Nebraska Newborn Screening Program, Practitioners Manual

Acknowledgements

Information from the former Practitioner's Manual has been posted on Nebraska's Newborn Screening Web Site www.dhhs.nnsp/NBS. This manual is designed to provide the newborn's Health Care Practitioner with the information they need to fulfill their responsibilities. Health Care Provider's, most often the newborn's Pediatrician or Family Physician providing pediatric care, has primary responsibility for educating the new parent about newborn screening, ensuring the screen is ordered and that any needed follow-up occurs. This manual prepares you for this and provides references to the resources you will need at the time you need them. The State Newborn Screening Program with the experts and stakeholders from the Nebraska Newborn Screening Advisory Committee are here to support you with patient education resources, guidelines and information about regulatory requirements. Nebraska's program is proud of its recognition for high quality follow-up assistance to Health Care Providers as well as it's mechanisms for continually monitoring and responding to ensure high quality throughout the system.

I wish to acknowledge the substantial contributions in reviewing and editing this manual by the members of the Nebraska Newborn Screening Advisory Committee and the Program Follow-up Coordinators Krystal Baumert and Karen Eveans, MD and Administrative Assistant, Jessica Davis. Special recognition goes to Pediatric Subspecialists John Colombo, MD, Kevin Corley, MD, James L. Harper, MD, Richard Lutz, MD, William Rizzo, MD for their expertise on the technical sections covering cystic fibrosis, the endocrine conditions, hemoglobinopathies, and metabolic conditions.

It is our sincere wish that this manual serve as a useful tool to Health Care Providers and invite comment at any time for the betterment of the system.

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For the most up to date information on Nebraska Newborn Screening Requirements Call (402) 471-6733 or go to
http://dhhs.ne.gov/publichealth/Pages/nsp_physicians.aspx

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Introduction

Nebraska was one of the original 30 states to participate in the phenylketonuria (PKU) trials in the 1960's. By 2018 Nebraska was screening for 32 conditions from the blood spot specimen. Proposals to screen for an ever increasing cadre of conditions undergo evidence-based review by the Federal Health and Human Services Secretary's Advisory Committee on Heritable Diseases in Newborns and Children (ACHDNC), as well as Nebraska's Newborn Screening Advisory Committee (NBSAC). The NBSAC also considers factors such as what resources must be in place for a state-wide screening system to successfully add any particular condition.

Purpose of this Manual

The purpose of this manual is to give clear and concise information to Nebraska practitioners involved with newborn screening. It gives information on screening practices and a general overview of each disorder. It includes a listing of many on-line resources available to newborn health care providers as additional tools and information. The regulations and the State Statute are included on the "Links and Resources" web page link.

The goal of the Nebraska Newborn Screening Program (NNSP) is to assure the timely and appropriate diagnosis and treatment of Nebraska newborns affected by the disorders on the screening panel. This manual is designed to enhance the practitioner's knowledge of the Nebraska Newborn Screening Program to facilitate screening of all infants for the specified conditions.

We encourage practitioners to use the term "newborn screen" or "bloodspot screen" rather than "PKU test" since many other disorders are included in the screening panel, and to avoid confusion with the point-of-care screening done for early hearing detection and intervention, and critical congenital heart disease.

General Overview of the Newborn Screening Program and the Health Care Provider's Role

Every baby born in Nebraska is required by law to be screened.

Parent education ideally occurs before the baby is born, sometime during the third trimester. Obstetric care providers are encouraged to introduce information about newborn screening during the third trimester. Copies of a one-page introductory parent information sheet can be obtained from the NNSP at no charge. The "Parent's Guide to Your Baby's Newborn Screening" brochure is also provided at all birthing facilities to give to new parents either at pre-admission or during the obstetric hospital stay. This is available in multiple languages. A video for parents "Newborn Screening, Protecting Your Baby's Health" has also been provided to all hospital mother/baby units, and is available in English and Spanish. Copies of any of these materials are available free of charge to practitioner's for use in their clinics.

After the baby is born in the hospital, newborn screening specimens are ordered. Five drops of blood are collected via heel stick on a standard filter paper form (the CARe form or Collection and Reporting Form). This should occur when the baby is 24 to 48 hours old, or immediately prior to discharge, whichever comes first. The form is allowed to dry at room temperature for about 4 hours and is shipped overnight to the testing laboratory within 24 hours of collection.

Once the testing laboratory receives the specimen, an accession number is assigned and all information is entered into the newborn screening database in the lab, and concurrently laboratory testing begins. In-lab turnaround time is generally about 1.5 days to complete all the testing, although some results are available earlier.

If all tests are normal, the lab sends the test results to the hospital. The hospital makes the test results available to the ordering physician.

If the specimen is determined to be unsatisfactory for testing, or if one or more tests are inconclusive or positive, the lab will notify the submitter and the newborn's physician (determined from the CARe form) which of the tests need to be repeated or need other confirmatory/diagnostic testing. The State Newborn Screening Program will also contact the baby's health care provider with further information for referral and diagnostic testing for positive screens and provide parent information sheets and Physician ACT (action) sheets. In urgent situations as determined by established protocols, the program will provide the information to the appropriate pediatric sub-specialist and connect them with the attending physician. Follow-up action by the health care provider should be done without delay to ensure the well-being and safety of the child.

On average, when all goes as planned (collection between 24-48 hours, overnight shipping, and in-lab testing and reporting), results are available between 4.5-5 days of age. Rapid turnaround time is critical to identify affected newborns and prevent neurological damage, other chronic health problems and death.

The State Newborn Screening Program follow-up personnel monitor and track every baby requiring any additional testing. They will stay connected with you via phone, fax and mail until necessary repeat or confirmatory testing has been completed and the program receives documentation of results with normal ranges, or verifying diagnosis and that treatment has begun. In most cases only a repeat screen is required. For most repeats we can track this via the laboratory data system. If repeat testing is conducted by another State newborn screening lab, or whenever any other confirmatory testing is required, the attending physician must report the results back to the newborn screening program including diagnosis and treatment information as appropriate.

Common Questions & Answers

Q: I have a parent who wishes to refuse the newborn blood-spot screening. Is there a waiver form they can sign?

A: No. Neb. Rev. Stat §§ 71-519 to 71-524 is explicit that "all newborns" born in Nebraska must be screened. The law holds the baby's attending physician responsible for ensuring the newborn screen is completed and that any needed follow-up occurs. In addition to the routine education you provide to parents about the benefits of newborn screening and the minimal risk of the heel stick procedure, it may be helpful to explain that the newborn screening law has been challenged several times in County, District, and Juvenile Court. Each time the ruling has been the baby must be screened. An appeal to the Nebraska Supreme Court also upheld the law that every newborn must be screened.

Q: I ordered the baby's newborn screen but I am not following this baby after discharge. Whose responsibility is it for follow-up?

A: The practice of having a Hospitalist or on-call physician or other health care provider see the baby in the hospital and order the newborn screen is becoming more common. The ordering physician is listed on the "Collection and Reporting Form" attached to the filter paper which functions as the laboratory test requisition. The form also requests the name and phone number of the baby's post discharge health care provider. If the baby is discharged without a post-discharge provider being documented, any follow-up will be the responsibility of the ordering physician. You are responsible for ensuring appropriate follow-up. If you know you will not be seeing the baby post-discharge you should ensure that upon discharge an attending physician or other health care provider is identified by the parents and recorded in the baby's medical record. When you are notified of an abnormal result after discharge and you are no longer seeing the baby, you should contact the identified baby's physician to ensure they know of the abnormal result, and the repeat or confirmatory testing that must be done. If the identified post-discharge physician denies knowledge of the patient, you should follow-up directly with the parents to ensure appropriate follow-up occurs. When personnel from the laboratory or state follow-up program contact you with abnormal screen results, you should notify them if you are not the baby's physician, of who is identified as the baby's post-discharge physician.

Q: I'm hearing concerns from parents about the Government keeping their baby's DNA? How can I respond with good information?

A: Different States have different practices on how long they keep any leftover dried blood spot specimens, as well as how they may be used. In Nebraska we do not keep the blood spots long term. They are kept for 90 days, and then in the following 30 day period they must be destroyed. Our laboratory ensures they are incinerated.

If during the short period that they are stored, someone wishes to use them for research, they can only do this if they first have IRB approval, parent/guardian consent and the approval of the Department of Health and Human Services' Chief Medical Officer. Only then can they be released, and only after the first 90-day storage period, because during that time the priority use of the specimen must be for the benefit of the baby. For example, some babies who are identified with hearing loss, benefit from further testing of the residual dried blood spot to identify if they had congenital cytomegalovirus (vs. acquired CMV) which may explain the hearing loss, and perhaps rule out other causes of syndromic hearing loss.

