**

## 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.

**Report that Pivalic acid was administered on the specimen collection card.**

## 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





# Requesting newborn bloodspot screening records

- If the child is younger than 6 years, request his or her newborn bloodspot screening records by faxing the child's full name, date of birth, kit number and mother's name (at the time of the child's birth) and date of birth on your letterhead to 503-693-5601.
- Records that are over 6 years old are outside of their record retention and should have been destroyed. It is unlikely that older records will be located. When requesting records older than 6 years, include a medical record release authorization signed by the patient, if over 18, or the parent or guardian.
- If you are requesting records for a baby who was born in another state, please contact that state's newborn bloodspot screening program to request results. Contact information for each state is provided by Baby's First Test at [www.babysfirsttest.org](http://www.babysfirsttest.org).
- Parents or legal guardians may request the infant's newborn bloodspot screening records by completing the form located at [www.bitly.com/get-phl-results](http://www.bitly.com/get-phl-results).





# Use, release, and retention of residual bloodspot specimens

**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.
- Program evaluation and quality improvement.
- Education activities required by Oregon Statute.

Residual bloodspot specimens will only be released by the OSPHL:

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

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



# Tips to avoid rejected specimens

**Improperly collected specimens compromise the accuracy of test results. When a specimen is rejected, a repeat collection will be required. This unnecessarily delays the screening of the newborn.**

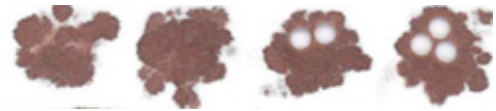
**Contact the OSPHL at 503-693-4174 to request more information about specimen collection or to request support from the NBS Education Coordinator.**

## Tips to avoid “Layered Blood” rejection

**Specimen front**



**Specimen back**

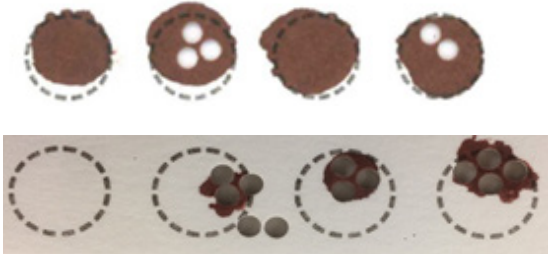


### Tips to avoid this type of rejection

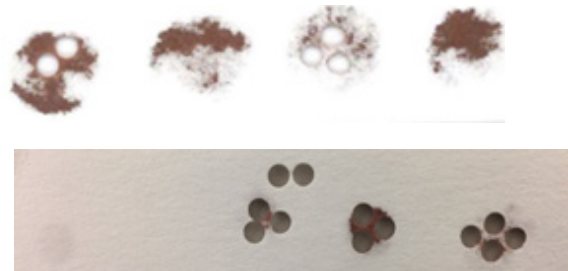
- Use the proper size lancet (< 2mm length).
- Allow a large drop to form on the heel before touching with the filter paper.
- Collect blood into one circle at a time.
- Do not apply additional blood to an incompletely filled circle.
- Do not apply blood to both sides of the filter paper.
- Do not compress the filter paper.

## Tips to avoid “Incomplete Saturation” or “Quantity Not Sufficient” rejection

**Specimen front**



**Specimen back**



### Tips to avoid this type of rejection

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

## Tips to avoid “Contaminated” rejection

**Specimen front**



**Specimen back**



### Tips to avoid this type of rejection

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



# 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)

### CF essentials

- **Screening test:** The first-tier immunoassay measures immunoreactive trypsinogen (IRT). For specimens with an elevated IRT on one (if sufficiently high) or both screening specimens, second-tier DNA screening for 34 common variants is performed.
- **Confirmatory test:** Sweat chloride testing and DNA mutation analysis
- **Validity:** A small percentage of cases (<10%) will be falsely negative. Most cases should be abnormal on the first screen. IRT may be falsely elevated in premature, stressed, or sick infants. IRT can be falsely low in infants with CF who are born with meconium ileus.
- **Treatment:** Comprehensive, multidisciplinary care, pancreatic enzyme replacement, soluble vitamin replacement, high-calorie/high-fat diet, airway clearance regimen, and new specific targeted therapies based on genotype. Refer to accredited Cystic Fibrosis 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.

Cystic fibrosis (CF) is a recessively inherited defect of the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Over 1,800 mutations of the CFTR protein have been identified, but a single mutation (F508del), accounts for ~86% of all the mutations worldwide. There are approximately 34,000 adults and children with CF in the United States. The incidence of CF in the United States is approximately 1:3,500 newborns but varies by ethnicity: 1:3,500 Caucasian Americans, 1:8,500 Hispanic Americans, 1:17,000 African Americans, 1:31,000 Asian Americans.

## Clinical features

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

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

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

## Causes of CF

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

## Laboratory tests

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

For specimens with an elevated IRT on one (if sufficiently high) or both screening specimens, second-tier DNA analysis is performed (34 mutations, including current ACMG/ACOG recommendations). Depending on results, further diagnostic and confirmatory testing will be required, including additional mutation analysis.

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.
- Infants with meconium ileus may not have an elevated IRT. If meconium ileus is present, then diagnostic testing should be performed regardless of NBS results. It is important to remember that all infants with meconium ileus should have routine newborn bloodspot screening specimens collected even if CF is suspected, as they should be screened for the other conditions on the screening panel.
- A small percentage of infants with CF may not have an elevated IRT. Thus, a normal IRT at birth does not completely rule out CF. Children with recurrent respiratory problems, failure to thrive, or other symptoms consistent with CF, should still be evaluated and undergo sweat chloride testing.

## Confirmatory testing

CF can be diagnosed by two different methods, sweat chloride testing and/or DNA mutation analysis. Sweat chloride testing remains the gold standard, as it is a concrete marker of CFTR dysfunction. A chloride value in the sweat of  $\geq 60$  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](http://www.cftr2.org)), and most are not included in standard multi-array DNA analyses. (10, 11) Confirmation of two CF-causing mutations confirm the diagnosis, while only one may indicate a carrier state, CFTR-related metabolic syndrome (CRMS), or an affected individual with a less common mutation on the second allele.

## Treatment

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

## Screening practice considerations

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

**Table 3: CF screening result summary**

Results	Likely causes	Actions
1 <sup>st</sup> and 2 <sup>nd</sup> IRT elevated on filter paper specimens	<ul style="list-style-type: none"><li>• Cystic fibrosis probable</li><li>• Possible false positive</li></ul>	<ul style="list-style-type: none"><li>• NBS coordinator faxes results.</li><li>• Medical consultant phones practitioner with follow-up recommendations.</li><li>• Sweat chloride testing needed to confirm/clarify diagnosis.</li></ul>
One or two CFTR mutations identified on 23-mutation DNA analysis	<ul style="list-style-type: none"><li>• If two mutations, CF probable</li><li>• If one mutation, possible CF carrier vs CRMS vs CF with rare 2<sup>nd</sup> mutation</li></ul>	<ul style="list-style-type: none"><li>• NBS coordinator faxes results.</li><li>• Medical consultant phones practitioner with follow-up recommendations.</li><li>• Sweat chloride testing needed to confirm/clarify diagnosis.</li></ul>

# Congenital Adrenal Hyperplasia (CAH)

## CAH essentials

- **Neonatal emergency:** 3/4 will develop salt wasting crisis, which can be fatal, in the first week to month of life.
- **Incidence:** 1:12,700 newborns
- **Screening test:** 17-OH-progesterone
- **Validity:** 70% identified on 1st screen  
30% on 2nd screen
- **Causes:** 21-hydroxylase deficiency or other inborn error of cortisol synthesis; recessive inheritance
- **Treatment:** Hydrocortisone and mineralocorticoids
- **False positives:** Occur more frequently in premature, low birth weight or sick infants
- **Outcome:** Early detection and treatment can be lifesaving. Chromosome analysis in infants with ambiguous genitalia will prevent gender misassignment (11). Ultimate outcome depends on severity of defect, days to treatment and adherence. Refer to pediatric endocrinologist.

CAH is an inherited defect of cortisol synthesis. The adrenal gland cannot make cortisol and overproduces male hormones. Without cortisol, infants are at risk for adrenal crisis and may be unable to regulate salt and fluids, and can die. The most common disorder is 21-hydroxylase deficiency.

## Clinical features (12)

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

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

## Causes of CAH

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

## Laboratory tests

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

## Confirmation

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

## Treatment

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

## Screening practice considerations

- This disorder 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. Collect heel stick specimens between 24–48 hours of life. Transport all specimens 4–12 hours after collection and no later than 24 hours.
- Female infants who are virilized or infants with ambiguous genitalia should be considered at risk for this condition, tested at birth and monitored for electrolyte abnormalities until the diagnosis is excluded.
- Male infants are not usually recognized at birth.
- About 30% of infants will be detected only on a second screen. (13–15)

**Table 4: CAH screening result summary**

Results	Likely causes	Actions
Elevated 17 OHP (17-hydroxyprogesterone)	<ul style="list-style-type: none"><li>• CAH probable</li><li>• False positive</li></ul>	Neonatal emergency; NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

# Primary Congenital Hypothyroidism (CH)

## CH essentials (16, 17)

- **Incidence:** 1:2,000 newborns
- **Screening test:** T4 (thyroxine) and TSH (thyroid stimulating hormone)
- **Validity:** 90% identified on 1st screen, 10% on 2nd screen
- **Causes:** Thyroid dysgenesis: 85%; hereditary inborn error of thyroid hormone biosynthesis: 15%
- **Treatment:** L-thyroxine normalize T4 by 2 weeks of treatment initiation; TSH by 1 month
- **False positives:** Early collection within 24 hours of birth; premature or ill infants
- **Outcome:** Can be normal, but depends on severity of thyroid deficit, days to treatment and adherence to treatment. Severely affected infants with just a 2-week delay in reaching a serum T4 >10 ug/dL may have up to a 10-point drop in IQ. (18)

Primary congenital hypothyroidism (CH) occurs in infants who are born without the ability to produce adequate amounts of thyroid hormone. Thyroid hormone is important for normal function of all of the body's organs and is essential for normal brain development. The incidence of congenital hypothyroidism is 1:2,300. CH is more common in Hispanic and Native American populations (1:700–2,000). There is a 2:1 female/male ratio, explanation unknown. Infants with Down's syndrome have increased risk of CH (1:140 newborns).

## Clinical features

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

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

## Causes of primary congenital hypothyroidism

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

Some cases of central or secondary (hypopituitary) hypothyroidism may also be detected (see Table 5). These newborns often have clinical features of other pituitary hormone deficiencies, such as hypoglycemia or small penis and undescended testes in male infants.

## Laboratory tests

The initial screening test is the T4 assay. Infants with T4 results of <10% are further tested by a screening TSH assay. Different combinations of results are possible; see (see Table 5).

When the infant's physician is notified that screening results are abnormal, blood should be collected by venipuncture as soon as possible for measurement of TSH and free T4 to confirm the abnormal screening results. In the case where the screening T4 is low and TSH is elevated, treatment can be started as soon as the serum is obtained, pending final confirmation. If the serum thyroid function tests confirm hypothyroidism, further diagnostic studies, such as a thyroid ultrasound examination or radionuclide scan and X-ray to assess skeletal maturation, may be performed to determine the type, age of onset and severity of hypothyroidism. Generally, these studies do not change management and thus are optional.

## Thyroid function in premature infants

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

## Treatment

The American Academy of Pediatrics (AAP) recommends that infants be managed in consultation with a pediatric endocrinologist. (16) Treatment of CH is effective if done correctly. L-tyroxine (brand or generic l-thyroxine), in pill form, is crushed, mixed with water or expressed breast milk and administered once daily. The recommended starting dose is 10–15 mcg/day of body weight daily, usually 37.5 mcg/day to 50 mcg/day. AAP recommendations for follow-up TSH and free T4 are as follows:

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

**Treatment goals:** Maintain serum free T4 in the upper half of the normal or 1.2–2.4 ng/dL for free T4 (normal range may vary with assay), and TSH normalized (<6  $\mu$ IU/mL). Clinical evaluations can occur less frequently. As infants grow, the dose of thyroxine is increased. Periodic developmental testing should be done on all patients. If treatment is started early and thyroid levels are monitored closely, development remains normal (19).

## Screening practice considerations

- Primary congenital hypothyroidism is common, occurring in approximately 1:2,000 newborns.
- Ninety percent of hypothyroid infants are detected on the first specimen; in 10% of cases, hypothyroidism develops in the weeks after birth and is detected on a second screening test as production of thyroid hormone decreases after birth. (20–21)
- Some infants (usually pre-term) will manifest a delayed rise in TSH, and so are also detected on the routine second or third screening test. Practitioners therefore must remain alert to clinical symptoms in premature and older infants despite normal initial screening.
- False positive results may occur if the specimen is collected within the first few hours after birth, as the TSH rises in response to the extra-uterine environment.
- Topical iodine use on the infant or a mother who is breastfeeding and taking iodine supplements may cause transient hypothyroidism. In addition, nursing mothers drinking “seaweed soup”, which has a high iodine content, may also cause hypothyroidism in the neonate; this will resolve if ingestion of seaweed soup is discontinued.

**Table 5: CH screening result summary**

Results	Likely causes	Actions
T4 low/TSH elevated	<ul style="list-style-type: none"> <li>• Hypothyroidism probable</li> <li>• False positive</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
T4 low/TSH slightly elevated	<ul style="list-style-type: none"> <li>• Mild hypothyroidism</li> <li>• Transient hypothyroidism seen with recovery from “hypothyroxinemia of prematurity”</li> <li>• False positive</li> </ul>	NWRNBS Program contacts practitioner by FAX and by mail requesting further testing.
T4 low/TSH normal (on two specimens unless premature)	<ul style="list-style-type: none"> <li>• Thyroid binding globulin (TBG) deficiency</li> <li>• Central or secondary (hypopituitary) hypothyroidism</li> <li>• Non-thyroidal illness syndrome (“sick euthyroid syndrome”) associated with prematurity or acute illness</li> <li>• False positive</li> </ul>	NWRNBS Program contacts practitioner by FAX and by mail requesting further testing.

## Sickle Cell Disease and other Hemoglobinopathies

### Sickle Cell Disease essentials

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

The primary goal of hemoglobinopathy screening is to detect clinically significant sickling hemoglobinopathies in the neonatal period, before symptoms occur. Newborn diagnosis of sickle cell disease, if coupled with family education and centralized comprehensive care, can markedly lower morbidity and mortality. (22)

Homozygous sickle cell disease (SCD) occurs when the recessive gene for hemoglobin S, sickle hemoglobin, is inherited homozygously or with a second gene for certain other hemoglobin variants, such as beta thalassemia or hemoglobin C. These doubly heterozygous conditions tend to be less severe than those who are homozygous for hemoglobin S, although all are potentially capable of producing severe complications. The disease incidence in a population depends on the population’s racial composition.

## Clinical features

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

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

## Laboratory tests

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

## Treatment

Infants with significant hemoglobinopathies should have a primary care provider and receive periodic evaluation 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.

## Carrier detection makes SCD screening different

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. If both parents are carriers of an autosomal recessive genetic trait, the risk of any infant of that couple being homozygous, and therefore having the disease, is 1:4.

## Screening practice considerations

- Newborn bloodspot screening for hemoglobinopathies is not done on the second specimen unless an abnormality has been identified on the first specimen. It is crucial to use the first kit for the first test; the cards are not interchangeable.
- Transfusion of red blood cells before collecting the newborn bloodspot screening specimen will invalidate the hemoglobinopathy test. Always obtain a specimen before any transfusion regardless of the infant's age.
- Some hemoglobinopathies, particularly some thalassemias, are not reliably detected by newborn 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.

# Amino Acid Conditions

## Hypermethioninemia

*Homocystinuria (cystathionine beta-synthase deficiency)\**

### Homocystinuria essentials

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

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

### Clinical features (23, 24)

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.

---

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

## Methionine adenosyltransferase (MAT) deficiency

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

### Laboratory test

Elevation of methionine is detected by tandem mass spectrometry (MS/MS).

### Treatment

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

### Screening practice considerations

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

**Table 6: Hypermethioninemia screening result summary**

Results	Likely causes	Actions
Methionine slightly elevated	<ul style="list-style-type: none"><li>• Homocystinuria/MAT deficiency possible</li><li>• Tyrosinemia, Type I, galactosemia</li><li>• Liver disease</li><li>• Parenteral nutrition</li><li>• High protein diet</li><li>• False positive</li></ul>	NWRNBS Program requests repeat filter paper specimen by mail.
Methionine elevated	<ul style="list-style-type: none"><li>• Homocystinuria/MAT deficiency probable</li><li>• Tyrosinemia, Type I</li><li>• Liver disease</li><li>• Parenteral nutrition</li><li>• High protein diet</li><li>• False positive</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations

# Phenylketonuria (PKU) and Hyperphenylalaninemia

## Hyperphenylalaninemia essentials

- **Incidence:** 1:16,300 births
- **Screening test:** Phenylalanine elevated by tandem mass spectrometry (MS/MS); phenylalanine/tyrosine ratio elevated
- **Confirmatory tests:** Quantitative amino acids; biopterins in blood and urine
- **Validity:** >99% on 1st screen
- **Treatment:** Low phenylalanine diet; biopterin supplementation
- **Outcome:** Normal if treated early and adherence is good

Detection of elevated phenylalanine levels requires urgent follow-up. The disorder is due to a recessively inherited enzyme defect in which the body cannot use the amino acid phenylalanine properly. All other metabolic processes are intact, but phenylalanine, which comes from all dietary protein, accumulates in the blood to toxic levels. All forms of hyperphenylalaninemias from mild to severe and including biopterin defects are inherited as autosomal recessive disorders.

## Clinical features

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

Phenylalanine starts rising after birth and often reaches abnormal levels within 24 hours of life. A phenylalanine/tyrosine ratio can also be used to identify cases.

## Variant forms of PKU (hyperphenylalaninemia)

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

Some forms of hyperphenylalaninemia are caused by defects of the cofactor biopterin metabolism and blood phenylalanine levels are variable. These patients have progressive neurological damage with seizures and steady deterioration that becomes noticeable sometime between 6 and 20 months of age despite early

treatment with a low phenylalanine diet. Definitive tests can differentiate these variant forms of PKU. In view of the severity of this group of diseases, all infants with persistently abnormal levels of phenylalanine must have testing by special blood and urine tests for bipterin abnormalities.

## Maternal PKU and hyperphenylalaninemia

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

## Laboratory tests

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

## Treatment (25–28)

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

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

## Screening practice considerations

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

**Table 7: PKU and hyperphenylalaninemia screening result summary**

Results	Likely causes	Actions
Phenylalanine elevated; Phe/Tyr elevated	<ul style="list-style-type: none"> <li>• PKU possible</li> <li>• Variants forms of PKU</li> <li>• Mother has PKU</li> <li>• False positive</li> <li>• Transient hyperphenylalaninemia</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

## Tyrosinemia type I, II, and Transient Tyrosinemia\*

### Tyrosinemia essentials

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

### Transient Tyrosinemia (29)

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

### Clinical features

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

\* Not all cases of tyrosinemia will be detected by newborn bloodspot screening.

## Treatment

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

## Tyrosinemia Type I (Hepatorenal Tyrosinemia) (30)

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

### Clinical features

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

### Treatment

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

## Tyrosinemia Type II (Occulocutaneous Tyrosinemia)

Tyrosinemia, Type II is caused by a deficiency of the enzyme tyrosine aminotransferase (TAT) and is inherited as an autosomal recessive trait. TAT deficiency is rare, with about 100 cases described worldwide, although more infants may be identified as MS/MS screening continues to be implemented. (31)

## Clinical features (20, 31)

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

## Treatment

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

## Laboratory tests

Tyrosinemia is detected using both tyrosine and succinylacetone measured by MS/MS. There is considerable overlap in tyrosine levels between normal infants, those with transient tyrosinemia and affected infants, making the tyrosine level itself not very specific. Succinylacetone is the unique marker for tyrosinemia type I.

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

## Screening practice considerations

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

**Table 8: Tyrosinemia screening result summary**

Results	Likely causes	Actions
Tyrosine elevated Succinoylacetone normal	<ul style="list-style-type: none"><li>• Transient tyrosinemia</li><li>• Tyrosinemia type II or III possible</li><li>• Liver disease</li><li>• Parenteral nutrition</li><li>• False positive</li></ul>	NWRNBS Program requests repeat filter paper by mail.
Succinolyacetone increased. Tyrosine normal.	<ul style="list-style-type: none"><li>• Tyrosinemia type I</li><li>• False positive</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations

# Fatty Acid Oxidation (FAO) Conditions

## FAO condition essentials

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

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

## Fatty Acid Oxidation conditions\*

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

---

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

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

## Clinical features (33, 34)

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

**Hepatic (35, 36):** No typical age of presentation, may occur on the first day of life through adulthood. Infants with MCAD can present with sudden cardio/pulmonary arrest before screening results are known. Precipitating factors are fasting and/or stress associated with intercurrent illness. Patients present with “Reyes-like” symptoms including vomiting, lethargy, hypoketotic hypoglycemia, mild hyperammonemia, hyperuricemia, hypocarnitinemia and abnormal liver function tests. Liver biopsy often shows steatosis. Hepatic presentation is common in MCAD, VLCAD, LCHAD, neonatal CPT I & II and mild CACT deficiency. Patients with LCHAD may develop retinal pigmentary changes and progressive visual loss in childhood despite early diagnosis and treatment.

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

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

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

## Treatment

Even with screening, some infants with FAO disorders may die before laboratory results are available. Treatment for MCAD and some other FAOs is extraordinarily simple once the diagnosis is suspected. Avoidance of fasting, particularly as infants and young children, is the primary treatment. Carnitine supplementation (100mg/kg/day) is used to provide a pathway for removal of toxic intermediate metabolites in some FAOs. With appropriate treatment hepatic, cardiac and muscular complications can be reduced or eliminated. Patients with these disorders may require IV support for fluid and calories during intercurrent infections or illnesses. With pre-symptomatic diagnosis and appropriate therapy, outcome can be normal for infants with MCAD. (37, 38) Outcomes for the other disorders are still being evaluated.

## Screening practice considerations

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

## Organic Acid Conditions (OA)

### OA condition essentials

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

The following OAs are screened for by MS/MS:

- Beta-ketothiolase deficiency
- Glutaric acidemia, type I (glutaryl-CoA dehydrogenase deficiency)
- Isobutyryl CoA dehydrogenase deficiency
- Isovaleric acidemia, (isovaleryl-CoA dehydrogenase deficiency)
- Malonic aciduria
- Maple syrup urine disease (branched chain alpha-ketoacid dehydrogenase deficiency)

- Methylmalonic acidemias, methylmalonyl CoA mutase deficiency and defects of B-12 metabolism
- Propionic acidemia
- 3-Hydroxy-3-methylglutaryl (HMG) CoA lyase deficiency
- 2-Methyl-3-hydroxybutyryl CoA dehydrogenase deficiency
- 2-Methylbutyryl CoA dehydrogenase deficiency (mitochondrial acetoacetyl-CoA thiolase deficiency)
- 3-Methylcrotonyl CoA carboxylase (3MCC) deficiency
- 3-Methylglutaconyl CoA hydratase deficiency (3-methyl-glutaconic aciduria, type I)
- Multiple carboxylase deficiency

## Clinical features (39, 41)

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

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

**Asymptomatic cases:** There are numerous reports of cases of isolated 3-methylcrotonyl-CoA carboxylase deficiency who have remained asymptomatic despite biochemical and/or enzymatic confirmation of the condition. The etiology of these variant presentations is not yet understood. Mild forms of methylmalonic acidemia have been found.

**Glutaric Acidemia, type I:** Glutaric Acidemia, Type I or GA I is an organic acidemia with clinical features unlike those described above. (40–42) In this disease, there is

an accumulation of glutaric acid and 3-hydroxy glutaric acid, which are believed to be toxic to cells, particularly in the central nervous system. The classic presentation is macrocephaly at or shortly after birth. Infants have a period of apparently normal development but may have soft neurological signs, like jitteriness, irritability and truncal hypotonia. Generally, between 6 and 18 months of age, patients will experience an acute encephalopathic episode resulting in damage to the basal ganglia and atrophy of the caudate and putamen. This occurs over the course of a few hours to a day and is irreversible and untreatable. Severe dystonia, dyskinesia and other neurological findings result, either in a static or slowly progressive form. These children are often misdiagnosed as having extra pyramidal cerebral palsy. Approximately 25% of GA I patients will present with motor delay, hypotonia, dystonia and dyskinesia that develop gradually during the first few years of life, without any apparent acute crisis. Intellect is relatively intact. Infants with GA I are prone to acute subdural and retinal hemorrhages after minor head trauma. This can be misdiagnosed as child abuse. Finally, 5% of all Amish patients have been completely asymptomatic without any crises and normal development. Neurological crises and symptoms rarely occur after 5 years of age.

## Laboratory tests

All these disorders are detected using MS/MS. Leucine can be elevated in infants receiving parenteral nutrition, usually along with other amino acid elevations. In a normal newborn, however, elevations of these compounds are unusual and require rapid follow-up. There is evidence that not all affected infants will be found by NBS. (43)

## Treatment

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

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

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

## Screening practice considerations

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

# Urea Cycle Conditions (UCD)

## Urea Cycle essentials

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

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

- Arginase deficiency
- Argininosuccinic aciduria (ASA)
- Citrullinemia, type I and II

They are inherited as autosomal recessive traits.

## Arginase deficiency (44)

### Clinical features

Arginase deficiency is associated with irritability, inconsolable crying, anorexia, vomiting and developmental delay in infancy. This progresses to spastic tetraplegia with lower limbs more severely affected than the upper, psychomotor 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.

## Citrullinemia, Type I (CTLN1) and Argininosuccinic Aciduria (ASA) (44, 46)

### Clinical features-neonatal onset

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

### Clinical features-late onset

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

### Asymptomatic cases

Newborn 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.

## Citrin Deficiency (Citrullinemia, Type II and Neonatal Intrahepatic Cholestasis [NICCD]) (47)

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

### Clinical features-neonatal onset

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

### Clinical features-late onset

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

### Laboratory tests

Elevations of citrulline and arginine are detected by MS/MS. The laboratory cutoff for citrulline is  $\leq 70 \mu\text{M/L}$ ; for arginine,  $\leq 110 \mu\text{M/L}$ ; argininosuccinic acid,  $\leq 1.50 \mu\text{M/L}$ . Transient elevations of plasma arginine and citrulline in the newborn are unusual unless the infant is premature and/or receiving parenteral nutrition.

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

### Treatment (Citrullinemia, Type I & ASA)

All patients with a neonatal presentation represent medical emergencies and outcomes may be variable. Patients with neonatal onset disease will typically require aggressive treatment with hemodialysis. All patients, both late onset and those rescued from neonatal hyperammonemia, will require treatment with low protein diets and medications to prevent hyperammonemia and remove toxic compounds. The outcome for patients rescued from prolonged neonatal hyperammonemia is extremely poor.

Brain damage is likely. Even patients treated prospectively from birth may not be unaffected. (46) Those with late onset disease fare better, and presymptomatic diagnosis and treatment may allow normal development.

## Treatment: NICCD and CTLN2

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

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

## Screening practice considerations

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

**Table 9: UCD screening result summary**

Results	Likely causes	Actions
Arginine >110 µM/L	<ul style="list-style-type: none"> <li>• Arginase deficiency possible</li> <li>• Transient argininemia</li> <li>• Liver disease</li> <li>• False positive</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
ASA >1.50 µM/L	<ul style="list-style-type: none"> <li>• Argininosuccinic aciduria possible</li> <li>• Liver disease</li> <li>• False positive</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
Citrulline >70 µM/L	<ul style="list-style-type: none"> <li>• Citrullinemia, argininosuccinic aciduria possible</li> <li>• Transient citrullinemia</li> <li>• Liver disease</li> <li>• False positive</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
Citrulline >120 µM/L on second specimen	<ul style="list-style-type: none"> <li>• Mild citrullinemia, argininosuccinic aciduria possible</li> <li>• Transient citrullinemia</li> <li>• Liver disease</li> <li>• False positive</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations

# Galactosemia

## Galactosemia essentials

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

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

## Clinical features (48)

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

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

## Laboratory tests

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

- **GALT activity:** The enzyme test depends upon fluorescence produced by the normal galactose enzyme cascade in red blood cells. A temporarily abnormal result (diminished or absent fluorescent activity) is found in some infants. The test may be persistently abnormal if the enzyme activity is <50% of normal. It does not differentiate milder variants from severe defects or G6PD.
- **Galactose:** Slight elevations can occur in normal neonates, but galactose metabolites are greatly elevated in infants with galactosemia if they are receiving a lactose-containing formula or breast milk. Liver disease may also cause an elevation of galactose metabolites. All infants with an abnormal GALT or who have been transfused will be screened for galactose.

## Treatment

Galactosemia is treated by dietary galactose restriction (usually accomplished in the infant period through the use of soy-based or partially hydrolyzed infant formulas). The diet must be followed for life and requires close supervision. Even with early diagnosis and strict dietary restrictions children with galactosemia are at risk for speech disorders, tremors, growth and developmental delays and in females, ovarian failure.

## Screening practice considerations

- The GALT test should be abnormal in virtually all severe classic galactosemic infants even if the specimen is obtained before lactose is ingested, unless the infant has been transfused. Obtain a specimen before any transfusion.
- The GALT enzyme is prone to degradation if the sample is delayed in the mail or exposed to excess temperature or humidity. This produces a false positive GALT result.
- Galactose accumulation depends on lactose ingestion so that blood galactose metabolites may be normal in infants being fed a soy-based formula.

**Table 10: Galactosemia screening result summary**

Results		Likely causes	Actions
GALT test	Galactose metabolites		
<3.5 u/dL	≥20 mg/dL	<ul style="list-style-type: none"> <li>• Severe galactosemia</li> <li>• Variant galactosemia</li> <li>• False positive</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
<3.5 u/dL	<20 mg/dL	<ul style="list-style-type: none"> <li>• Severe galactosemia with little lactose intake</li> <li>• Variant galactosemia</li> <li>• Other enzyme defects in red blood cells</li> <li>• Improperly handled sample (heat damage or transit delay)</li> </ul>	Contact by mail or fax if infant is ≥48 hours old. Contact by fax if <48 hours old or if not on lactose.

## Biotinidase Deficiency

### Biotinidase deficiency essentials

- **Incidence:** 1:60,000 births
- **Screening test:** Biotinidase qualitative colorimetric enzyme assay
- **Confirmatory tests:** Quantitative biotinidase enzyme assay
- **Validity:** 100% found on 1st screen
- **Treatment:** 5-10 mg biotin/day
- **Outcome:** Excellent if compliant with biotin therapy This recessively inherited disorder affects the cells' ability to recycle the vitamin-cofactor biotin, which impairs the function of mitochondrial carboxylases.

### Clinical features (49, 50)

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

Infants with partial deficiency (5–10%) have been identified through newborn 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.

## Laboratory tests

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

## Treatment

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

Screening practice considerations

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

**Table 11: Biotinidase deficiency screening result summary**

Results	Likely causes	Actions
Color change does not occur	<ul style="list-style-type: none"><li>• Biotinidase deficiency possible</li><li>• False positive</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

# Severe Combined Immunodeficiency (SCID)

## SCID essentials

- **Incidence:** 1:50,000-1:100,000 births
- **Screening test:** Polymerase chain reaction to detect T-cell Receptor Excision Circles (TRECs)
- **Confirmatory tests:** CBC, lymphocyte subset flow cytometry
- **Validity:** The TREC assay used by Oregon to evaluate specimens for SCID and other t-cell lymphopenia has a positive predictive value greater than 0.7.
- **Treatment:** Bone marrow transplant, gene therapy or enzyme replacement
- **Outcome:** Good if treated within first 3 months of life

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

## Clinical features

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

## Causes of SCID

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

## Laboratory tests

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

## Confirmation

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

## Treatment

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

# Lysosomal Storage Disorders (LSDs)

## What are LSDs?

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

## Which LSDs are being tested:

Disorder	Enzyme	Gene
Fabry Disease	Alpha Galactosidase A	<i>GLA</i>
Gaucher Disease	Acid Beta-Glucosidase	<i>GBA</i>
Mucopolysaccharidosis Type 1 (MPS-1)	Alpha-L-Iduronidase	<i>IDUA</i>
Pompe Disease	Acid Alpha-Glucosidase	<i>GAA</i>

## How are LSDs diagnosed?

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

# Fabry Disease

## Fabry disease essentials (53, 54)

- **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.
- **Screening test:** Tandem mass spectrometry (MS/MS) to detect Alpha-galactosidase A (GLA) enzyme followed by second-tier DNA analysis of the *GLA* gene.
- **Confirmatory test:** GLA enzyme activity in plasma and leukocytes, possible assistance via DNA analysis of family members.
- **Validity:** Published false positive rate is 0.27% for the NeoLSD kit (PerkinElmer) used to measure enzyme activity

- **Treatment:** Enzyme replacement administered via infusion and in some cases oral chaperone therapy.
- **Outcome in early diagnosis:** Affected individuals will typically not develop symptoms for years to decades. Outcomes are improved with frequent monitoring and intervention to halt and prevent further progression of disease.

While Fabry disease is a disorder primarily affecting males, female heterozygotes can also be symptomatic and may be detected via newborn bloodspot screening. Some will remain asymptomatic throughout most of their life, others may benefit from early intervention.

## Clinical features

Mutations in *GLA* result in reduced formation of alpha-galactosidase A (GLA), 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.

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. In heterozygous females, milder symptoms later in life are expected but they can display a classic disease presentation.

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).

## Causes of Fabry disease

Fabry disease is inherited in an X-linked manner. In affected males, the infant's mother is an obligate heterozygote. Female carriers may have varying presentations due to random X-chromosome inactivation. The most severely impacted females likely express X chromosome with pathogenic *GLA* variant in the affected organs. Rarely, de novo pathogenic variants arise spontaneously.

## Laboratory tests

The screening test measures activity of GLA enzyme. A low enzyme on first valid specimen will trigger DNA analysis of the *GLA* gene (second tier). Depending on results, further diagnostic and confirmatory testing may be required.

There are several issues to keep in mind regarding abnormal enzyme tests:

- Reduced enzyme alone is not diagnostic of the disease. Diagnosis must be confirmed by DNA analysis and subsequent diagnostic testing.
- Unlike in most autosomal recessive disorders, there may be significant clinical implications for family members of infants with significant *GLA* variants. In some cases, family testing may assist in diagnosis and/or prognosis discussions.

## Confirmatory testing

In males, confirmation of the diagnosis after newborn bloodspot screening is made by measurement of GLA enzyme activity in plasma and leukocytes. Measurement in both are recommended due to inconsistent reductions seen in some DNA variants. A GLA enzyme  $< 1\%$  is consistent with classic disease and  $> 1\%$  but below the unaffected range is consistent with atypical disease. For females, measurement of GLA is unreliable and does not predict prognosis or severity.

DNA results from the newborn bloodspot screen assists in confirmation of diagnosis but may not be definitive if the variant is of uncertain significance. In some cases a mature, maternal adult family member can be tested. If that family member shares the variant detected in the newborn but has no features of Fabry disease then development of disease is considered unlikely.

## Treatment

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.

## Carrier detection

Screening may identify female Fabry disease heterozygotes as discussed above, but not all female heterozygotes will be detected on newborn bloodspot screening.

## Screening practice considerations

- GLA enzyme is not valid in screens collected in infants before 20 hours of life.
- GLA enzyme is measured in one valid specimen only. Normal GLA enzymes are not repeated on the 2<sup>nd</sup> or other subsequent specimens.

**Table 12: Fabry disease screening result summary for male newborns**

Results in MALE Newborn	Likely causes	Actions
GLA enzyme low, DNA analysis detects no and/or benign variant	<ul style="list-style-type: none"><li>• False positive</li></ul>	NWRNBS Program will report by letter regarding test results and any other recommendations.
GLA enzyme low, DNA analysis detects hemizygous variant of uncertain significance	<ul style="list-style-type: none"><li>• False positive</li><li>• Fabry disease</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
GLA enzyme low, DNA analysis detects hemizygous likely pathogenic or pathogenic variant	<ul style="list-style-type: none"><li>• Fabry disease</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

**Table 13: Fabry disease screening result summary for female newborns**

Results in FEMALE Newborn	Likely causes	Actions
GLA enzyme low, DNA analysis detects no and/or benign variant(s)	<ul style="list-style-type: none"><li>• False positive</li></ul>	NWRNBS Program will report by letter regarding test results and any other recommendations.
GLA enzyme low, DNA analysis detects variant(s) of uncertain significance	<ul style="list-style-type: none"><li>• False positive</li><li>• Heterozygous Fabry disease carrier</li><li>• Fabry disease</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
GLA enzyme low, DNA analysis detects likely pathogenic and/or pathogenic variant(s)	<ul style="list-style-type: none"><li>• Heterozygous Fabry disease carrier</li><li>• Fabry disease</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

# Pompe Disease

## Disease essentials (55–57)

- **Incidence:** Estimated between 1 in 28,000 to 1 in 40,000
- **Screening test:** Tandem mass spectrometry (MS/MS) to detect acid alpha-glucosidase (GAA) enzyme followed by second-tier DNA analysis of the *GAA* gene.
- **Confirmatory tests:** Creatine kinase (CK), aspartate transaminase (AST), alanine transaminase (ALT), acid alpha-glucosidase (GAA) enzyme in blood and urinary glucotetrasaccharide (Hex4)
- **Validity:** Published false positive rate is 0.12% for the NeoLSD kit (PerkinElmer) used to measure enzyme activity
- **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)
- **Treatment:** Enzyme replacement administered via infusion and supportive therapy.
- **Outcome in early diagnosis:** Significant improvements are expected in cardiac and respiratory function in infantile-onset forms with early treatment including prolonged lifespan. Improvement in long-term muscle function is expected in later-onset forms.

The presence of pseudodeficiency DNA variants in *GAA* will result in lower measured GAA enzyme on traditional assays but does not impact the actual function of the enzyme in vivo. Presence of pseudodeficiency variants is not associated with any clinical features of disease but will result in false positive screens and blood tests.

## Clinical features

Mutations in *GAA* result in reduced formation of acid alpha-glucosidase (GAA), the lysosomal enzyme responsible for processing of 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 and 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 lifespans 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.

## Causes of disease

Pompe disease is inherited in an autosomal recessive manner resulting in insufficient GAA enzyme.

## Laboratory tests

The screening test measures activity of GAA enzyme. A low enzyme on first valid specimen will trigger DNA analysis of the *GAA* gene (second tier). Depending on results, further diagnostic and confirmatory testing may be required.

There are several issues to keep in mind regarding abnormal enzyme tests:

- Reduced enzyme alone is not diagnostic of the disease. Diagnosis must be confirmed by DNA analysis and diagnostic testing.
- Presence of one or more pseudodeficiency variants will often result in a false positive screen. DNA testing may be able to clarify these cases before further testing or referral to specialist need to be pursued.

## 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 IOPD and 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.

**Table 14: Pompe disease variants, onset, and affected populations**

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

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.

## Treatment

Currently available treatment is enzyme replacement therapy (ERT) initiated prior to the development of tissue and organ damage in order 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.

## Screening practice considerations

- GAA enzyme is not valid in screens collected in infants before 20 hours of life.
- GAA enzyme is measured in one valid specimen only. Normal GAA enzymes are not repeated on the 2<sup>nd</sup> or other subsequent specimens.

**Table 15: Pompe disease screening result summary**

Results	Likely causes	Actions
GAA enzyme low, DNA analysis detects no and/or benign variant(s) including pseudodeficiency.	<ul style="list-style-type: none"> <li>• False positive</li> </ul>	NWRNBS Program will report by letter regarding test results and any other recommendations.
GAA enzyme low, DNA analysis detects heterozygous variant of uncertain significance, likely pathogenic or pathogenic variant	<ul style="list-style-type: none"> <li>• False positive</li> <li>• Pompe disease carrier</li> </ul>	NWRNBS Program will report by letter regarding test results and any other recommendations.
GAA enzyme low, DNA analysis detects homozygous or compound heterozygous variants of uncertain significance	<ul style="list-style-type: none"> <li>• Pompe disease carrier</li> <li>• Pompe disease</li> </ul>	NNBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
GAA enzyme low, DNA analysis detects homozygous or compound heterozygous likely pathogenic or pathogenic variants	<ul style="list-style-type: none"> <li>• Pompe disease carrier (if inherited in cis-)</li> <li>• Pompe disease (inherited in trans-)</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

# Mucopolysaccharidosis Type I (MPS I)

## Disease essentials (58, 59)

- **Incidence:** Estimated between 1 in 87,000 to 1 in 185,000
- **Screening test:** Tandem mass spectrometry (MS/MS) to detect alpha-L-iduronidase (IDUA) enzyme followed by second-tier DNA analysis of the *IDUA* gene.
- **Confirmatory test:** Alpha-L-iduronidase (IDUA) enzyme, glycosaminoglycans (GAGs) (aka mucopolysaccharides or MPS) in blood and/or urine.
- **Validity:** Published false positive rate is 0.07% for the NeoLSD kit (PerkinElmer) used to measure enzyme activity
- **False positives:** Can occur in heterozygous carriers and in presence of pseudodeficiency variants (particularly common in individuals of African ancestry).
- **Treatment:** Hematopoietic stem cell transplantation (HSCT) in severe disease and/or enzyme replacement therapy (ERT)
- **Outcome in early diagnosis:** In severe disease with early HSCT, or attenuated disease with early ERT, significant improvements expected in lifespan and overall disease burden.

The presence of pseudodeficiency DNA variants in *IDUA* will result in lower measured IDUA enzyme on traditional assays but does not impact the actual function of the enzyme in vivo. Presence of pseudodeficiency variants is not associated with any clinical features of disease but will result in false positive screens and blood tests.

## Clinical features

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.

## Causes of disease

MPS I is inherited in an autosomal recessive manner resulting in insufficient IDUA enzyme.

## Laboratory tests

The screening test measures activity of IDUA enzyme. A low enzyme on first valid specimen will trigger DNA analysis of the *IDUA* gene (second tier). Depending on results, further diagnostic and confirmatory testing may be required.

There are several issues to keep in mind regarding abnormal enzyme tests:

- Reduced enzyme alone is not diagnostic of the disease. Diagnosis must be confirmed by DNA analysis and diagnostic testing.
- Presence of one or more pseudodeficiency variants will often result in a false positive screen. DNA testing may be able to clarify these cases before further testing or referral to specialist need to be pursued.

## Confirmatory testing

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.

## 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.

## Screening practice considerations

- IDUA enzyme is not valid in screens collected in infants before 20 hours of life.
- IDUA enzyme is measured in one valid specimen only. Normal IDUA enzymes are not repeated on the 2nd or other subsequent specimens.

**Table 16: MPS I screening result summary**

Results	Likely causes	Actions
IDUA enzyme low, DNA analysis detects no and/or benign variant(s) including pseudodeficiency.	<ul style="list-style-type: none"> <li>• False positive</li> </ul>	NNWRNBS Program will report by letter regarding test results and any other recommendations.
IDUA enzyme low, DNA analysis detects heterozygous variant of uncertain significance, likely pathogenic or pathogenic variant	<ul style="list-style-type: none"> <li>• False positive</li> <li>• MPS I carrier</li> </ul>	NNWRNBS Program will report by letter regarding test results and any other recommendations.
IDUA enzyme low, DNA analysis detects homozygous or compound heterozygous variants of uncertain significance	<ul style="list-style-type: none"> <li>• MPS I carrier</li> <li>• MPS I</li> </ul>	NNBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
IDUA enzyme low, DNA analysis detects homozygous or compound heterozygous likely pathogenic or pathogenic variants	<ul style="list-style-type: none"> <li>• MPS I carrier (if inherited in cis-)</li> <li>• MPS I (inherited in trans-)</li> </ul>	NNBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

# Gaucher Disease

## Disease essentials (60, 61)

- **Incidence:** In the U.S, estimated at 1 in 40,000. In the Ashkenazi Jewish population prevalence is 1:855 individuals.
- **Screening test:** Tandem mass spectrometry (MS/MS) to detect acid beta-glucocerebrosidase (GBA) enzyme followed by second-tier DNA analysis of the *GBA* gene.
- **Confirmatory test:** Glucocerebrosidase (GBA) enzyme and chitotriosidase activity
- **Validity:** Published false positive rate is 0.07% for the NeoLSD kit (PerkinElmer) used to measure enzyme activity

- **False positives:** May occur in unaffected, heterozygous carriers
- **Treatment:** Enzyme replacement administered via infusion and in some cases oral substrate reduction therapy (SRT).
- **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.

## Clinical features

Mutations in *GBA* result in reduced formation of acid beta-glucocerebrosidase (GBA), 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.

## Causes of disease

Gaucher disease is inherited in an autosomal recessive manner resulting in insufficient GBA enzyme.

## Laboratory tests

The screening test measures activity of GBA enzyme. A low enzyme on first valid specimen will trigger DNA analysis of the *GBA* gene (second tier). Depending on results, further diagnostic and confirmatory testing may be required.

An important issue to keep in mind regarding abnormal enzyme tests:

- Reduced enzyme alone is not diagnostic of the disease. Diagnosis must be confirmed by DNA analysis and diagnostic testing.

## Confirmatory testing

Diagnosis of Gaucher disease is established by presence of biallelic pathogenic variants in GBA along with reduced GBA enzyme consistent with disease on diagnostic testing.

DNA analysis often assists in determining disease type in newborns identified by screening. Certain variants in the homozygous or compound heterozygous state can predict specific Gaucher disease type. The presence of at least one copy of the common variant, p.Asn409Ser or N409S (historically known as “N370S”) is protective against CNS disease.

**Table 17: Gaucher disease variants, disease types and affected population**

<b>GBA Pathogenic Variants</b>	<b>Gaucher disease type expected</b>	<b>Affected Individuals</b>
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%

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.

## 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.

## Screening practice considerations

- GBA enzyme is not valid in screens collected in infants before 20 hours of life.
- GBA enzyme is measured in one valid specimen only. Normal GBA enzymes are not repeated on the 2nd or other subsequent specimens.

**Table 18: Gaucher disease screening result summary**

Results	Likely causes	Actions
GBA enzyme low, DNA analysis detects no and/or benign variant(s)	<ul style="list-style-type: none"> <li>False positive</li> </ul>	NWRNBS Program will report by letter regarding test results and any other recommendations.
GBA enzyme low, DNA analysis detects heterozygous variant of uncertain significance, likely pathogenic or pathogenic variant	<ul style="list-style-type: none"> <li>False positive</li> <li>Gaucher disease carrier</li> </ul>	NWRNBS Program will report by letter regarding test results and any other recommendations.
GBA enzyme low, DNA analysis detects homozygous or compound heterozygous variants of uncertain significance	<ul style="list-style-type: none"> <li>Gaucher disease carrier</li> <li>Gaucher disease</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
GBA enzyme low, DNA analysis detects homozygous or compound heterozygous likely pathogenic or pathogenic variants	<ul style="list-style-type: none"> <li>Gaucher disease carrier (if inherited in cis-)</li> <li>Gaucher disease (inherited in trans-)</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

## Spinal Muscular Atrophy (SMA)

**Screening for Spinal Muscular Atrophy (SMA) is anticipated to begin June 1, 2022.**

### SMA essentials (62–71)

- **Incidence:** 1:11,000 births
- **Screening test:** Polymerase chain reaction to detect deletion of exon 7 of the *SMN1* gene. 95% of cases are due to deletions in the *SMN1* gene.
- **Confirmatory tests:** Sequencing to identify deletions/mutations in the *SMN1* gene and copy number variants in the *SMN2* gene
- **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.
- **Outcome:** Can vary depending on type of SMA.

SMA, attributed to variants in the *SMN1* gene, is an autosomal recessive condition that progressively destroys motor neurons—nerve cells in the brain stem and spinal cord that control essential skeletal muscle activity such as speaking, walking, breathing, and swallowing, leading to muscle weakness and atrophy. Motor neurons control movement in the arms, legs, chest, face, throat and tongue. When there are disruptions in the signals between motor neurons and muscles, the muscles weaken, begin wasting away and develop twitching (called fasciculations).

## Clinical features

There is a wide range of impairment seen in SMA caused by defects in the *SMN1* gene, from onset before birth with breathing difficulties at birth to mild weakness in adults. Accordingly, SMA can be classified into four types, based on highest motor milestone achieved.

**Table 19: SMA types and clinical features**

Type	Other Name	Life Span	Motor	Clinical features
<b>SMA type 0</b> 0 copies of <i>SMN2</i>	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.
<b>SMA type I</b> 1-2 copies of <i>SMN2</i>	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 of the spine) or other skeletal abnormalities as they get older.
<b>SMA type II</b> 3 copies of <i>SMN2</i>	The	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.
<b>SMA type III</b> 3-4 copies of <i>SMN2</i>	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.
<b>SMA type IV</b> 4-6 copies of <i>SMN2</i>		Normal	Normal	Onset after 21 years of age, with mild to moderate proximal muscle weakness and other symptoms.

## Causes of SMA

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*.

## Laboratory tests

Screening is based on real time PCR that detects *SMN1* deletions.

## Confirmation

- Molecular Genetic testing of *SMN1* gene.
- Deletion/duplication analysis for exon 7 of *SMN1* and sequencing of *SMN1* if exon 7 is fully present
- Copy Number Variants may be assessed on *SMN2* as there is a correlation between the *SMN2* copy number and severity of disease.

**Table 20: SMN2 copy number and SMA clinical phenotype (62)**

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

Adapted from Calucho et al [2018] (69)

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] (70) 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.

## Screening Practice Considerations

Immediate referral to a pediatric neurologist is recommended.

## Treatment

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 mutated *SMN1* gene. This therapy is approved for patients with SMA under the age of 2 years.

Infants may receive gene therapy.

## X-Linked Adrenoleukodystrophy (X-ALD)

**Screening for X-Linked Adrenoleukodystrophy (X-ALD) is anticipated to begin on or before January 1, 2023.**

### X-ALD essentials (72–75)

- **Prevalence/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.
- **Screening test:** Tandem mass spectrometry (MS/MS) to detect C26:0 lysophosphatidylcholine (C26:0-LPC).
- **Confirmatory test:** Very long chain fatty acids in serum and DNA analysis of *ABCD1* gene.
- **Validity:** To be determined
- **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.
- **Outcome in early diagnosis:** 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.

While X-ALD is a disorder primarily affecting males, female heterozygotes can also develop symptoms in adulthood and may be detected via newborn bloodspot screening.

### Clinical features

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:

- 1) **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.
- 2) **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.
- 3) **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 the majority of 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.

## Causes of X-ALD

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.

## Laboratory tests

The screening test measures C26:0-LPC. An elevated level on first valid specimen will trigger request for repeat or referral. Depending on results, further diagnostic and confirmatory testing may be required.

There are several issues to keep in mind regarding abnormal C26:0-LPC:

- 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.

## 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 may not be reliable in ruling out or confirming the disorder. Biochemical and molecular testing cannot predict clinical outcomes, so careful monitoring of adrenocortical function and brain imaging are required throughout life for males.

## Monitoring for adrenal insufficiency

Male infants/children with confirmed ALD also undergo testing for adrenal insufficiency. This is unlikely to be present in the neonatal period. Monitoring in childhood is performed by measuring fasting morning plasma ACTH and serum cortisol levels.

## Treatment

Individuals identified by newborn bloodspot screening are not expected to require treatment in infancy. However, baseline evaluations and regular monitoring will be conducted once the diagnosis is confirmed.

As of this publication, the primary available treatments for X-ALD are:

- 1) **Corticosteroid Therapy:** A large number of 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.
- 2) **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 on the basis of 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.

## Carrier detection

Screening may identify female X-ALD disease heterozygotes as discussed above, but not all female heterozygotes will be detected on newborn bloodspot screening.

## Screening practice considerations

C26:0-LPC is not valid in screens collected in infants before 24 hours of life.

**Table 21: X-ALD screening result summary for male newborns**

Results in MALE Newborn	Likely causes	Actions
Elevated C26:0-LPC	<ul style="list-style-type: none"><li>• X-ALD or other disorder of VLCFAs likely</li><li>• False positive</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations

**Table 22: X-ALD screening result summary for female newborns**

Results in FEMALE Newborn	Likely causes	Actions
Elevated C26:0-LPC	<ul style="list-style-type: none"><li>• Heterozygous X-ALD variant present</li><li>• Other disorder of VLCFAs</li><li>• False positive</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

# References

1. Pass KA, Lane PA, Fernhoff PM, Hinton CF, Panny SR, Parks JS, et al. US newborn screening system guidelines II: follow-up of children, diagnosis, management, and evaluation. Statement of the Council of Regional Networks for Genetic Services (CORN). *J Pediatr*. 2000 Oct;137(4 Suppl): S1-46.
2. American College of Medical Genetics Newborn Screening Expert Group. Newborn screening: toward a uniform screening panel and system--executive summary. *Pediatrics*. 2006 May;117(5 Pt 2): S296-307.
3. National Newborn Screening and Genetics Resource Center, Austin TX website: [genes-r-us.uthscsa.edu](http://genes-r-us.uthscsa.edu)
4. Arn PH. Newborn screening: current status. *Health Affairs*. 2007;26(2):559-566.
5. Tuerck JM, Buist NR, Skeels MR, Miyahira RS, Beach PG. Computerized surveillance of errors in newborn screening practice. *Am J Public Health*. 1987 Dec;77(12):1528-31.
6. CLSI. Newborn screening for preterm, low birth weight, and sick newborns; approved guidelines. CLSI NBS03-A. Wayne, PA: Clinical and Laboratory Standards Institute; October 2009.
7. Kosciuk RL, Farrell PM, Kosorok MR, Zaremba KM, Laxova A, Lai HC, et al. Cognitive function of children with cystic fibrosis: deleterious effect of early malnutrition. *Pediatrics*. 2004 Jun;113(6):1549-58.
8. Sontag MK, Hammond KB, Zielenski J, Wagener JS, Accurso FJ. Two-tiered immunoreactive trypsinogen-based newborn screening for cystic fibrosis in Colorado: screening efficacy and diagnostic outcomes. *J Pediatr*. 2005 Sep;147(3 Suppl): S83-8.
9. 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.
10. 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.
11. 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.

12. 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.
13. 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.
14. 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.
15. 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.
16. 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
17. 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
18. Selva KA, Harper A, Downs A, Blasco PA, Lafranchi SH. Neurodevelopmental outcomes in congenital hypothyroidism: comparison of initial T4 dose and time to reach target T4 and TSH. *J Pediatr*. 2005 Dec;147(6):775-80.
19. Van Vliet G. Neonatal hypothyroidism: treatment and outcome. *Thyroid*. 1999 Jan;9(1):79-84.
20. LaFranchi SH, Hanna CE, Krainz PL, Skeels MR, Miyahira RS, Sesser DE. Screening for congenital hypothyroidism with specimen collection at two time periods: results of the Northwest Regional Screening Program. *Pediatrics*. 1985 Nov;76(5):734-40.
21. 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.
22. Dick MC. Standards for the management of sickle cell disease in children. *Arch Dis Child Educ Pract Ed*. 2008 Dec;93(6):169-76.

23. 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.
24. 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.
25. Channon S, Goodman G, Zlotowitz S, Mockler C, Lee PJ. Effects of dietary management of phenylketonuria on long-term cognitive outcome. *Arch Dis Child.* 2007 Mar;92(3):213-8.
26. Cleary MA, Feillet F, White FJ, Vidailhet M, Macdonald A, Grimsley A, et al. Randomised controlled trial of essential fatty acid supplementation in phenylketonuria. *Eur J Clin Nutr.* 2006 Jul;60(7):915-20.
27. Fiege B, Blau N. Assessment of tetrahydrobiopterin (BH4) responsiveness in phenylketonuria. *J Pediatr.* 2007 Jun;150(6):627-30.
28. Matalon R, Michals-Matalon K, Bhatia G, Grechanina E, Novikov P, McDonald JD, et al. Large neutral amino acids in the treatment of phenylketonuria (PKU). *J Inherit Metab Dis.* 2006 Dec;29(6):732-8.
29. Scott CR. The genetic tyrosinemias. *Am J Med Genet C Semin Med Genet.* 2006 May 15;142(2):121-6.
30. Koelink CJ, van Hasselt P, van der Ploeg A, van den Heuvel-Eibrink MM, Wijburg FA, Bijleveld CM, et al. Tyrosinemia type I treated by NTBC: how does AFP predict liver cancer? *Mol Genet Metab.* 2006 Dec;89(4):310-5.
31. Tsai CP, Lin PY, Lee NC, Niu DM, Lee SM, Hsu WM. Corneal lesion as the initial manifestation of tyrosinemia type II. *J Chin Med Assoc.* 2006 Jun;69(6):286-8.
32. Magera MJ, Gunawardena ND, Hahn SH, Tortorelli S, Mitchell GA, Goodman SI, et al. Quantitative determination of succinylacetone in dried blood spots for newborn screening of tyrosinemia type I. *Mol Genet Metab.* 2006 May;88(1):16-21.
33. Shekhawat PS, Matern D, Strauss AW. Fetal fatty acid oxidation disorders, their effect on maternal health and neonatal outcome: impact of expanded newborn screening on their diagnosis and management. *Pediatr Res.* 2005 May;57(5 Pt 2):78R-86R.
34. Wilcken B, Haas M, Joy P, Wiley V, Chaplin M, Black C, et al. Outcome of neonatal screening for medium-chain acyl-CoA dehydrogenase deficiency in Australia: a cohort study. *Lancet.* 2007 Jan 6;369(9555):37-42.

35. Wilcox RL, Nelson CC, Stenzel P, Steiner RD. Postmortem screening for fatty acid oxidation disorders by analysis of Guthrie cards with tandem mass spectrometry in sudden unexpected death in infancy. *J Pediatr*. 2002 Dec;141(6):833-6.
36. Van Wassenhaer AG, Kok JH, Dekker FW, de Vijlder JJ. Thyroid function in very preterm infants: influences of gestational age and disease. *Pediatr Res*. 1997 Nov;42(5):604-9
37. 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.
38. 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.
39. Deodato F, Boenzi S, Santorelli FM, Dionisi-Vici C. Methylmalonic and propionic aciduria. *Am J Med Genet C Semin Med Genet*. 2006 May 15;142(2):104-12.
40. Andersson HC, Marble M, Shapira E. Long-term outcome in treated combined methylmalonic acidemia and homocystinemia. *Genet Med*. 1999 May-Jun;1(4):146-50.
41. Hedlund GL, Longo N, Pasquali M. Glutaric acidemia type 1. *Am J Med Genet C Semin Med Genet*. 2006 May 15;142(2):86-94.
42. 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.
43. 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.
44. 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.
45. 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.
46. Gropman AL, Batshaw ML. Cognitive outcome in urea cycle disorders. *Mol Genet Metab*. 2004;81 (Suppl 1): S58-62. 47.
47. Ohura T, Kobayashi K, Tazawa Y, Abukawa D, Sakamoto O, Tsuchiya S, et al. Clinical pictures of 75 patients with neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD). *J Inherit Metab Dis*. 2007 ;30(2):139-44.

48. Bosch AM. Classical galactosaemia revisited. *J Inherit Metab Dis*. 2006;29(4):516- 25.
49. Wolf B. Biotinidase Deficiency: New Directions and Practical Concerns. *Curr Treat Options Neurol*. 2003;5(4):321-328.
50. American Academy of Pediatrics. Newborn Screening Expands: Recommendations for Pediatricians and Medical Homes – Implications for the System. *Pediatrics*. 2008;121: 192-217.
51. Dick MC. Standards for the management of sickle cell disease in children. *Arch Dis Child Educ Pract Ed*. 2008 Dec;93(6):169-76.
52. Hopkins PV, Klug T, Vermette L, Raburn-Miller J, Kiesling J, Rogers S. Incidence of 4 Lysosomal Storage Disorders From 4 Years of Newborn Screening. *JAMA Pediatr*. 2018;172(7): 696-697.
53. Newborn screening act sheet Fabry disease: Decreased alpha-galactosidase a [Internet]. Mayo Clinic Laboratories. 2020 [cited 2022 Apr 19]. Available from: [https://www.mayocliniclabs.com/~media/it-mmfiles/special-instructions/Newborn\\_Screening\\_Act\\_Sheet\\_Fabry\\_Disease-Decreased\\_Alpha-Galactosidase\\_A.pdf](https://www.mayocliniclabs.com/~media/it-mmfiles/special-instructions/Newborn_Screening_Act_Sheet_Fabry_Disease-Decreased_Alpha-Galactosidase_A.pdf)
54. Mehta A, Hughes DA. Fabry Disease. In: GeneReviews® [Internet]. University of Washington, Seattle; 2002 Aug 5 [Updated 2022 Jan 27] [cited 2022 Apr 19]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1292>
55. Bodamer OA, Scott CR, Giugliani R, on behalf of the Pompe Disease Newborn Screening Working Group. Newborn Screening for Pompe Disease. *Pediatrics* [Internet]. 2017 July 1 [cited 2022 Apr 20];140(Supplement\_1):S4–13. Available from: [https://publications.aap.org/pediatrics/article/140/Supplement\\_1/S4/179258/Newborn-Screening-for-Pompe-Disease](https://publications.aap.org/pediatrics/article/140/Supplement_1/S4/179258/Newborn-Screening-for-Pompe-Disease)
56. Dasouki M, Jawdat O, Almadhoun O, Pasnoor M, McVey AL, Abuzinadah A, et al. Pompe disease: literature review and case series. *Neurol Clinics* [Internet]. 2014 Aug. [cited 2022 Apr 20];32(3):751–76, ix. Available from: <http://dx.doi.org/10.1016/j.ncl.2014.04.010>
57. Leslie N, Bailey L. Pompe Disease. In: GeneReviews® [Internet]. University of Washington, Seattle; 2007 Aug 31 [Updated 2017 May 11] [cited 2022 Apr 20]. Available from: <https://www.ncbi.nlm.nih.gov/sites/books/NBK1261/>
58. Clarke LA. Mucopolysaccharidosis Type I. In: GeneReviews® [Internet]. University of Washington, Seattle; 2002 Oct 31 [Updated 2021 Feb 25] [cited 2022 Apr 20]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1162/>

59. Duke University for the Maternal and Child Health Bureau of the Health and Resources and Services Administration. Newborn Screening for Mucopolysaccharidosis Type I [Internet]. 2018 Jun 27. Available from: <https://www.hrsa.gov/sites/default/files/hrsa/advisory-committees/heritable-disorders/rusp/previous-nominations/mps-i-27-june-2018.pdf>
60. Pastores GM, Hughes DA. Gaucher Disease. In: GeneReviews® [Internet]. University of Washington, Seattle; 2000 Jul 27 [Updated 2018 Jun 21] [cited 2022 Apr 20]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1269>
61. OMIM® - Online Mendelian Inheritance in Man® entry - # 230800 - GAUCHER DISEASE, TYPE I; GD1 [Internet]. Omim.org. [cited 2022 Apr 20]. Available from: <https://www.omim.org/entry/230800>
62. Prior TW, Leach ME, Finanger E. Spinal Muscular Atrophy. In: GeneReviews® [Internet]. University of Washington, Seattle; 2020 Feb 24 [Updated 2020 Dec 3] [cited 2022 Apr 21]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1352/>
63. Mercuri E, Finkel RS, Muntoni F, Wirth B, Montes J, Main M, et al. Diagnosis and management of spinal muscular atrophy: part 1: recommendations for diagnosis, rehabilitation, orthopedic and nutritional care. *Neuromuscular Disorders*. [Internet]. 2018 Feb 1 [cited 2022 Apr 21];28(2):103–15. Available from: <http://dx.doi.org/10.1016/j.nmd.2017.11.005>
64. Chng SY, Wong YQ, Hui JH, Wong HK, Ong HT, Goh DY. Pulmonary function and scoliosis in children with spinal muscular atrophy types II and III. *Journal of Pediatrics and Child Health* [Internet]. 2003 Dec. [cited 2022 Apr 21];39(9):673–6. Available from: <http://dx.doi.org/10.1046/j.1440-1754.2003.00266.x>
65. Kolb SJ, Coffey CS, Yankey JW, Krossschell K, Arnold WD, Rutkove SB, et al. Natural history of infantile-onset spinal muscular atrophy. *Annals of Neurology* [Internet]. 2017 Dec. [cited 2022 Apr 21];82(6):883–91. Available from: <http://dx.doi.org/10.1002/ana.25101>
66. Wadman RI, Stam M, Gijzen M, Lemmink HH, Snoeck IN, Wijngaarde CA, et al. Association of motor milestones, SMN2 copy and outcome in spinal muscular atrophy types 0-4. *Journal of Neurology, Neurosurgery & Psychiatry* [Internet]. 2017 Apr 1;88(4):365–7. Available from: <http://dx.doi.org/10.1136/jnnp-2016-314292>
67. Montes J, McDermott MP, Mirek E, Mazzone ES, Main M, Glanzman AM, et al. Ambulatory function in spinal muscular atrophy: Age-related patterns of progression. *PLOS ONE* [Internet]. 2018 Jun 26 [cited 2022 Apr 21];13(6):e0199657. Available from: <http://dx.doi.org/10.1371/journal.pone.0199657>

68. Finkel RS, Mercuri E, Meyer OH, Simonds AK, Schroth MK, Graham RJ, et al. Diagnosis and management of spinal muscular atrophy: Part 2: Pulmonary and acute care; medications, supplements and immunizations; other organ systems; and ethics. *Neuromuscular Disorders* [Internet]. 2018 Mar 1 [cited 2022 Apr 21];28(3):197–207. Available from: <http://dx.doi.org/10.1016/j.nmd.2017.11.004>
69. Calucho M, Bernal S, Alías L, March F, Venceslá A, Rodríguez-Álvarez FJ, et al. Correlation between SMA type and SMN2 copy number revisited: An analysis of 625 unrelated Spanish patients and a compilation of 2834 reported cases. *Neuromuscular Disorders* [Internet]. 2018 Mar 1 [cited 2022 Apr 21];28(3):208–15. Available from: <http://dx.doi.org/10.1016/j.nmd.2018.01.003>
70. Prior TW, Swoboda KJ, Scott HD, Hejmanowski AQ. Homozygous SMN1 deletions in unaffected family members and modification of the phenotype by SMN2. *American Journal of Medical Genetics, Part A* [Internet]. 2004 Aug 24 [cited 2022 Apr 21];130A(3):307–10. Available from: <http://dx.doi.org/10.1002/ajmg.a.30251>
71. Chien YH, Chiang SC, Weng WC, Lee NC, Lin CJ, Hsieh WS, et al. Presymptomatic diagnosis of spinal muscular atrophy through newborn bloodspot screening. *The Journal of Pediatrics*. 2017;190:124–9.
72. 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>
73. Lee S, Clinard K, Young SP, Rehder CW, Fan Z, Calikoglu AS, et al. Evaluation of X-linked adrenoleukodystrophy newborn screening in North Carolina. *JAMA Network Open* [Internet]. 2020 Jan 31 [cited 2022 Apr 21];3(1):e1920356. Available from: <http://dx.doi.org/10.1001/jamanetworkopen.2019.20356>
74. Raymond GV, Moser AB, Fatemi A. X-Linked Adrenoleukodystrophy. In: *GeneReviews®* [Internet]. University of Washington, Seattle; 1999 Mar 26 [Updated 2018 Feb 15] [cited 2022 Apr 21]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1315/>
75. Wiens K, Berry SA, Choi H, Gaviglio A, Gupta A, Hietala A, et al. A report on state-wide implementation of newborn screening for X-linked Adrenoleukodystrophy. *American Journal of Medical Genetics, Part A* [Internet]. 2019 May 10 [cited 2022 Apr 21];179(7):1205–13. Available from: <http://dx.doi.org/10.1002/ajmg.a.61171>

# Northwest Regional Newborn Bloodspot Screening Program

*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  
Phone: 503-693-4174  
Fax: 503-693-5601



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

OHA 8189 (Rev. 06/2022)

AMEND: 333-024-1025

NOTICE FILED DATE: 03/30/2022

RULE SUMMARY: Amend OAR 333-024-1025 – Revise Oregon Newborn Bloodspot Screening Practitioner’s Manual to the 12th Edition, publishing in 2022. Key changes within include but are not limited to the following:

- Addition of section describing the Northwest Regional Newborn Bloodspot Screening Advisory Board
- Updates to “information about newborn bloodspot screening medical conditions” section to:
  - o Align descriptions with current clinical understanding and practice
  - o Add sections for SMA and X-ALD
- Various edits for clarity and consistency.