If you are caring for a baby born and screened in another State and parents have questions about residual blood spot storage, you can advise the parent's to check with that State's Newborn Screening Program about their policies for storage, use and disposal of residual dried blood spots. Contacts for all State Programs can be found at the national newborn screening resources center NewSTEPs.

Q: How important is it to get the specimen collected at 24-48 hours of age?

A: Every attempt is made to control variables that can affect test results in population screening in order to improve the sensitivity and specificity of results. In the case of timing, almost all reference ranges are based on specimens collected between 24-48 hours of age. For some conditions such as congenital primary hypothyroidism, the newborn has substantial changes in thyroxin (T4) and TSH during the first 24 hours, with recognizable patterns of normal and abnormal results in the next 24 hour period. For amino acidopathies such as PKU where phenylalanine and phenylalanine to tyrosine ratios are important indicators, it is important to get past the initial catabolic phase. Due to the sensitivity of tandem mass spectrometry testing, it is no longer important that the baby have 24 hours of feedings in order to detect elevations of phenylalanine suspicious of PKU. Many of the conditions screened for can affect the baby within days or weeks, so rapid intervention is essential, and collecting the specimen at the right time can help this.

Q:What if a specimen still hasn't been collected by 48 hours of age?

A: It is still essential to collect a newborn screen. Although the longer one waits, the higher the risk of delayed diagnosis and treatment for affected newborns and hence the increased risk for irreversible neurological damage, other health problems and infant death.

Q: I have a patient who delivered their baby at home and has not been to the hospital. What do I need to do?

A: All out of hospital births must also be screened. Order the newborn screen immediately and help the parent's navigate where to go. All birthing facilities have the ability to collect the specimens.

Q: What are the key things that can help me explain the importance of newborn screening to parents of my patients?

A: Babies with these conditions usually don't have signs and symptoms that are recognizable until the internal damage has begun. Therefore the blood test is necessary for early detection and treatment.

We screen for these conditions because there are treatments available, and if started early enough we can prevent serious problems like neurological damage and infant death, and at a minimum in other cases we can reduce the effects of the condition.

The parents might not have any family history (or known family history) for any of these conditions. In fact, in most cases when we find a new baby with one of these conditions, they do not have any known family history.

Q: I had a patient worried about their child's insurability if they have one of the conditions detected. What should I tell them?

A: Insurability may be an issue whether the child's condition is detected early or detected later. If the child is detected early, treated, and damage is prevented, or if the child isn't screened, presents with signs and symptoms (such as a metabolic crisis) and suffers severe neurological damage requiring a lifetime of care the insurance issue will probably be the same.

Q: Some parents react strongly with anxiety when we have them bring baby back for repeat or confirmatory testing. How can I balance the need for them to respond rapidly, with reassurance to minimize their anxiety?

A: Helping parents to understand the concept that "screening" is like casting a large net to catch a few fish might be useful. Finding those few babies that are affected but are difficult to find because they look so healthy at birth is the goal. So, sometimes there will be "false positives" or results that when they get retested, come back normal the second time. On the other hand, if the screen result was correct, and is verified by repeat or confirmatory/diagnostic testing, this is the best chance to help their baby live a healthy life, by finding out early and getting treatment. The more you understand about the special considerations for each test (see "Special Considerations" following the description of each screened condition), the better prepared you will be to answer further questions, and help explain other reasons why "false positives" sometimes occur.

Q: Can I get results STAT if I have a concern about a baby?

A: It is perfectly acceptable, and preferable to contact the newborn screening program (402) 471-0374 or laboratory (412) 220-2300 if you have a sick baby and suspect a possible condition on the screening panel. The laboratory will work to prioritize those requests for processing if possible and contact you with the results.

RECOMMENDED ON-LINE RESOURCES FOR PROFESSIONALS

Nebraska Newborn Screening Program Website (www.dhhs.ne.nsp)

- Find program overview, sections for Primary Care Physicians, Hospitals/NICU's, Parents, publications and more.

American College of Medical Genetics ACT Sheets: (http://www.acmg.net)

- Condition specific Action sheets to help physicians respond to positive newborn screen result

National Library of Medicine Genetic Home Resource pages: (http://ghr.nlm.nih.gov)

- Includes specific descriptions of screened disorders, genotype, phenotype

NewSTEPS (https://www.newsteps.org/)

Nebraska Speakers, Expert Resources Available to You

Contact the Nebraska Newborn Screening Program for Contact Information

Topic	Person	Affiliation
Cystic Fibrosis	John Colombo, MD	Director Cystic Fibrosis Center, member of NBS Advisory Committee
Endocrine conditions on screening panel	Zoe González-García, MD	Pediatric Endocrinologist, UNMC/Children's, member NBS Advisory Committee
Endocrine conditions on screening panel	Richard Lutz, MD	Geneticist, Pediatric Endocrinologist, UNMC/Children's member of NBS Advisory Committee
Hemoglobinopathies on screening panel	James L. Harper, MD	Pediatric Hematologist, UNMC/Childrens, member of NBS Advisory Committee
Newborn screening **Metabolic conditions on screening panel** **Endocrine conditions on screening panel** **Use of MS/MS in NBS**	Richard Lutz, MD	Board certified Pediatrics, Medical Genetics, Pediatric Endocrinology
Lysosomal Storage Diseases	William Rizzo, MD	Board certified Pediatrics, Biochemical Genetics
Metabolic conditions on screening panel	William Rizzo, MD	Board certified Pediatrics, Biochemical Genetics
NICU practices and newborn screening	Khalid Awad, MD	Board certified Pediatrics, Neonatology Perinatology,Medical Director, Methodist Women's Hospital NICU, Member NBS Advisory Committee
Newborn Screening Practices, Regulation requirements	Julie Luedtke	Program Manager, Nebraska Newborn Screening Program (402) 471-6733
Severe Combine Immune Deficiencies (SCID)	Hana Niebur, MD	Pediatric Immunology Specialist, Children's Specialty Physicians, Member of NBS Advisory Committee
X-ALD (Adrenoleukodystrophies)	William Rizzo,	Board certified Pediatrics, Biochemical Genetics

DISORDERS ON NEBRASKA's NBS PANEL

The following table (1) shows conditions in Nebraska's screening panel and which tests are used. Estimated incidence rates are derived from the National Newborn Screening and Genetics Resource Center data reported by states of confirmed cases from 2008 (the most recent year available at the time of publication). Those rates assume 4,255,198 births screened. In some cases State screening data reported did not reflect the full year as the particular State did not begin screening for a particular condition until sometime in the year.

For perspective, using the pre-July 2018 panel of 29 conditions screened, Nebraska screens approximately 26,000-27,000 babies per year, and identifies 50-60 newborns with clinically significant conditions each year. As of July 2018, Nebraska screens for 32 diseases.

Table I – Summary of Disorders Screened

Information Related to Disorders Included on the Nebraska's Screening Panel

Condition	Testing for	Effects if Not Treated	Treatment
AMINO ACID CONDITIONS including: - Argininosuccinic Acidemia (ASA) - Citrullinemia (CIT) - Homocystinuria (HCY) - Isovaleric Acidemia (IVA) - Maple Syrup Urine Disease (MSUD) - Methylmalonic Acidemia (MUT) or (Cbl A and B) - Phenylketonuria (PKU) - Propionic Acidemia (PA) - Tyrosinemia (TYR) - 3-Methylcrotonyl-CoA Carboxylase Deficiency (3-MCC)	Tandem Mass Spectrometry Amino Acid Profile	Varies depending on condition. Failure to thrive, metabolic acidosis, vision problems, skeletal problems, severe developmental disabilities, seizures, and possibly death.	Special metabolic formula and diet.
Biotinidase Deficiency	p-aminobenzoate	Developmental disabilities, seizures, deafness, blindness, skin rash	Daily oral Rx Biotin

Condition	Testing for	Effects if Not Treated	Treatment
Congenital Adrenal Hyperplasia (CAH)	Steroid 17-alpha hydroxyprogesterone levels/reflex to extracted 17-OHP on subset of specimens	Variable: ambiguous genitalia, adrenal "salt-wasting" crisis with possible mortality	Steroid replacement and monitoring by pediatric Endocrinologist appropriate emergency intervention
Congenital Primary Hypothyroidism (CPH)	Thyroxin (T_4) reflex to Thyroid Stimulating Hormone (TSH) on T4's in lowest 10%	Severe developmental disabilities and growth	Thyroid Hormone treatment
Cystic Fibrosis (CF)	Immunoreactive trypsinogen (IRT) levels/reflex to DNA on subset of specimens	Variable: pancreatic insufficiency, failure to thrive, decreased pulmonary function, respiratory infection risk , possible mortality	Management by Accredited CF Center Team
Fatty Acid Conditions including: - Carnitine Update Defect - Medium Chain Acyl Co-A Dehydrogenase Deficiency (MCAD) - Long-chain Hydroxyacyl-CoA Dehydrogenase Deficiency (LCHAD) - Trifunctional Protein Deficiency (TFP) - Very Long-chain Acycl-CoA Dehydrogenase Deficiency (VLCAD)	Tandem Mass Spectrometry Acylcarnitine Profile	Hypoglycemia, vomiting, coma, possible seizures & possible death. Possible developmental disability if survive metabolic crisis.	Prevent fasting, follow low-fat diet and carnitine supplements. If illness presents, hospitalization to prevent metabolic crisis.
Galactosemia	Total galactose & uridyl-transferase	Septicemia, cataracts, developmental disabilities, cirrhosis, ovarian failure, death if untreated	Lactose-free diet

Condition	Testing for	Effects if Not Treated	Treatment
Hemoglobinopathies Including Sickle Cell Disease, Sickle-Hemoglobin C Disease, and Sickle Beta Thalassemia	Hemoglobins F, A, S & C	Anemia, septicemia, painful crisis, acute chest syndrome, splenomegaly, stroke, high mortality rate	Penicillin prophylaxis, folic acid, parent education and counseling.
Lysosomal Storage Diseases: Mucopolysaccharidosis Type 1 (MPS-I) Pompe Disease (PD)	Deficiency of: Alpha L-Iduronidase (IDUA) Deficiency of: Lysosomal acid alpha-glucosidase (GAA)	MPS-I: Hurler's Syndrome most severe form with neurocognitive impairment, skeletal abnormalities, heart & lung disease and other problems. Spectrum disease includes Hurler-Scheie, and Scheie as the most mildly affected. Pompe: Infantile onset skeletal and cardiac muscle damage. Spectrum for childhood to adult onset.	Enzyme replacement therapy and hematopoeitic stem cell transplantation Enzyme Replacement Therapy
ORGANIC ACID CONDITIONS including: - Beta-ketothiolase Deficiency (BKT) - Glutaric Acidemia type 1 (GA1) - 3-Hydroxy 3-Methyl Glutaric Aciduria HMG)	Tandem Mass Spectrometry Amino Acid and Acylcarnitine profies	Metabolic crisis which includes: very low blood sugar, vomiting, possible seizures, coma and possible death Developmental problems may occur if the child has and survives the above. May also include heart problems.	Close monitoring with a metabolic specialist, special formulas, and diet.
Severe Combined Immune Deficiency (SCID)	T-cell receptor excision circles as a marker of T-cell production.	May suffer from repeated infections, death if untreated	Early intervention to prevent infection and bone marrow stem cell transplant.
VITAMIN METABOLISM CONDITIONS including: - Multiple Carboxylase Deficiency (MCD) - (Cbl A and B) amino acid and vitamin disorder	Tandem Mass Spectrometry Amino Acid and Acylcarnitine profiles Beutler and Baluda Enzyme Reation Units	Varies by condition but can include, mental retardation, seizures, nerve and brain cell damage and possibly death.	Vitamin supplementation (pharmaceutical doses) and monitoring by metabolic specialist.

- (Biotinidase Deficiency is also in this category but not screened by tandem mass spectrometry)			
X-Linked Adrenoleukodystrophy	Mass Spectrometry C26:O LPC	Varies by type. Childhood Cerebral form rapid demyelination, neurologic symptoms, spastic quadriplegia, seizures, fatal in childhood. Other forms involve adrenal glands or adult onset forms also can be fatal.	MRI monitoring for earliest signs of demylination, then Allogenic Hematopoietic Stem Cell Transplant/

Table II Summary of Test Result Interpretations and Recommended Protocol

Condition Screened and cut-offs	Interpretation of result (WNL = within normal limits)	Practitioner's response
Biotinidase Deficiency		
>8.1-16 ERU	Inconclusive for biotinidase deficiency.	Specimen has low enzyme activity but not in the typical profound deficiency range. Possible heat denatured specimen, profound, partial, carrier or false positive.
≤ 8 ERU	Positive for biotinidase deficiency.	Obtain a repeat dried blood spot specimen. Specimen has substantially low enzyme activity on initial screen, or continued low enzyme activity on second screen. Order a confirmatory serum specimen (with unrelated control specimen) per NBS program instructions. Consult with pediatric metabolic specialist.
Congenital Adrenal Hyperplasia		
Every specimen collected at > 24 hours is tested for 17-OHP and reference ranges are adjusted based on birthweight.	Specimen has an elevated 17-OHP above the critical cut-off and the extracted 17-OHP is pending and birthweight is > 2500 g. POSITIVE.	**Possible neonatal emergency**. Immediately assess the infant for signs of salt-wasting, admit to hospital for observation, consult with pediatric endocrinologist, and monitor electrolytes. Order Steroid Profile designated by specialist. Extracted 17-OHP from dried blood spot will be reported as soon as available.
Some reflex to an extracted-17OHP to eliminate some of the noise from interfering substances often associated with prematurity.	Specimen has an elevated 17-OHP above the critical cutoff, but the infant is < 2500 grams. Extracted 17- OHP is pending INCONCLUSIVE.	Request parent bring baby to clinic visit for assessment the day of notification. Consult with pediatric endocrinologist. Recommend Steroid Profile testing. If VLBW 17-OHP1 acceptable as requires less blood. Extracted 17-OHP will be reported out as soon as available. [If elevated, amended report of POSITIVE will be made, if normal amended report of WNL will be made.]
There are several classifications of abnormal results reported out, with specific protocols recommended for each. These	Specimen has an elevated 17-OHP but not above the critical cut-off,	Recommend repeat dried blood spot testing.

are designed to minimize anxiety and cost associated with high false positives that can be common with the CAH assay.	extracted 17-OHP is elevated, and baby's birthweight is <1500 g. INCONCLUSIVE. Specimen has an elevated 17-OHP but not above the critical cut-off and extracted 17-OHP is elevated, and the baby's birthweight is >1500g. INCONCLUSIVE.	Recommend repeat dried blood spot testing.
Congenital Primary Hypothyroidism All newborn specimens collected at > 24 hours are tested for T4. The lowest 10% of T4's reflex to TSH. If TSH ≥ 20 ng/mL is reported out abnormal.	T4 lowest 10% of run. TSH ≥ 20 mIU/L. Positive screen result for congenital primary hypothyroidism.	Obtain serum FT4 and TSH. Consult with pediatric endocrinology. May suggest nuclear thyroid studies.
Cystic Fibrosis All specimens collected at >24 hours are tested for immunoreactive-trypsinogen (IRT). Those collected < 12 days with elevated IRT's > 98.2% of the run collected ≥ 12 days with elevated IRT's > 80 ng/mL reflex to look for most common mutation associated with CF, ΔF508. If present, reflexes again to larger mutation panel. Note: If baby presents with Meconium Ileus or other bowel obstruction: report to screening	IRT> 98.2% of the run, no ΔF508 and IRT ≥ 130 ng/DL. (Inconclusive) IRT> 98.2% of the run and one copy of ΔF508. (Inconclusive) IRT> 98.2% of the run and two copies of ΔF508, or one copy ΔF508 with another mutation from the 39 + 4 CFTR mutation panel. (POSITIVE) MI: IRT results are often low, even though these babies are at increased risk of having cystic fibrosis.	Inconclusive screen for CF. Obtain repeat DBS. At least a carrier. Refer to Accredited CF Center for sweat testing and evaluation. POSITIVE for Cystic Fibrosis. Refer to Accredited CF Center for sweat chloride testing and evaluation. Report MI to laboratory. Testing will proceed on the mutation panel. Consider consult or referral to CF Specialist for evaluation.

lab. Test will automatically reflex to 39 + 4 CFTR mutation panel.	The IRT is not considered a reliable screen for babies with MI or other bowel obstruction. (2 mutations Positive; 1 or 0 mutations Inconclusive)	
Galactosemia The screen for galactosemia tests for galactose and GALT (uridyl transferase). Gal > 15 positive. Results assessed in conjunction with GALT.	Elevated galactose with normal GALT. Inconclusive screen result for Galactosemia, possible Duarte homozygote, Galactokinase Deficiency, or false positive. - - - - - - - - - - - - Elevated galactose with low GALT enzyme activity. Positive screen result for Galactosemia.	Obtain repeat dried blood spot specimen. **POTENTIAL NEONATAL EMERGENCY- Consult Pediatric Metabolic Specialist**. Interrupt breast or cow's milk formula feedings. Collect confirmatory whole blood and order galactose-1-phosphate uridyltransferase assay. Assess infant. May wish to check urine for reducing sugars. Consult pediatric metabolic specialist. Introduce powder-based soy formula after confirmatory specimen collected.
Hemoglobinopathies Results reported out in order of highest quantity to lowest. F= fetal hgb. A= Adult hgb. S= Sickle hgb. C= Hgb. C Barts= Barts hgb. V= all other variants. The screening lab reflexes to DNA when variants are identified by isoelectric focusing.	Anything other than FA is not a normal hgb. screen result.	Collect and order a newborn hemoglobinopathy electrophoresis confirmatory specimen. Most of the findings will be indicative of trait or carrier status. However, screen results of: FS, FSC, FC, FS+ Barts, FSA, F only, and FE are all likely clinically significant. FA + Bart's could involve a clinically significant thalassemia and should be followed to determine if one, two or three gene deletion. Repeat the screen if clinically significant hemoglobins are identified.