CHANGES TO RULE:

### 333-024-1025

Newborn Screening: Persons Responsible for Ensuring that Second, Third and Repeat Specimens are Collected and Submitted

(1) The following, in order of priority, are responsible for ensuring that the second specimens, and when applicable, third or repeat specimens, are collected and submitted in accordance with this rule:¶

(a) A facility or practitioner responsible for the care of an infant at any time during the first six months of life. ¶

(b) A parent or legal guardian.¶

(2) The persons described in section (1) of this rule must ensure that specimens are collected within the timeframes and in the manner described in OAR 333-024-1030 to 333-024-1040, and in accordance with the instructions provided by the Oregon State Public Health Laboratory available in the Oregon Newborn Bloodspot Screening Practitioner's Manual (Practitioner's Manual), 11<sup>th</sup> Edition; 2019~~22~~ found at [www.healthoregon.org/nbs](http://www.healthoregon.org/nbs), unless the infant is exempt pursuant to OAR 333-024-1050. ¶

(3) A person who is responsible for collecting and submitting the second or third specimen must either obtain the remaining specimen card(s) from the person who collected and submitted the first specimen or obtain a single specimen card from the Oregon State Public Health Laboratory as described in OAR 333-024-1100.

Statutory/Other Authority: ORS 413.014, 433.285, 431A.750

Statutes/Other Implemented: ORS 433.285, 433.290, 433.295

RULE ATTACHMENTS DO NOT SHOW CHANGES. PLEASE CONTACT AGENCY REGARDING CHANGES.

# Oregon Newborn Bloodspot Screening Practitioner's Manual



Northwest Regional Newborn Bloodspot Screening Program

# Newborn Bloodspot Screening Practitioner's Manual

## **Oregon Health Authority**

### **Public Health Division**

John L. Fontana, PhD, (HCLD) ABB

Patrice Held, PhD, FACMG

Sheri Hearn, MPH

Sarah Humphrey King, CHES

Sara Etienne, BS

Kristi Murphy, MS GC

## **Oregon Health & Science University**

Kara Connelly, MD

Erika Finanger, MD

Cary Harding, MD

Shyam Joshi, MD

Kim Kripps MD

Stephen L. LaFranchi, MD

Mike Powers, MD

Sarah Viall, PPCNP-BC

Leah Wessenberg, FNP

Trisha Wong, MD

Melinda Wu, MD

Amy Yang, MD

Tyler Yates, MD

12th Edition, 2022

# Table of Contents

» Table of Contents .....	3	» Tips to avoid rejected specimens .....	34
» Introduction .....	5	» Information about newborn bloodspot screening medical conditions .....	36
» Advisory Board .....	7	» Cystic Fibrosis (CF) .....	36
» Definitions .....	8	» Congenital Adrenal Hyperplasia (CAH) .....	39
» Newborn bloodspot screening responsibilities in Oregon .....	9	» Primary Congenital Hypothyroidism (CH) .....	41
» Education services .....	10	» Sickle Cell Disease and other Hemoglobinopathies .....	44
» Fee exemption for Oregon births .....	10	» Amino Acid Conditions .....	46
» Parent refusal to have the infant screened in Oregon .....	10	» Fatty Acid Oxidation (FAO) Conditions .....	53
» Medical conditions on the newborn bloodspot screening panel .....	11	» Organic Acid Conditions (OA) .....	55
» Newborn bloodspot screening kits .....	17	» Urea Cycle Conditions (UCD) .....	58
» Timing for specimen collection .....	18	» Galactosemia .....	62
» Patient demographic information .....	21	» Biotinidase Deficiency .....	64
» Heel-stick specimen collection instructions .....	23	» Severe Combined Immunodeficiency (SCID) .....	65
» Specimen transport .....	26	» Lysosomal Storage Disorders (LSDs) .....	67
» Reporting of results .....	28	» Fabry Disease .....	67
» Situations that may impact newborn bloodspot screening results .....	30	» Pompe Disease .....	71
» Requesting newborn bloodspot screening records .....	32	» Mucopolysaccharidosis Type I (MPS I) .....	74
» Use, release, and retention of residual bloodspot specimens .....	33	» Gaucher Disease .....	76
		» Spinal Muscular Atrophy (SMA) .....	79
		» X-Linked Adrenoleukodystrophy (X-ALD) .....	82
		» References .....	86



### **Acknowledgment**

We are indebted to the newborn bloodspot screening state coordinators, medical consultants, and practitioners for their assistance and advice.

### **Recommended citation**

NW Regional Newborn Bloodspot Screening Program.  
Newborn Bloodspot Screening Practitioner's Manual, 12th Edition; 2022.



# 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 OSPHL and abnormal results are referred to the NBS Follow-up Team.

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

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

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

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 as of 2018, some lysosomal storage disorders. The OSPHL 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.

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

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

**Newborn 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.**



# 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 for the review and creation of legislative reports based on members' respective areas of expertise. The Board assists NWRNBS with strategic planning and the development of policies, priorities and services related to newborn bloodspot screening. The Board's role also includes reviewing conditions to be recommended for the addition or removal from the test panel of diseases. In all activities, The Board considers the newborn screening system as a whole, to improve health outcomes for all infants and their families. The Board is comprised of 13 partners within the newborn bloodspot screening community. The members include representatives of hospitals, birth centers, families, insurance, midwifery, nursing, pediatrics and other perspectives.

If you are interested in participating with the NWRNBS Advisory Board, please send an email to [nbs.advisoryboard@dhsosha.state.or.us](mailto:nbs.advisoryboard@dhsosha.state.or.us).



# 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 filter paper collection device, attached demographic form and other items provided by the Oregon State Public Health Laboratory for the the purposes of collection and submission of specimens for newborn bloodspot screening.

**“Practitioner”** means: the person who takes responsibility for the delivery or health care of an infant born in Oregon and is one of the following:

- a) A physician licensed under ORS 677;
- b) A naturopathic physician licensed under ORS 685;
- c) Advanced practice registered nurse licensed under ORS 678;
- d) A chiropractic physician licensed under ORS chapter 684; or
- e) A direct entry midwife licensed under ORS 687.

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

**“Specimen”** means: a blood specimen obtained from an infant by means of capillary puncture or skin puncture (heel stick) that has spotted onto the newborn bloodspot screening kit and allowed to air dry.



# Newborn bloodspot screening responsibilities in Oregon

**This section describes the responsibilities for successful newborn bloodspot screening in Oregon. Practitioner's 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.
- **Oregon State Public Health Laboratory (OSPHL) and NBS Follow-up Team:** The laboratory is responsible for testing, record keeping, ensuring quality of laboratory methods, notifying providers of results, tracking abnormal and unresolved results, and providing educational materials.
- **Oregon Health & Science University (OHSU) subspecialty programs:** These partners are responsible for providing consultation services to practitioners and the OSPHL.

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

**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 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 Education Coordinator. In addition, education resources are made available to practitioners and parents at [www.bitly.com/nbs-resource](http://www.bitly.com/nbs-resource).

## Fee exemption for Oregon births

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

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

## Parent refusal to have the infant screened in Oregon

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

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

**NBS Follow-up Team**

**Fax: 503-693-5601**



# Medical conditions on the newborn bloodspot screening panel

Oregon newborns are screened for the following medical conditions recommended by the Advisory Committee on Heritable Disorders in Newborns and Children and the Northwest Regional Newborn Bloodspot Screening (NWRNBS) Program Advisory Board. More information on these medical conditions is available at the end of this manual and at:

- Baby's First Test: <http://babysfirsttest.org/>
- The Oregon State Public Health Laboratory: [www.healthoregon.org/nbs](http://www.healthoregon.org/nbs)
- The American College of Medical Genetics (ACMG): [ACT Sheets and Algorithms](#).
- Western States Regional Genetics Network: [www.newbornscreening.info](http://www.newbornscreening.info)

**Newborn bloodspot screening is not diagnostic. Both false negative and false positive results may occur. Confirmatory testing is required for diagnosis.**

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

Medical Condition	Analyte(s) tested for	Incidence in NW region	Symptoms <i>if not treated</i>	Common Medical Treatment
<b>Organic Acid Disorders</b>				
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	1 per 148,000	Vomiting; lethargy; acidosis possibly resulting in coma, death	Protein-restricted diet; carnitine and glycine therapy

Medical Condition	Analyte(s) tested for	Incidence in NW region	Symptoms <i>if not treated</i>	Common Medical Treatment
3-methylcrotonyl CoA carboxylase deficiency (3MCC)	C5OH	1 per 51,000	Most have been asymptomatic	None, except carnitine therapy if deficient
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
Multiple carboxylase deficiency (MCD)	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	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	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	Rare, less than 1 per 300,000	None to severe cardiomyopathy	Carnitine therapy; protein restriction; avoid fasting
2-methylbutyryl CoA dehydrogenase deficiency (2MBC)	C5	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

Medical Condition	Analyte(s) tested for	Incidence in NW region	Symptoms <i>if not treated</i>	Common Medical Treatment
<b>Fatty Acid Oxidation Disorders</b>				
Carnitine uptake deficiency (CUD)	C0	1 per 116,000	Hypoglycemia; cardiomyopathy	Carnitine therapy
Medium chain acyl-CoA dehydrogenase deficiency (MCAD)*	C6, C8, C10, 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, 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)*	C14:1, C16, C16OH, C18, C18OH	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)	C14:1, C16, C16OH, C18, C18OH	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	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, C14, C16, C18:1	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

Medical Condition	Analyte(s) tested for	Incidence in NW region	Symptoms <i>if not treated</i>	Common Medical Treatment
Carnitine palmitoyl transferase deficiency, type II (CPT-II)*	C16, C18, C18:1	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
Carnitine acylcarnitine translocase deficiency (CACT)	C16, C18, C18:1	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
<b>Amino Acid Disorders</b>				
Argininosuccinate lyase deficiency (Arginosuccinic aciduria; 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	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	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

Medical Condition	Analyte(s) tested for	Incidence in NW region	Symptoms <i>if not treated</i>	Common Medical Treatment
<b>Endocrine Disorders</b>				
Primary congenital hypothyroidism	Thyroid hormone T4 and Second tier TSH	1 per 2,300	Intellectual and developmental disability; other brain damage; growth delay	Thyroid hormone
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
<b>Pulmonary Disorders</b>				
Cystic fibrosis (CF)	Immunoreactive Trypsinogen (IRT)	1 per 6,500	Lung disease; growth failure	Pulmonary therapy; prevent infection; replace digestive enzymes
<b>Other Metabolic Disorders</b>				
Biotinidase deficiency	Biotinidase	1 per 1.05 million	Intellectual and developmental disability; seizures; skin rash; alopecia; hearing loss; death	Biotin therapy
Classic galactosemia (GALT)*	Galactosemia enzyme (GALT)	1 per 95,000	Neurodevelopmental impairment; liver disease; cataracts; Gram-negative sepsis in newborns	Galactose-restricted diet
<b>Hemoglobin Disorders</b>				
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
<b>Immunology Disorders</b>				
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

Medical Condition	Analyte(s) tested for	Incidence in NW region	Symptoms <i>if not treated</i>	Common Medical Treatment
<b>Lysosomal Storage Disorders</b>				
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
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
<b>Other Conditions</b>				
Spinal muscular atrophy (SMA) <sup>2†</sup>	Exon 7 of the <i>SMN1</i> 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) <sup>‡</sup>	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.

<sup>†</sup> Screening for this condition is anticipated to begin June 1, 2022.

<sup>‡</sup> Screening for this condition is anticipated to begin on or before January 1, 2023.

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](http://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 here: [www.nmhealth.org/about/phd/fhb/cms/nbgs/](http://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.

**Newborn bloodspot screening kits are pre-coded for the facility or practitioner that ordered the kit and should not be loaned to, or borrowed from, other facilities.**

**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 2nd specimen to be matched easily by the screening lab to the data from the 1st specimen. This matching system helps to link the data from newborn 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 in neonatal intensive care units (NICU). Each specimen in the kit has a barcode and a kit number that allow the 2nd specimen and 3rd specimen to be matched easily by the screening lab to the data from the 1st specimen. This matching system helps to ensure that newborn 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 1st specimen should be written on the single kit to help with matching the data for the infant. These kits will also be used when the OSPHL requests a repeat specimen.



# 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 time lines established below, collect and submit the bloodspot as soon as possible up to six months of age.

## 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 age but before 48 hours of age and a second specimen must be collected between 10 and 14 days of age as shown in [Table 2](#).

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

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

## Infants admitted to the NICU

For babies that require admission to a neonatal intensive care unit, collect the first specimen **as soon as possible after 24 hours of age but before 36 hours of age**. If the infant is being transfused, collect the specimen prior to transfusion regardless of the age of the infant. If an infant is transfused prior to 24 hours of age the second specimen must be collected at 48-72 hours of age. If the infant is **not** transfused prior to 24 hours of age the second specimen must be collected between 10 and 14 days of age. A third specimen must be collected at approximately 1 month, but no sooner than 28 days after birth.

For infants that are discharged or transferred after the first specimen (or second specimen) is collected, the remaining collection cards from the triple kit must be routed to the provider who will collect these specimens.

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

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

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

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

## Early discharge

If a family is requesting an early discharge, collect the 1st specimen before they leave your care. Some infants may not return for routine postnatal care. Please be certain to check the 'early discharge' box on the demographic portion of the card, to alert the lab that this is the reason for the early collection.

## Baby expires

In many cases, blood spot specimens from an infant who expired are a valuable resource for the family.

We recommend that you collect a newborn bloodspot screening specimen either at the typical screening interval, or sooner if needed and in consideration for the wishes of the baby's guardians.

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

- **Calling 503-693-4174 or**
- **Faxing the infant's information to 503-693-5601**

# Older infants

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

Table 2: Age of infant at specimen collection

	Collection Kit	First specimen	Second specimen	Third specimen
Routine Birth	Double Kit	As soon as possible after 24 hours of age but before 48 hours of life	10–14 days of age	Not Collected
NICU infants transfused prior to 24 hours of age	Triple Kit	Prior to transfusion	48–72 hours after birth	~ 1 month, no sooner than 28 days
NICU infants <u>not</u> transfused prior to 24 hours of age	Triple Kit	As soon as possible after 24 hours of age but before 36 hours of age and prior to transfusion	10–14 days of age	~ 1 month, no sooner than 28 days





# Patient demographic information

## **Incomplete demographic information may result in your specimen not being tested.**

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

Accurate and complete patient, provider, and specimen collection information must be provided on every collection card to allow for rapid follow-up if results are abnormal. This information is required by Clinical Laboratory Improvement Amendments of 1988 (CLIA) and must be legible.

The person performing the collection must:

1. Verify that the collection kit will not expire before all parts of the kit can be tested by the laboratory. If a double kit will expire within 1 month of the collection, please use a different kit. The expiration date is on the spine and the back of the kit as well as on the top of the filter paper portion.
2. Identify the infant and match with the correct screening kit. Make sure to select the correct kit part (1st, 2nd or 3rd) depending on the specimen being collected.

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

**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.**

3. **ALL** demographic fields must be filled in before collecting the specimen (see figure 2).
  - a. If the birth mother will not be maintaining custody of the infant, provide the name, address and phone number for the infant's guardian in the "Mother" fields. This information may be used to locate the infant for follow-up.
  - b. Labels may be used to provide demographic information. They must be included on all layers of the screening kit. They must not cover demographic information fields that will be hand-written.

**Provide the name and contact information for the provider who is responsible for the infant's medical care and treatment *after discharge* in the "Send report to PCP / Clinic" field. This practitioner will be sent screening results and follow-up information. Do not use the resident, attending, or on-call provider!**

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


RETURN TO: OREGON STATE PUBLIC HEALTH LAB  
 PO BOX 275, PORTLAND, OR 97207-0275 (503)693-4174

7070717  
W161

LOT

**1st Newborn Screening**

**SPECIMEN OR** SN



\* 1 1 7 5 0 6 8 4 0 5 \*

DO NOT WRITE  
IN THIS SPACE

Baby's Last Name		Baby's First Name		Send Report to PCP/Clinic:		CODE	
<input type="checkbox"/> Single Birth, or <input type="checkbox"/> Multi-Birth A B C D E F Circle One				Address/Telephone Number			
Sex: M F		ID Chart #		Birth Date ( _ / _ / _ )		Time of Day (circle one) ( : ) am pm	
Food Source Last 24 Hours: <input checked="" type="checkbox"/> Breast, <input checked="" type="checkbox"/> Soy Formula, <input checked="" type="checkbox"/> NPO		Check all that apply		Specimen Date ( _ / _ / _ )		Time of Day (circle one) ( : ) am pm	
<input checked="" type="checkbox"/> Lactose Formula, <input type="checkbox"/> Other _____		<input type="checkbox"/> Other Factors: <input type="checkbox"/> Early discharge <input type="checkbox"/> TPN		Transfusion Last RBC Transfusion Date / / OR <input type="checkbox"/> None		Birth Wt. Lbs. oz _____ gms Present Wt. Lbs. oz _____ gms	
Hosp. or Hosp. CODE/Submitter ID and Address				Baby's Race: <input checked="" type="checkbox"/> White, <input type="checkbox"/> Black, <input type="checkbox"/> Amer. Ind./Native, <input type="checkbox"/> Unknown/Other, <input type="checkbox"/> Asian / Pacific Islander		Alaskan Native Hispanic? No Yes	
				Mother's Last Name		Mother's First Name	
				Mother's Address-Number & Street			
				City		State Zip Code	
Specimen taken by:				Telephone Number:			
				Specimen reference #			



# Heel-stick specimen collection instructions\*

Each facility or medical provider must establish a procedure for staff performing newborn bloodspot screening specimen collections. Resources are available from the Clinical Laboratory Standards Institute that can help with creating or updating your procedure.

**Contact the OSPHL  
at 503-693-4174  
for assistance with  
specimen collection.**

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

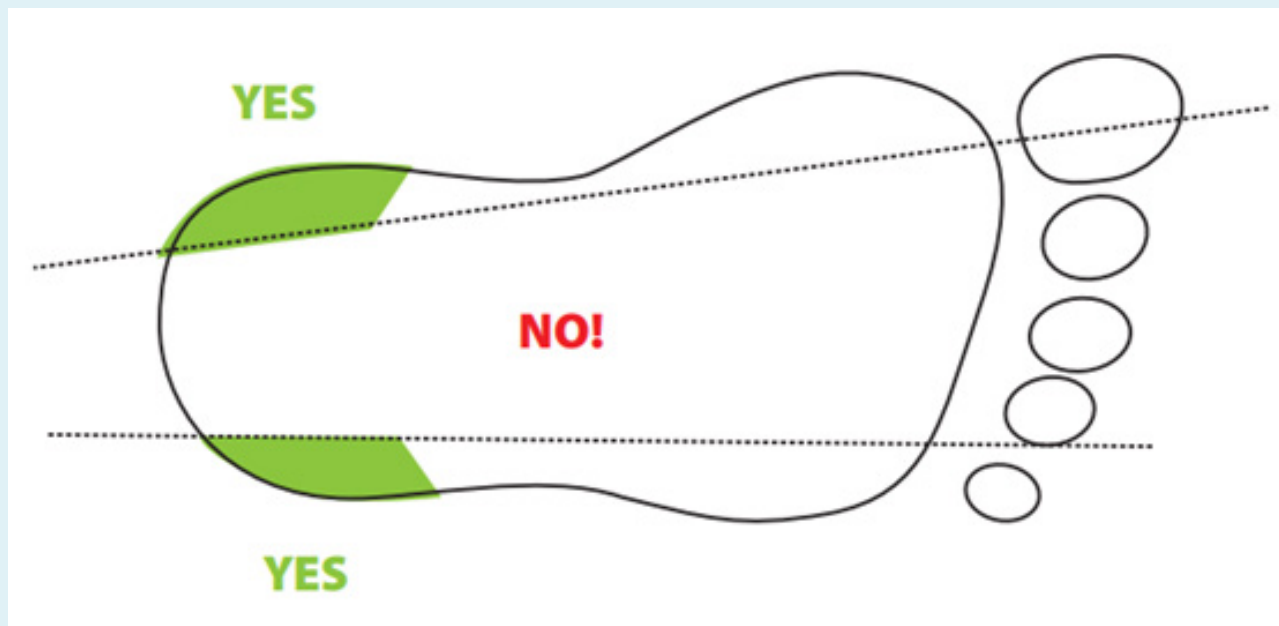
A training detailing proper collection and helpful resources can be found at [www.bitly.com/nbs-resource](http://www.bitly.com/nbs-resource). Tips to avoid rejected specimens are provided later in this manual.

1. Use a scalpel bladed lancet manufactured specifically for heel stick collection from an infant. Do not use a lancet longer than 2.0 mm. Do not use capillary tubes or other collection devices.
2. Select a lance site on the infant's heel (see Figure 3). Cleanse lance site with alcohol and air dry. Do not use betadine, iodine, lotion, or essential oils on the baby prior to collecting the specimen.
3. Perform lancing on the most medial or most lateral portion of the plantar surface of the heel.

---

\* These recommendations conform to CLSI publication NBS01-A6.

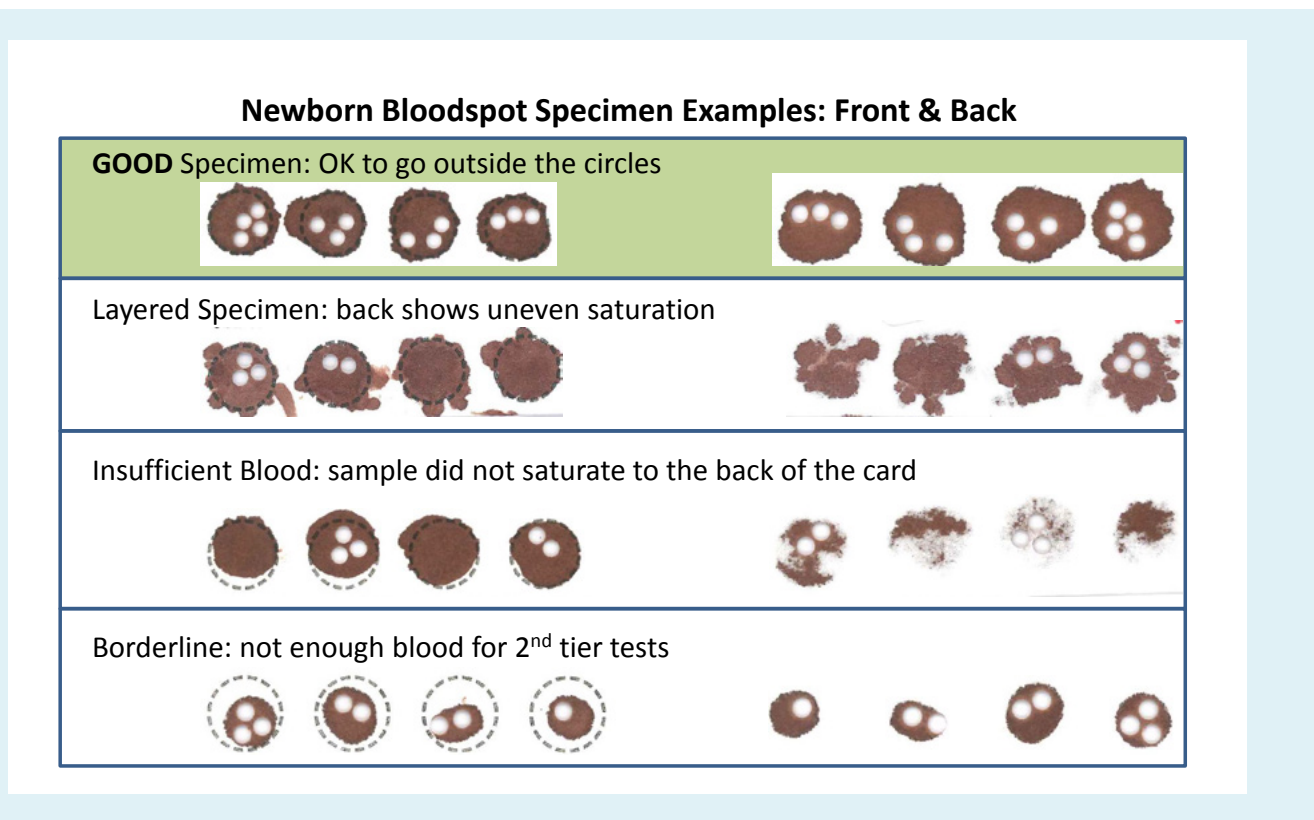
Figure 3: Recommendation for heel puncture site in newborns



4. Lance the heel with the sterile scalpel bladed lancet. Wipe away the first drop of blood to remove tissue fluids. Do not “milk” or squeeze the heel.
5. Allow a single drop of blood to collect on the heel that is large enough to fill a collection circle. Do not layer multiple spots of blood on top of one another.
6. Touch the filter paper gently to the drop of blood. Only apply blood to one side of the filter paper (it doesn’t matter which side is used).
7. Allow the blood to soak through the filter paper so that the blood spot looks similar on both the front and back of the collection kit.



Figure 4: Newborn bloodspot specimen examples



Complete, even saturation of the filter paper is essential for accurate testing. The filter paper is calibrated to absorb a specific quantity of blood. Incomplete, uneven saturation or layering of the blood alters the quantity of blood used for testing and will lead to inaccurate test results. This figure is also available at: [www.bitly.com/nbs-example](http://www.bitly.com/nbs-example).

8. Collect the blood in all five circles, repeating instructions 5 through 7. If blood flow is not sufficient, re-lance the heel. It is better to fill three circles completely than to fill four circles inadequately.
9. Air dry specimens at room temperature for between 3 and 4 hours in a **horizontal** position with the blood spots exposed. Hanging wet specimens vertically will cause heavier red cells to migrate to the dependent end of the circle resulting in uneven saturation.
10. Do not expose the specimen to excess heat or humidity at any time. Do not dry on a heater, in a microwave, with a hair dryer or in sunlight. Do not place in plastic bags, leave in a hot mailbox or in a hot car. These practices can destroy some proteins and enzymes that are required for accurate test results.
11. Ensure that the specimen is completely dry before transporting.

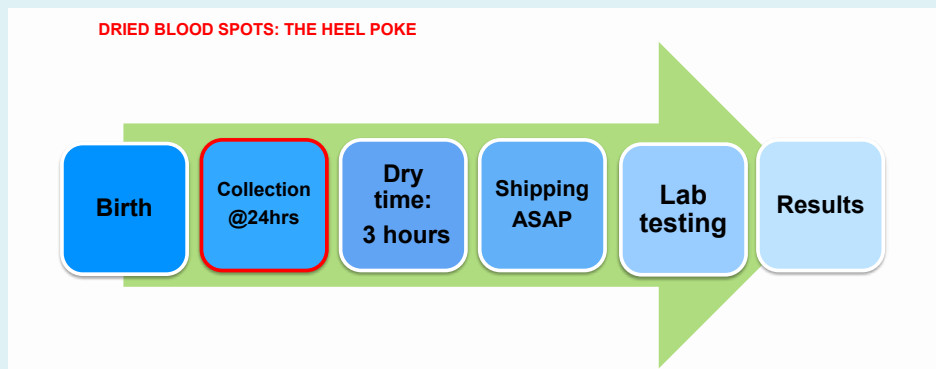


# Specimen transport

**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.**

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

**Figure 5: Newborn bloodspot screening process**



Specimens should be sent as soon as they are dried (between 3 and 4 hours) and no later than 24 hours after collection.

1. Keep a record of the specimens that are sent, including the kit numbers. A packing list or manifest should be included with the shipment.
2. Insert the dried specimen(s) into an envelope. Do not put specimens in plastic bags or containers. Do not compress the specimens.
3. Send the specimens no later than 24 hours after collection.
4. All specimens must be sent by express mail, courier or another timely delivery mechanism. Specimens should be received by the OSPHL within 48 hours of collection.

5. Send the specimens to:

Oregon State Public Health Laboratory  
Newborn Bloodspot Screening Program  
7202 NE Evergreen Parkway, Suite 100  
Hillsboro, OR 97124

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

Prompt transit is essential for identifying infants who may be impacted by a screening condition within one week of birth. 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 promptly. Delays within a facility may be from inefficient internal processes, slow courier services, simple forgetfulness, or, most dangerously, batching specimens.

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





# Reporting of results

## Results are available online

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

## Results reporting

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

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

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.

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

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

Calling 503-693-4174 or

Faxing the infant’s information to 503-693-5601



## I did not receive my newborn bloodspot screening results!

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

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

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



# 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. These include:

### Primary Congenital Hypothyroidism (CH)

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

### Lysosomal Storage Disorders (LSD)

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

## Parenteral nutrition and carnitine therapy

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 cell transfusions

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

**Report that a transfusion occurred on the specimen collection card.**

## 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.

**Report that Pivalic acid was administered on the specimen collection card.**

## 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





# Requesting newborn bloodspot screening records

- If the child is younger than 6 years, request his or her newborn bloodspot screening records by faxing the child's full name, date of birth, kit number and mother's name (at the time of the child's birth) and date of birth on your letterhead to 503-693-5601.
- Records that are over 6 years old are outside of their record retention and should have been destroyed. It is unlikely that older records will be located. When requesting records older than 6 years, include a medical record release authorization signed by the patient, if over 18, or the parent or guardian.
- If you are requesting records for a baby who was born in another state, please contact that state's newborn bloodspot screening program to request results. Contact information for each state is provided by Baby's First Test at [www.babysfirsttest.org](http://www.babysfirsttest.org).
- Parents or legal guardians may request the infant's newborn bloodspot screening records by completing the form located at [www.bitly.com/get-phl-results](http://www.bitly.com/get-phl-results).





# Use, release, and retention of residual bloodspot specimens

**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.
- Program evaluation and quality improvement.
- Education activities required by Oregon Statute.

Residual bloodspot specimens will only be released by the OSPHL:

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

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



# Tips to avoid rejected specimens

**Improperly collected specimens compromise the accuracy of test results. When a specimen is rejected, a repeat collection will be required. This unnecessarily delays the screening of the newborn.**

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

## Tips to avoid “Layered Blood” rejection

**Specimen front**



**Specimen back**

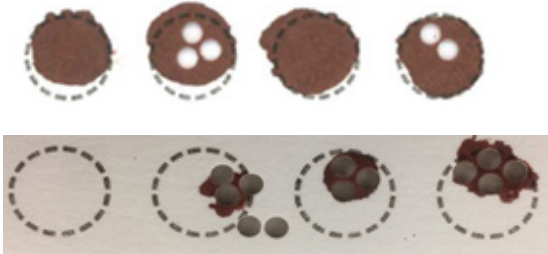


### Tips to avoid this type of rejection

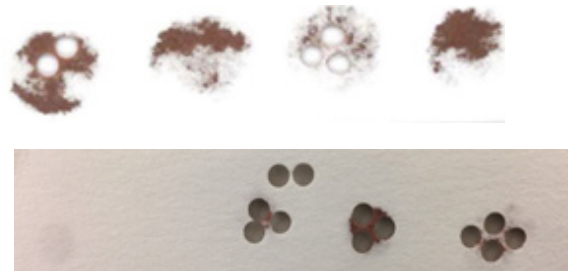
- Use the proper size lancet (< 2mm length).
- Allow a large drop to form on the heel before touching with the filter paper.
- Collect blood into one circle at a time.
- Do not apply additional blood to an incompletely filled circle.
- Do not apply blood to both sides of the filter paper.
- Do not compress the filter paper.

## Tips to avoid “Incomplete Saturation” or “Quantity Not Sufficient” rejection

**Specimen front**



**Specimen back**



### Tips to avoid this type of rejection

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

## Tips to avoid “Contaminated” rejection

**Specimen front**



**Specimen back**



### Tips to avoid this type of rejection

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



# 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)

### CF essentials

- **Screening test:** The first-tier immunoassay measures immunoreactive trypsinogen (IRT). For specimens with an elevated IRT on one (if sufficiently high) or both screening specimens, second-tier DNA screening for 34 common variants is performed.
- **Confirmatory test:** Sweat chloride testing and DNA mutation analysis
- **Validity:** A small percentage of cases (<10%) will be falsely negative. Most cases should be abnormal on the first screen. IRT may be falsely elevated in premature, stressed, or sick infants. IRT can be falsely low in infants with CF who are born with meconium ileus.
- **Treatment:** Comprehensive, multidisciplinary care, pancreatic enzyme replacement, soluble vitamin replacement, high-calorie/high-fat diet, airway clearance regimen, and new specific targeted therapies based on genotype. Refer to accredited Cystic Fibrosis 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.