Condition Screened and cut-offs	Interpretation of result (WNL = within normal limits)	Practitioner's response
Amino acidopathies: ASA CIT HCY MSUD PKU TYR **Fatty acidopathies:** CUD MCAD LCHAD TFP VLCAD **Organic acidopathies:** BKT GAI IVA MMA(mutase) MMA(Cbl A&B) MCD PA 3-MCC HMG	All are screened by MS/MS. The acylcarnitine profile and amino acid profiles look for markers and relative ratio's of markers to determine results that are out of range. Results are reported out at: 1) Slight elevation 2) Above normal or moderate elevation, needs urgent report 3) Substantially elevated 4) Repeat specimen remains above normal	1) When result is reported out as slightly elevated, obtain a repeat dried blood spot newborn screening specimen. Newborn screening regulations require repeat specimens to be collected within 48 hours. 2) Result is reported that levels are elevated and the lab recommends obtaining a repeat dried blood spot newborn screening specimen within 48 hours. The NBS follow-up program recommends these be collected sooner. 3) When result is reported out as substantially elevated, this is a high alert situation. The newborn screening follow-up program will put you in touch with the pediatric metabolic specialist on service at that time 24/7. You will also receive a copy of a link to an ACT (action) sheet with recommendations for immediate next steps. Various confirmatory tests are recommended depending on the suspected condition, quantitative plasma amino acids, urine organic acids, etc. The metabolic specialist will be able to assist with recommending confirmatory tests, as well provide guidance on any needed immediate assessment of the infant. It is recommended these patients be referred to the pediatric metabolic clinic, for diagnosis and management. -Refer to laboratory report for recommended response.
Lysosomal Storage Disease: MPS-I	Enzyme alpha iduronidase (IDUA) level low on first > 24 hour specimen; Inconclusive. Low IDUA on repeat screen will reflex to	Collect a repeat dried blood spot screen.

	sequencing. Results will be reported out upon completion of sequencing	Consult with/refer to pediatric metabolic specialist
Lysosomal Storage Disease: Pompe	Enzyme acid alpha glucosidase (GAA) level low on first > 24 hour screen; Inconclusive Low GAA level on repeat screen will reflex to sequencing the GAA gene.	Collect repeat dried blood spot screen Evaluate cardiac status immediately (chest x-ray, ECG, ECHO) and for hypotonia. Order blood test for creatine kinase, and consult pediatric metabolic specialist. Do not wait for sequencing results to assess these things.
X-ALD	Lysophosphatidylcholine (C26:0) elevated on first > 24 hour screen and on reflex second tier FIA/MS/MS test. Inconclusive. C26:0 elevated on repeat screen which is done via FIA/MS/MS and will reflex to sequencing the ABCD1 gene. Results will be reported out upon completion of sequencing.	Collect repeat dried blood spot screen. Consult with and refer to pediatric metabolic specialist.

This is a condensed table. Please refer to the regulations (see appendix) for complete duties and responsibilities.

PLEASE NOTE:
THESE TESTS ARE SCREENING TESTS.
USUALLY ABNORMAL RESULTS NEED FULL EVALUATION AND CONSULTATION BEFORE A DIAGNOSIS IS CONFIRMED OR TREATMENT IS STARTED.

Treatment or intervention before confirmatory or diagnostic test results are available is ONLY recommended when positive screen results are found for MCAD (counseling on avoiding fasting), Galactosemia (soy formula) and Congenital Adrenal Hyperplasia (possible hospitalization). The pediatric endocrinologist will determine if treatment for congenital primary hypothyroidism should begin before the results of diagnostic tests are received.

BIOTINIDASE DEFICIENCY

Biotinidase is an enzyme that releases biotin, an essential vitamin cofactor, from a bound form so that it can be used by the body. Deficiency of the enzyme results in improper functioning of several other enzyme systems dependent on biotin, leading to neurological damage. This recessively inherited disorder affects the regeneration of biotin and leads to greatly reduced carboxylase activity. Nebraska's incidence of Biotinidase Deficiency has been strikingly higher than worldwide. Internationally the incidence of profound biotinidase deficiency is reported at 1:112,000 and partial deficiency at 1:129,0001. Nebraska's incidence of 52/474,833 births screened between 1991-2009 was 1:9,131 for partial and profound deficiency combined.

Clinical Features

The symptoms of biotinidase deficiency are variable with respect to age of onset, frequency and severity. Infants with biotinidase deficiency appear normal at birth but develop one or more of the following symptoms after the first few weeks or months of life: ataxia, seizures, hearing loss, alopecia, developmental delay, skin rash, or metabolic acidosis which can result in coma and death. Individuals with partial deficiency (a variant form) may also be at risk for development of symptoms. Family studies are indicated when an affected newborn is identified.

Laboratory Tests

Detection of enzyme activity is made by a qualitative colorimetric assay. In the presence of the enzyme, a color change occurs. Via chromatography a semi-quantitative measure converts to a value referenced in units of Enzyme Reactive Units.

Confirmation and Treatment

Consultation with a pediatric metabolic specialist should be made for confirmation and diagnosis (see Medical Consultants). Upon notification of a positive screening result the physician should collect or cause to be collected 0.5ml (minimum) of serum. The specimen must be frozen (sent on dry ice) and should be tested for biotinidase enzyme activity. A control specimen from an unrelated donor should be collected, handled and shipped at the same time. The acute symptoms of biotinidase deficiency will completely disappear with administration of pharmacological doses of biotin. If given early enough in an infant's life, the prognosis for normal growth and development is good. If children are not detected until neurological damage has occurred, treatment with biotin can reduce further damage but not reverse the damage already done.

ABNORMAL TEST RESULT	LIKELY CAUSES	Health Care Provider Actions
< 8.0 ERU (Positive) 8.1-<16 ERU (Inconclusive)	-Biotinidase deficiency -Partial Biotinidase deficiency -Enzyme in specimen denatured by heat and/or humidity -False positive	-POSTIVE: Notify parents, and obtain confirmatory serum specimen per program protocol (specifics will be communicated via phone.) Consult with pediatric metabolic specialist. Report lab tests, diagnosis and treatment to NNSP. -INCONCLUSIVE: Notify parents, and obtain repeat dried blood spot testing. Most often levels will normalize on repeat. If on repeat levels are still low, consult with and/or refer to pediatric metabolic specialist.

Screening Practice Considerations

Detection of the deficiency does not depend on timing or type of feeding because it is an enzyme test. It should therefore be detected on the first specimen unless the infant has been transfused. ***ALWAYS OBTAIN A NEWBORN SCREENING SPECIMEN PRIOR TO A TRANSFUSION.*** The enzyme is prone to damage if the sample is delayed in shipping or exposed to high temperatures resulting in a possible false positive test.

If the blood spot specimen is heavily applied, layered or double spotted, it may be deemed unsatisfactory for testing biotinidase deficiency because of the risk of a false negative result and a repeat specimen will be required.

Prompt confirmatory testing is required even if there is evidence to suggest that one of the situations associated with false positive screens is present. These situations can include heat-damaged specimen. The suspicion of this does not exclude the possibility of disease.

CONGENITAL ADRENAL HYPERPLASIA
(Salt-Wasting)

Congenital Adrenal Hyperplasia (classic/severe salt-wasting type due to 21-hydroxylase deficiency) is an inherited disorder of the adrenal cortex. Lack of adequate adrenal cortisol and aldosterone, and increased androgen production. The pituitary gland controls the adrenal glands. When the adrenals are not producing enough of their main hormone "cortisol", the pituitary pumps out adrenal-stimulating hormone (ACTH). The adrenals make Cortisol, Aldosterone (the salt retaining hormone) and Androgens all from cholesterol. In CAH the enzyme necessary to produce both Cortisol and the salt retaining hormone is missing. This results in insufficient cortisol and aldosterone. The feedback loop tells the pituitary not enough of these are being made, so it produces more adrenal stimulating hormone resulting in an overproduction of androgens, resulting in virilization of female newborns.