Cystic fibrosis (CF) is a recessively inherited defect of the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Over 1,800 mutations of the CFTR protein have been identified, but a single mutation (F508del), accounts for ~86% of all the mutations worldwide. There are approximately 34,000 adults and children with CF in the United States. The incidence of CF in the United States is approximately 1:3,500 newborns but varies by ethnicity: 1:3,500 Caucasian Americans, 1:8,500 Hispanic Americans, 1:17,000 African Americans, 1:31,000 Asian Americans.

## Clinical features

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

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

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

## Causes of CF

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

## Laboratory tests

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

For specimens with an elevated IRT on one (if sufficiently high) or both screening specimens, second-tier DNA analysis is performed (34 mutations, including current ACMG/ACOG recommendations). Depending on results, further diagnostic and confirmatory testing will be required, including additional mutation analysis.

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.
- Infants with meconium ileus may not have an elevated IRT. If meconium ileus is present, then diagnostic testing should be performed regardless of NBS results. It is important to remember that all infants with meconium ileus should have routine newborn bloodspot screening specimens collected even if CF is suspected, as they should be screened for the other conditions on the screening panel.
- A small percentage of infants with CF may not have an elevated IRT. Thus, a normal IRT at birth does not completely rule out CF. Children with recurrent respiratory problems, failure to thrive, or other symptoms consistent with CF, should still be evaluated and undergo sweat chloride testing.

## Confirmatory testing

CF can be diagnosed by two different methods, sweat chloride testing and/or DNA mutation analysis. Sweat chloride testing remains the gold standard, as it is a concrete marker of CFTR dysfunction. A chloride value in the sweat of  $\geq 60$  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](http://www.cftr2.org)), and most are not included in standard multi-array DNA analyses. (10, 11) Confirmation of two CF-causing mutations confirm the diagnosis, while only one may indicate a carrier state, CFTR-related metabolic syndrome (CRMS), or an affected individual with a less common mutation on the second allele.

## Treatment

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

## Screening practice considerations

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

**Table 3: CF screening result summary**

Results	Likely causes	Actions
1 <sup>st</sup> and 2 <sup>nd</sup> IRT elevated on filter paper specimens	<ul style="list-style-type: none"><li>• Cystic fibrosis probable</li><li>• Possible false positive</li></ul>	<ul style="list-style-type: none"><li>• NBS coordinator faxes results.</li><li>• Medical consultant phones practitioner with follow-up recommendations.</li><li>• Sweat chloride testing needed to confirm/clarify diagnosis.</li></ul>
One or two CFTR mutations identified on 23-mutation DNA analysis	<ul style="list-style-type: none"><li>• If two mutations, CF probable</li><li>• If one mutation, possible CF carrier vs CRMS vs CF with rare 2<sup>nd</sup> mutation</li></ul>	<ul style="list-style-type: none"><li>• NBS coordinator faxes results.</li><li>• Medical consultant phones practitioner with follow-up recommendations.</li><li>• Sweat chloride testing needed to confirm/clarify diagnosis.</li></ul>

# Congenital Adrenal Hyperplasia (CAH)

## CAH essentials

- **Neonatal emergency:** 3/4 will develop salt wasting crisis, which can be fatal, in the first week to month of life.
- **Incidence:** 1:12,700 newborns
- **Screening test:** 17-OH-progesterone
- **Validity:** 70% identified on 1st screen  
30% on 2nd screen
- **Causes:** 21-hydroxylase deficiency or other inborn error of cortisol synthesis; recessive inheritance
- **Treatment:** Hydrocortisone and mineralocorticoids
- **False positives:** Occur more frequently in premature, low birth weight or sick infants
- **Outcome:** Early detection and treatment can be lifesaving. Chromosome analysis in infants with ambiguous genitalia will prevent gender misassignment (11). Ultimate outcome depends on severity of defect, days to treatment and adherence. Refer to pediatric endocrinologist.

CAH is an inherited defect of cortisol synthesis. The adrenal gland cannot make cortisol and overproduces male hormones. Without cortisol, infants are at risk for adrenal crisis and may be unable to regulate salt and fluids, and can die. The most common disorder is 21-hydroxylase deficiency.

## Clinical features (12)

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

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

## Causes of CAH

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

## Laboratory tests

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

## Confirmation

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

## Treatment

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

## Screening practice considerations

- This disorder 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. Collect heel stick specimens between 24–48 hours of life. Transport all specimens 4–12 hours after collection and no later than 24 hours.
- Female infants who are virilized or infants with ambiguous genitalia should be considered at risk for this condition, tested at birth and monitored for electrolyte abnormalities until the diagnosis is excluded.
- Male infants are not usually recognized at birth.
- About 30% of infants will be detected only on a second screen. (13–15)

**Table 4: CAH screening result summary**

Results	Likely causes	Actions
Elevated 17 OHP (17-hydroxyprogesterone)	<ul style="list-style-type: none"><li>• CAH probable</li><li>• False positive</li></ul>	Neonatal emergency; NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

# Primary Congenital Hypothyroidism (CH)

## CH essentials (16, 17)

- **Incidence:** 1:2,000 newborns
- **Screening test:** T4 (thyroxine) and TSH (thyroid stimulating hormone)
- **Validity:** 90% identified on 1st screen, 10% on 2nd screen
- **Causes:** Thyroid dysgenesis: 85%; hereditary inborn error of thyroid hormone biosynthesis: 15%
- **Treatment:** L-thyroxine normalize T4 by 2 weeks of treatment initiation; TSH by 1 month
- **False positives:** Early collection within 24 hours of birth; premature or ill infants
- **Outcome:** Can be normal, but depends on severity of thyroid deficit, days to treatment and adherence to treatment. Severely affected infants with just a 2-week delay in reaching a serum T4 >10 ug/dL may have up to a 10-point drop in IQ. (18)

Primary congenital hypothyroidism (CH) occurs in infants who are born without the ability to produce adequate amounts of thyroid hormone. Thyroid hormone is important for normal function of all of the body's organs and is essential for normal brain development. The incidence of congenital hypothyroidism is 1:2,300. CH is more common in Hispanic and Native American populations (1:700–2,000). There is a 2:1 female/male ratio, explanation unknown. Infants with Down's syndrome have increased risk of CH (1:140 newborns).

## Clinical features

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

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

## Causes of primary congenital hypothyroidism

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

Some cases of central or secondary (hypopituitary) hypothyroidism may also be detected (see Table 5). These newborns often have clinical features of other pituitary hormone deficiencies, such as hypoglycemia or small penis and undescended testes in male infants.

## Laboratory tests

The initial screening test is the T4 assay. Infants with T4 results of <10% are further tested by a screening TSH assay. Different combinations of results are possible; see (see Table 5).

When the infant's physician is notified that screening results are abnormal, blood should be collected by venipuncture as soon as possible for measurement of TSH and free T4 to confirm the abnormal screening results. In the case where the screening T4 is low and TSH is elevated, treatment can be started as soon as the serum is obtained, pending final confirmation. If the serum thyroid function tests confirm hypothyroidism, further diagnostic studies, such as a thyroid ultrasound examination or radionuclide scan and X-ray to assess skeletal maturation, may be performed to determine the type, age of onset and severity of hypothyroidism. Generally, these studies do not change management and thus are optional.

## Thyroid function in premature infants

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

## Treatment

The American Academy of Pediatrics (AAP) recommends that infants be managed in consultation with a pediatric endocrinologist. (16) Treatment of CH is effective if done correctly. L-tyroxine (brand or generic l-thyroxine), in pill form, is crushed, mixed with water or expressed breast milk and administered once daily. The recommended starting dose is 10–15 mcg/day of body weight daily, usually 37.5 mcg/day to 50 mcg/day. AAP recommendations for follow-up TSH and free T4 are as follows:

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

**Treatment goals:** Maintain serum free T4 in the upper half of the normal or 1.2–2.4 ng/dL for free T4 (normal range may vary with assay), and TSH normalized (<6  $\mu$ IU/mL). Clinical evaluations can occur less frequently. As infants grow, the dose of thyroxine is increased. Periodic developmental testing should be done on all patients. If treatment is started early and thyroid levels are monitored closely, development remains normal (19).

## Screening practice considerations

- Primary congenital hypothyroidism is common, occurring in approximately 1:2,000 newborns.
- Ninety percent of hypothyroid infants are detected on the first specimen; in 10% of cases, hypothyroidism develops in the weeks after birth and is detected on a second screening test as production of thyroid hormone decreases after birth. (20–21)
- Some infants (usually pre-term) will manifest a delayed rise in TSH, and so are also detected on the routine second or third screening test. Practitioners therefore must remain alert to clinical symptoms in premature and older infants despite normal initial screening.
- False positive results may occur if the specimen is collected within the first few hours after birth, as the TSH rises in response to the extra-uterine environment.
- Topical iodine use on the infant or a mother who is breastfeeding and taking iodine supplements may cause transient hypothyroidism. In addition, nursing mothers drinking “seaweed soup”, which has a high iodine content, may also cause hypothyroidism in the neonate; this will resolve if ingestion of seaweed soup is discontinued.

**Table 5: CH screening result summary**

Results	Likely causes	Actions
T4 low/TSH elevated	<ul style="list-style-type: none"> <li>• Hypothyroidism probable</li> <li>• False positive</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
T4 low/TSH slightly elevated	<ul style="list-style-type: none"> <li>• Mild hypothyroidism</li> <li>• Transient hypothyroidism seen with recovery from “hypothyroxinemia of prematurity”</li> <li>• False positive</li> </ul>	NWRNBS Program contacts practitioner by FAX and by mail requesting further testing.
T4 low/TSH normal (on two specimens unless premature)	<ul style="list-style-type: none"> <li>• Thyroid binding globulin (TBG) deficiency</li> <li>• Central or secondary (hypopituitary) hypothyroidism</li> <li>• Non-thyroidal illness syndrome (“sick euthyroid syndrome”) associated with prematurity or acute illness</li> <li>• False positive</li> </ul>	NWRNBS Program contacts practitioner by FAX and by mail requesting further testing.

## Sickle Cell Disease and other Hemoglobinopathies

### Sickle Cell Disease essentials

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

The primary goal of hemoglobinopathy screening is to detect clinically significant sickling hemoglobinopathies in the neonatal period, before symptoms occur. Newborn diagnosis of sickle cell disease, if coupled with family education and centralized comprehensive care, can markedly lower morbidity and mortality. (22)

Homozygous sickle cell disease (SCD) occurs when the recessive gene for hemoglobin S, sickle hemoglobin, is inherited homozygously or with a second gene for certain other hemoglobin variants, such as beta thalassemia or hemoglobin C. These doubly heterozygous conditions tend to be less severe than those who are homozygous for hemoglobin S, although all are potentially capable of producing severe complications. The disease incidence in a population depends on the population’s racial composition.

## Clinical features

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

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

## Laboratory tests

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

## Treatment

Infants with significant hemoglobinopathies should have a primary care provider and receive periodic evaluation 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.

## Carrier detection makes SCD screening different

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. If both parents are carriers of an autosomal recessive genetic trait, the risk of any infant of that couple being homozygous, and therefore having the disease, is 1:4.

## Screening practice considerations

- Newborn bloodspot screening for hemoglobinopathies is not done on the second specimen unless an abnormality has been identified on the first specimen. It is crucial to use the first kit for the first test; the cards are not interchangeable.
- Transfusion of red blood cells before collecting the newborn bloodspot screening specimen will invalidate the hemoglobinopathy test. Always obtain a specimen before any transfusion regardless of the infant's age.
- Some hemoglobinopathies, particularly some thalassemias, are not reliably detected by newborn 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.

# Amino Acid Conditions

## Hypermethioninemia

*Homocystinuria (cystathionine beta-synthase deficiency)\**

### Homocystinuria essentials

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

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

### Clinical features (23, 24)

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.

---

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

## Methionine adenosyltransferase (MAT) deficiency

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

### Laboratory test

Elevation of methionine is detected by tandem mass spectrometry (MS/MS).

### Treatment

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

### Screening practice considerations

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

**Table 6: Hypermethioninemia screening result summary**

Results	Likely causes	Actions
Methionine slightly elevated	<ul style="list-style-type: none"><li>• Homocystinuria/MAT deficiency possible</li><li>• Tyrosinemia, Type I, galactosemia</li><li>• Liver disease</li><li>• Parenteral nutrition</li><li>• High protein diet</li><li>• False positive</li></ul>	NWRNBS Program requests repeat filter paper specimen by mail.
Methionine elevated	<ul style="list-style-type: none"><li>• Homocystinuria/MAT deficiency probable</li><li>• Tyrosinemia, Type I</li><li>• Liver disease</li><li>• Parenteral nutrition</li><li>• High protein diet</li><li>• False positive</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations

# Phenylketonuria (PKU) and Hyperphenylalaninemia

## Hyperphenylalaninemia essentials

- **Incidence:** 1:16,300 births
- **Screening test:** Phenylalanine elevated by tandem mass spectrometry (MS/MS); phenylalanine/tyrosine ratio elevated
- **Confirmatory tests:** Quantitative amino acids; bipterins in blood and urine
- **Validity:** >99% on 1st screen
- **Treatment:** Low phenylalanine diet; bipterin supplementation
- **Outcome:** Normal if treated early and adherence is good

Detection of elevated phenylalanine levels requires urgent follow-up. The disorder is due to a recessively inherited enzyme defect in which the body cannot use the amino acid phenylalanine properly. All other metabolic processes are intact, but phenylalanine, which comes from all dietary protein, accumulates in the blood to toxic levels. All forms of hyperphenylalaninemias from mild to severe and including bipterin defects are inherited as autosomal recessive disorders.

## Clinical features

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

Phenylalanine starts rising after birth and often reaches abnormal levels within 24 hours of life. A phenylalanine/tyrosine ratio can also be used to identify cases.

## Variant forms of PKU (hyperphenylalaninemia)

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

Some forms of hyperphenylalaninemia are caused by defects of the cofactor bipterin metabolism and blood phenylalanine levels are variable. These patients have progressive neurological damage with seizures and steady deterioration that becomes noticeable sometime between 6 and 20 months of age despite early

treatment with a low phenylalanine diet. Definitive tests can differentiate these variant forms of PKU. In view of the severity of this group of diseases, all infants with persistently abnormal levels of phenylalanine must have testing by special blood and urine tests for bipterin abnormalities.

## Maternal PKU and hyperphenylalaninemia

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

## Laboratory tests

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

## Treatment (25–28)

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

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

## Screening practice considerations

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

**Table 7: PKU and hyperphenylalaninemia screening result summary**

Results	Likely causes	Actions
Phenylalanine elevated; Phe/Tyr elevated	<ul style="list-style-type: none"> <li>• PKU possible</li> <li>• Variants forms of PKU</li> <li>• Mother has PKU</li> <li>• False positive</li> <li>• Transient hyperphenylalaninemia</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

## Tyrosinemia type I, II, and Transient Tyrosinemia\*

### Tyrosinemia essentials

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

### Transient Tyrosinemia (29)

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

### Clinical features

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

\* Not all cases of tyrosinemia will be detected by newborn bloodspot screening.

## Treatment

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

## Tyrosinemia Type I (Hepatorenal Tyrosinemia) (30)

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

### Clinical features

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

### Treatment

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

## Tyrosinemia Type II (Occulocutaneous Tyrosinemia)

Tyrosinemia, Type II is caused by a deficiency of the enzyme tyrosine aminotransferase (TAT) and is inherited as an autosomal recessive trait. TAT deficiency is rare, with about 100 cases described worldwide, although more infants may be identified as MS/MS screening continues to be implemented. (31)

## Clinical features (20, 31)

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

## Treatment

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

## Laboratory tests

Tyrosinemia is detected using both tyrosine and succinylacetone measured by MS/MS. There is considerable overlap in tyrosine levels between normal infants, those with transient tyrosinemia and affected infants, making the tyrosine level itself not very specific. Succinylacetone is the unique marker for tyrosinemia type I.

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

## Screening practice considerations

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

**Table 8: Tyrosinemia screening result summary**

Results	Likely causes	Actions
Tyrosine elevated Succinoylacetone normal	<ul style="list-style-type: none"><li>• Transient tyrosinemia</li><li>• Tyrosinemia type II or III possible</li><li>• Liver disease</li><li>• Parenteral nutrition</li><li>• False positive</li></ul>	NWRNBS Program requests repeat filter paper by mail.
Succinolyacetone increased. Tyrosine normal.	<ul style="list-style-type: none"><li>• Tyrosinemia type I</li><li>• False positive</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations

# Fatty Acid Oxidation (FAO) Conditions

## FAO condition essentials

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

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

## Fatty Acid Oxidation conditions\*

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

---

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

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

## Clinical features (33, 34)

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

**Hepatic (35, 36):** No typical age of presentation, may occur on the first day of life through adulthood. Infants with MCAD can present with sudden cardio/pulmonary arrest before screening results are known. Precipitating factors are fasting and/or stress associated with intercurrent illness. Patients present with “Reyes-like” symptoms including vomiting, lethargy, hypoketotic hypoglycemia, mild hyperammonemia, hyperuricemia, hypocarnitinemia and abnormal liver function tests. Liver biopsy often shows steatosis. Hepatic presentation is common in MCAD, VLCAD, LCHAD, neonatal CPT I & II and mild CACT deficiency. Patients with LCHAD may develop retinal pigmentary changes and progressive visual loss in childhood despite early diagnosis and treatment.

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

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

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

## Treatment

Even with screening, some infants with FAO disorders may die before laboratory results are available. Treatment for MCAD and some other FAOs is extraordinarily simple once the diagnosis is suspected. Avoidance of fasting, particularly as infants and young children, is the primary treatment. Carnitine supplementation (100mg/kg/day) is used to provide a pathway for removal of toxic intermediate metabolites in some FAOs. With appropriate treatment hepatic, cardiac and muscular complications can be reduced or eliminated. Patients with these disorders may require IV support for fluid and calories during intercurrent infections or illnesses. With pre-symptomatic diagnosis and appropriate therapy, outcome can be normal for infants with MCAD. (37, 38) Outcomes for the other disorders are still being evaluated.

## Screening practice considerations

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

## Organic Acid Conditions (OA)

### OA condition essentials

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

The following OAs are screened for by MS/MS:

- Beta-ketothiolase deficiency
- Glutaric acidemia, type I (glutaryl-CoA dehydrogenase deficiency)
- Isobutyryl CoA dehydrogenase deficiency
- Isovaleric acidemia, (isovaleryl-CoA dehydrogenase deficiency)
- Malonic aciduria
- Maple syrup urine disease (branched chain alpha-ketoacid dehydrogenase deficiency)

- Methylmalonic acidemias, methylmalonyl CoA mutase deficiency and defects of B-12 metabolism
- Propionic acidemia
- 3-Hydroxy-3-methylglutaryl (HMG) CoA lyase deficiency
- 2-Methyl-3-hydroxybutyryl CoA dehydrogenase deficiency
- 2-Methylbutyryl CoA dehydrogenase deficiency (mitochondrial acetoacetyl-CoA thiolase deficiency)
- 3-Methylcrotonyl CoA carboxylase (3MCC) deficiency
- 3-Methylglutaconyl CoA hydratase deficiency (3-methyl-glutaconic aciduria, type I)
- Multiple carboxylase deficiency

## Clinical features (39, 41)

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

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

**Asymptomatic cases:** There are numerous reports of cases of isolated 3-methylcrotonyl-CoA carboxylase deficiency who have remained asymptomatic despite biochemical and/or enzymatic confirmation of the condition. The etiology of these variant presentations is not yet understood. Mild forms of methylmalonic acidemia have been found.

**Glutaric Acidemia, type I:** Glutaric Acidemia, Type I or GA I is an organic acidemia with clinical features unlike those described above. (40–42) In this disease, there is

an accumulation of glutaric acid and 3-hydroxy glutaric acid, which are believed to be toxic to cells, particularly in the central nervous system. The classic presentation is macrocephaly at or shortly after birth. Infants have a period of apparently normal development but may have soft neurological signs, like jitteriness, irritability and truncal hypotonia. Generally, between 6 and 18 months of age, patients will experience an acute encephalopathic episode resulting in damage to the basal ganglia and atrophy of the caudate and putamen. This occurs over the course of a few hours to a day and is irreversible and untreatable. Severe dystonia, dyskinesia and other neurological findings result, either in a static or slowly progressive form. These children are often misdiagnosed as having extra pyramidal cerebral palsy. Approximately 25% of GA I patients will present with motor delay, hypotonia, dystonia and dyskinesia that develop gradually during the first few years of life, without any apparent acute crisis. Intellect is relatively intact. Infants with GA I are prone to acute subdural and retinal hemorrhages after minor head trauma. This can be misdiagnosed as child abuse. Finally, 5% of all Amish patients have been completely asymptomatic without any crises and normal development. Neurological crises and symptoms rarely occur after 5 years of age.

## Laboratory tests

All these disorders are detected using MS/MS. Leucine can be elevated in infants receiving parenteral nutrition, usually along with other amino acid elevations. In a normal newborn, however, elevations of these compounds are unusual and require rapid follow-up. There is evidence that not all affected infants will be found by NBS. (43)

## Treatment

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

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

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

## Screening practice considerations

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

# Urea Cycle Conditions (UCD)

## Urea Cycle essentials

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

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

- Arginase deficiency
- Argininosuccinic aciduria (ASA)
- Citrullinemia, type I and II

They are inherited as autosomal recessive traits.

## Arginase deficiency (44)

### Clinical features

Arginase deficiency is associated with irritability, inconsolable crying, anorexia, vomiting and developmental delay in infancy. This progresses to spastic tetraplegia with lower limbs more severely affected than the upper, psychomotor 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.

## Citrullinemia, Type I (CTLN1) and Argininosuccinic Aciduria (ASA) (44, 46)

### Clinical features-neonatal onset

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

### Clinical features-late onset

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

### Asymptomatic cases

Newborn 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.

## Citrin Deficiency (Citrullinemia, Type II and Neonatal Intrahepatic Cholestasis [NICCD]) (47)

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

### Clinical features-neonatal onset

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

### Clinical features-late onset

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

### Laboratory tests

Elevations of citrulline and arginine are detected by MS/MS. The laboratory cutoff for citrulline is  $\leq 70 \mu\text{M/L}$ ; for arginine,  $\leq 110 \mu\text{M/L}$ ; argininosuccinic acid,  $\leq 1.50 \mu\text{M/L}$ . Transient elevations of plasma arginine and citrulline in the newborn are unusual unless the infant is premature and/or receiving parenteral nutrition.

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

### Treatment (Citrullinemia, Type I & ASA)

All patients with a neonatal presentation represent medical emergencies and outcomes may be variable. Patients with neonatal onset disease will typically require aggressive treatment with hemodialysis. All patients, both late onset and those rescued from neonatal hyperammonemia, will require treatment with low protein diets and medications to prevent hyperammonemia and remove toxic compounds. The outcome for patients rescued from prolonged neonatal hyperammonemia is extremely poor.

Brain damage is likely. Even patients treated prospectively from birth may not be unaffected. (46) Those with late onset disease fare better, and presymptomatic diagnosis and treatment may allow normal development.

## Treatment: NICCD and CTLN2

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

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

## Screening practice considerations

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

**Table 9: UCD screening result summary**

Results	Likely causes	Actions
Arginine >110 µM/L	<ul style="list-style-type: none"> <li>• Arginase deficiency possible</li> <li>• Transient argininemia</li> <li>• Liver disease</li> <li>• False positive</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
ASA >1.50 µM/L	<ul style="list-style-type: none"> <li>• Argininosuccinic aciduria possible</li> <li>• Liver disease</li> <li>• False positive</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
Citrulline >70 µM/L	<ul style="list-style-type: none"> <li>• Citrullinemia, argininosuccinic aciduria possible</li> <li>• Transient citrullinemia</li> <li>• Liver disease</li> <li>• False positive</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
Citrulline >120 µM/L on second specimen	<ul style="list-style-type: none"> <li>• Mild citrullinemia, argininosuccinic aciduria possible</li> <li>• Transient citrullinemia</li> <li>• Liver disease</li> <li>• False positive</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations

# Galactosemia

## Galactosemia essentials

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

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

## Clinical features (48)

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

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

## Laboratory tests

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

- **GALT activity:** The enzyme test depends upon fluorescence produced by the normal galactose enzyme cascade in red blood cells. A temporarily abnormal result (diminished or absent fluorescent activity) is found in some infants. The test may be persistently abnormal if the enzyme activity is <50% of normal. It does not differentiate milder variants from severe defects or G6PD.
- **Galactose:** Slight elevations can occur in normal neonates, but galactose metabolites are greatly elevated in infants with galactosemia if they are receiving a lactose-containing formula or breast milk. Liver disease may also cause an elevation of galactose metabolites. All infants with an abnormal GALT or who have been transfused will be screened for galactose.

## Treatment

Galactosemia is treated by dietary galactose restriction (usually accomplished in the infant period through the use of soy-based or partially hydrolyzed infant formulas). The diet must be followed for life and requires close supervision. Even with early diagnosis and strict dietary restrictions children with galactosemia are at risk for speech disorders, tremors, growth and developmental delays and in females, ovarian failure.

## Screening practice considerations

- The GALT test should be abnormal in virtually all severe classic galactosemic infants even if the specimen is obtained before lactose is ingested, unless the infant has been transfused. Obtain a specimen before any transfusion.
- The GALT enzyme is prone to degradation if the sample is delayed in the mail or exposed to excess temperature or humidity. This produces a false positive GALT result.
- Galactose accumulation depends on lactose ingestion so that blood galactose metabolites may be normal in infants being fed a soy-based formula.

**Table 10: Galactosemia screening result summary**

Results		Likely causes	Actions
GALT test	Galactose metabolites		
<3.5 u/dL	≥20 mg/dL	<ul style="list-style-type: none"> <li>• Severe galactosemia</li> <li>• Variant galactosemia</li> <li>• False positive</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
<3.5 u/dL	<20 mg/dL	<ul style="list-style-type: none"> <li>• Severe galactosemia with little lactose intake</li> <li>• Variant galactosemia</li> <li>• Other enzyme defects in red blood cells</li> <li>• Improperly handled sample (heat damage or transit delay)</li> </ul>	Contact by mail or fax if infant is ≥48 hours old. Contact by fax if <48 hours old or if not on lactose.

## Biotinidase Deficiency

### Biotinidase deficiency essentials

- **Incidence:** 1:60,000 births
- **Screening test:** Biotinidase qualitative colorimetric enzyme assay
- **Confirmatory tests:** Quantitative biotinidase enzyme assay
- **Validity:** 100% found on 1st screen
- **Treatment:** 5-10 mg biotin/day
- **Outcome:** Excellent if compliant with biotin therapy This recessively inherited disorder affects the cells' ability to recycle the vitamin-cofactor biotin, which impairs the function of mitochondrial carboxylases.

### Clinical features (49, 50)

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

Infants with partial deficiency (5–10%) have been identified through newborn 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.

## Laboratory tests

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

## Treatment

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

Screening practice considerations

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

**Table 11: Biotinidase deficiency screening result summary**

Results	Likely causes	Actions
Color change does not occur	<ul style="list-style-type: none"><li>• Biotinidase deficiency possible</li><li>• False positive</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

# Severe Combined Immunodeficiency (SCID)

## SCID essentials

- **Incidence:** 1:50,000-1:100,000 births
- **Screening test:** Polymerase chain reaction to detect T-cell Receptor Excision Circles (TRECs)
- **Confirmatory tests:** CBC, lymphocyte subset flow cytometry
- **Validity:** The TREC assay used by Oregon to evaluated specimens for SCID and other t-cell lymphopenia has a positive predictive value greater than 0.7.
- **Treatment:** Bone marrow transplant, gene therapy or enzyme replacement
- **Outcome:** Good if treated within first 3 months of life

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

## Clinical features

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

## Causes of SCID

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

## Laboratory tests

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

## Confirmation

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

## Treatment

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

# Lysosomal Storage Disorders (LSDs)

## What are LSDs?

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

## Which LSDs are being tested:

Disorder	Enzyme	Gene
Fabry Disease	Alpha Galactosidase A	<i>GLA</i>
Gaucher Disease	Acid Beta-Glucosidase	<i>GBA</i>
Mucopolysaccharidosis Type 1 (MPS-1)	Alpha-L-Iduronidase	<i>IDUA</i>
Pompe Disease	Acid Alpha-Glucosidase	<i>GAA</i>

## How are LSDs diagnosed?

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

# Fabry Disease

## Fabry disease essentials (53, 54)

- **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.
- **Screening test:** Tandem mass spectrometry (MS/MS) to detect Alpha-galactosidase A (GLA) enzyme followed by second-tier DNA analysis of the *GLA* gene.
- **Confirmatory test:** GLA enzyme activity in plasma and leukocytes, possible assistance via DNA analysis of family members.
- **Validity:** Published false positive rate is 0.27% for the NeoLSD kit (PerkinElmer) used to measure enzyme activity

- **Treatment:** Enzyme replacement administered via infusion and in some cases oral chaperone therapy.
- **Outcome in early diagnosis:** Affected individuals will typically not develop symptoms for years to decades. Outcomes are improved with frequent monitoring and intervention to halt and prevent further progression of disease.

While Fabry disease is a disorder primarily affecting males, female heterozygotes can also be symptomatic and may be detected via newborn bloodspot screening. Some will remain asymptomatic throughout most of their life, others may benefit from early intervention.

## Clinical features

Mutations in *GLA* result in reduced formation of alpha-galactosidase A (GLA), 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.

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. In heterozygous females, milder symptoms later in life are expected but they can display a classic disease presentation.

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).

## Causes of Fabry disease

Fabry disease is inherited in an X-linked manner. In affected males, the infant's mother is an obligate heterozygote. Female carriers may have varying presentations due to random X-chromosome inactivation. The most severely impacted females likely express X chromosome with pathogenic *GLA* variant in the affected organs. Rarely, de novo pathogenic variants arise spontaneously.

## Laboratory tests

The screening test measures activity of GLA enzyme. A low enzyme on first valid specimen will trigger DNA analysis of the *GLA* gene (second tier). Depending on results, further diagnostic and confirmatory testing may be required.

There are several issues to keep in mind regarding abnormal enzyme tests:

- Reduced enzyme alone is not diagnostic of the disease. Diagnosis must be confirmed by DNA analysis and subsequent diagnostic testing.
- Unlike in most autosomal recessive disorders, there may be significant clinical implications for family members of infants with significant *GLA* variants. In some cases, family testing may assist in diagnosis and/or prognosis discussions.

## Confirmatory testing

In males, confirmation of the diagnosis after newborn bloodspot screening is made by measurement of GLA enzyme activity in plasma and leukocytes. Measurement in both are recommended due to inconsistent reductions seen in some DNA variants. A GLA enzyme  $< 1\%$  is consistent with classic disease and  $> 1\%$  but below the unaffected range is consistent with atypical disease. For females, measurement of GLA is unreliable and does not predict prognosis or severity.

DNA results from the newborn bloodspot screen assists in confirmation of diagnosis but may not be definitive if the variant is of uncertain significance. In some cases a mature, maternal adult family member can be tested. If that family member shares the variant detected in the newborn but has no features of Fabry disease then development of disease is considered unlikely.

## Treatment

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.

## Carrier detection

Screening may identify female Fabry disease heterozygotes as discussed above, but not all female heterozygotes will be detected on newborn bloodspot screening.

## Screening practice considerations

- GLA enzyme is not valid in screens collected in infants before 20 hours of life.
- GLA enzyme is measured in one valid specimen only. Normal GLA enzymes are not repeated on the 2<sup>nd</sup> or other subsequent specimens.

**Table 12: Fabry disease screening result summary for male newborns**

Results in MALE Newborn	Likely causes	Actions
GLA enzyme low, DNA analysis detects no and/or benign variant	<ul style="list-style-type: none"><li>• False positive</li></ul>	NWRNBS Program will report by letter regarding test results and any other recommendations.
GLA enzyme low, DNA analysis detects hemizygous variant of uncertain significance	<ul style="list-style-type: none"><li>• False positive</li><li>• Fabry disease</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
GLA enzyme low, DNA analysis detects hemizygous likely pathogenic or pathogenic variant	<ul style="list-style-type: none"><li>• Fabry disease</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

**Table 13: Fabry disease screening result summary for female newborns**

Results in FEMALE Newborn	Likely causes	Actions
GLA enzyme low, DNA analysis detects no and/or benign variant(s)	<ul style="list-style-type: none"><li>• False positive</li></ul>	NWRNBS Program will report by letter regarding test results and any other recommendations.
GLA enzyme low, DNA analysis detects variant(s) of uncertain significance	<ul style="list-style-type: none"><li>• False positive</li><li>• Heterozygous Fabry disease carrier</li><li>• Fabry disease</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
GLA enzyme low, DNA analysis detects likely pathogenic and/or pathogenic variant(s)	<ul style="list-style-type: none"><li>• Heterozygous Fabry disease carrier</li><li>• Fabry disease</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

# Pompe Disease

## Disease essentials (55–57)

- **Incidence:** Estimated between 1 in 28,000 to 1 in 40,000
- **Screening test:** Tandem mass spectrometry (MS/MS) to detect acid alpha-glucosidase (GAA) enzyme followed by second-tier DNA analysis of the *GAA* gene.
- **Confirmatory tests:** Creatine kinase (CK), aspartate transaminase (AST), alanine transaminase (ALT), acid alpha-glucosidase (GAA) enzyme in blood and urinary glucotetrasaccharide (Hex4)
- **Validity:** Published false positive rate is 0.12% for the NeoLSD kit (PerkinElmer) used to measure enzyme activity
- **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)
- **Treatment:** Enzyme replacement administered via infusion and supportive therapy.
- **Outcome in early diagnosis:** Significant improvements are expected in cardiac and respiratory function in infantile-onset forms with early treatment including prolonged lifespan. Improvement in long-term muscle function is expected in later-onset forms.