Clinical Features

Salt losers can experience acute dehydration, very low blood pressure, nausea and vomiting, and failure to thrive. The levels of sodium chloride and glucose in the blood fall and the potassium rises. Female infants may be detected clinically by ambiguous genitalia. Males are at highest risk of being undetected without screening. Long term management with pediatric endocrinology is recommended to monitor steroid levels, precocious puberty, rapid early bone growth which may result in short stature. Infants with Congenital Adrenal Hyperplasia are at risk for life-threatening adrenal crises, shock, and death in males and females. Emergency treatment of hospitalization, IV hydrocortisone and IV glucose may be indicated.

Lab Test

The screening test for CAH is an immunoassay for 17-OHP (17-hydroxyprogesterone), and normal reference ranges, cut-offs, and critical cut-offs are based on stratified birthweight ranges, as lower birth weight newborns tend to have higher 17-OHP's on average. While the assay for 17- OHP is considered the state of the art for newborn screening, it tends to have higher false positive rates than for other conditions screened for, particularly in premature low birth weight babies. Therefore elevated 17-OHP's reflex to an extracted 17-OHP assay designed to reduce some of the interference or "noise" associated with 17-OHP's in blood spot specimens especially from premature newborns. Because the premature/LBW babies more frequently have abnormal results, the lab algorithm has "preliminary" reports to alert the NICU providers to monitor electrolytes, without unnecessarily triggering confirmatory tests. Specimens collected at less than 24 hours of age will not be tested for CAH.

ABNORMAL TEST RESULTS	LIKELY CAUSES	Health Care Provider Actions
17-OHP above the critical cut-off for weight range and baby is > 2500g. POSTIVE report sent, Extracted assay to be run.	(applies to all abnormal results) Salt-wasting CAH -Simple virilizing CAH -False positive -Prematurity or stress	-POSTIVE: Possible neonatal emergency. Consult with pediatric endocrinologist. Notify parents, evaluate newborn immediately and admit to hospital for monitoring. Order confirmatory serum specimen for steroid profile and monitor electrolytes. Ensure steroid profile is tested at laboratory with established neonatal reference ranges. Report lab tests, diagnosis and treatment to NNSP.
17-OHP above the critical cut-off for weight range but baby is < 2500 g. Preliminary positive reported. Extracted assay run first. If elevated, report amended to Positive. If normal report amended to WNL.		-PRELIMINARY POSITIVE: If NICU admission monitor electrolytes, watch for signs/symptoms. If discharged to home contact parents and assess status. Contact pediatric endocrinologist if concerns. If Positive after extracted result is reported, follow steps as above for POSITIVE.
17-OHP above reference range, but not above the critical cut-off. Extracted 17-OHP above reference range. Inconclusive.		INCONCLUSIVE. Obtain repeat dried blood spot specimen. Assess infant.

Confirmation & Treatment

For POSITIVE screen results the infant should be assessed immediately. If the baby is not already in the NICU or has been discharged to home, they should be admitted and evaluated. Serum electrolytes should be monitored. IV hydrocortisone, fluids and glucose may be recommended. Contact the pediatric endocrinologist on service. A steroid profile to confirm should be ordered.

Screening Practice Considerations

NOTE: The screening test for CAH cannot detect all forms of CAH (e.g. 11-hydroxylase deficiency), and may not detect all simple virilizing forms.

While abnormal screen results in premature or low birth weight infants may be more common and be associated with stress or prematurity, treatment of POSITIVE screen results should be treated as urgently as for full term newborns.

Clinical Considerations:

Ambiguous genitalia in females who may appear to be male with non-palpable testes, may be present in a newborn with congenital adrenal hyperplasia, salt –wasting or simple virilizing. Because less than 24 hour specimens result in an unacceptably high abnormal rate, these specimens are no longer tested for CAH. Therefore obtaining a repeat after 24 hours becomes critical, as does vigilance to watch for the signs and symptoms.

CONGENITAL PRIMARY HYPOTHYROIDISM

Congenital primary hypothyroidism, or CH or CPH, is the most common disorder detected by newborn screening. It is caused by inadequate production of thyroid hormone. Thyroid hormone is important for the normal functioning of all of the body's organs and is essential for normal brain development. The incidence of congenital primary hypothyroidism is estimated at 1:3,000 to 1:4,000 live births.

The most common causes of CPH are total or partial failure of the thyroid gland to develop (aplasia or hypoplasia), or its development in an abnormal location (an ectopic gland). The screening program is not designed to detect less common causes of hypothyroidism such as those caused by pituitary insufficiency.

Clinical Features

Deficiency of thyroid hormone in an infant may result in intellectual disability and growth retardation if it is not diagnosed and treated early in life. Many infants with CPH who are untreated may appear clinically normal before 3 months of age, by which time some brain damage has usually occurred.

When symptoms or signs are present, they may include prolonged neonatal jaundice, constipation, lethargy and poor muscle tone, feeding problems, a large tongue, puffy face, large fontanelle, distended abdomen, and umbilical hernia. However, these signs and symptoms are non-specific for CH, are found in fewer than 30% of neonates with CH, and may be present in infants without the condition. Therefore, in the newborn, clinical signs and symptoms are not reliable indicators of CH.

Laboratory Tests

The primary screening test for thyroxine (T4) level is performed for every newborn. Samples in the lowest 10 percentile of T4 results then reflex to test for TSH using a second punch from the same sample. Specimens collected at less than 24 hours of life will not be tested for T4/TSH due to the expected physiologic surge of the TSH levels. The confirmatory test is a Free T4 and TSH immunoassay performed on a serum sample.

ABNORMAL TEST RESULTS	LIKELY CAUSES	Health Care Provider Actions
T4 low/TSH elevated	-Hypothyroidism -False positive -Prematurity (transient hypothyroidism or hypothyroidism of prematurity)0	-POSTIVE: Notify parents, and obtain confirmatory serum specimen. Ensure specimen is tested at laboratory with established neonatal reference ranges for Free T_4 and TSH. Consult with pediatric endocrinologist. Report lab tests, diagnosis and treatment to NNSP.
T4 low/ TSH normal	•Thyroid Binding Globulin Deficiency (TBG) deficiency •False positive •Hypothalamic or Pituitary gland problems with secondary or tertiary hypothyroidism •Prematurity	For purposes of screening for congenital primary hypothyroidism, this result is normal.
T4 high/ TSH elevated	-Hyperthyroidism (extraordinarily rare in infants)	For purposes of screening for congenital primary hypothyroidism this result is normal.

Different combinations of screening test results are possible:

Thyroid Function in Premature Infants

In premature infants, there is a physiological reduction in blood T4 levels. This is generally thought to be due to immaturity of the hypothalamus- pituitary-thyroid axis. This is not due to a low thyroid binding globulin (TBG) and the TSH levels may or may not be elevated. These cases need special observation and follow-up to ensure that the low T4 levels rise to the normal range as the infant matures, but this may take several weeks.

Confirmation and Treatment

Consultation with a pediatric endocrinologist should be made for confirmation and diagnosis. (See Medical Consultants). Upon notification of a positive screening result the physician should order Serum FreeT4 and TSH testing to be collected - 200µl of serum specimen. Treatment of congenital primary hypothyroidism is simple and effective. Thyroid hormone in pill form, is crushed, mixed with food and administered once daily. Alternatively, it can be compounded into liquid formulation, but this is only stable for 1 week, and may not suspend uniformly. It is strongly encouraged that infants diagnosed with CPH be followed by a pediatric endocrinologist for exams and blood T4 and TSH levels to assist in regulating the thyroxine dosage, and in managing the "challenge" to rule out transient hypothyroidism. Infants and children should also undergo periodic developmental testing. In Nebraska patients age 0-3 can obtain developmental testing through the Early Development Network (the Early Intervention Part C program).

Screening Practice Considerations

Congenital primary hypothyroidism is the most common disorder detected by the program. Detection does not depend on nutritional factors. A blood transfusion may alter the values; therefore ***THE NEWBORN SCREENING SPECIMEN SHOULD ALWAYS BE COLLECTED PRIOR TO A BLOOD TRANSFUSION.***

The normal newborn demonstrates a TSH surge in the first hours of life as an adaptation to the extra-uterine environment. To be valid, a specimen must be collected when the infant is at least 24 to 48 hours of age. Less than 24 hour specimens will not be tested for CPH. If an infant's specimen is collected "early" (less than 24 hours of age) a repeat specimen must be collected within seven (7) days of age but preferably between 24-48 hours of life. Most often early collections are due to premature, low birth weight or sick newborns being admitted to the NICU. In these cases a repeat screen needs to be collected at 48-72 hours.

Prompt confirmatory testing is required even if there is evidence to suggest that one of the situations associated with false positive screens is present. These situations can include early specimen collection and prematurity. The presence of prematurity does not exclude the possibility of disease.