The presence of pseudodeficiency DNA variants in *GAA* will result in lower measured GAA enzyme on traditional assays but does not impact the actual function of the enzyme in vivo. Presence of pseudodeficiency variants is not associated with any clinical features of disease but will result in false positive screens and blood tests.

## Clinical features

Mutations in *GAA* result in reduced formation of acid alpha-glucosidase (GAA), the lysosomal enzyme responsible for processing of 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 and 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 lifespans 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.

## Causes of disease

Pompe disease is inherited in an autosomal recessive manner resulting in insufficient GAA enzyme.

## Laboratory tests

The screening test measures activity of GAA enzyme. A low enzyme on first valid specimen will trigger DNA analysis of the *GAA* gene (second tier). Depending on results, further diagnostic and confirmatory testing may be required.

There are several issues to keep in mind regarding abnormal enzyme tests:

- Reduced enzyme alone is not diagnostic of the disease. Diagnosis must be confirmed by DNA analysis and diagnostic testing.
- Presence of one or more pseudodeficiency variants will often result in a false positive screen. DNA testing may be able to clarify these cases before further testing or referral to specialist need to be pursued.

## 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 IOPD and 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.

**Table 14: Pompe disease variants, onset, and affected populations**

<b>GAA Pathogenic Variant</b>	<b>Associated with (IOPD or LOPD)</b>	<b>Commonly Affected Populations</b>
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

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.

## Treatment

Currently available treatment is enzyme replacement therapy (ERT) initiated prior to the development of tissue and organ damage in order 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.

## Screening practice considerations

- GAA enzyme is not valid in screens collected in infants before 20 hours of life.
- GAA enzyme is measured in one valid specimen only. Normal GAA enzymes are not repeated on the 2<sup>nd</sup> or other subsequent specimens.

**Table 15: Pompe disease screening result summary**

Results	Likely causes	Actions
GAA enzyme low, DNA analysis detects no and/or benign variant(s) including pseudodeficiency.	<ul style="list-style-type: none"> <li>• False positive</li> </ul>	NWRNBS Program will report by letter regarding test results and any other recommendations.
GAA enzyme low, DNA analysis detects heterozygous variant of uncertain significance, likely pathogenic or pathogenic variant	<ul style="list-style-type: none"> <li>• False positive</li> <li>• Pompe disease carrier</li> </ul>	NWRNBS Program will report by letter regarding test results and any other recommendations.
GAA enzyme low, DNA analysis detects homozygous or compound heterozygous variants of uncertain significance	<ul style="list-style-type: none"> <li>• Pompe disease carrier</li> <li>• Pompe disease</li> </ul>	NNBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
GAA enzyme low, DNA analysis detects homozygous or compound heterozygous likely pathogenic or pathogenic variants	<ul style="list-style-type: none"> <li>• Pompe disease carrier (if inherited in cis-)</li> <li>• Pompe disease (inherited in trans-)</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

# Mucopolysaccharidosis Type I (MPS I)

## Disease essentials (58, 59)

- **Incidence:** Estimated between 1 in 87,000 to 1 in 185,000
- **Screening test:** Tandem mass spectrometry (MS/MS) to detect alpha-L-iduronidase (IDUA) enzyme followed by second-tier DNA analysis of the *IDUA* gene.
- **Confirmatory test:** Alpha-L-iduronidase (IDUA) enzyme, glycosaminoglycans (GAGs) (aka mucopolysaccharides or MPS) in blood and/or urine.
- **Validity:** Published false positive rate is 0.07% for the NeoLSD kit (PerkinElmer) used to measure enzyme activity
- **False positives:** Can occur in heterozygous carriers and in presence of pseudodeficiency variants (particularly common in individuals of African ancestry).
- **Treatment:** Hematopoietic stem cell transplantation (HSCT) in severe disease and/or enzyme replacement therapy (ERT)
- **Outcome in early diagnosis:** In severe disease with early HSCT, or attenuated disease with early ERT, significant improvements expected in lifespan and overall disease burden.

The presence of pseudodeficiency DNA variants in *IDUA* will result in lower measured IDUA enzyme on traditional assays but does not impact the actual function of the enzyme in vivo. Presence of pseudodeficiency variants is not associated with any clinical features of disease but will result in false positive screens and blood tests.

## Clinical features

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.

## Causes of disease

MPS I is inherited in an autosomal recessive manner resulting in insufficient IDUA enzyme.

## Laboratory tests

The screening test measures activity of IDUA enzyme. A low enzyme on first valid specimen will trigger DNA analysis of the *IDUA* gene (second tier). Depending on results, further diagnostic and confirmatory testing may be required.

There are several issues to keep in mind regarding abnormal enzyme tests:

- Reduced enzyme alone is not diagnostic of the disease. Diagnosis must be confirmed by DNA analysis and diagnostic testing.
- Presence of one or more pseudodeficiency variants will often result in a false positive screen. DNA testing may be able to clarify these cases before further testing or referral to specialist need to be pursued.

## Confirmatory testing

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.

## 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.

## Screening practice considerations

- IDUA enzyme is not valid in screens collected in infants before 20 hours of life.
- IDUA enzyme is measured in one valid specimen only. Normal IDUA enzymes are not repeated on the 2nd or other subsequent specimens.

**Table 16: MPS I screening result summary**

Results	Likely causes	Actions
IDUA enzyme low, DNA analysis detects no and/or benign variant(s) including pseudodeficiency.	<ul style="list-style-type: none"> <li>• False positive</li> </ul>	NNWRNBS Program will report by letter regarding test results and any other recommendations.
IDUA enzyme low, DNA analysis detects heterozygous variant of uncertain significance, likely pathogenic or pathogenic variant	<ul style="list-style-type: none"> <li>• False positive</li> <li>• MPS I carrier</li> </ul>	NNWRNBS Program will report by letter regarding test results and any other recommendations.
IDUA enzyme low, DNA analysis detects homozygous or compound heterozygous variants of uncertain significance	<ul style="list-style-type: none"> <li>• MPS I carrier</li> <li>• MPS I</li> </ul>	NNBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
IDUA enzyme low, DNA analysis detects homozygous or compound heterozygous likely pathogenic or pathogenic variants	<ul style="list-style-type: none"> <li>• MPS I carrier (if inherited in cis-)</li> <li>• MPS I (inherited in trans-)</li> </ul>	NNBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

## Gaucher Disease

### Disease essentials (60, 61)

- **Incidence:** In the U.S, estimated at 1 in 40,000. In the Ashkenazi Jewish population prevalence is 1:855 individuals.
- **Screening test:** Tandem mass spectrometry (MS/MS) to detect acid beta-glucocerebrosidase (GBA) enzyme followed by second-tier DNA analysis of the *GBA* gene.
- **Confirmatory test:** Glucocerebrosidase (GBA) enzyme and chitotriosidase activity
- **Validity:** Published false positive rate is 0.07% for the NeoLSD kit (PerkinElmer) used to measure enzyme activity

- **False positives:** May occur in unaffected, heterozygous carriers
- **Treatment:** Enzyme replacement administered via infusion and in some cases oral substrate reduction therapy (SRT).
- **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.

## Clinical features

Mutations in *GBA* result in reduced formation of acid beta-glucocerebrosidase (GBA), 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.

## Causes of disease

Gaucher disease is inherited in an autosomal recessive manner resulting in insufficient GBA enzyme.

## Laboratory tests

The screening test measures activity of GBA enzyme. A low enzyme on first valid specimen will trigger DNA analysis of the *GBA* gene (second tier). Depending on results, further diagnostic and confirmatory testing may be required.

An important issue to keep in mind regarding abnormal enzyme tests:

- Reduced enzyme alone is not diagnostic of the disease. Diagnosis must be confirmed by DNA analysis and diagnostic testing.

## Confirmatory testing

Diagnosis of Gaucher disease is established by presence of biallelic pathogenic variants in GBA along with reduced GBA enzyme consistent with disease on diagnostic testing.

DNA analysis often assists in determining disease type in newborns identified by screening. Certain variants in the homozygous or compound heterozygous state can predict specific Gaucher disease type. The presence of at least one copy of the common variant, p.Asn409Ser or N409S (historically known as “N370S”) is protective against CNS disease.

**Table 17: Gaucher disease variants, disease types and affected population**

<b>GBA Pathogenic Variants</b>	<b>Gaucher disease type expected</b>	<b>Affected Individuals</b>
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%

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.

## 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.

## Screening practice considerations

- GBA enzyme is not valid in screens collected in infants before 20 hours of life.
- GBA enzyme is measured in one valid specimen only. Normal GBA enzymes are not repeated on the 2nd or other subsequent specimens.

**Table 18: Gaucher disease screening result summary**

Results	Likely causes	Actions
GBA enzyme low, DNA analysis detects no and/or benign variant(s)	<ul style="list-style-type: none"> <li>False positive</li> </ul>	NWRNBS Program will report by letter regarding test results and any other recommendations.
GBA enzyme low, DNA analysis detects heterozygous variant of uncertain significance, likely pathogenic or pathogenic variant	<ul style="list-style-type: none"> <li>False positive</li> <li>Gaucher disease carrier</li> </ul>	NWRNBS Program will report by letter regarding test results and any other recommendations.
GBA enzyme low, DNA analysis detects homozygous or compound heterozygous variants of uncertain significance	<ul style="list-style-type: none"> <li>Gaucher disease carrier</li> <li>Gaucher disease</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
GBA enzyme low, DNA analysis detects homozygous or compound heterozygous likely pathogenic or pathogenic variants	<ul style="list-style-type: none"> <li>Gaucher disease carrier (if inherited in cis-)</li> <li>Gaucher disease (inherited in trans-)</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

## Spinal Muscular Atrophy (SMA)

**Screening for Spinal Muscular Atrophy (SMA) is anticipated to begin June 1, 2022.**

### SMA essentials (62–71)

- **Incidence:** 1:11,000 births
- **Screening test:** Polymerase chain reaction to detect deletion of exon 7 of the *SMN1* gene. 95% of cases are due to deletions in the *SMN1* gene.
- **Confirmatory tests:** Sequencing to identify deletions/mutations in the *SMN1* gene and copy number variants in the *SMN2* gene
- **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.
- **Outcome:** Can vary depending on type of SMA.

SMA, attributed to variants in the *SMN1* gene, is an autosomal recessive condition that progressively destroys motor neurons—nerve cells in the brain stem and spinal cord that control essential skeletal muscle activity such as speaking, walking, breathing, and swallowing, leading to muscle weakness and atrophy. Motor neurons control movement in the arms, legs, chest, face, throat and tongue. When there are disruptions in the signals between motor neurons and muscles, the muscles weaken, begin wasting away and develop twitching (called fasciculations).

## Clinical features

There is a wide range of impairment seen in SMA caused by defects in the *SMN1* gene, from onset before birth with breathing difficulties at birth to mild weakness in adults. Accordingly, SMA can be classified into four types, based on highest motor milestone achieved.

**Table 19: SMA types and clinical features**

Type	Other Name	Life Span	Motor	Clinical features
<b>SMA type 0</b> 0 copies of <i>SMN2</i>	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.
<b>SMA type I</b> 1-2 copies of <i>SMN2</i>	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 of the spine) or other skeletal abnormalities as they get older.
<b>SMA type II</b> 3 copies of <i>SMN2</i>	The	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.
<b>SMA type III</b> 3-4 copies of <i>SMN2</i>	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.
<b>SMA type IV</b> 4-6 copies of <i>SMN2</i>		Normal	Normal	Onset after 21 years of age, with mild to moderate proximal muscle weakness and other symptoms.

## Causes of SMA

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*.

## Laboratory tests

Screening is based on real time PCR that detects *SMN1* deletions.

## Confirmation

- Molecular Genetic testing of *SMN1* gene.
- Deletion/duplication analysis for exon 7 of *SMN1* and sequencing of *SMN1* if exon 7 is fully present
- Copy Number Variants may be assessed on *SMN2* as there is a correlation between the *SMN2* copy number and severity of disease.

**Table 20: SMN2 copy number and SMA clinical phenotype (62)**

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

Adapted from Calucho et al [2018] (69)

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] (70) 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.

## Screening Practice Considerations

Immediate referral to a pediatric neurologist is recommended.

## Treatment

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 mutated *SMN1* gene. This therapy is approved for patients with SMA under the age of 2 years.

Infants may receive gene therapy.

## X-Linked Adrenoleukodystrophy (X-ALD)

**Screening for X-Linked Adrenoleukodystrophy (X-ALD) is anticipated to begin on or before January 1, 2023.**

### X-ALD essentials (72–75)

- **Prevalence/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.
- **Screening test:** Tandem mass spectrometry (MS/MS) to detect C26:0 lysophosphatidylcholine (C26:0-LPC).
- **Confirmatory test:** Very long chain fatty acids in serum and DNA analysis of *ABCD1* gene.
- **Validity:** To be determined
- **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.
- **Outcome in early diagnosis:** 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.

While X-ALD is a disorder primarily affecting males, female heterozygotes can also develop symptoms in adulthood and may be detected via newborn bloodspot screening.

### Clinical features

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:

- 1) **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.
- 2) **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.
- 3) **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 the majority of 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.

## Causes of X-ALD

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.

## Laboratory tests

The screening test measures C26:0-LPC. An elevated level on first valid specimen will trigger request for repeat or referral. Depending on results, further diagnostic and confirmatory testing may be required.

There are several issues to keep in mind regarding abnormal C26:0-LPC:

- 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.

## 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 may not be reliable in ruling out or confirming the disorder. Biochemical and molecular testing cannot predict clinical outcomes, so careful monitoring of adrenocortical function and brain imaging are required throughout life for males.

## Monitoring for adrenal insufficiency

Male infants/children with confirmed ALD also undergo testing for adrenal insufficiency. This is unlikely to be present in the neonatal period. Monitoring in childhood is performed by measuring fasting morning plasma ACTH and serum cortisol levels.

## Treatment

Individuals identified by newborn bloodspot screening are not expected to require treatment in infancy. However, baseline evaluations and regular monitoring will be conducted once the diagnosis is confirmed.

As of this publication, the primary available treatments for X-ALD are:

- 1) **Corticosteroid Therapy:** A large number of 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.
- 2) **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 on the basis of 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.

## Carrier detection

Screening may identify female X-ALD disease heterozygotes as discussed above, but not all female heterozygotes will be detected on newborn bloodspot screening.

## Screening practice considerations

C26:0-LPC is not valid in screens collected in infants before 24 hours of life.

**Table 21: X-ALD screening result summary for male newborns**

Results in MALE Newborn	Likely causes	Actions
Elevated C26:0-LPC	<ul style="list-style-type: none"><li>• X-ALD or other disorder of VLCFAs likely</li><li>• False positive</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations

**Table 22: X-ALD screening result summary for female newborns**

Results in FEMALE Newborn	Likely causes	Actions
Elevated C26:0-LPC	<ul style="list-style-type: none"><li>• Heterozygous X-ALD variant present</li><li>• Other disorder of VLCFAs</li><li>• False positive</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

# References

1. Pass KA, Lane PA, Fernhoff PM, Hinton CF, Panny SR, Parks JS, et al. US newborn screening system guidelines II: follow-up of children, diagnosis, management, and evaluation. Statement of the Council of Regional Networks for Genetic Services (CORN). *J Pediatr*. 2000 Oct;137(4 Suppl): S1-46.
2. American College of Medical Genetics Newborn Screening Expert Group. Newborn screening: toward a uniform screening panel and system--executive summary. *Pediatrics*. 2006 May;117(5 Pt 2): S296-307.
3. National Newborn Screening and Genetics Resource Center, Austin TX website: [genes-r-us.uthscsa.edu](http://genes-r-us.uthscsa.edu)
4. Arn PH. Newborn screening: current status. *Health Affairs*. 2007;26(2):559-566.
5. Tuerck JM, Buist NR, Skeels MR, Miyahira RS, Beach PG. Computerized surveillance of errors in newborn screening practice. *Am J Public Health*. 1987 Dec;77(12):1528-31.
6. CLSI. Newborn screening for preterm, low birth weight, and sick newborns; approved guidelines. CLSI NBS03-A. Wayne, PA: Clinical and Laboratory Standards Institute; October 2009.
7. Kosciuk RL, Farrell PM, Kosorok MR, Zaremba KM, Laxova A, Lai HC, et al. Cognitive function of children with cystic fibrosis: deleterious effect of early malnutrition. *Pediatrics*. 2004 Jun;113(6):1549-58.
8. Sontag MK, Hammond KB, Zielenski J, Wagener JS, Accurso FJ. Two-tiered immunoreactive trypsinogen-based newborn screening for cystic fibrosis in Colorado: screening efficacy and diagnostic outcomes. *J Pediatr*. 2005 Sep;147(3 Suppl): S83-8.
9. 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.
10. 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.
11. 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.

12. 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.
13. 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.
14. 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.
15. 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.
16. 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
17. 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
18. Selva KA, Harper A, Downs A, Blasco PA, Lafranchi SH. Neurodevelopmental outcomes in congenital hypothyroidism: comparison of initial T4 dose and time to reach target T4 and TSH. *J Pediatr*. 2005 Dec;147(6):775-80.
19. Van Vliet G. Neonatal hypothyroidism: treatment and outcome. *Thyroid*. 1999 Jan;9(1):79-84.
20. LaFranchi SH, Hanna CE, Krainz PL, Skeels MR, Miyahira RS, Sesser DE. Screening for congenital hypothyroidism with specimen collection at two time periods: results of the Northwest Regional Screening Program. *Pediatrics*. 1985 Nov;76(5):734-40.
21. 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.
22. Dick MC. Standards for the management of sickle cell disease in children. *Arch Dis Child Educ Pract Ed*. 2008 Dec;93(6):169-76.

23. 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.
24. 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.
25. Channon S, Goodman G, Zlotowitz S, Mockler C, Lee PJ. Effects of dietary management of phenylketonuria on long-term cognitive outcome. *Arch Dis Child.* 2007 Mar;92(3):213-8.
26. Cleary MA, Feillet F, White FJ, Vidailhet M, Macdonald A, Grimsley A, et al. Randomised controlled trial of essential fatty acid supplementation in phenylketonuria. *Eur J Clin Nutr.* 2006 Jul;60(7):915-20.
27. Fiege B, Blau N. Assessment of tetrahydrobiopterin (BH4) responsiveness in phenylketonuria. *J Pediatr.* 2007 Jun;150(6):627-30.
28. Matalon R, Michals-Matalon K, Bhatia G, Grechanina E, Novikov P, McDonald JD, et al. Large neutral amino acids in the treatment of phenylketonuria (PKU). *J Inherit Metab Dis.* 2006 Dec;29(6):732-8.
29. Scott CR. The genetic tyrosinemias. *Am J Med Genet C Semin Med Genet.* 2006 May 15;142(2):121-6.
30. Koelink CJ, van Hasselt P, van der Ploeg A, van den Heuvel-Eibrink MM, Wijburg FA, Bijleveld CM, et al. Tyrosinemia type I treated by NTBC: how does AFP predict liver cancer? *Mol Genet Metab.* 2006 Dec;89(4):310-5.
31. Tsai CP, Lin PY, Lee NC, Niu DM, Lee SM, Hsu WM. Corneal lesion as the initial manifestation of tyrosinemia type II. *J Chin Med Assoc.* 2006 Jun;69(6):286-8.
32. Magera MJ, Gunawardena ND, Hahn SH, Tortorelli S, Mitchell GA, Goodman SI, et al. Quantitative determination of succinylacetone in dried blood spots for newborn screening of tyrosinemia type I. *Mol Genet Metab.* 2006 May;88(1):16-21.
33. Shekhawat PS, Matern D, Strauss AW. Fetal fatty acid oxidation disorders, their effect on maternal health and neonatal outcome: impact of expanded newborn screening on their diagnosis and management. *Pediatr Res.* 2005 May;57(5 Pt 2):78R-86R.
34. Wilcken B, Haas M, Joy P, Wiley V, Chaplin M, Black C, et al. Outcome of neonatal screening for medium-chain acyl-CoA dehydrogenase deficiency in Australia: a cohort study. *Lancet.* 2007 Jan 6;369(9555):37-42.

35. Wilcox RL, Nelson CC, Stenzel P, Steiner RD. Postmortem screening for fatty acid oxidation disorders by analysis of Guthrie cards with tandem mass spectrometry in sudden unexpected death in infancy. *J Pediatr*. 2002 Dec;141(6):833-6.
36. Van Wassenhaer AG, Kok JH, Dekker FW, de Vijlder JJ. Thyroid function in very preterm infants: influences of gestational age and disease. *Pediatr Res*. 1997 Nov;42(5):604-9
37. 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.
38. 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.
39. Deodato F, Boenzi S, Santorelli FM, Dionisi-Vici C. Methylmalonic and propionic aciduria. *Am J Med Genet C Semin Med Genet*. 2006 May 15;142(2):104-12.
40. Andersson HC, Marble M, Shapira E. Long-term outcome in treated combined methylmalonic acidemia and homocystinemia. *Genet Med*. 1999 May-Jun;1(4):146-50.
41. Hedlund GL, Longo N, Pasquali M. Glutaric acidemia type 1. *Am J Med Genet C Semin Med Genet*. 2006 May 15;142(2):86-94.
42. 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.
43. 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.
44. 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.
45. 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.
46. Gropman AL, Batshaw ML. Cognitive outcome in urea cycle disorders. *Mol Genet Metab*. 2004;81 (Suppl 1): S58-62. 47.
47. Ohura T, Kobayashi K, Tazawa Y, Abukawa D, Sakamoto O, Tsuchiya S, et al. Clinical pictures of 75 patients with neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD). *J Inherit Metab Dis*. 2007 ;30(2):139-44.

48. Bosch AM. Classical galactosaemia revisited. *J Inherit Metab Dis*. 2006;29(4):516- 25.
49. Wolf B. Biotinidase Deficiency: New Directions and Practical Concerns. *Curr Treat Options Neurol*. 2003;5(4):321-328.
50. American Academy of Pediatrics. Newborn Screening Expands: Recommendations for Pediatricians and Medical Homes – Implications for the System. *Pediatrics*. 2008;121: 192-217.
51. Dick MC. Standards for the management of sickle cell disease in children. *Arch Dis Child Educ Pract Ed*. 2008 Dec;93(6):169-76.
52. Hopkins PV, Klug T, Vermette L, Raburn-Miller J, Kiesling J, Rogers S. Incidence of 4 Lysosomal Storage Disorders From 4 Years of Newborn Screening. *JAMA Pediatr*. 2018;172(7): 696-697.
53. Newborn screening act sheet Fabry disease: Decreased alpha-galactosidase a [Internet]. Mayo Clinic Laboratories. 2020 [cited 2022 Apr 19]. Available from: [https://www.mayocliniclabs.com/~media/it-mmfiles/special-instructions/Newborn\\_Screening\\_Act\\_Sheet\\_Fabry\\_Disease-Decreased\\_Alpha-Galactosidase\\_A.pdf](https://www.mayocliniclabs.com/~media/it-mmfiles/special-instructions/Newborn_Screening_Act_Sheet_Fabry_Disease-Decreased_Alpha-Galactosidase_A.pdf)
54. Mehta A, Hughes DA. Fabry Disease. In: GeneReviews® [Internet]. University of Washington, Seattle; 2002 Aug 5 [Updated 2022 Jan 27] [cited 2022 Apr 19]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1292>
55. Bodamer OA, Scott CR, Giugliani R, on behalf of the Pompe Disease Newborn Screening Working Group. Newborn Screening for Pompe Disease. *Pediatrics* [Internet]. 2017 July 1 [cited 2022 Apr 20];140(Supplement\_1):S4–13. Available from: [https://publications.aap.org/pediatrics/article/140/Supplement\\_1/S4/179258/Newborn-Screening-for-Pompe-Disease](https://publications.aap.org/pediatrics/article/140/Supplement_1/S4/179258/Newborn-Screening-for-Pompe-Disease)
56. Dasouki M, Jawdat O, Almadhoun O, Pasnoor M, McVey AL, Abuzinadah A, et al. Pompe disease: literature review and case series. *Neurol Clinics* [Internet]. 2014 Aug. [cited 2022 Apr 20];32(3):751–76, ix. Available from: <http://dx.doi.org/10.1016/j.ncl.2014.04.010>
57. Leslie N, Bailey L. Pompe Disease. In: GeneReviews® [Internet]. University of Washington, Seattle; 2007 Aug 31 [Updated 2017 May 11] [cited 2022 Apr 20]. Available from: <https://www.ncbi.nlm.nih.gov/sites/books/NBK1261/>
58. Clarke LA. Mucopolysaccharidosis Type I. In: GeneReviews® [Internet]. University of Washington, Seattle; 2002 Oct 31 [Updated 2021 Feb 25] [cited 2022 Apr 20]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1162/>

59. Duke University for the Maternal and Child Health Bureau of the Health and Resources and Services Administration. Newborn Screening for Mucopolysaccharidosis Type I [Internet]. 2018 Jun 27. Available from: <https://www.hrsa.gov/sites/default/files/hrsa/advisory-committees/heritable-disorders/rusp/previous-nominations/mps-i-27-june-2018.pdf>
60. Pastores GM, Hughes DA. Gaucher Disease. In: GeneReviews® [Internet]. University of Washington, Seattle; 2000 Jul 27 [Updated 2018 Jun 21] [cited 2022 Apr 20]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1269>
61. OMIM® - Online Mendelian Inheritance in Man® entry - # 230800 - GAUCHER DISEASE, TYPE I; GD1 [Internet]. Omim.org. [cited 2022 Apr 20]. Available from: <https://www.omim.org/entry/230800>
62. Prior TW, Leach ME, Finanger E. Spinal Muscular Atrophy. In: GeneReviews® [Internet]. University of Washington, Seattle; 2020 Feb 24 [Updated 2020 Dec 3] [cited 2022 Apr 21]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1352/>
63. Mercuri E, Finkel RS, Muntoni F, Wirth B, Montes J, Main M, et al. Diagnosis and management of spinal muscular atrophy: part 1: recommendations for diagnosis, rehabilitation, orthopedic and nutritional care. *Neuromuscular Disorders*. [Internet]. 2018 Feb 1 [cited 2022 Apr 21];28(2):103–15. Available from: <http://dx.doi.org/10.1016/j.nmd.2017.11.005>
64. Chng SY, Wong YQ, Hui JH, Wong HK, Ong HT, Goh DY. Pulmonary function and scoliosis in children with spinal muscular atrophy types II and III. *Journal of Pediatrics and Child Health* [Internet]. 2003 Dec. [cited 2022 Apr 21];39(9):673–6. Available from: <http://dx.doi.org/10.1046/j.1440-1754.2003.00266.x>
65. Kolb SJ, Coffey CS, Yankey JW, Krossschell K, Arnold WD, Rutkove SB, et al. Natural history of infantile-onset spinal muscular atrophy. *Annals of Neurology* [Internet]. 2017 Dec. [cited 2022 Apr 21];82(6):883–91. Available from: <http://dx.doi.org/10.1002/ana.25101>
66. Wadman RI, Stam M, Gijzen M, Lemmink HH, Snoeck IN, Wijngaarde CA, et al. Association of motor milestones, SMN2 copy and outcome in spinal muscular atrophy types 0-4. *Journal of Neurology, Neurosurgery & Psychiatry* [Internet]. 2017 Apr 1;88(4):365–7. Available from: <http://dx.doi.org/10.1136/jnnp-2016-314292>
67. Montes J, McDermott MP, Mirek E, Mazzone ES, Main M, Glanzman AM, et al. Ambulatory function in spinal muscular atrophy: Age-related patterns of progression. *PLOS ONE* [Internet]. 2018 Jun 26 [cited 2022 Apr 21];13(6):e0199657. Available from: <http://dx.doi.org/10.1371/journal.pone.0199657>

68. Finkel RS, Mercuri E, Meyer OH, Simonds AK, Schroth MK, Graham RJ, et al. Diagnosis and management of spinal muscular atrophy: Part 2: Pulmonary and acute care; medications, supplements and immunizations; other organ systems; and ethics. *Neuromuscular Disorders* [Internet]. 2018 Mar 1 [cited 2022 Apr 21];28(3):197–207. Available from: <http://dx.doi.org/10.1016/j.nmd.2017.11.004>
69. Calucho M, Bernal S, Alías L, March F, Venceslá A, Rodríguez-Álvarez FJ, et al. Correlation between SMA type and SMN2 copy number revisited: An analysis of 625 unrelated Spanish patients and a compilation of 2834 reported cases. *Neuromuscular Disorders* [Internet]. 2018 Mar 1 [cited 2022 Apr 21];28(3):208–15. Available from: <http://dx.doi.org/10.1016/j.nmd.2018.01.003>
70. Prior TW, Swoboda KJ, Scott HD, Hejmanowski AQ. Homozygous SMN1 deletions in unaffected family members and modification of the phenotype by SMN2. *American Journal of Medical Genetics, Part A* [Internet]. 2004 Aug 24 [cited 2022 Apr 21];130A(3):307–10. Available from: <http://dx.doi.org/10.1002/ajmg.a.30251>
71. Chien YH, Chiang SC, Weng WC, Lee NC, Lin CJ, Hsieh WS, et al. Presymptomatic diagnosis of spinal muscular atrophy through newborn bloodspot screening. *The Journal of Pediatrics*. 2017;190:124–9.
72. 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>
73. Lee S, Clinard K, Young SP, Rehder CW, Fan Z, Calikoglu AS, et al. Evaluation of X-linked adrenoleukodystrophy newborn screening in North Carolina. *JAMA Network Open* [Internet]. 2020 Jan 31 [cited 2022 Apr 21];3(1):e1920356. Available from: <http://dx.doi.org/10.1001/jamanetworkopen.2019.20356>
74. Raymond GV, Moser AB, Fatemi A. X-Linked Adrenoleukodystrophy. In: *GeneReviews®* [Internet]. University of Washington, Seattle; 1999 Mar 26 [Updated 2018 Feb 15] [cited 2022 Apr 21]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1315/>
75. Wiens K, Berry SA, Choi H, Gaviglio A, Gupta A, Hietala A, et al. A report on state-wide implementation of newborn screening for X-linked Adrenoleukodystrophy. *American Journal of Medical Genetics, Part A* [Internet]. 2019 May 10 [cited 2022 Apr 21];179(7):1205–13. Available from: <http://dx.doi.org/10.1002/ajmg.a.61171>

# Northwest Regional Newborn Bloodspot Screening Program

*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  
Phone: 503-693-4174  
Fax: 503-693-5601



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

OHA 8189 (Rev. 06/2022)

AMEND: 333-024-1040

NOTICE FILED DATE: 03/30/2022

RULE SUMMARY: Amend OAR 333-024-1040 – Revise Oregon Newborn Bloodspot Screening Practitioner’s Manual to the 12th Edition, publishing in 2022. Key changes within include but are not limited to the following:

- Addition of section describing the Northwest Regional Newborn Bloodspot Screening Advisory Board
- Updates to “information about newborn bloodspot screening medical conditions” section to:
  - o Align descriptions with current clinical understanding and practice
  - o Add sections for SMA and X-ALD
- Various edits for clarity and consistency.

CHANGES TO RULE:

### 333-024-1040

#### Newborn Screening: Manner of Submitting Specimens

A person responsible for submitting specimens to the Oregon State Public Health Laboratory under OAR 333-024-1020 and OAR 333-024-1025 must: ¶

(1) Collect the specimens:¶

(a) Using kits available from the Oregon State Public Health Laboratory; and¶

(b) According to instructions provided by the Oregon State Public Health Laboratory, which can be viewed in the Oregon Newborn Bloodspot Screening Practitioner's Manual (Practitioner's Manual), ~~11~~12th Edition; ~~2019~~2022 found at [www.healthoregon.org/nbs](http://www.healthoregon.org/nbs).¶

(2) Provide the Oregon State Public Health Laboratory with information that identifies the individual or individuals who are responsible for the medical care and treatment of the infant and for responding to testing results generated by newborn screening.¶

(3) Send specimens for newborn screening to the Oregon State Public Health Laboratory within 24 hours of collection and drying in accordance with the shipping instructions provided by the Oregon State Public Health Laboratory, which can be viewed in the Oregon Newborn Bloodspot Screening Practitioner's Manual (Practitioner's Manual), ~~11~~12th Edition; ~~2019~~2022 found at [www.healthoregon.org/nbs](http://www.healthoregon.org/nbs).¶

(4) Ensure that specimens for newborn screening are sent via courier, express mail, or other timely delivery mechanism.

Statutory/Other Authority: ORS 413.014, 433.285, 431A.750

Statutes/Other Implemented: ORS 433.285, 433.290, 433.295

RULE ATTACHMENTS DO NOT SHOW CHANGES. PLEASE CONTACT AGENCY REGARDING CHANGES.

# Oregon Newborn Bloodspot Screening Practitioner's Manual



Northwest Regional Newborn Bloodspot Screening Program

# Newborn Bloodspot Screening Practitioner's Manual

## **Oregon Health Authority**

### **Public Health Division**

John L. Fontana, PhD, (HCLD) ABB

Patrice Held, PhD, FACMG

Sheri Hearn, MPH

Sarah Humphrey King, CHES

Sara Etienne, BS

Kristi Murphy, MS GC

## **Oregon Health & Science University**

Kara Connelly, MD

Erika Finanger, MD

Cary Harding, MD

Shyam Joshi, MD

Kim Kripps MD

Stephen L. LaFranchi, MD

Mike Powers, MD

Sarah Viall, PPCNP-BC

Leah Wessenberg, FNP

Trisha Wong, MD

Melinda Wu, MD

Amy Yang, MD

Tyler Yates, MD

12th Edition, 2022

# Table of Contents

» Table of Contents .....	3	» Tips to avoid rejected specimens .....	34
» Introduction .....	5	» Information about newborn bloodspot screening medical conditions .....	36
» Advisory Board .....	7	» Cystic Fibrosis (CF) .....	36
» Definitions .....	8	» Congenital Adrenal Hyperplasia (CAH) .....	39
» Newborn bloodspot screening responsibilities in Oregon .....	9	» Primary Congenital Hypothyroidism (CH) .....	41
» Education services .....	10	» Sickle Cell Disease and other Hemoglobinopathies .....	44
» Fee exemption for Oregon births .....	10	» Amino Acid Conditions .....	46
» Parent refusal to have the infant screened in Oregon .....	10	» Fatty Acid Oxidation (FAO) Conditions .....	53
» Medical conditions on the newborn bloodspot screening panel .....	11	» Organic Acid Conditions (OA) .....	55
» Newborn bloodspot screening kits .....	17	» Urea Cycle Conditions (UCD) .....	58
» Timing for specimen collection .....	18	» Galactosemia .....	62
» Patient demographic information .....	21	» Biotinidase Deficiency .....	64
» Heel-stick specimen collection instructions .....	23	» Severe Combined Immunodeficiency (SCID) .....	65
» Specimen transport .....	26	» Lysosomal Storage Disorders (LSDs) .....	67
» Reporting of results .....	28	» Fabry Disease .....	67
» Situations that may impact newborn bloodspot screening results .....	30	» Pompe Disease .....	71
» Requesting newborn bloodspot screening records .....	32	» Mucopolysaccharidosis Type I (MPS I) .....	74
» Use, release, and retention of residual bloodspot specimens .....	33	» Gaucher Disease .....	76
		» Spinal Muscular Atrophy (SMA) .....	79
		» X-Linked Adrenoleukodystrophy (X-ALD) .....	82
		» References .....	86



### **Acknowledgment**

We are indebted to the newborn bloodspot screening state coordinators, medical consultants, and practitioners for their assistance and advice.

### **Recommended citation**

NW Regional Newborn Bloodspot Screening Program.  
Newborn Bloodspot Screening Practitioner's Manual, 12th Edition; 2022.



# 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 OSPHL and abnormal results are referred to the NBS Follow-up Team.

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

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

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

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 as of 2018, some lysosomal storage disorders. The OSPHL 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.

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

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

**Newborn 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.**



# 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 for the review and creation of legislative reports based on members' respective areas of expertise. The Board assists NWRNBS with strategic planning and the development of policies, priorities and services related to newborn bloodspot screening. The Board's role also includes reviewing conditions to be recommended for the addition or removal from the test panel of diseases. In all activities, The Board considers the newborn screening system as a whole, to improve health outcomes for all infants and their families. The Board is comprised of 13 partners within the newborn bloodspot screening community. The members include representatives of hospitals, birth centers, families, insurance, midwifery, nursing, pediatrics and other perspectives.

If you are interested in participating with the NWRNBS Advisory Board, please send an email to [nbs.advisoryboard@dhsosha.state.or.us](mailto:nbs.advisoryboard@dhsosha.state.or.us).



# 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 filter paper collection device, attached demographic form and other items provided by the Oregon State Public Health Laboratory for the the purposes of collection and submission of specimens for newborn bloodspot screening.

**“Practitioner”** means: the person who takes responsibility for the delivery or health care of an infant born in Oregon and is one of the following:

- a) A physician licensed under ORS 677;
- b) A naturopathic physician licensed under ORS 685;
- c) Advanced practice registered nurse licensed under ORS 678;
- d) A chiropractic physician licensed under ORS chapter 684; or
- e) A direct entry midwife licensed under ORS 687.

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

**“Specimen”** means: a blood specimen obtained from an infant by means of capillary puncture or skin puncture (heel stick) that has spotted onto the newborn bloodspot screening kit and allowed to air dry.



# Newborn bloodspot screening responsibilities in Oregon

**This section describes the responsibilities for successful newborn bloodspot screening in Oregon. Practitioner's 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.
- **Oregon State Public Health Laboratory (OSPHL) and NBS Follow-up Team:** The laboratory is responsible for testing, record keeping, ensuring quality of laboratory methods, notifying providers of results, tracking abnormal and unresolved results, and providing educational materials.
- **Oregon Health & Science University (OHSU) subspecialty programs:** These partners are responsible for providing consultation services to practitioners and the OSPHL.

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

**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 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 Education Coordinator. In addition, education resources are made available to practitioners and parents at [www.bitly.com/nbs-resource](http://www.bitly.com/nbs-resource).

## Fee exemption for Oregon births

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

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

## Parent refusal to have the infant screened in Oregon

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

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

**NBS Follow-up Team**

**Fax: 503-693-5601**



# Medical conditions on the newborn bloodspot screening panel

Oregon newborns are screened for the following medical conditions recommended by the Advisory Committee on Heritable Disorders in Newborns and Children and the Northwest Regional Newborn Bloodspot Screening (NWRNBS) Program Advisory Board. More information on these medical conditions is available at the end of this manual and at:

- Baby's First Test: <http://babysfirsttest.org/>
- The Oregon State Public Health Laboratory: [www.healthoregon.org/nbs](http://www.healthoregon.org/nbs)
- The American College of Medical Genetics (ACMG): [ACT Sheets and Algorithms](#).
- Western States Regional Genetics Network: [www.newbornscreening.info](http://www.newbornscreening.info)

**Newborn bloodspot screening is not diagnostic. Both false negative and false positive results may occur. Confirmatory testing is required for diagnosis.**

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

Medical Condition	Analyte(s) tested for	Incidence in NW region	Symptoms <i>if not treated</i>	Common Medical Treatment
<b>Organic Acid Disorders</b>				
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	1 per 148,000	Vomiting; lethargy; acidosis possibly resulting in coma, death	Protein-restricted diet; carnitine and glycine therapy

Medical Condition	Analyte(s) tested for	Incidence in NW region	Symptoms <i>if not treated</i>	Common Medical Treatment
3-methylcrotonyl CoA carboxylase deficiency (3MCC)	C5OH	1 per 51,000	Most have been asymptomatic	None, except carnitine therapy if deficient
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
Multiple carboxylase deficiency (MCD)	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	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	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	Rare, less than 1 per 300,000	None to severe cardiomyopathy	Carnitine therapy; protein restriction; avoid fasting
2-methylbutyryl CoA dehydrogenase deficiency (2MBC)	C5	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

Medical Condition	Analyte(s) tested for	Incidence in NW region	Symptoms <i>if not treated</i>	Common Medical Treatment
<b>Fatty Acid Oxidation Disorders</b>				
Carnitine uptake deficiency (CUD)	C0	1 per 116,000	Hypoglycemia; cardiomyopathy	Carnitine therapy
Medium chain acyl-CoA dehydrogenase deficiency (MCAD)*	C6, C8, C10, 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, 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)*	C14:1, C16, C16OH, C18, C18OH	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)	C14:1, C16, C16OH, C18, C18OH	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	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, C14, C16, C18:1	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

Medical Condition	Analyte(s) tested for	Incidence in NW region	Symptoms <i>if not treated</i>	Common Medical Treatment
Carnitine palmitoyl transferase deficiency, type II (CPT-II)*	C16, C18, C18:1	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
Carnitine acylcarnitine translocase deficiency (CACT)	C16, C18, C18:1	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
<b>Amino Acid Disorders</b>				
Argininosuccinate lyase deficiency (Arginosuccinic aciduria; 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	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	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

Medical Condition	Analyte(s) tested for	Incidence in NW region	Symptoms <i>if not treated</i>	Common Medical Treatment
<b>Endocrine Disorders</b>				
Primary congenital hypothyroidism	Thyroid hormone T4 and Second tier TSH	1 per 2,300	Intellectual and developmental disability; other brain damage; growth delay	Thyroid hormone
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
<b>Pulmonary Disorders</b>				
Cystic fibrosis (CF)	Immunoreactive Trypsinogen (IRT)	1 per 6,500	Lung disease; growth failure	Pulmonary therapy; prevent infection; replace digestive enzymes
<b>Other Metabolic Disorders</b>				
Biotinidase deficiency	Biotinidase	1 per 1.05 million	Intellectual and developmental disability; seizures; skin rash; alopecia; hearing loss; death	Biotin therapy
Classic galactosemia (GALT)*	Galactosemia enzyme (GALT)	1 per 95,000	Neurodevelopmental impairment; liver disease; cataracts; Gram-negative sepsis in newborns	Galactose-restricted diet
<b>Hemoglobin Disorders</b>				
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
<b>Immunology Disorders</b>				
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

Medical Condition	Analyte(s) tested for	Incidence in NW region	Symptoms <i>if not treated</i>	Common Medical Treatment
<b>Lysosomal Storage Disorders</b>				
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
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
<b>Other Conditions</b>				
Spinal muscular atrophy (SMA) <sup>2†</sup>	Exon 7 of the <i>SMN1</i> 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) <sup>‡</sup>	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.

<sup>†</sup> Screening for this condition is anticipated to begin June 1, 2022.

<sup>‡</sup> Screening for this condition is anticipated to begin on or before January 1, 2023.

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](http://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 here: [www.nmhealth.org/about/phd/fhb/cms/nbgs/](http://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.

**Newborn bloodspot screening kits are pre-coded for the facility or practitioner that ordered the kit and should not be loaned to, or borrowed from, other facilities.**

**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 2nd specimen to be matched easily by the screening lab to the data from the 1st specimen. This matching system helps to link the data from newborn 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 in neonatal intensive care units (NICU). Each specimen in the kit has a barcode and a kit number that allow the 2nd specimen and 3rd specimen to be matched easily by the screening lab to the data from the 1st specimen. This matching system helps to ensure that newborn 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 1st specimen should be written on the single kit to help with matching the data for the infant. These kits will also be used when the OSPHL requests a repeat specimen.



# 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 time lines established below, collect and submit the bloodspot as soon as possible up to six months of age.

## 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 age but before 48 hours of age and a second specimen must be collected between 10 and 14 days of age as shown in [Table 2](#).

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

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

## Infants admitted to the NICU

For babies that require admission to a neonatal intensive care unit, collect the first specimen **as soon as possible after 24 hours of age but before 36 hours of age**. If the infant is being transfused, collect the specimen prior to transfusion regardless of the age of the infant. If an infant is transfused prior to 24 hours of age the second specimen must be collected at 48-72 hours of age. If the infant is **not** transfused prior to 24 hours of age the second specimen must be collected between 10 and 14 days of age. A third specimen must be collected at approximately 1 month, but no sooner than 28 days after birth.

For infants that are discharged or transferred after the first specimen (or second specimen) is collected, the remaining collection cards from the triple kit must be routed to the provider who will collect these specimens.

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

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

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

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

## Early discharge

If a family is requesting an early discharge, collect the 1st specimen before they leave your care. Some infants may not return for routine postnatal care. Please be certain to check the 'early discharge' box on the demographic portion of the card, to alert the lab that this is the reason for the early collection.

## Baby expires

In many cases, blood spot specimens from an infant who expired are a valuable resource for the family.

We recommend that you collect a newborn bloodspot screening specimen either at the typical screening interval, or sooner if needed and in consideration for the wishes of the baby's guardians.

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

- **Calling 503-693-4174 or**
- **Faxing the infant's information to 503-693-5601**

# Older infants

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

Table 2: Age of infant at specimen collection

	Collection Kit	First specimen	Second specimen	Third specimen
Routine Birth	Double Kit	As soon as possible after 24 hours of age but before 48 hours of life	10–14 days of age	Not Collected
NICU infants transfused prior to 24 hours of age	Triple Kit	Prior to transfusion	48–72 hours after birth	~ 1 month, no sooner than 28 days
NICU infants <u>not</u> transfused prior to 24 hours of age	Triple Kit	As soon as possible after 24 hours of age but before 36 hours of age and prior to transfusion	10–14 days of age	~ 1 month, no sooner than 28 days





# Patient demographic information

## **Incomplete demographic information may result in your specimen not being tested.**

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

Accurate and complete patient, provider, and specimen collection information must be provided on every collection card to allow for rapid follow-up if results are abnormal. This information is required by Clinical Laboratory Improvement Amendments of 1988 (CLIA) and must be legible.

The person performing the collection must:

1. Verify that the collection kit will not expire before all parts of the kit can be tested by the laboratory. If a double kit will expire within 1 month of the collection, please use a different kit. The expiration date is on the spine and the back of the kit as well as on the top of the filter paper portion.
2. Identify the infant and match with the correct screening kit. Make sure to select the correct kit part (1st, 2nd or 3rd) depending on the specimen being collected.

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

**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.**

3. **ALL** demographic fields must be filled in before collecting the specimen (see figure 2).
  - a. If the birth mother will not be maintaining custody of the infant, provide the name, address and phone number for the infant's guardian in the "Mother" fields. This information may be used to locate the infant for follow-up.
  - b. Labels may be used to provide demographic information. They must be included on all layers of the screening kit. They must not cover demographic information fields that will be hand-written.


**Provide the name and contact information for the provider who is responsible for the infant's medical care and treatment *after discharge* in the "Send report to PCP / Clinic" field. This practitioner will be sent screening results and follow-up information. Do not use the resident, attending, or on-call provider!**

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

RETURN TO: OREGON STATE PUBLIC HEALTH LAB  
 PO BOX 275, PORTLAND, OR 97207-0275 (503)693-4174

7070717  
 W161  
 LOT

**1st** Newborn Screening  
**SPECIMEN OR**

  
 SN \* 1 1 7 5 0 6 8 4 0 5 \*

DO NOT WRITE  
 IN THIS SPACE

Baby's Last Name		Baby's First Name		Send Report to PCP/Clinic:		CODE	
( ) Single Birth, or ( ) Multi-Birth A B C D E F Circle One				Address/Telephone Number			
Sex: M F		ID Chart #		Birth Date ( / / )		Time of Day (circle one) : am pm	
Food Source Last 24 Hours:		<input checked="" type="checkbox"/> Breast, <input checked="" type="checkbox"/> Soy Formula, <input checked="" type="checkbox"/> NPO <input checked="" type="checkbox"/> Lactose Formula, <input checked="" type="checkbox"/> Other		Specimen Date ( / / )		Time of Day (circle one) : am pm	
Other Factors:		Transfusion		Baby's Race:		Birth Wt. Lbs. oz	
<input type="checkbox"/> Early discharge <input type="checkbox"/> TPN		Last RBC Transfusion Date / / OR <input type="checkbox"/> None		<input checked="" type="checkbox"/> White, <input checked="" type="checkbox"/> Black, <input checked="" type="checkbox"/> Amer. Ind./Native <input checked="" type="checkbox"/> Unknown/Other, <input checked="" type="checkbox"/> Asian / Pacific Islander		Present Wt. Lbs. oz gms	
Hosp. or Hosp. CODE/Submitter ID and Address				Mother's Last Name		First Name	
				Mother's Address-Number & Street		Mother's Birth Date	
				City		State Zip Code	
Specimen taken by:				Telephone Number:			
				Specimen reference #			



# Heel-stick specimen collection instructions\*

Each facility or medical provider must establish a procedure for staff performing newborn bloodspot screening specimen collections. Resources are available from the Clinical Laboratory Standards Institute that can help with creating or updating your procedure.

**Contact the OSPHL  
at 503-693-4174  
for assistance with  
specimen collection.**

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

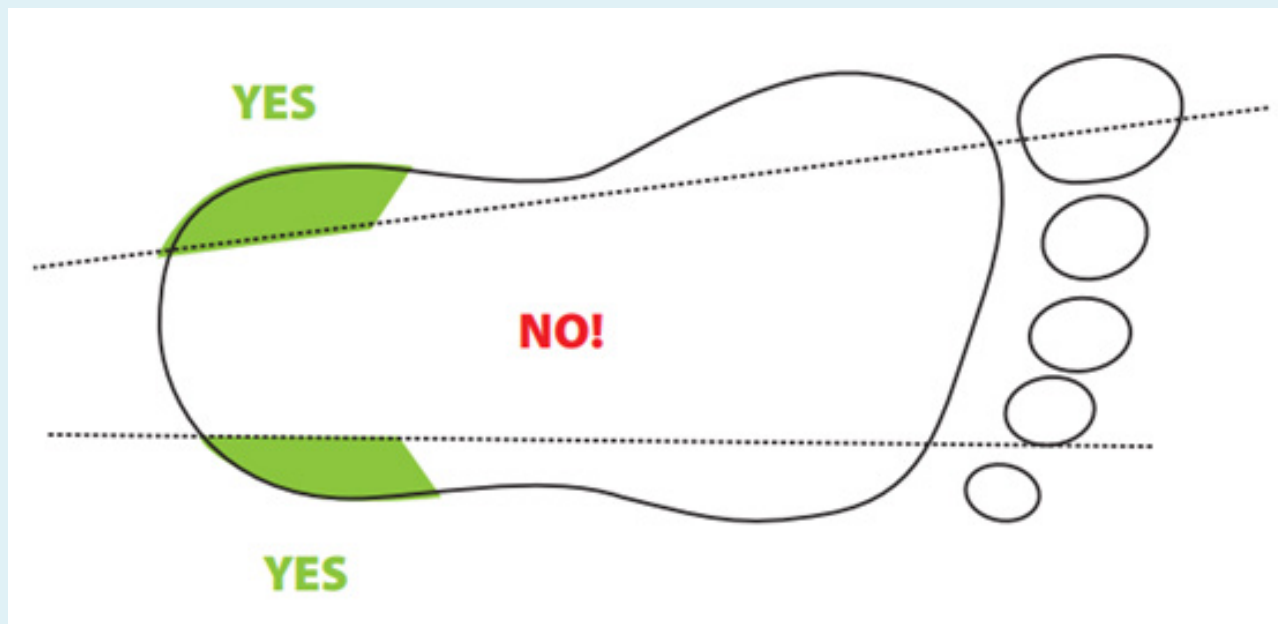
A training detailing proper collection and helpful resources can be found at [www.bitly.com/nbs-resource](http://www.bitly.com/nbs-resource). Tips to avoid rejected specimens are provided later in this manual.

1. Use a scalpel bladed lancet manufactured specifically for heel stick collection from an infant. Do not use a lancet longer than 2.0 mm. Do not use capillary tubes or other collection devices.
2. Select a lance site on the infant's heel (see Figure 3). Cleanse lance site with alcohol and air dry. Do not use betadine, iodine, lotion, or essential oils on the baby prior to collecting the specimen.
3. Perform lancing on the most medial or most lateral portion of the plantar surface of the heel.

---

\* These recommendations conform to CLSI publication NBS01-A6.

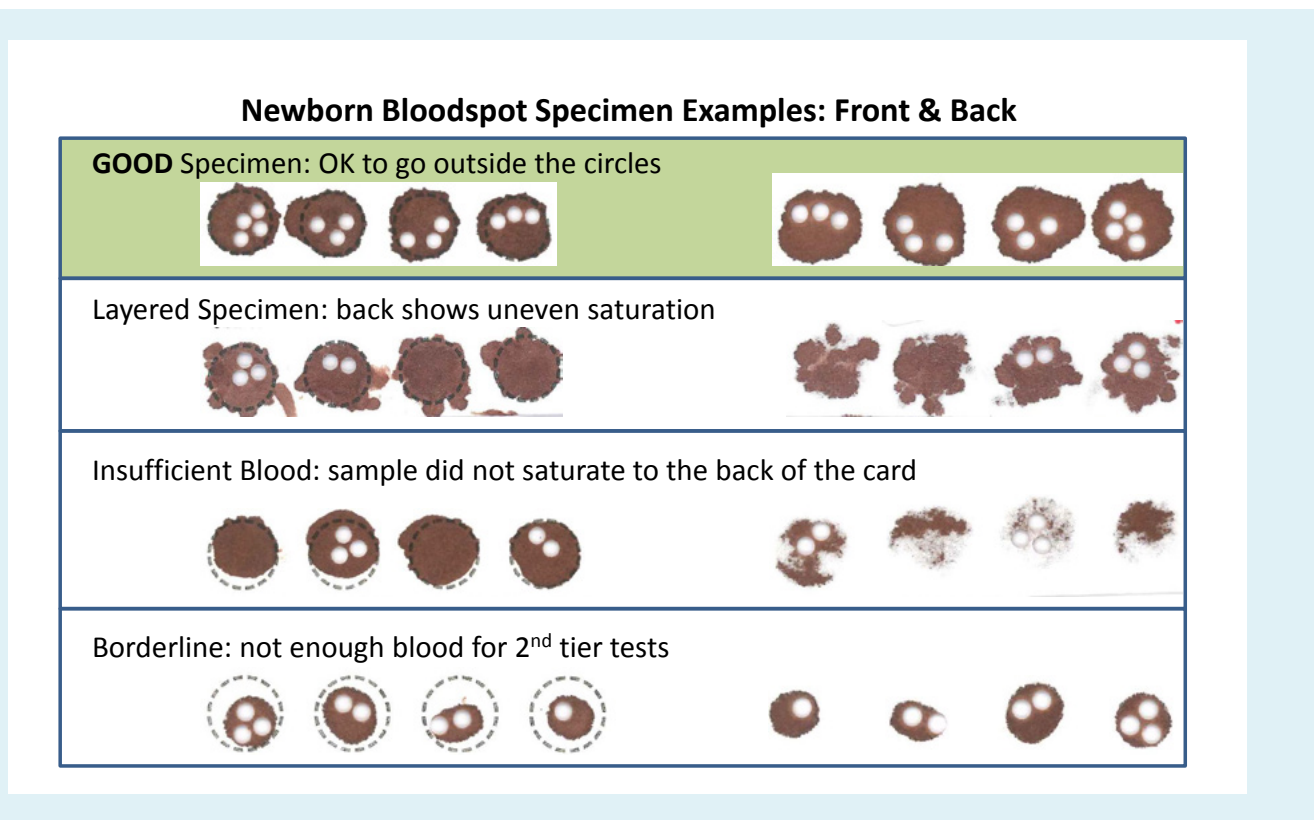
Figure 3: Recommendation for heel puncture site in newborns



4. Lance the heel with the sterile scalpel bladed lancet. Wipe away the first drop of blood to remove tissue fluids. Do not “milk” or squeeze the heel.
5. Allow a single drop of blood to collect on the heel that is large enough to fill a collection circle. Do not layer multiple spots of blood on top of one another.
6. Touch the filter paper gently to the drop of blood. Only apply blood to one side of the filter paper (it doesn’t matter which side is used).
7. Allow the blood to soak through the filter paper so that the blood spot looks similar on both the front and back of the collection kit.



Figure 4: Newborn bloodspot specimen examples



Complete, even saturation of the filter paper is essential for accurate testing. The filter paper is calibrated to absorb a specific quantity of blood. Incomplete, uneven saturation or layering of the blood alters the quantity of blood used for testing and will lead to inaccurate test results. This figure is also available at: [www.bitly.com/nbs-example](http://www.bitly.com/nbs-example).

8. Collect the blood in all five circles, repeating instructions 5 through 7. If blood flow is not sufficient, re-lance the heel. It is better to fill three circles completely than to fill four circles inadequately.
9. Air dry specimens at room temperature for between 3 and 4 hours in a **horizontal** position with the blood spots exposed. Hanging wet specimens vertically will cause heavier red cells to migrate to the dependent end of the circle resulting in uneven saturation.
10. Do not expose the specimen to excess heat or humidity at any time. Do not dry on a heater, in a microwave, with a hair dryer or in sunlight. Do not place in plastic bags, leave in a hot mailbox or in a hot car. These practices can destroy some proteins and enzymes that are required for accurate test results.
11. Ensure that the specimen is completely dry before transporting.

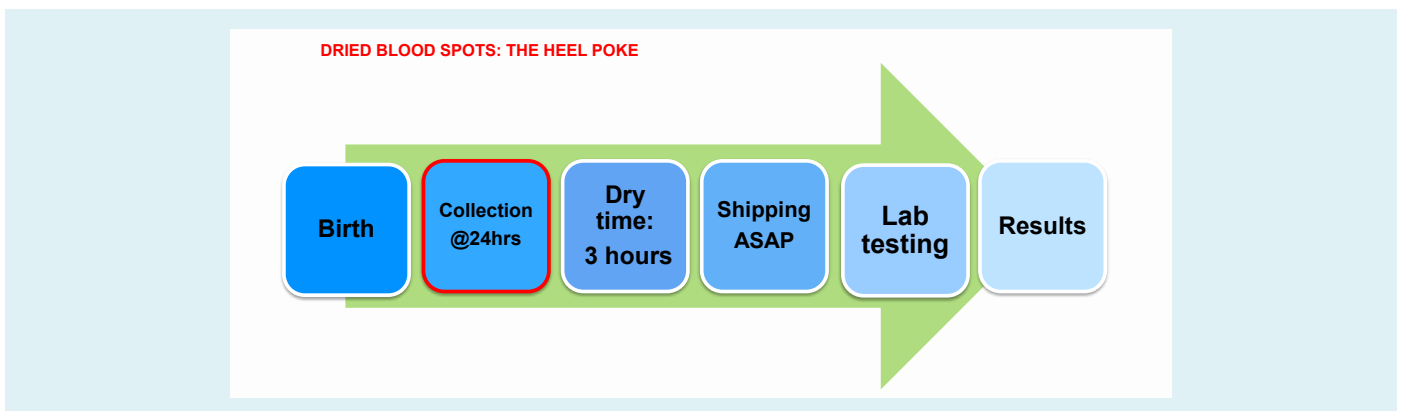


# Specimen transport

**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.**

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

**Figure 5: Newborn bloodspot screening process**



Specimens should be sent as soon as they are dried (between 3 and 4 hours) and no later than 24 hours after collection.

1. Keep a record of the specimens that are sent, including the kit numbers. A packing list or manifest should be included with the shipment.
2. Insert the dried specimen(s) into an envelope. Do not put specimens in plastic bags or containers. Do not compress the specimens.
3. Send the specimens no later than 24 hours after collection.
4. All specimens must be sent by express mail, courier or another timely delivery mechanism. Specimens should be received by the OSPHL within 48 hours of collection.

5. Send the specimens to:

Oregon State Public Health Laboratory  
Newborn Bloodspot Screening Program  
7202 NE Evergreen Parkway, Suite 100  
Hillsboro, OR 97124

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

Prompt transit is essential for identifying infants who may be impacted by a screening condition within one week of birth. 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 promptly. Delays within a facility may be from inefficient internal processes, slow courier services, simple forgetfulness, or, most dangerously, batching specimens.

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





# Reporting of results

## Results are available online

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

## Results reporting

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

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

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.

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

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

Calling 503-693-4174 or

Faxing the infant’s information to 503-693-5601



## I did not receive my newborn bloodspot screening results!

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

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

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



# 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. These include:

### Primary Congenital Hypothyroidism (CH)

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

### Lysosomal Storage Disorders (LSD)

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

## Parenteral nutrition and carnitine therapy

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 cell transfusions

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

**Report that a transfusion occurred on the specimen collection card.**

## 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.

**Report that Pivalic acid was administered on the specimen collection card.**

## 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





# Requesting newborn bloodspot screening records

- If the child is younger than 6 years, request his or her newborn bloodspot screening records by faxing the child's full name, date of birth, kit number and mother's name (at the time of the child's birth) and date of birth on your letterhead to 503-693-5601.
- Records that are over 6 years old are outside of their record retention and should have been destroyed. It is unlikely that older records will be located. When requesting records older than 6 years, include a medical record release authorization signed by the patient, if over 18, or the parent or guardian.
- If you are requesting records for a baby who was born in another state, please contact that state's newborn bloodspot screening program to request results. Contact information for each state is provided by Baby's First Test at [www.babysfirsttest.org](http://www.babysfirsttest.org).
- Parents or legal guardians may request the infant's newborn bloodspot screening records by completing the form located at [www.bitly.com/get-phl-results](http://www.bitly.com/get-phl-results).





# Use, release, and retention of residual bloodspot specimens

**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.
- Program evaluation and quality improvement.
- Education activities required by Oregon Statute.

Residual bloodspot specimens will only be released by the OSPHL:

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

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



# Tips to avoid rejected specimens

**Improperly collected specimens compromise the accuracy of test results. When a specimen is rejected, a repeat collection will be required. This unnecessarily delays the screening of the newborn.**

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

## Tips to avoid “Layered Blood” rejection

**Specimen front**



**Specimen back**

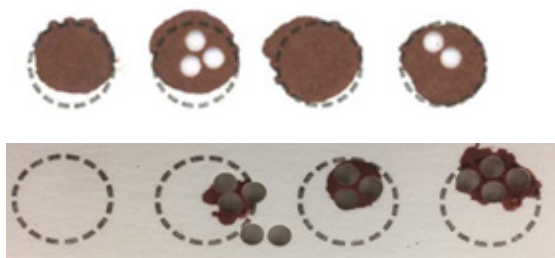


### Tips to avoid this type of rejection

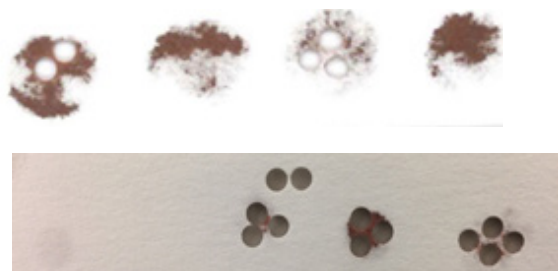
- Use the proper size lancet (< 2mm length).
- Allow a large drop to form on the heel before touching with the filter paper.
- Collect blood into one circle at a time.
- Do not apply additional blood to an incompletely filled circle.
- Do not apply blood to both sides of the filter paper.
- Do not compress the filter paper.

## Tips to avoid “Incomplete Saturation” or “Quantity Not Sufficient” rejection

**Specimen front**



**Specimen back**

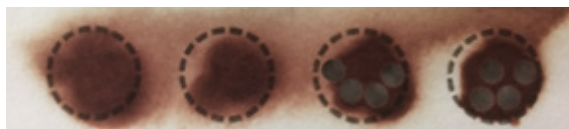


### Tips to avoid this type of rejection

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

## Tips to avoid “Contaminated” rejection

**Specimen front**



**Specimen back**



### Tips to avoid this type of rejection

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



# 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)

### CF essentials

- **Screening test:** The first-tier immunoassay measures immunoreactive trypsinogen (IRT). For specimens with an elevated IRT on one (if sufficiently high) or both screening specimens, second-tier DNA screening for 34 common variants is performed.
- **Confirmatory test:** Sweat chloride testing and DNA mutation analysis
- **Validity:** A small percentage of cases (<10%) will be falsely negative. Most cases should be abnormal on the first screen. IRT may be falsely elevated in premature, stressed, or sick infants. IRT can be falsely low in infants with CF who are born with meconium ileus.
- **Treatment:** Comprehensive, multidisciplinary care, pancreatic enzyme replacement, soluble vitamin replacement, high-calorie/high-fat diet, airway clearance regimen, and new specific targeted therapies based on genotype. Refer to accredited Cystic Fibrosis 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.

Cystic fibrosis (CF) is a recessively inherited defect of the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Over 1,800 mutations of the CFTR protein have been identified, but a single mutation (F508del), accounts for ~86% of all the mutations worldwide. There are approximately 34,000 adults and children with CF in the United States. The incidence of CF in the United States is approximately 1:3,500 newborns but varies by ethnicity: 1:3,500 Caucasian Americans, 1:8,500 Hispanic Americans, 1:17,000 African Americans, 1:31,000 Asian Americans.

## Clinical features

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

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

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

## Causes of CF

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

## Laboratory tests

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

For specimens with an elevated IRT on one (if sufficiently high) or both screening specimens, second-tier DNA analysis is performed (34 mutations, including current ACMG/ACOG recommendations). Depending on results, further diagnostic and confirmatory testing will be required, including additional mutation analysis.

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.
- Infants with meconium ileus may not have an elevated IRT. If meconium ileus is present, then diagnostic testing should be performed regardless of NBS results. It is important to remember that all infants with meconium ileus should have routine newborn bloodspot screening specimens collected even if CF is suspected, as they should be screened for the other conditions on the screening panel.
- A small percentage of infants with CF may not have an elevated IRT. Thus, a normal IRT at birth does not completely rule out CF. Children with recurrent respiratory problems, failure to thrive, or other symptoms consistent with CF, should still be evaluated and undergo sweat chloride testing.

## Confirmatory testing

CF can be diagnosed by two different methods, sweat chloride testing and/or DNA mutation analysis. Sweat chloride testing remains the gold standard, as it is a concrete marker of CFTR dysfunction. A chloride value in the sweat of  $\geq 60$  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](http://www.cftr2.org)), and most are not included in standard multi-array DNA analyses. (10, 11) Confirmation of two CF-causing mutations confirm the diagnosis, while only one may indicate a carrier state, CFTR-related metabolic syndrome (CRMS), or an affected individual with a less common mutation on the second allele.

## Treatment

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

## Screening practice considerations

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

**Table 3: CF screening result summary**

Results	Likely causes	Actions
1 <sup>st</sup> and 2 <sup>nd</sup> IRT elevated on filter paper specimens	<ul style="list-style-type: none"><li>• Cystic fibrosis probable</li><li>• Possible false positive</li></ul>	<ul style="list-style-type: none"><li>• NBS coordinator faxes results.</li><li>• Medical consultant phones practitioner with follow-up recommendations.</li><li>• Sweat chloride testing needed to confirm/clarify diagnosis.</li></ul>
One or two CFTR mutations identified on 23-mutation DNA analysis	<ul style="list-style-type: none"><li>• If two mutations, CF probable</li><li>• If one mutation, possible CF carrier vs CRMS vs CF with rare 2<sup>nd</sup> mutation</li></ul>	<ul style="list-style-type: none"><li>• NBS coordinator faxes results.</li><li>• Medical consultant phones practitioner with follow-up recommendations.</li><li>• Sweat chloride testing needed to confirm/clarify diagnosis.</li></ul>

# Congenital Adrenal Hyperplasia (CAH)

## CAH essentials

- **Neonatal emergency:** 3/4 will develop salt wasting crisis, which can be fatal, in the first week to month of life.
- **Incidence:** 1:12,700 newborns
- **Screening test:** 17-OH-progesterone
- **Validity:** 70% identified on 1st screen  
30% on 2nd screen
- **Causes:** 21-hydroxylase deficiency or other inborn error of cortisol synthesis; recessive inheritance
- **Treatment:** Hydrocortisone and mineralocorticoids
- **False positives:** Occur more frequently in premature, low birth weight or sick infants
- **Outcome:** Early detection and treatment can be lifesaving. Chromosome analysis in infants with ambiguous genitalia will prevent gender misassignment (11). Ultimate outcome depends on severity of defect, days to treatment and adherence. Refer to pediatric endocrinologist.