PLEASE NOTE: This screening test is not designed to detect TBG deficiency or causes of hypothyroidism other than congenital primary hypothyroidism. In addition, some infants develop late onset of congenital primary hypothyroidism. If TBG deficiency or other forms of hypothyroidism is suspected, or in the presence of clinical symptoms, appropriate exams and studies are indicated. Therefore, in the presence of clinical symptoms evaluation for hypothyroidism should be performed despite normal newborn screening results.

CYSTIC FIBROSIS

The cystic fibrosis transmembrane conductance regulator (CFTR) protein regulates chloride transport that is important for function of lungs, upper respiratory tract, pancreas, liver, sweat glands, and genitourinary tract. CF affects multiple body systems and is associated with progressive damage to respiratory and digestive systems. On the NBS panel CF is one of the most common conditions that is screened for with an incidence rate of approximately 1:3500 white infants, 1:7,000 Hispanic infants, and 1:15,000 black infants. Early diagnosis with newborn screening and comprehensive treatment through an Accredited CF Center is associated with improved growth, improved pulmonary function, reduced hospitalizations, and reduced costs.

Clinical Features

Prior to screening infants/children would present with meconium ileus, failure to thrive, recurrent cough, wheezing and chronic abdominal pain. Deficient chloride transport in lungs causes production of abnormally thick mucous leading to airway obstruction, neutrophil dominated inflammation and recurrent and progressive pulmonary infections. Pancreatic insufficiency is found in 80 – 90% of cases. Most males are infertile. More than 1500 mutations in the CFTR gene have been identified, but the ΔF508 mutation is associated with 70% of patients from European ancestry, and is highly associated with pancreatic insufficiency.

Lab test

The primary screening test for CF is an assay for Immuno-reactive trypsinogen (IRT). When specimens collected at < 12 days have IRT's in the top 1.2% of the run and in specimens collected > 12 days with IRT's greater than 80 ng/mL, the testing reflexes to DNA analysis for the ΔF508 mutation. If no ΔF508 is found, a repeat dried blood spot is recommended if the IRT is >130. If one copy of ΔF508 is found the test reflexes again on a new punch from the initial or same specimen to look at a 39 +4 CFTR mutation panel of some of the more commonly found mutations. If one or two mutations are found, referral to an Accredited CF Center and sweat testing is recommended. Specimens collected at less than 24 hours of life will not be tested for CF due to the high rate of inconclusive reports due to commonly elevated IRT levels in < 24 hour specimens.

ABNORMAL TEST RESULTS	LIKELY CAUSES	Health Care Provider Actions
Inconclusive due to elevated IRT but no copies ΔF508 mutation	IRT elevation due to other causes - asphyxia, Trisomy, prematurity, liver or GI dysfunction, CF carrier state, CFTR-Related Metabolic Syndrome (CRMS), CF	Repeat NBS to recheck IRT as directed
Inconclusive due to repeat elevated IRT and one or less mutations	IRT elevation due to other causes - asphyxia, Trisomy, prematurity, liver or GI dysfunction, CF carrier state, CRMS, CF	Refer to Accredited CF Center for evaluation
Inconclusive due to elevated IRT with one copy of ΔF508 mutation	CF carrier state, CRMS, CF, lab error	Refer to Accredited CF Center for evaluation and sweat testing
Inconclusive due to meconium ileus/bowel obstruction with one or less mutations	Bowel obstruction without CF, CF carrier state, CF	Refer to Accredited CF Center for evaluation and sweat testing
Positive due to elevated IRT and two CFTR mutations or meconium ileus with two CFTR mutations	CRMS, CF carrier state, CF	Refer to Accredited CF Center for evaluation and sweat testing

Confirmation and Treatment

Any newborn with a persistently elevated IRT (on repeat) or elevated IRT with one or more mutations should be referred to an Accredited CF Center for evaluation, sweat testing and diagnosis. Diagnosed patients should receive comprehensive care including genetic counseling, pulmonology, nutrition, radiology and infectious disease services.

Special Considerations

Meconium Ileus: If a baby is found to have meconium ileus (not meconium staining of amniotic fluid) or other bowel obstruction, the newborn screening program and laboratory should be notified immediately. The IRT assay for newborns with MI or other bowel obstruction is known to often have suppressed levels and is therefore not reliable for screening. For babies with MI or other bowel obstruction, notifying the newborn screening laboratory will trigger testing for mutations on the 39 + 4 CFTR mutation panel.

It is especially important to recognize that screening for Cystic Fibrosis has somewhat lower sensitivity than other conditions on the screening panel. Health Care Providers should consider evaluation for cystic fibrosis in patients presenting with symptoms, even if newborn screening results were normal. In addition each State NBS Program has different screening algorithms with different levels of sensitivity and specificity, so if the infant or child was born outside Nebraska, a different screening algorithm may have been used.

There are numerous reasons for elevated IRT in newborns, including specimen collection at < 24°, asphyxia, stress, Trisomies 13, 18, 21, septicemia, congenital cytomegalovirus, obstructive liver disease, biliary atresia. African American babies tend to have higher numbers of elevated IRT's but a lower incidence of diagnosed cystic fibrosis.

NOTE: Regarding the Screening Algorithm:

Based on new information learned after the publication of CLSI guidelines for newborn screening for CF, the Program, Laboratory and CF Representatives of the NBS Advisory Committee evaluated prior data, and determined that on specimens collected at 12 days of age or later, a different cut-off was in order to more safely identify newborns with CF. The effective date of this change: 4/02/2012

GALACTOSEMIA

Galactosemia is an autosomal recessive inherited metabolic disorder. Abnormal galactose metabolism can be caused by the deficiency of any of the three enzymes of the galactose catabolic pathway: galactose-1-phosphate uridyl transferase, galactokinase, or UDP-galactose-4-epimerase. Clinically, deficiency of galactose-1-phosphate uridyl transferase has become synonymous with classical galactosemia. Because Nebraska's screening test is for galactose and GALT (transferase), the targeted condition for screening is galactosemia associated with the deficient enzyme galactose-1-phosphate uridyl transferase. Classic Galactosemia (transferase) occurs in approximately 1:47,000 infants.

Galactose is a component of lactose, the principle carbohydrate of mammalian milk and most non-soy commercial infant formulas. Lactose is hydrolyzed to glucose and galactose in the intestine. The body cannot break down the galactose without the proper enzymes. The galactose builds up in the body and causes cellular damage and even death. The incidence of galactosemia is estimated at 1:60,000 to 1:80,000 live births.

Clinical Features

The severe form of this disease is due to almost total deficiency of galactose-1- phosphate uridyl transferase enzyme activity in all cells of the body. The early clinical features of severe galactosemia include liver dysfunction manifest as jaundice and hypoglycemia; neurological findings of irritability and seizures; and gastrointestinal findings of poor feeding, failure to thrive, vomiting, and diarrhea. Death may result from gram-negative sepsis within one to two weeks of birth. If the infant is untreated and survives the neonatal period, cataracts, cirrhosis, Fanconi syndrome, and neurological damage are usual developments.

There are several genetic variants characterized by a less severe reduction in enzyme activity (e.g. Duarte Variant). Most of these cases are asymptomatic and are detected because of a persisting abnormality in the enzyme test. However, some of these cases may benefit from dietary therapy. For this reason, infants with elevated galactose and normal GALT require further testing and should be evaluated by a pediatric metabolic specialist.

Laboratory Test

The Beutler and Baluda qualitative screening test is used. Dried blood spots are incubated with galactose-1-phosphate, uridine diphosphoglucose, and NADP. In the presence of galactose-1-phosphate uridyl transferase, a sequence of enzyme reactions proceeds with generation of NADPH which is detected by fluorescence or colorimetrically.

LAB RESULTS	LIKELY CAUSES	Health Care Provider Actions
POSITIVE - Elevated Galactose, Low Uridyl transferase	-Severe galactosemia -Improperly collected sample (heat damage or transit delay) -Mild galactosemia variant -Other enzyme defect in RBC's -False positive	POSITIVE: Potential Neonatal Emergency. Consult with pediatric metabolic specialist. Contact parents and obtain serum confirmatory test for galactose-1-phosphate uridyl-transferase. Interrupt breast or mammalian milk formula and initiate powder- based soy formula. Report lab tests, diagnosis and treatment to NNSP.
Inconclusive - Elevated Galactose, Normal GALT	(same as above)	Inconclusive: Contact parents and obtain repeat dried blood spot specimen testing. Most often repeats will be normal. If galactose is still elevated on repeat, refer to pediatric metabolic specialist.