CAH is an inherited defect of cortisol synthesis. The adrenal gland cannot make cortisol and overproduces male hormones. Without cortisol, infants are at risk for adrenal crisis and may be unable to regulate salt and fluids, and can die. The most common disorder is 21-hydroxylase deficiency.

## Clinical features (12)

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

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

## Causes of CAH

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

## Laboratory tests

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

## Confirmation

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

## Treatment

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

## Screening practice considerations

- This disorder 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. Collect heel stick specimens between 24–48 hours of life. Transport all specimens 4–12 hours after collection and no later than 24 hours.
- Female infants who are virilized or infants with ambiguous genitalia should be considered at risk for this condition, tested at birth and monitored for electrolyte abnormalities until the diagnosis is excluded.
- Male infants are not usually recognized at birth.
- About 30% of infants will be detected only on a second screen. (13–15)

**Table 4: CAH screening result summary**

Results	Likely causes	Actions
Elevated 17 OHP (17-hydroxyprogesterone)	<ul style="list-style-type: none"><li>• CAH probable</li><li>• False positive</li></ul>	Neonatal emergency; NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

# Primary Congenital Hypothyroidism (CH)

## CH essentials (16, 17)

- **Incidence:** 1:2,000 newborns
- **Screening test:** T4 (thyroxine) and TSH (thyroid stimulating hormone)
- **Validity:** 90% identified on 1st screen, 10% on 2nd screen
- **Causes:** Thyroid dysgenesis: 85%; hereditary inborn error of thyroid hormone biosynthesis: 15%
- **Treatment:** L-thyroxine normalize T4 by 2 weeks of treatment initiation; TSH by 1 month
- **False positives:** Early collection within 24 hours of birth; premature or ill infants
- **Outcome:** Can be normal, but depends on severity of thyroid deficit, days to treatment and adherence to treatment. Severely affected infants with just a 2-week delay in reaching a serum T4 >10 ug/dL may have up to a 10-point drop in IQ. (18)

Primary congenital hypothyroidism (CH) occurs in infants who are born without the ability to produce adequate amounts of thyroid hormone. Thyroid hormone is important for normal function of all of the body's organs and is essential for normal brain development. The incidence of congenital hypothyroidism is 1:2,300. CH is more common in Hispanic and Native American populations (1:700–2,000). There is a 2:1 female/male ratio, explanation unknown. Infants with Down's syndrome have increased risk of CH (1:140 newborns).

## Clinical features

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

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

## Causes of primary congenital hypothyroidism

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

Some cases of central or secondary (hypopituitary) hypothyroidism may also be detected (see Table 5). These newborns often have clinical features of other pituitary hormone deficiencies, such as hypoglycemia or small penis and undescended testes in male infants.

## Laboratory tests

The initial screening test is the T4 assay. Infants with T4 results of <10% are further tested by a screening TSH assay. Different combinations of results are possible; see (see Table 5).

When the infant's physician is notified that screening results are abnormal, blood should be collected by venipuncture as soon as possible for measurement of TSH and free T4 to confirm the abnormal screening results. In the case where the screening T4 is low and TSH is elevated, treatment can be started as soon as the serum is obtained, pending final confirmation. If the serum thyroid function tests confirm hypothyroidism, further diagnostic studies, such as a thyroid ultrasound examination or radionuclide scan and X-ray to assess skeletal maturation, may be performed to determine the type, age of onset and severity of hypothyroidism. Generally, these studies do not change management and thus are optional.

## Thyroid function in premature infants

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

## Treatment

The American Academy of Pediatrics (AAP) recommends that infants be managed in consultation with a pediatric endocrinologist. (16) Treatment of CH is effective if done correctly. L-tyroxine (brand or generic l-thyroxine), in pill form, is crushed, mixed with water or expressed breast milk and administered once daily. The recommended starting dose is 10–15 mcg/day of body weight daily, usually 37.5 mcg/day to 50 mcg/day. AAP recommendations for follow-up TSH and free T4 are as follows:

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

**Treatment goals:** Maintain serum free T4 in the upper half of the normal or 1.2–2.4 ng/dL for free T4 (normal range may vary with assay), and TSH normalized (<6  $\mu$ IU/mL). Clinical evaluations can occur less frequently. As infants grow, the dose of thyroxine is increased. Periodic developmental testing should be done on all patients. If treatment is started early and thyroid levels are monitored closely, development remains normal (19).

## Screening practice considerations

- Primary congenital hypothyroidism is common, occurring in approximately 1:2,000 newborns.
- Ninety percent of hypothyroid infants are detected on the first specimen; in 10% of cases, hypothyroidism develops in the weeks after birth and is detected on a second screening test as production of thyroid hormone decreases after birth. (20–21)
- Some infants (usually pre-term) will manifest a delayed rise in TSH, and so are also detected on the routine second or third screening test. Practitioners therefore must remain alert to clinical symptoms in premature and older infants despite normal initial screening.
- False positive results may occur if the specimen is collected within the first few hours after birth, as the TSH rises in response to the extra-uterine environment.
- Topical iodine use on the infant or a mother who is breastfeeding and taking iodine supplements may cause transient hypothyroidism. In addition, nursing mothers drinking “seaweed soup”, which has a high iodine content, may also cause hypothyroidism in the neonate; this will resolve if ingestion of seaweed soup is discontinued.

**Table 5: CH screening result summary**

Results	Likely causes	Actions
T4 low/TSH elevated	<ul style="list-style-type: none"> <li>• Hypothyroidism probable</li> <li>• False positive</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
T4 low/TSH slightly elevated	<ul style="list-style-type: none"> <li>• Mild hypothyroidism</li> <li>• Transient hypothyroidism seen with recovery from “hypothyroxinemia of prematurity”</li> <li>• False positive</li> </ul>	NWRNBS Program contacts practitioner by FAX and by mail requesting further testing.
T4 low/TSH normal (on two specimens unless premature)	<ul style="list-style-type: none"> <li>• Thyroid binding globulin (TBG) deficiency</li> <li>• Central or secondary (hypopituitary) hypothyroidism</li> <li>• Non-thyroidal illness syndrome (“sick euthyroid syndrome”) associated with prematurity or acute illness</li> <li>• False positive</li> </ul>	NWRNBS Program contacts practitioner by FAX and by mail requesting further testing.

## Sickle Cell Disease and other Hemoglobinopathies

### Sickle Cell Disease essentials

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

The primary goal of hemoglobinopathy screening is to detect clinically significant sickling hemoglobinopathies in the neonatal period, before symptoms occur. Newborn diagnosis of sickle cell disease, if coupled with family education and centralized comprehensive care, can markedly lower morbidity and mortality. (22)

Homozygous sickle cell disease (SCD) occurs when the recessive gene for hemoglobin S, sickle hemoglobin, is inherited homozygously or with a second gene for certain other hemoglobin variants, such as beta thalassemia or hemoglobin C. These doubly heterozygous conditions tend to be less severe than those who are homozygous for hemoglobin S, although all are potentially capable of producing severe complications. The disease incidence in a population depends on the population’s racial composition.

## Clinical features

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

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

## Laboratory tests

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

## Treatment

Infants with significant hemoglobinopathies should have a primary care provider and receive periodic evaluation 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.

## Carrier detection makes SCD screening different

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. If both parents are carriers of an autosomal recessive genetic trait, the risk of any infant of that couple being homozygous, and therefore having the disease, is 1:4.

## Screening practice considerations

- Newborn bloodspot screening for hemoglobinopathies is not done on the second specimen unless an abnormality has been identified on the first specimen. It is crucial to use the first kit for the first test; the cards are not interchangeable.
- Transfusion of red blood cells before collecting the newborn bloodspot screening specimen will invalidate the hemoglobinopathy test. Always obtain a specimen before any transfusion regardless of the infant's age.
- Some hemoglobinopathies, particularly some thalassemias, are not reliably detected by newborn 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.

# Amino Acid Conditions

## Hypermethioninemia

*Homocystinuria (cystathionine beta-synthase deficiency)\**

### Homocystinuria essentials

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

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

### Clinical features (23, 24)

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.

---

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

## Methionine adenosyltransferase (MAT) deficiency

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

### Laboratory test

Elevation of methionine is detected by tandem mass spectrometry (MS/MS).

### Treatment

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

### Screening practice considerations

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

**Table 6: Hypermethioninemia screening result summary**

Results	Likely causes	Actions
Methionine slightly elevated	<ul style="list-style-type: none"><li>• Homocystinuria/MAT deficiency possible</li><li>• Tyrosinemia, Type I, galactosemia</li><li>• Liver disease</li><li>• Parenteral nutrition</li><li>• High protein diet</li><li>• False positive</li></ul>	NWRNBS Program requests repeat filter paper specimen by mail.
Methionine elevated	<ul style="list-style-type: none"><li>• Homocystinuria/MAT deficiency probable</li><li>• Tyrosinemia, Type I</li><li>• Liver disease</li><li>• Parenteral nutrition</li><li>• High protein diet</li><li>• False positive</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations

# Phenylketonuria (PKU) and Hyperphenylalaninemia

## Hyperphenylalaninemia essentials

- **Incidence:** 1:16,300 births
- **Screening test:** Phenylalanine elevated by tandem mass spectrometry (MS/MS); phenylalanine/tyrosine ratio elevated
- **Confirmatory tests:** Quantitative amino acids; biopterins in blood and urine
- **Validity:** >99% on 1st screen
- **Treatment:** Low phenylalanine diet; biopterin supplementation
- **Outcome:** Normal if treated early and adherence is good

Detection of elevated phenylalanine levels requires urgent follow-up. The disorder is due to a recessively inherited enzyme defect in which the body cannot use the amino acid phenylalanine properly. All other metabolic processes are intact, but phenylalanine, which comes from all dietary protein, accumulates in the blood to toxic levels. All forms of hyperphenylalaninemias from mild to severe and including biopterin defects are inherited as autosomal recessive disorders.

## Clinical features

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

Phenylalanine starts rising after birth and often reaches abnormal levels within 24 hours of life. A phenylalanine/tyrosine ratio can also be used to identify cases.

## Variant forms of PKU (hyperphenylalaninemia)

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

Some forms of hyperphenylalaninemia are caused by defects of the cofactor biopterin metabolism and blood phenylalanine levels are variable. These patients have progressive neurological damage with seizures and steady deterioration that becomes noticeable sometime between 6 and 20 months of age despite early

treatment with a low phenylalanine diet. Definitive tests can differentiate these variant forms of PKU. In view of the severity of this group of diseases, all infants with persistently abnormal levels of phenylalanine must have testing by special blood and urine tests for bipterin abnormalities.

## Maternal PKU and hyperphenylalaninemia

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

## Laboratory tests

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

## Treatment (25–28)

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

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

## Screening practice considerations

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

**Table 7: PKU and hyperphenylalaninemia screening result summary**

Results	Likely causes	Actions
Phenylalanine elevated; Phe/Tyr elevated	<ul style="list-style-type: none"> <li>• PKU possible</li> <li>• Variants forms of PKU</li> <li>• Mother has PKU</li> <li>• False positive</li> <li>• Transient hyperphenylalaninemia</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

## Tyrosinemia type I, II, and Transient Tyrosinemia\*

### Tyrosinemia essentials

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

### Transient Tyrosinemia (29)

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

### Clinical features

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

\* Not all cases of tyrosinemia will be detected by newborn bloodspot screening.

## Treatment

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

## Tyrosinemia Type I (Hepatorenal Tyrosinemia) (30)

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

### Clinical features

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

### Treatment

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

## Tyrosinemia Type II (Occulocutaneous Tyrosinemia)

Tyrosinemia, Type II is caused by a deficiency of the enzyme tyrosine aminotransferase (TAT) and is inherited as an autosomal recessive trait. TAT deficiency is rare, with about 100 cases described worldwide, although more infants may be identified as MS/MS screening continues to be implemented. (31)

## Clinical features (20, 31)

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

## Treatment

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

## Laboratory tests

Tyrosinemia is detected using both tyrosine and succinylacetone measured by MS/MS. There is considerable overlap in tyrosine levels between normal infants, those with transient tyrosinemia and affected infants, making the tyrosine level itself not very specific. Succinylacetone is the unique marker for tyrosinemia type I.

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

## Screening practice considerations

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

**Table 8: Tyrosinemia screening result summary**

Results	Likely causes	Actions
Tyrosine elevated Succinoylacetone normal	<ul style="list-style-type: none"><li>• Transient tyrosinemia</li><li>• Tyrosinemia type II or III possible</li><li>• Liver disease</li><li>• Parenteral nutrition</li><li>• False positive</li></ul>	NWRNBS Program requests repeat filter paper by mail.
Succinolyacetone increased. Tyrosine normal.	<ul style="list-style-type: none"><li>• Tyrosinemia type I</li><li>• False positive</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations

# Fatty Acid Oxidation (FAO) Conditions

## FAO condition essentials

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

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

## Fatty Acid Oxidation conditions\*

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

---

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

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

## Clinical features (33, 34)

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

**Hepatic (35, 36):** No typical age of presentation, may occur on the first day of life through adulthood. Infants with MCAD can present with sudden cardio/pulmonary arrest before screening results are known. Precipitating factors are fasting and/or stress associated with intercurrent illness. Patients present with “Reyes-like” symptoms including vomiting, lethargy, hypoketotic hypoglycemia, mild hyperammonemia, hyperuricemia, hypocarnitinemia and abnormal liver function tests. Liver biopsy often shows steatosis. Hepatic presentation is common in MCAD, VLCAD, LCHAD, neonatal CPT I & II and mild CACT deficiency. Patients with LCHAD may develop retinal pigmentary changes and progressive visual loss in childhood despite early diagnosis and treatment.

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

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

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

## Treatment

Even with screening, some infants with FAO disorders may die before laboratory results are available. Treatment for MCAD and some other FAOs is extraordinarily simple once the diagnosis is suspected. Avoidance of fasting, particularly as infants and young children, is the primary treatment. Carnitine supplementation (100mg/kg/day) is used to provide a pathway for removal of toxic intermediate metabolites in some FAOs. With appropriate treatment hepatic, cardiac and muscular complications can be reduced or eliminated. Patients with these disorders may require IV support for fluid and calories during intercurrent infections or illnesses. With pre-symptomatic diagnosis and appropriate therapy, outcome can be normal for infants with MCAD. (37, 38) Outcomes for the other disorders are still being evaluated.

## Screening practice considerations

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

## Organic Acid Conditions (OA)

### OA condition essentials

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

The following OAs are screened for by MS/MS:

- Beta-ketothiolase deficiency
- Glutaric acidemia, type I (glutaryl-CoA dehydrogenase deficiency)
- Isobutyryl CoA dehydrogenase deficiency
- Isovaleric acidemia, (isovaleryl-CoA dehydrogenase deficiency)
- Malonic aciduria
- Maple syrup urine disease (branched chain alpha-ketoacid dehydrogenase deficiency)

- Methylmalonic acidemias, methylmalonyl CoA mutase deficiency and defects of B-12 metabolism
- Propionic acidemia
- 3-Hydroxy-3-methylglutaryl (HMG) CoA lyase deficiency
- 2-Methyl-3-hydroxybutyryl CoA dehydrogenase deficiency
- 2-Methylbutyryl CoA dehydrogenase deficiency (mitochondrial acetoacetyl-CoA thiolase deficiency)
- 3-Methylcrotonyl CoA carboxylase (3MCC) deficiency
- 3-Methylglutaconyl CoA hydratase deficiency (3-methyl-glutaconic aciduria, type I)
- Multiple carboxylase deficiency

## Clinical features (39, 41)

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

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

**Asymptomatic cases:** There are numerous reports of cases of isolated 3-methylcrotonyl-CoA carboxylase deficiency who have remained asymptomatic despite biochemical and/or enzymatic confirmation of the condition. The etiology of these variant presentations is not yet understood. Mild forms of methylmalonic acidemia have been found.

**Glutaric Acidemia, type I:** Glutaric Acidemia, Type I or GA I is an organic acidemia with clinical features unlike those described above. (40–42) In this disease, there is

an accumulation of glutaric acid and 3-hydroxy glutaric acid, which are believed to be toxic to cells, particularly in the central nervous system. The classic presentation is macrocephaly at or shortly after birth. Infants have a period of apparently normal development but may have soft neurological signs, like jitteriness, irritability and truncal hypotonia. Generally, between 6 and 18 months of age, patients will experience an acute encephalopathic episode resulting in damage to the basal ganglia and atrophy of the caudate and putamen. This occurs over the course of a few hours to a day and is irreversible and untreatable. Severe dystonia, dyskinesia and other neurological findings result, either in a static or slowly progressive form. These children are often misdiagnosed as having extra pyramidal cerebral palsy. Approximately 25% of GA I patients will present with motor delay, hypotonia, dystonia and dyskinesia that develop gradually during the first few years of life, without any apparent acute crisis. Intellect is relatively intact. Infants with GA I are prone to acute subdural and retinal hemorrhages after minor head trauma. This can be misdiagnosed as child abuse. Finally, 5% of all Amish patients have been completely asymptomatic without any crises and normal development. Neurological crises and symptoms rarely occur after 5 years of age.

## Laboratory tests

All these disorders are detected using MS/MS. Leucine can be elevated in infants receiving parenteral nutrition, usually along with other amino acid elevations. In a normal newborn, however, elevations of these compounds are unusual and require rapid follow-up. There is evidence that not all affected infants will be found by NBS. (43)

## Treatment

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

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

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

## Screening practice considerations

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

# Urea Cycle Conditions (UCD)

## Urea Cycle essentials

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

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

- Arginase deficiency
- Argininosuccinic aciduria (ASA)
- Citrullinemia, type I and II

They are inherited as autosomal recessive traits.

## Arginase deficiency (44)

### Clinical features

Arginase deficiency is associated with irritability, inconsolable crying, anorexia, vomiting and developmental delay in infancy. This progresses to spastic tetraplegia with lower limbs more severely affected than the upper, psychomotor 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.

## Citrullinemia, Type I (CTLN1) and Argininosuccinic Aciduria (ASA) (44, 46)

### Clinical features-neonatal onset

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

### Clinical features-late onset

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

### Asymptomatic cases

Newborn 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.

## Citrin Deficiency (Citrullinemia, Type II and Neonatal Intrahepatic Cholestasis [NICCD]) (47)

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

### Clinical features-neonatal onset

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

### Clinical features-late onset

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

### Laboratory tests

Elevations of citrulline and arginine are detected by MS/MS. The laboratory cutoff for citrulline is  $\leq 70 \mu\text{M/L}$ ; for arginine,  $\leq 110 \mu\text{M/L}$ ; argininosuccinic acid,  $\leq 1.50 \mu\text{M/L}$ . Transient elevations of plasma arginine and citrulline in the newborn are unusual unless the infant is premature and/or receiving parenteral nutrition.

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

### Treatment (Citrullinemia, Type I & ASA)

All patients with a neonatal presentation represent medical emergencies and outcomes may be variable. Patients with neonatal onset disease will typically require aggressive treatment with hemodialysis. All patients, both late onset and those rescued from neonatal hyperammonemia, will require treatment with low protein diets and medications to prevent hyperammonemia and remove toxic compounds. The outcome for patients rescued from prolonged neonatal hyperammonemia is extremely poor.

Brain damage is likely. Even patients treated prospectively from birth may not be unaffected. (46) Those with late onset disease fare better, and presymptomatic diagnosis and treatment may allow normal development.

## Treatment: NICCD and CTLN2

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

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

## Screening practice considerations

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

**Table 9: UCD screening result summary**

Results	Likely causes	Actions
Arginine >110 µM/L	<ul style="list-style-type: none"> <li>• Arginase deficiency possible</li> <li>• Transient argininemia</li> <li>• Liver disease</li> <li>• False positive</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
ASA >1.50 µM/L	<ul style="list-style-type: none"> <li>• Argininosuccinic aciduria possible</li> <li>• Liver disease</li> <li>• False positive</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
Citrulline >70 µM/L	<ul style="list-style-type: none"> <li>• Citrullinemia, argininosuccinic aciduria possible</li> <li>• Transient citrullinemia</li> <li>• Liver disease</li> <li>• False positive</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
Citrulline >120 µM/L on second specimen	<ul style="list-style-type: none"> <li>• Mild citrullinemia, argininosuccinic aciduria possible</li> <li>• Transient citrullinemia</li> <li>• Liver disease</li> <li>• False positive</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations

# Galactosemia

## Galactosemia essentials

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

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

## Clinical features (48)

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

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

## Laboratory tests

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

- **GALT activity:** The enzyme test depends upon fluorescence produced by the normal galactose enzyme cascade in red blood cells. A temporarily abnormal result (diminished or absent fluorescent activity) is found in some infants. The test may be persistently abnormal if the enzyme activity is <50% of normal. It does not differentiate milder variants from severe defects or G6PD.
- **Galactose:** Slight elevations can occur in normal neonates, but galactose metabolites are greatly elevated in infants with galactosemia if they are receiving a lactose-containing formula or breast milk. Liver disease may also cause an elevation of galactose metabolites. All infants with an abnormal GALT or who have been transfused will be screened for galactose.

## Treatment

Galactosemia is treated by dietary galactose restriction (usually accomplished in the infant period through the use of soy-based or partially hydrolyzed infant formulas). The diet must be followed for life and requires close supervision. Even with early diagnosis and strict dietary restrictions children with galactosemia are at risk for speech disorders, tremors, growth and developmental delays and in females, ovarian failure.

## Screening practice considerations

- The GALT test should be abnormal in virtually all severe classic galactosemic infants even if the specimen is obtained before lactose is ingested, unless the infant has been transfused. Obtain a specimen before any transfusion.
- The GALT enzyme is prone to degradation if the sample is delayed in the mail or exposed to excess temperature or humidity. This produces a false positive GALT result.
- Galactose accumulation depends on lactose ingestion so that blood galactose metabolites may be normal in infants being fed a soy-based formula.

**Table 10: Galactosemia screening result summary**

Results		Likely causes	Actions
GALT test	Galactose metabolites		
<3.5 u/dL	≥20 mg/dL	<ul style="list-style-type: none"> <li>• Severe galactosemia</li> <li>• Variant galactosemia</li> <li>• False positive</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
<3.5 u/dL	<20 mg/dL	<ul style="list-style-type: none"> <li>• Severe galactosemia with little lactose intake</li> <li>• Variant galactosemia</li> <li>• Other enzyme defects in red blood cells</li> <li>• Improperly handled sample (heat damage or transit delay)</li> </ul>	Contact by mail or fax if infant is ≥48 hours old. Contact by fax if <48 hours old or if not on lactose.

## Biotinidase Deficiency

### Biotinidase deficiency essentials

- **Incidence:** 1:60,000 births
- **Screening test:** Biotinidase qualitative colorimetric enzyme assay
- **Confirmatory tests:** Quantitative biotinidase enzyme assay
- **Validity:** 100% found on 1st screen
- **Treatment:** 5-10 mg biotin/day
- **Outcome:** Excellent if compliant with biotin therapy This recessively inherited disorder affects the cells' ability to recycle the vitamin-cofactor biotin, which impairs the function of mitochondrial carboxylases.

### Clinical features (49, 50)

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

Infants with partial deficiency (5–10%) have been identified through newborn 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.

## Laboratory tests

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

## Treatment

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

Screening practice considerations

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

**Table 11: Biotinidase deficiency screening result summary**

Results	Likely causes	Actions
Color change does not occur	<ul style="list-style-type: none"><li>• Biotinidase deficiency possible</li><li>• False positive</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

# Severe Combined Immunodeficiency (SCID)

## SCID essentials

- **Incidence:** 1:50,000-1:100,000 births
- **Screening test:** Polymerase chain reaction to detect T-cell Receptor Excision Circles (TRECs)
- **Confirmatory tests:** CBC, lymphocyte subset flow cytometry
- **Validity:** The TREC assay used by Oregon to evaluate specimens for SCID and other t-cell lymphopenia has a positive predictive value greater than 0.7.
- **Treatment:** Bone marrow transplant, gene therapy or enzyme replacement
- **Outcome:** Good if treated within first 3 months of life

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

## Clinical features

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

## Causes of SCID

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

## Laboratory tests

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

## Confirmation

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

## Treatment

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

# Lysosomal Storage Disorders (LSDs)

## What are LSDs?

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

## Which LSDs are being tested:

Disorder	Enzyme	Gene
Fabry Disease	Alpha Galactosidase A	<i>GLA</i>
Gaucher Disease	Acid Beta-Glucosidase	<i>GBA</i>
Mucopolysaccharidosis Type 1 (MPS-1)	Alpha-L-Iduronidase	<i>IDUA</i>
Pompe Disease	Acid Alpha-Glucosidase	<i>GAA</i>

## How are LSDs diagnosed?

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

# Fabry Disease

## Fabry disease essentials (53, 54)

- **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.
- **Screening test:** Tandem mass spectrometry (MS/MS) to detect Alpha-galactosidase A (GLA) enzyme followed by second-tier DNA analysis of the *GLA* gene.
- **Confirmatory test:** GLA enzyme activity in plasma and leukocytes, possible assistance via DNA analysis of family members.
- **Validity:** Published false positive rate is 0.27% for the NeoLSD kit (PerkinElmer) used to measure enzyme activity

- **Treatment:** Enzyme replacement administered via infusion and in some cases oral chaperone therapy.
- **Outcome in early diagnosis:** Affected individuals will typically not develop symptoms for years to decades. Outcomes are improved with frequent monitoring and intervention to halt and prevent further progression of disease.

While Fabry disease is a disorder primarily affecting males, female heterozygotes can also be symptomatic and may be detected via newborn bloodspot screening. Some will remain asymptomatic throughout most of their life, others may benefit from early intervention.

## Clinical features

Mutations in *GLA* result in reduced formation of alpha-galactosidase A (GLA), 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.

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. In heterozygous females, milder symptoms later in life are expected but they can display a classic disease presentation.

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).

## Causes of Fabry disease

Fabry disease is inherited in an X-linked manner. In affected males, the infant's mother is an obligate heterozygote. Female carriers may have varying presentations due to random X-chromosome inactivation. The most severely impacted females likely express X chromosome with pathogenic *GLA* variant in the affected organs. Rarely, de novo pathogenic variants arise spontaneously.

## Laboratory tests

The screening test measures activity of GLA enzyme. A low enzyme on first valid specimen will trigger DNA analysis of the *GLA* gene (second tier). Depending on results, further diagnostic and confirmatory testing may be required.

There are several issues to keep in mind regarding abnormal enzyme tests:

- Reduced enzyme alone is not diagnostic of the disease. Diagnosis must be confirmed by DNA analysis and subsequent diagnostic testing.
- Unlike in most autosomal recessive disorders, there may be significant clinical implications for family members of infants with significant *GLA* variants. In some cases, family testing may assist in diagnosis and/or prognosis discussions.

## Confirmatory testing

In males, confirmation of the diagnosis after newborn bloodspot screening is made by measurement of GLA enzyme activity in plasma and leukocytes. Measurement in both are recommended due to inconsistent reductions seen in some DNA variants. A GLA enzyme  $< 1\%$  is consistent with classic disease and  $> 1\%$  but below the unaffected range is consistent with atypical disease. For females, measurement of GLA is unreliable and does not predict prognosis or severity.

DNA results from the newborn bloodspot screen assists in confirmation of diagnosis but may not be definitive if the variant is of uncertain significance. In some cases a mature, maternal adult family member can be tested. If that family member shares the variant detected in the newborn but has no features of Fabry disease then development of disease is considered unlikely.

## Treatment

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.

## Carrier detection

Screening may identify female Fabry disease heterozygotes as discussed above, but not all female heterozygotes will be detected on newborn bloodspot screening.

## Screening practice considerations

- GLA enzyme is not valid in screens collected in infants before 20 hours of life.
- GLA enzyme is measured in one valid specimen only. Normal GLA enzymes are not repeated on the 2<sup>nd</sup> or other subsequent specimens.

**Table 12: Fabry disease screening result summary for male newborns**

Results in MALE Newborn	Likely causes	Actions
GLA enzyme low, DNA analysis detects no and/or benign variant	<ul style="list-style-type: none"><li>• False positive</li></ul>	NWRNBS Program will report by letter regarding test results and any other recommendations.
GLA enzyme low, DNA analysis detects hemizygous variant of uncertain significance	<ul style="list-style-type: none"><li>• False positive</li><li>• Fabry disease</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
GLA enzyme low, DNA analysis detects hemizygous likely pathogenic or pathogenic variant	<ul style="list-style-type: none"><li>• Fabry disease</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

**Table 13: Fabry disease screening result summary for female newborns**

Results in FEMALE Newborn	Likely causes	Actions
GLA enzyme low, DNA analysis detects no and/or benign variant(s)	<ul style="list-style-type: none"><li>• False positive</li></ul>	NWRNBS Program will report by letter regarding test results and any other recommendations.
GLA enzyme low, DNA analysis detects variant(s) of uncertain significance	<ul style="list-style-type: none"><li>• False positive</li><li>• Heterozygous Fabry disease carrier</li><li>• Fabry disease</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
GLA enzyme low, DNA analysis detects likely pathogenic and/or pathogenic variant(s)	<ul style="list-style-type: none"><li>• Heterozygous Fabry disease carrier</li><li>• Fabry disease</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

# Pompe Disease

## Disease essentials (55–57)

- **Incidence:** Estimated between 1 in 28,000 to 1 in 40,000
- **Screening test:** Tandem mass spectrometry (MS/MS) to detect acid alpha-glucosidase (GAA) enzyme followed by second-tier DNA analysis of the *GAA* gene.
- **Confirmatory tests:** Creatine kinase (CK), aspartate transaminase (AST), alanine transaminase (ALT), acid alpha-glucosidase (GAA) enzyme in blood and urinary glucotetrasaccharide (Hex4)
- **Validity:** Published false positive rate is 0.12% for the NeoLSD kit (PerkinElmer) used to measure enzyme activity
- **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)
- **Treatment:** Enzyme replacement administered via infusion and supportive therapy.
- **Outcome in early diagnosis:** Significant improvements are expected in cardiac and respiratory function in infantile-onset forms with early treatment including prolonged lifespan. Improvement in long-term muscle function is expected in later-onset forms.

The presence of pseudodeficiency DNA variants in *GAA* will result in lower measured GAA enzyme on traditional assays but does not impact the actual function of the enzyme in vivo. Presence of pseudodeficiency variants is not associated with any clinical features of disease but will result in false positive screens and blood tests.

## Clinical features

Mutations in *GAA* result in reduced formation of acid alpha-glucosidase (GAA), the lysosomal enzyme responsible for processing of 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 and 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 lifespans 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.

## Causes of disease

Pompe disease is inherited in an autosomal recessive manner resulting in insufficient GAA enzyme.

## Laboratory tests

The screening test measures activity of GAA enzyme. A low enzyme on first valid specimen will trigger DNA analysis of the *GAA* gene (second tier). Depending on results, further diagnostic and confirmatory testing may be required.

There are several issues to keep in mind regarding abnormal enzyme tests:

- Reduced enzyme alone is not diagnostic of the disease. Diagnosis must be confirmed by DNA analysis and diagnostic testing.
- Presence of one or more pseudodeficiency variants will often result in a false positive screen. DNA testing may be able to clarify these cases before further testing or referral to specialist need to be pursued.

## 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 IOPD and 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.

**Table 14: Pompe disease variants, onset, and affected populations**

<b>GAA Pathogenic Variant</b>	<b>Associated with (IOPD or LOPD)</b>	<b>Commonly Affected Populations</b>
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

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.

## Treatment

Currently available treatment is enzyme replacement therapy (ERT) initiated prior to the development of tissue and organ damage in order 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.

## Screening practice considerations

- GAA enzyme is not valid in screens collected in infants before 20 hours of life.
- GAA enzyme is measured in one valid specimen only. Normal GAA enzymes are not repeated on the 2<sup>nd</sup> or other subsequent specimens.

**Table 15: Pompe disease screening result summary**

Results	Likely causes	Actions
GAA enzyme low, DNA analysis detects no and/or benign variant(s) including pseudodeficiency.	<ul style="list-style-type: none"> <li>• False positive</li> </ul>	NWRNBS Program will report by letter regarding test results and any other recommendations.
GAA enzyme low, DNA analysis detects heterozygous variant of uncertain significance, likely pathogenic or pathogenic variant	<ul style="list-style-type: none"> <li>• False positive</li> <li>• Pompe disease carrier</li> </ul>	NWRNBS Program will report by letter regarding test results and any other recommendations.
GAA enzyme low, DNA analysis detects homozygous or compound heterozygous variants of uncertain significance	<ul style="list-style-type: none"> <li>• Pompe disease carrier</li> <li>• Pompe disease</li> </ul>	NNBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
GAA enzyme low, DNA analysis detects homozygous or compound heterozygous likely pathogenic or pathogenic variants	<ul style="list-style-type: none"> <li>• Pompe disease carrier (if inherited in cis-)</li> <li>• Pompe disease (inherited in trans-)</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

# Mucopolysaccharidosis Type I (MPS I)

## Disease essentials (58, 59)

- **Incidence:** Estimated between 1 in 87,000 to 1 in 185,000
- **Screening test:** Tandem mass spectrometry (MS/MS) to detect alpha-L-iduronidase (IDUA) enzyme followed by second-tier DNA analysis of the *IDUA* gene.
- **Confirmatory test:** Alpha-L-iduronidase (IDUA) enzyme, glycosaminoglycans (GAGs) (aka mucopolysaccharides or MPS) in blood and/or urine.
- **Validity:** Published false positive rate is 0.07% for the NeoLSD kit (PerkinElmer) used to measure enzyme activity
- **False positives:** Can occur in heterozygous carriers and in presence of pseudodeficiency variants (particularly common in individuals of African ancestry).
- **Treatment:** Hematopoietic stem cell transplantation (HSCT) in severe disease and/or enzyme replacement therapy (ERT)
- **Outcome in early diagnosis:** In severe disease with early HSCT, or attenuated disease with early ERT, significant improvements expected in lifespan and overall disease burden.

The presence of pseudodeficiency DNA variants in *IDUA* will result in lower measured IDUA enzyme on traditional assays but does not impact the actual function of the enzyme in vivo. Presence of pseudodeficiency variants is not associated with any clinical features of disease but will result in false positive screens and blood tests.

## Clinical features

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.

## Causes of disease

MPS I is inherited in an autosomal recessive manner resulting in insufficient IDUA enzyme.

## Laboratory tests

The screening test measures activity of IDUA enzyme. A low enzyme on first valid specimen will trigger DNA analysis of the *IDUA* gene (second tier). Depending on results, further diagnostic and confirmatory testing may be required.

There are several issues to keep in mind regarding abnormal enzyme tests:

- Reduced enzyme alone is not diagnostic of the disease. Diagnosis must be confirmed by DNA analysis and diagnostic testing.
- Presence of one or more pseudodeficiency variants will often result in a false positive screen. DNA testing may be able to clarify these cases before further testing or referral to specialist need to be pursued.

## Confirmatory testing

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.

## 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.

## Screening practice considerations

- IDUA enzyme is not valid in screens collected in infants before 20 hours of life.
- IDUA enzyme is measured in one valid specimen only. Normal IDUA enzymes are not repeated on the 2nd or other subsequent specimens.

**Table 16: MPS I screening result summary**

Results	Likely causes	Actions
IDUA enzyme low, DNA analysis detects no and/or benign variant(s) including pseudodeficiency.	<ul style="list-style-type: none"> <li>• False positive</li> </ul>	NNWRNBS Program will report by letter regarding test results and any other recommendations.
IDUA enzyme low, DNA analysis detects heterozygous variant of uncertain significance, likely pathogenic or pathogenic variant	<ul style="list-style-type: none"> <li>• False positive</li> <li>• MPS I carrier</li> </ul>	NNWRNBS Program will report by letter regarding test results and any other recommendations.
IDUA enzyme low, DNA analysis detects homozygous or compound heterozygous variants of uncertain significance	<ul style="list-style-type: none"> <li>• MPS I carrier</li> <li>• MPS I</li> </ul>	NNBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
IDUA enzyme low, DNA analysis detects homozygous or compound heterozygous likely pathogenic or pathogenic variants	<ul style="list-style-type: none"> <li>• MPS I carrier (if inherited in cis-)</li> <li>• MPS I (inherited in trans-)</li> </ul>	NNBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

# Gaucher Disease

## Disease essentials (60, 61)

- **Incidence:** In the U.S, estimated at 1 in 40,000. In the Ashkenazi Jewish population prevalence is 1:855 individuals.
- **Screening test:** Tandem mass spectrometry (MS/MS) to detect acid beta-glucocerebrosidase (GBA) enzyme followed by second-tier DNA analysis of the *GBA* gene.
- **Confirmatory test:** Glucocerebrosidase (GBA) enzyme and chitotriosidase activity
- **Validity:** Published false positive rate is 0.07% for the NeoLSD kit (PerkinElmer) used to measure enzyme activity

- **False positives:** May occur in unaffected, heterozygous carriers
- **Treatment:** Enzyme replacement administered via infusion and in some cases oral substrate reduction therapy (SRT).
- **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.

## Clinical features

Mutations in *GBA* result in reduced formation of acid beta-glucocerebrosidase (GBA), 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.

## Causes of disease

Gaucher disease is inherited in an autosomal recessive manner resulting in insufficient GBA enzyme.

## Laboratory tests

The screening test measures activity of GBA enzyme. A low enzyme on first valid specimen will trigger DNA analysis of the *GBA* gene (second tier). Depending on results, further diagnostic and confirmatory testing may be required.

An important issue to keep in mind regarding abnormal enzyme tests:

- Reduced enzyme alone is not diagnostic of the disease. Diagnosis must be confirmed by DNA analysis and diagnostic testing.

## Confirmatory testing

Diagnosis of Gaucher disease is established by presence of biallelic pathogenic variants in GBA along with reduced GBA enzyme consistent with disease on diagnostic testing.

DNA analysis often assists in determining disease type in newborns identified by screening. Certain variants in the homozygous or compound heterozygous state can predict specific Gaucher disease type. The presence of at least one copy of the common variant, p.Asn409Ser or N409S (historically known as “N370S”) is protective against CNS disease.

**Table 17: Gaucher disease variants, disease types and affected population**

<b>GBA Pathogenic Variants</b>	<b>Gaucher disease type expected</b>	<b>Affected Individuals</b>
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%

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.

## 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.

## Screening practice considerations

- GBA enzyme is not valid in screens collected in infants before 20 hours of life.
- GBA enzyme is measured in one valid specimen only. Normal GBA enzymes are not repeated on the 2nd or other subsequent specimens.

**Table 18: Gaucher disease screening result summary**

Results	Likely causes	Actions
GBA enzyme low, DNA analysis detects no and/or benign variant(s)	<ul style="list-style-type: none"> <li>False positive</li> </ul>	NWRNBS Program will report by letter regarding test results and any other recommendations.
GBA enzyme low, DNA analysis detects heterozygous variant of uncertain significance, likely pathogenic or pathogenic variant	<ul style="list-style-type: none"> <li>False positive</li> <li>Gaucher disease carrier</li> </ul>	NWRNBS Program will report by letter regarding test results and any other recommendations.
GBA enzyme low, DNA analysis detects homozygous or compound heterozygous variants of uncertain significance	<ul style="list-style-type: none"> <li>Gaucher disease carrier</li> <li>Gaucher disease</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.
GBA enzyme low, DNA analysis detects homozygous or compound heterozygous likely pathogenic or pathogenic variants	<ul style="list-style-type: none"> <li>Gaucher disease carrier (if inherited in cis-)</li> <li>Gaucher disease (inherited in trans-)</li> </ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

## Spinal Muscular Atrophy (SMA)

**Screening for Spinal Muscular Atrophy (SMA) is anticipated to begin June 1, 2022.**

### SMA essentials (62–71)

- **Incidence:** 1:11,000 births
- **Screening test:** Polymerase chain reaction to detect deletion of exon 7 of the *SMN1* gene. 95% of cases are due to deletions in the *SMN1* gene.
- **Confirmatory tests:** Sequencing to identify deletions/mutations in the *SMN1* gene and copy number variants in the *SMN2* gene
- **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.
- **Outcome:** Can vary depending on type of SMA.

SMA, attributed to variants in the *SMN1* gene, is an autosomal recessive condition that progressively destroys motor neurons—nerve cells in the brain stem and spinal cord that control essential skeletal muscle activity such as speaking, walking, breathing, and swallowing, leading to muscle weakness and atrophy. Motor neurons control movement in the arms, legs, chest, face, throat and tongue. When there are disruptions in the signals between motor neurons and muscles, the muscles weaken, begin wasting away and develop twitching (called fasciculations).

## Clinical features

There is a wide range of impairment seen in SMA caused by defects in the *SMN1* gene, from onset before birth with breathing difficulties at birth to mild weakness in adults. Accordingly, SMA can be classified into four types, based on highest motor milestone achieved.

**Table 19: SMA types and clinical features**

Type	Other Name	Life Span	Motor	Clinical features
<b>SMA type 0</b> 0 copies of <i>SMN2</i>	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.
<b>SMA type I</b> 1-2 copies of <i>SMN2</i>	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 of the spine) or other skeletal abnormalities as they get older.
<b>SMA type II</b> 3 copies of <i>SMN2</i>	The	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.
<b>SMA type III</b> 3-4 copies of <i>SMN2</i>	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.
<b>SMA type IV</b> 4-6 copies of <i>SMN2</i>		Normal	Normal	Onset after 21 years of age, with mild to moderate proximal muscle weakness and other symptoms.

## Causes of SMA

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*.

## Laboratory tests

Screening is based on real time PCR that detects *SMN1* deletions.

## Confirmation

- Molecular Genetic testing of *SMN1* gene.
- Deletion/duplication analysis for exon 7 of *SMN1* and sequencing of *SMN1* if exon 7 is fully present
- Copy Number Variants may be assessed on *SMN2* as there is a correlation between the *SMN2* copy number and severity of disease.

**Table 20: SMN2 copy number and SMA clinical phenotype (62)**

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

Adapted from Calucho et al [2018] (69)

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] (70) 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.

## Screening Practice Considerations

Immediate referral to a pediatric neurologist is recommended.

## Treatment

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 mutated *SMN1* gene. This therapy is approved for patients with SMA under the age of 2 years.

Infants may receive gene therapy.

## X-Linked Adrenoleukodystrophy (X-ALD)

**Screening for X-Linked Adrenoleukodystrophy (X-ALD) is anticipated to begin on or before January 1, 2023.**

### X-ALD essentials (72–75)

- **Prevalence/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.
- **Screening test:** Tandem mass spectrometry (MS/MS) to detect C26:0 lysophosphatidylcholine (C26:0-LPC).
- **Confirmatory test:** Very long chain fatty acids in serum and DNA analysis of *ABCD1* gene.
- **Validity:** To be determined
- **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.
- **Outcome in early diagnosis:** 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.

While X-ALD is a disorder primarily affecting males, female heterozygotes can also develop symptoms in adulthood and may be detected via newborn bloodspot screening.

### Clinical features

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:

- 1) **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.
- 2) **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.
- 3) **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 the majority of 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.

## Causes of X-ALD

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.

## Laboratory tests

The screening test measures C26:0-LPC. An elevated level on first valid specimen will trigger request for repeat or referral. Depending on results, further diagnostic and confirmatory testing may be required.

There are several issues to keep in mind regarding abnormal C26:0-LPC:

- 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.

## 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 may not be reliable in ruling out or confirming the disorder. Biochemical and molecular testing cannot predict clinical outcomes, so careful monitoring of adrenocortical function and brain imaging are required throughout life for males.

## Monitoring for adrenal insufficiency

Male infants/children with confirmed ALD also undergo testing for adrenal insufficiency. This is unlikely to be present in the neonatal period. Monitoring in childhood is performed by measuring fasting morning plasma ACTH and serum cortisol levels.

## Treatment

Individuals identified by newborn bloodspot screening are not expected to require treatment in infancy. However, baseline evaluations and regular monitoring will be conducted once the diagnosis is confirmed.

As of this publication, the primary available treatments for X-ALD are:

- 1) **Corticosteroid Therapy:** A large number of 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.
- 2) **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 on the basis of 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.

## Carrier detection

Screening may identify female X-ALD disease heterozygotes as discussed above, but not all female heterozygotes will be detected on newborn bloodspot screening.

## Screening practice considerations

C26:0-LPC is not valid in screens collected in infants before 24 hours of life.

**Table 21: X-ALD screening result summary for male newborns**

Results in MALE Newborn	Likely causes	Actions
Elevated C26:0-LPC	<ul style="list-style-type: none"><li>• X-ALD or other disorder of VLCFAs likely</li><li>• False positive</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations

**Table 22: X-ALD screening result summary for female newborns**

Results in FEMALE Newborn	Likely causes	Actions
Elevated C26:0-LPC	<ul style="list-style-type: none"><li>• Heterozygous X-ALD variant present</li><li>• Other disorder of VLCFAs</li><li>• False positive</li></ul>	NBS coordinator faxes results. Medical consultant phones practitioner with follow-up recommendations.

# References

1. Pass KA, Lane PA, Fernhoff PM, Hinton CF, Panny SR, Parks JS, et al. US newborn screening system guidelines II: follow-up of children, diagnosis, management, and evaluation. Statement of the Council of Regional Networks for Genetic Services (CORN). *J Pediatr*. 2000 Oct;137(4 Suppl): S1-46.
2. American College of Medical Genetics Newborn Screening Expert Group. Newborn screening: toward a uniform screening panel and system--executive summary. *Pediatrics*. 2006 May;117(5 Pt 2): S296-307.
3. National Newborn Screening and Genetics Resource Center, Austin TX website: [genes-r-us.uthscsa.edu](http://genes-r-us.uthscsa.edu)
4. Arn PH. Newborn screening: current status. *Health Affairs*. 2007;26(2):559-566.
5. Tuerck JM, Buist NR, Skeels MR, Miyahira RS, Beach PG. Computerized surveillance of errors in newborn screening practice. *Am J Public Health*. 1987 Dec;77(12):1528-31.
6. CLSI. Newborn screening for preterm, low birth weight, and sick newborns; approved guidelines. CLSI NBS03-A. Wayne, PA: Clinical and Laboratory Standards Institute; October 2009.
7. Kosciuk RL, Farrell PM, Kosorok MR, Zaremba KM, Laxova A, Lai HC, et al. Cognitive function of children with cystic fibrosis: deleterious effect of early malnutrition. *Pediatrics*. 2004 Jun;113(6):1549-58.
8. Sontag MK, Hammond KB, Zielenski J, Wagener JS, Accurso FJ. Two-tiered immunoreactive trypsinogen-based newborn screening for cystic fibrosis in Colorado: screening efficacy and diagnostic outcomes. *J Pediatr*. 2005 Sep;147(3 Suppl): S83-8.
9. 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.
10. 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.
11. 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.

12. 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.
13. 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.
14. 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.
15. 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.
16. 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
17. 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
18. Selva KA, Harper A, Downs A, Blasco PA, Lafranchi SH. Neurodevelopmental outcomes in congenital hypothyroidism: comparison of initial T4 dose and time to reach target T4 and TSH. *J Pediatr*. 2005 Dec;147(6):775-80.
19. Van Vliet G. Neonatal hypothyroidism: treatment and outcome. *Thyroid*. 1999 Jan;9(1):79-84.
20. LaFranchi SH, Hanna CE, Krainz PL, Skeels MR, Miyahira RS, Sesser DE. Screening for congenital hypothyroidism with specimen collection at two time periods: results of the Northwest Regional Screening Program. *Pediatrics*. 1985 Nov;76(5):734-40.
21. 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.
22. Dick MC. Standards for the management of sickle cell disease in children. *Arch Dis Child Educ Pract Ed*. 2008 Dec;93(6):169-76.

23. 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.
24. 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.
25. Channon S, Goodman G, Zlotowitz S, Mockler C, Lee PJ. Effects of dietary management of phenylketonuria on long-term cognitive outcome. *Arch Dis Child.* 2007 Mar;92(3):213-8.
26. Cleary MA, Feillet F, White FJ, Vidailhet M, Macdonald A, Grimsley A, et al. Randomised controlled trial of essential fatty acid supplementation in phenylketonuria. *Eur J Clin Nutr.* 2006 Jul;60(7):915-20.
27. Fiege B, Blau N. Assessment of tetrahydrobiopterin (BH4) responsiveness in phenylketonuria. *J Pediatr.* 2007 Jun;150(6):627-30.
28. Matalon R, Michals-Matalon K, Bhatia G, Grechanina E, Novikov P, McDonald JD, et al. Large neutral amino acids in the treatment of phenylketonuria (PKU). *J Inherit Metab Dis.* 2006 Dec;29(6):732-8.
29. Scott CR. The genetic tyrosinemias. *Am J Med Genet C Semin Med Genet.* 2006 May 15;142(2):121-6.
30. Koelink CJ, van Hasselt P, van der Ploeg A, van den Heuvel-Eibrink MM, Wijburg FA, Bijleveld CM, et al. Tyrosinemia type I treated by NTBC: how does AFP predict liver cancer? *Mol Genet Metab.* 2006 Dec;89(4):310-5.
31. Tsai CP, Lin PY, Lee NC, Niu DM, Lee SM, Hsu WM. Corneal lesion as the initial manifestation of tyrosinemia type II. *J Chin Med Assoc.* 2006 Jun;69(6):286-8.
32. Magera MJ, Gunawardena ND, Hahn SH, Tortorelli S, Mitchell GA, Goodman SI, et al. Quantitative determination of succinylacetone in dried blood spots for newborn screening of tyrosinemia type I. *Mol Genet Metab.* 2006 May;88(1):16-21.
33. Shekhawat PS, Matern D, Strauss AW. Fetal fatty acid oxidation disorders, their effect on maternal health and neonatal outcome: impact of expanded newborn screening on their diagnosis and management. *Pediatr Res.* 2005 May;57(5 Pt 2):78R-86R.
34. Wilcken B, Haas M, Joy P, Wiley V, Chaplin M, Black C, et al. Outcome of neonatal screening for medium-chain acyl-CoA dehydrogenase deficiency in Australia: a cohort study. *Lancet.* 2007 Jan 6;369(9555):37-42.

35. Wilcox RL, Nelson CC, Stenzel P, Steiner RD. Postmortem screening for fatty acid oxidation disorders by analysis of Guthrie cards with tandem mass spectrometry in sudden unexpected death in infancy. *J Pediatr*. 2002 Dec;141(6):833-6.
36. Van Wassenhaer AG, Kok JH, Dekker FW, de Vijlder JJ. Thyroid function in very preterm infants: influences of gestational age and disease. *Pediatr Res*. 1997 Nov;42(5):604-9
37. 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.
38. 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.
39. Deodato F, Boenzi S, Santorelli FM, Dionisi-Vici C. Methylmalonic and propionic aciduria. *Am J Med Genet C Semin Med Genet*. 2006 May 15;142(2):104-12.
40. Andersson HC, Marble M, Shapira E. Long-term outcome in treated combined methylmalonic acidemia and homocystinemia. *Genet Med*. 1999 May-Jun;1(4):146-50.
41. Hedlund GL, Longo N, Pasquali M. Glutaric acidemia type 1. *Am J Med Genet C Semin Med Genet*. 2006 May 15;142(2):86-94.
42. 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.
43. 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.
44. 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.
45. 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.
46. Gropman AL, Batshaw ML. Cognitive outcome in urea cycle disorders. *Mol Genet Metab*. 2004;81 (Suppl 1): S58-62. 47.
47. Ohura T, Kobayashi K, Tazawa Y, Abukawa D, Sakamoto O, Tsuchiya S, et al. Clinical pictures of 75 patients with neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD). *J Inherit Metab Dis*. 2007 ;30(2):139-44.

48. Bosch AM. Classical galactosaemia revisited. *J Inherit Metab Dis*. 2006;29(4):516- 25.
49. Wolf B. Biotinidase Deficiency: New Directions and Practical Concerns. *Curr Treat Options Neurol*. 2003;5(4):321-328.
50. American Academy of Pediatrics. Newborn Screening Expands: Recommendations for Pediatricians and Medical Homes – Implications for the System. *Pediatrics*. 2008;121: 192-217.
51. Dick MC. Standards for the management of sickle cell disease in children. *Arch Dis Child Educ Pract Ed*. 2008 Dec;93(6):169-76.
52. Hopkins PV, Klug T, Vermette L, Raburn-Miller J, Kiesling J, Rogers S. Incidence of 4 Lysosomal Storage Disorders From 4 Years of Newborn Screening. *JAMA Pediatr*. 2018;172(7): 696-697.
53. Newborn screening act sheet Fabry disease: Decreased alpha-galactosidase a [Internet]. Mayo Clinic Laboratories. 2020 [cited 2022 Apr 19]. Available from: [https://www.mayocliniclabs.com/~media/it-mmfiles/special-instructions/Newborn\\_Screening\\_Act\\_Sheet\\_Fabry\\_Disease-Decreased\\_Alpha-Galactosidase\\_A.pdf](https://www.mayocliniclabs.com/~media/it-mmfiles/special-instructions/Newborn_Screening_Act_Sheet_Fabry_Disease-Decreased_Alpha-Galactosidase_A.pdf)
54. Mehta A, Hughes DA. Fabry Disease. In: GeneReviews® [Internet]. University of Washington, Seattle; 2002 Aug 5 [Updated 2022 Jan 27] [cited 2022 Apr 19]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1292>
55. Bodamer OA, Scott CR, Giugliani R, on behalf of the Pompe Disease Newborn Screening Working Group. Newborn Screening for Pompe Disease. *Pediatrics* [Internet]. 2017 July 1 [cited 2022 Apr 20];140(Supplement\_1):S4–13. Available from: [https://publications.aap.org/pediatrics/article/140/Supplement\\_1/S4/179258/Newborn-Screening-for-Pompe-Disease](https://publications.aap.org/pediatrics/article/140/Supplement_1/S4/179258/Newborn-Screening-for-Pompe-Disease)
56. Dasouki M, Jawdat O, Almadhoun O, Pasnoor M, McVey AL, Abuzinadah A, et al. Pompe disease: literature review and case series. *Neurol Clinics* [Internet]. 2014 Aug. [cited 2022 Apr 20];32(3):751–76, ix. Available from: <http://dx.doi.org/10.1016/j.ncl.2014.04.010>
57. Leslie N, Bailey L. Pompe Disease. In: GeneReviews® [Internet]. University of Washington, Seattle; 2007 Aug 31 [Updated 2017 May 11] [cited 2022 Apr 20]. Available from: <https://www.ncbi.nlm.nih.gov/sites/books/NBK1261/>
58. Clarke LA. Mucopolysaccharidosis Type I. In: GeneReviews® [Internet]. University of Washington, Seattle; 2002 Oct 31 [Updated 2021 Feb 25] [cited 2022 Apr 20]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1162/>

59. Duke University for the Maternal and Child Health Bureau of the Health and Resources and Services Administration. Newborn Screening for Mucopolysaccharidosis Type I [Internet]. 2018 Jun 27. Available from: <https://www.hrsa.gov/sites/default/files/hrsa/advisory-committees/heritable-disorders/rusp/previous-nominations/mps-i-27-june-2018.pdf>
60. Pastores GM, Hughes DA. Gaucher Disease. In: GeneReviews® [Internet]. University of Washington, Seattle; 2000 Jul 27 [Updated 2018 Jun 21] [cited 2022 Apr 20]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1269>
61. OMIM® - Online Mendelian Inheritance in Man® entry - # 230800 - GAUCHER DISEASE, TYPE I; GD1 [Internet]. Omim.org. [cited 2022 Apr 20]. Available from: <https://www.omim.org/entry/230800>
62. Prior TW, Leach ME, Finanger E. Spinal Muscular Atrophy. In: GeneReviews® [Internet]. University of Washington, Seattle; 2020 Feb 24 [Updated 2020 Dec 3] [cited 2022 Apr 21]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1352/>
63. Mercuri E, Finkel RS, Muntoni F, Wirth B, Montes J, Main M, et al. Diagnosis and management of spinal muscular atrophy: part 1: recommendations for diagnosis, rehabilitation, orthopedic and nutritional care. *Neuromuscular Disorders*. [Internet]. 2018 Feb 1 [cited 2022 Apr 21];28(2):103–15. Available from: <http://dx.doi.org/10.1016/j.nmd.2017.11.005>
64. Chng SY, Wong YQ, Hui JH, Wong HK, Ong HT, Goh DY. Pulmonary function and scoliosis in children with spinal muscular atrophy types II and III. *Journal of Pediatrics and Child Health* [Internet]. 2003 Dec. [cited 2022 Apr 21];39(9):673–6. Available from: <http://dx.doi.org/10.1046/j.1440-1754.2003.00266.x>
65. Kolb SJ, Coffey CS, Yankey JW, Krossschell K, Arnold WD, Rutkove SB, et al. Natural history of infantile-onset spinal muscular atrophy. *Annals of Neurology* [Internet]. 2017 Dec. [cited 2022 Apr 21];82(6):883–91. Available from: <http://dx.doi.org/10.1002/ana.25101>
66. Wadman RI, Stam M, Gijzen M, Lemmink HH, Snoeck IN, Wijngaarde CA, et al. Association of motor milestones, SMN2 copy and outcome in spinal muscular atrophy types 0-4. *Journal of Neurology, Neurosurgery & Psychiatry* [Internet]. 2017 Apr 1;88(4):365–7. Available from: <http://dx.doi.org/10.1136/jnnp-2016-314292>
67. Montes J, McDermott MP, Mirek E, Mazzone ES, Main M, Glanzman AM, et al. Ambulatory function in spinal muscular atrophy: Age-related patterns of progression. *PLOS ONE* [Internet]. 2018 Jun 26 [cited 2022 Apr 21];13(6):e0199657. Available from: <http://dx.doi.org/10.1371/journal.pone.0199657>

68. Finkel RS, Mercuri E, Meyer OH, Simonds AK, Schroth MK, Graham RJ, et al. Diagnosis and management of spinal muscular atrophy: Part 2: Pulmonary and acute care; medications, supplements and immunizations; other organ systems; and ethics. *Neuromuscular Disorders* [Internet]. 2018 Mar 1 [cited 2022 Apr 21];28(3):197–207. Available from: <http://dx.doi.org/10.1016/j.nmd.2017.11.004>
69. Calucho M, Bernal S, Alías L, March F, Venceslá A, Rodríguez-Álvarez FJ, et al. Correlation between SMA type and SMN2 copy number revisited: An analysis of 625 unrelated Spanish patients and a compilation of 2834 reported cases. *Neuromuscular Disorders* [Internet]. 2018 Mar 1 [cited 2022 Apr 21];28(3):208–15. Available from: <http://dx.doi.org/10.1016/j.nmd.2018.01.003>
70. Prior TW, Swoboda KJ, Scott HD, Hejmanowski AQ. Homozygous SMN1 deletions in unaffected family members and modification of the phenotype by SMN2. *American Journal of Medical Genetics, Part A* [Internet]. 2004 Aug 24 [cited 2022 Apr 21];130A(3):307–10. Available from: <http://dx.doi.org/10.1002/ajmg.a.30251>
71. Chien YH, Chiang SC, Weng WC, Lee NC, Lin CJ, Hsieh WS, et al. Presymptomatic diagnosis of spinal muscular atrophy through newborn bloodspot screening. *The Journal of Pediatrics*. 2017;190:124–9.
72. 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>
73. Lee S, Clinard K, Young SP, Rehder CW, Fan Z, Calikoglu AS, et al. Evaluation of X-linked adrenoleukodystrophy newborn screening in North Carolina. *JAMA Network Open* [Internet]. 2020 Jan 31 [cited 2022 Apr 21];3(1):e1920356. Available from: <http://dx.doi.org/10.1001/jamanetworkopen.2019.20356>
74. Raymond GV, Moser AB, Fatemi A. X-Linked Adrenoleukodystrophy. In: *GeneReviews®* [Internet]. University of Washington, Seattle; 1999 Mar 26 [Updated 2018 Feb 15] [cited 2022 Apr 21]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1315/>
75. Wiens K, Berry SA, Choi H, Gaviglio A, Gupta A, Hietala A, et al. A report on state-wide implementation of newborn screening for X-linked Adrenoleukodystrophy. *American Journal of Medical Genetics, Part A* [Internet]. 2019 May 10 [cited 2022 Apr 21];179(7):1205–13. Available from: <http://dx.doi.org/10.1002/ajmg.a.61171>

# Northwest Regional Newborn Bloodspot Screening Program

*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  
Phone: 503-693-4174  
Fax: 503-693-5601



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

OHA 8189 (Rev. 06/2022)

AMEND: 333-024-1070

NOTICE FILED DATE: 03/30/2022

RULE SUMMARY: Amend OAR 333-024-1070:

Beginning June 1, 2022:

- Clarify names of disorders tested,
- Edit test method used to identify lysosomal storage disorders and cystic fibrosis to align with current laboratory testing performed, and
- Add testing for spinal muscular atrophy (SMA).

Beginning on or before January 1, 2023, add testing for X-linked Adrenoleukodystrophy (X-ALD).

CHANGES TO RULE:

### 333-024-1070

Newborn Screening: The Newborn Screening Panel and Methods of Testing

(1) Every properly collected specimen submitted for newborn screening will be tested by the Oregon State Public Health Laboratory or, at the discretion of the Oregon State Public Health Laboratory, another CLIA certified laboratory.¶

(2) Newborn sScreening specimens will be tested for the medical conditions listed ~~below~~ in subsections (3) through (11), using the methods listed below. At its discretion, and consistent with CLIA standards, the Oregon State Public Health Laboratory may use an equivalent or better alternative method.¶

(3) Metabolic dDisorders:¶

(a) Organic aAcid dDisorders. Method: Quantitative measurement of amino acids by tandem mass spectrometry.¶

(A) Propionic acidemia (PA);¶

(B) Methylmalonic acidemia (MMA);¶

(C) Isovaleric acidemia (IVA);¶

(D) 3-methylcrotonyl CoA carboxylase deficiency (3MCC);¶

(E) 3-hHydroxy-3-mMethylglutaryl-CoA lyase deficiency ic Aciduria (HMG);¶

(F) ~~Multiple~~ Holocarboxylase dSynthase Deficiency (MCD);¶

(G) Beta-ketothiolase deficiency (BKT);¶

(H) Glutaric acidemia, Type I (GA-I);¶

(I) Malonic acidemia (MAL);¶

(J) Isobutyryl-CoA dehydrogenase deficiency (IBD) glycinuria;¶

(K) 2-mMethylbutyryl-CoA dehydrogenase deficiency (2MBC) glycinuria;¶

(L) 3-mMethylglutaconyl-CoA hydratase deficiency (3MGH); and¶

~~(M) 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency (2M3HBA)ic aciduria; and¶~~

(M) 2-methyl-3-hydroxybutyric aciduria.¶

(b) Fatty acid oxidation disorders. Method: Quantitative measurement of acylcarnitines by tandem mass spectrometry.¶

(A) Carnitine uptake defect (CUD);¶

(B) Medium chain acyl-CoA dehydrogenase deficiency (MCAD);¶

(C) Very long chain acyl-CoA dehydrogenase deficiency (VLCAD);¶

(D) Long chain 3 hydroxyacyl-CoA dehydrogenase deficiency (LCHAD);¶

(E) Trifunctional protein deficiency (TFP);¶

(F) Short chain acyl-CoA dehydrogenase deficiency (SCAD);¶

(G) Glutaric acidemia Type II (GA2);¶

(H) Carnitine palmitoyl transferase deficiency, Types I and II (CPT I and CPT II); and ¶

(I) Carnitine acylcarnitine translocase deficiency.¶

(c) Amino acid disorders. Method: Quantitative measurement of amino acids by tandem mass spectrometry.¶

(A) Argininosuccinate lyase deficiency ~~(e.g. Argininosuccinic aciduria or ASA);~~¶

(B) Citrullinemia, Type I (CIT);¶

(C) Maple syrup urine disease (MSUD);¶

(D) Homocystinuria (HCY);¶

(E) Phenylketonuria (PKU);¶

(F) Tyrosinemia, Types I, II, and III; and¶

(G) Arginase deficiencyemia (ARG).¶

(4) Endocrine disorders:¶

(a) Primary congenital hypothyroidism (CH). Method: Fluorescent immunoassay of thyroxine (T4) with secondary assay of thyroid stimulating hormone (thyrotropin or TSH).¶

(b) Congenital adrenal hyperplasia (CAH). Method: Fluorescent immunoassay of 17-alpha hydroxyprogesterone (17-OHP).¶

(5) Cystic fibrosis. Method: Primary screening by fluorescent immunoassay for the presence or absence of quantification of immunoreactive trypsinogen with second tier PCR testing amplification followed by allele-specific probe hybridization for common cystic fibrosis genotype mutations.¶

(6) Biotinidase deficiency. Method: Colorimetric or fluorometric assay for biotinidase activity or ~~fluorescent immunoassay~~.¶

(7) Classic Galactosemia. Method: Fluorescent immunoassay for the presence or absence of detectable galactose uridyl transferase in erythrocytes and galactose levels.¶

(8) Sick cell anemia and other hemoglobin disorders. Method: Primary screening for sickling hemoglobin by isoelectric focusing and confirmation by high performance liquid chromatography to detect hemoglobin variants.¶

(9) Severe combined immunodeficiency disease (SCID). Method: PCR to detect the absence or presence of T-cell receptor excision circles.¶

(10) Lysosomal storage diseases. Method: ~~Measurement of the activity of lysosomal storage enzymes by quantitative fluorometric enzymatic activity assay or~~ Quantitative measurement of enzyme levels by tandem mass spectrometry with second tier ~~test for specific analytes using~~ by tandem mass spectrometry, PCR, enzymatic assay or DNA sequencing.¶

(a) Pompe (glycogen storage disease Type II);¶

(b) Mucopolysaccharidosis Type I (MPS I);¶

(c) Fabry (alphagalactosidase A deficiency); and,¶

(d) Gaucher (glucocerebrosidase deficiency).¶

(11) ~~Newborn screening results may identify medical conditions, commonly referred to as "secondary~~ Spinal Muscular Atrophy (SMA). Method: PCR to detect presence or absence of the SMN1 gene. ¶

(12) Beginning on or before January 1, 2023, Newborn Screening specimens will also be tested for X-linked Adrenoleukodystrophy (X-ALD). Method: tandem mass spectrometry. ¶

(13) Newborn screening results may identify other medical conditions", that are not listed above. ~~Any secondary~~ Other medical conditions that ~~is~~ are identified during routine newborn screening will be included in a result report as described in OAR 333-024-1080. 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 ~~for a secondary condition that is identified in these circumstances~~.

Statutory/Other Authority: ORS 413.014, 433.285, 431A.750

Statutes/Other Implemented: ORS 433.285, 433.290, 433.295