Confirmation and Treatment

Consultation with a pediatric metabolic specialist should be made for confirmation and diagnosis (see Medical Consultants). Upon notification of a positive screening result the physician should direct the family to interrupt breast feeding and stop the feeding of lactose or galactose containing formulas. A powder-based soy formula is recommended. The physician should collect or cause to be collected 2 ml. of whole blood, lithium heparnized (green top) and order a confirmatory test for galactose- 1-phosphate uridyl-transferase. The galactosemia syndromes are treated by a rigid dietary exclusion of all galactose. Exclusion of milk and milk products alone does not constitute a galactose-restricted diet, as galactose is found in other foods as well. A galactose-free diet requires close supervision and monitoring. Referral to a pediatric metabolic specialist is strongly recommended.

Screening Practice Considerations

The Beutler test should be abnormal in all severe (classical) galactosemic infants - even if the specimen is collected before lactose is ingested - unless the infant has been transfused. ALWAYS OBTAIN A NEWBORN SCREENING SPECIMEN PRIOR TO A TRANSFUSION.

Galactosemia can kill quickly. It should be considered in any infant with non-glucose reducing substances in the urine. If galactosemia is suspected, immediately consult with a pediatric metabolic physician. In some cases the specialist may recommend testing urine for reducing sugars, in addition to the confirmatory test.

NOTE: Galactose reacts with “Clinitest” and with most blood “glucose” methods, such as Somogyi-Nelson, etc., which measure total blood sugars (but not with glucose oxidase methods, i.e. Clinistix). Galactosemia produces a positive Clinitest and a negative Clinistix result.

Prompt confirmatory testing is required even if there is evidence to suggest that a situation associated with false positive screens is present. One of these situations can include heat-damaged specimen. The presence of this does not exclude the possibility of disease.

If screening test results are positive for galactosemia, the physician shall take the child off milk and then collect or cause to be collected a new specimen for confirmatory tests. A powder based soy formula is recommended.

HEMOGLOBINOPATHIES

Hemoglobins are reported in order of quantity (e.g., FAS = F>A>S).

F = fetal hemoglobin
A = adult hemoglobin
S = sickle hemoglobin
V = unidentified migrating band variant
C = hemoglobin C

Most clinically significant hemoglobinopathies are inherited defects of the beta (β) globin chain of adult hemoglobin. Red blood cells of newborns have a predominance of fetal hemoglobin which does not contain β globin. For this reason, clinical signs and symptoms of β globin abnormalities are usually not apparent at birth but become evident as adult hemoglobin replaces fetal hemoglobin.

Most of the hemoglobinopathies detected by newborn screening are the result of single amino acid substitutions in the β globin and are inherited as autosomal recessive disorders. Persons with two abnormal β globin genes (homozygotes or double heterozygotes) have "disease." Individuals with one abnormal β globin gene (heterozygotes) have "trait" and are carriers for "disease." That is, individuals can pass on "trait" or potential for "disease" to offspring.

Thalassemias are caused by the decreased synthesis of the globin chains. Infants with α thalassemia may be identified by newborn screening because Barts hemoglobin (composed of four gamma chains) is detected. β thalassemia genes may interact with genes for β globin variants and produce serious hemoglobinopathies, but may not be detectable at birth..

The frequency of hemoglobinopathies varies among ethnic groups in the United States. Sickle hemoglobin is found in higher frequencies in descendants of people from Africa, Italy, Greece, Turkey, Arabia, and India.

Hemoglobin C occurs predominantly (but not exclusively) among descendants of people from central and western Africa. Hemoglobin E is common in persons of Southeast Asian ancestry. Many thalassemia genes originated in west and central Africa, Italy, Greece, Asia, Africa, and the Pacific Islands. Overall sickle cell disease occur in 1:2500 to 1:2000 US newborns and is estimated to occur in 1:346 black infants, and in 1:1114 Hispanic infants in the Eastern US.

Clinical Features

Sickle cell disease occurs in persons homozygous for the sickle gene (sickle cell anemia), in compound heterozygotes for sickle hemoglobin and hemoglobin C (hemoglobin SC disease), compound heterozygotes for sickle hemoglobin and β thalassemia (sickle-β thalassemia), (also possible SE, SD, and SO arab disease).

Infants with sickle cell disease may present with dactylitis, fever and sepsis, jaundice, anemia, or splenic sequestration (which may be life threatening in a small child) at any time after a few months of age. Other symptoms may occur as the disease progresses, such as recurrent pain, acute chest syndrome, and stroke. These disorders vary in severity, and some clinical features are not present in all affected individuals.

In other hemoglobin diseases, clinical features are influenced by the type of hemoglobin variant. Homozygous hemoglobin C or hemoglobin E show only mild hemolytic anemia. Persons with thalassemias have varying degrees of microcytic hypochromic anemia, and the severe forms (E-β thalassemia) may have hemolysis and be transfusion dependent.

Comprehensive medical care coordinated with a pediatric hematologist or pediatrician specializing in hemoglobinopathies reduces mortality in infancy and early childhood.

Most hemoglobin traits (heterozygotes) are associated with few or no clinical problems in childhood. Thus, the value of trait or carrier detection is the opportunity to educate families, to test other family members, and to provide genetic counseling.

TEST RESULTS	**LIKELY CAUSES**	**Health Care Provider Actions**
FA	-Normal	- Report normal result to parents
FAS	-Sickle cell trait -Sickle cell anemia after blood transfusion	-Confirmatory testing -Offer genetic counseling to family -Report lab results and diagnosis to NNSP
FSA	-S-β thalassemia	-Repeat dried blood spot to confirm -Offer genetic counseling to family -Referral to pediatric subspecialist -Report lab results, diagnosis and treatment to NNSP
FAC	-Hemoglobin C trait	-Confirmatory testing -Offer genetic counseling to family -Report lab results and diagnosis to NNSP
FAV	-Possibilities include many hemoglobin traits	-Confirmatory testing -Offer genetic counseling to family -Report lab results and diagnosis to NNSP
FA + Barts‡	-Possibilities include hemoglobin Barts which is indicative of α thalassemia	-Confirmatory testing -Offer genetic counseling to family -Consider referral to pediatric subspecialist in hemoglobinopathies -Report lab results, diagnosis and treatment to NNSP

TEST RESULTS	LIKELY CAUSES	Health Care Provider Actions
F only	-Premature infant -β thalassemia major -Hereditary persistent fetal hemoglobin	-Confirmatory testing -Offer genetic counseling to family -Referral to pediatric subspecialist in hemoglobinopathies -Report lab results, diagnosis and treatment to NNSP
AF Predominant A	-Transfused infant	-Normal matured infant -If this was on initial specimen, verify transfusion. If no screen before transfusion, re-screen at 120 days post transfusion.
FV (no A)	-Possibilities include homozygous Variant or Variant beta thalassemia	-Confirmatory testing -Offer genetic counseling to family -Referral to pediatric subspecialist in hemoglobinopathies -Report lab results, diagnosis and treatment to NNSP
FS (no A)	-Sickle cell anemia -Sickle-β thalassemia	-Repeat dried blood spot to confirm -Offer genetic counseling to family -Referral to pediatric subspecialist -Report lab results, diagnosis and treatment to NNSP
FSC (no A)	-Sickle-hemoglobin C disease	-Repeat dried blood spot to confirm -Offer genetic counseling to family -Referral to pediatric subspecialist -Report lab results, diagnosis and treatment to NNSP
FC (no A)	-Homozygous hemoglobin C	-Repeat dried blood spot to confirm -Offer genetic counseling to family -Referral to pediatric subspecialist -Report lab results, diagnosis and treatment to NNSP

Laboratory Tests

Screening is performed by isoelectric focusing (IEF) of a hemolysate prepared from a dried blood spot. Hemoglobin "bands" are identified by the migration in the electrophoretic field. The newborn screening test is highly sensitive and specific. When screen results are positive for an abnormal hemoglobin, a new punch from the same specimen is tested at the screening laboratory for DNA associated with hemoglobins S, C, E, D, and O arab. If there is evidence of a possible β thalassemia, a panel of 3 possible β thalassemia mutations is checked. However there are many other mutations that cause β thalassemia.

Barts hemoglobin is a fast migrating band that is present at birth in infants with α thalassemia and generally disappears as fetal hemoglobin production decreases. Thus, infants with an unidentified fast band on the screening tests and with a normal confirmatory test at later age should be suspected of having α thalassemia, especially if they have microcytosis and are of Asian, Mediterranean, or African ancestry. However, the absence of an unidentified fast band on the screening test does not exclude the possibility of α thalassemia.

Confirmation and Treatment

Consultation with a pediatric hematologist should be made for confirmation and diagnosis for clinically significant hemoglobinopathies. (see Medical Consultants). Upon notification of a positive hemoglobinopathy screening result the physician should collect or cause to be collected 0.5 ml (purple top) EDTA whole blood and order a confirmatory hemoglobinopathy test. It is important to obtain confirmation results and a diagnosis by 2 months of age for some clinically significant hemoglobinopathies (such as sickle cell disease) in order to initiate prophylactic treatment. Treatment for hemoglobin diseases is determined by the type and severity of the hemoglobin disorder. Since the screening lab reflexes to DNA when clinically significant hemoglobins are found (e.g. FS, FC, FSC, FSA) a repeat screen on a new specimen will suffice for confirmation. The screening lab does not charge for these repeats on clinically significant hemoglobinopathies.

Screening Practice Considerations

The laboratory tests should detect most abnormal hemoglobin subtypes, even if the specimen is collected before 24 hours of age, unless the infant has had an exchange transfusion. Blood transfusions may cause false negative results. ***ALWAYS OBTAIN A NEWBORN SCREENING SPECIMEN PRIOR TO A TRANSFUSION.***

The primary purpose of hemoglobinopathy screening is the identification of infants with sickle cell diseases for whom early intervention has been shown to markedly reduce morbidity and mortality. The screening test is not diagnostic and confirmation of all abnormal results should be done.

The most commonly identified abnormal phenotype through newborn screening is FAS which is presumably positive for sickle cell trait. Parents of infants with sickle cell trait have a chance of having an infant with sickle cell disease if both parents carry the gene for sickle hemoglobin. Further testing of both parents combined with genetic counseling can apprise parents of their chances in future pregnancies of having children affected with clinically significant disease. Every infant with a presumptive positive hemoglobinopathy screening result, must have confirmatory testing done in a timely manner.

Hemoglobinopathies are complex disorders, and practitioners are strongly encouraged to consult a pediatric hematologist and follow-up resources for more information concerning abnormal screening results and appropriate follow-up and treatment.

MCAD

Medium Chain Acyl Co-A Dehydrogenase Deficiency*

*This practitioner's manual describes one condition from each of the 3 classifications (AA's, FAO's, and OA's) of disorders screened for by tandem mass spectrometry (MS/MS). MCAD is an example of a Fatty Acid Oxidation (FAO) disorder detectable by this screen. While other FAO's may have similar presentations and characteristics, each are distinct. For more information on each condition we recommend referencing the ACMG's ACT sheets at http://www.ACMG.net , Genetic Home Reference at http://ghr.nlm.nih.gov and tables included in "Toward a Uniform Newborn Screening Panel" Genetics in Medicine, Vol 8, Supp 1, May 2006

MCAD is the most common of the 10 disorders of mitochondrial fatty acid oxidation identified to date. Prior to universal screening MCAD was later found to be the cause of death in infants and children in some cases of SIDS and Reye syndrome. MCAD is found almost exclusively in individuals from northwestern European origin, with incidence approximately 1:15,011. Classic presentation includes episodes of hypoketotic hypoglycemia triggered by fasting because of the inability to break down fats to ketone bodies for an energy source. Medium chain acylcarnitines accumulate in the mitochondria inhibiting mitochondrial β-oxidation. Fat accumulates in the liver during acute episodes. Secondary to the accumulation of fatty acids in the central nervous system, encephalopathy and cerebral edema occur. The combination of hypoglycemia and toxic effects of the fatty acids or metabolites contributes to coma.

Clinical Features

During periods of fasting, (e.g. gastrointestinal or upper intestinal viral infection, rarely neonatally when full breast feedings not yet established, or extended periods of time between feedings) episodes of vomiting and lethargy, may be followed by seizures, coma and death. Infants who survive the metabolic crisis may have developmental disabilities, speech and language delay, behavioral problems, ADHD, proximal muscle weakness, chronic seizure disorder, cerebral palsy and failure to thrive. Newborns appear normal at birth. Avoidance of fasting more than 4 hours during neonatal period, IV glucose and carnitine during times of viral illness and monitoring by a metabolic specialist can avoid the devastating outcomes. Some patients may receive supplemental carnitine.

Maternal HELLP syndrome

A higher suspicion of MCAD or other fatty acid oxidation disorders should occur when the mother is known to have HELLP syndrome (Hemolytic anemia, elevated liver enzymes and low platelet count) or acute fatty liver disease.

Laboratory Test

The screening test for MCAD is MS/MS analysis of acylcarnitines. Elevations in the C8 (octanoylcarnitine), or C8 plus elevated ratios of C8:C6 and C8:C10 are abnormal , indicating MCAD. As with all MS/MS results the interpretation will be reported out as slightly elevated, abnormal or substantially elevated. Specimens with abnormal or substantially elevated C8 results reflex to DNA for the most common mutation known to be associated with MCAD, A985G.

ABNORMAL TEST RESULT	LIKELY CAUSES	Health Care Provider Actions
Mild elevation of C8 Abnormal level of C8 and or C8:C6 or C8:C10 Substantial elevation of C8 and or C8:C6 or C8:C10 possibly with A986G mutation	MCAD False positive	-Obtain repeat dried blood spot specimen testing within 48 hours -Obtain repeat dried blood spot specimen testing immediately -Obtain quantitative plasma acylcarnitines, urine organic acids. -Consult with and refer to pediatric metabolic specialist -Report confirmatory lab results, diagnosis and treatment to NNSP

Confirmation and Treatment

Consultation with a pediatric metabolic specialist should be made for confirmation and diagnosis (see Medical Consultants). Upon notification of a positive screening result (substantial elevation), the physician should order quantitative plasma acylcarnitines and urine organic acids. Parents should be counseled to not allow the infant to go more than four (4) hours between feedings. The child should be admitted to the hospital for signs/symptoms of viral illness. With early and proper treatment the devastating outcomes can be avoided.

Special Considerations

MCAD is a relatively easy, inexpensive and effectively treated condition by preventive measures. MCAD like all conditions presents as a spectrum of disease, even within families with multiple members affected. One example of a family with several affected children had one child die from the first episode before 2 years of age, and other children as old as 10 years never having had an episode. The genotype/phenotype correlation is not yet well understood, nor are modifying factors.

MPS-I Mucopolysaccharidosis type I

MPS-I

MPSI is a lysosomal storage disease that is caused by deficiency of alpha-L-iduronidase and buildup of glycosaminoglycans (GAGs, formerly known as mucopolysaccharides) in tissues. The disease affects approximately 1 in 100,000 individuals. Historically, MPSI has three major phenotypes based on clinical severity, although there is a spectrum with some overlap between the clinical presentations. Accumulation of GAGs in tissues is responsible for the clinical symptoms, which are related to the degree of enzyme deficiency caused by mutations in the *IDUA* gene. Incidence is thought to be approximately 1:100,000, but as more programs adopt screening for this disease we will have a better assessment of the true incidence.

Clinical Features

Historically, MPSI has three major phenotypes based on clinical severity, although there is a spectrum with some overlap between the clinical presentations. In classical Hurler syndrome (MPSI-H), infants develop hepatosplenomegaly, course facial features, hirsutism, macroglossia, corneal clouding, bony abnormalities with contractures and spinal gibbus, short stature, macrocephaly, hydrocephalus, cardiac valve thickening, and cognitive delay. Progressive spinal stenosis may lead to neurologic symptoms. Many of these features develop during the first year of life and become worse over time, with survival only into the second decade. At the other extreme is classical Scheie syndrome (MPSI-S) with very mild bone abnormalities, normal intellect, and minimal organomegaly. An intermediate form of MPSI called Hurler/Scheie syndrome (MPSI-H/S) has an attenuated Hurler-like severity with onset of clinical features after several years of age, but lacking developmental or intellectual disability. Most MPSI patients exhibit the severe and intermediate phenotypes.

Laboratory Tests

Newborn screening for MPSI has the ability to detect all forms of MPSI by measuring alpha-L-iduronidase enzyme activity (IDUA) in dried blood spots. An initial screen result with a low IDUA level is reported as inconclusive and a repeat dried blood spot is requested. If the repeat screen result also has low IDUA, the specimen will reflex to sequencing of the IDUA gene. Those results will be available in approximately 7-10 days and will be reported out with the repeat IDUA level.

Confirmation and Treatment

Confirmation of an abnormal newborn screen relies on measurement of enzyme activity in leukocytes, finding excessive GAG excretion in urine, and screening the *IDUA* gene for disease-causing mutations. Treatment for MPSI requires a multidisciplinary approach. Symptomatic care is needed for the multi-organ involvement of these patients, including specialists in cardiology, orthopedics, otolaryngology, neurology and neurosurgery. For patients with Hurler syndrome (MPSI-H), hematopoietic stem cell transplantation (i.e. bone marrow or cord blood) should preferably be done before symptoms develop or worsen by the end of the first year of life. Transplantation will halt progression of visceral symptoms and maintain cognitive abilities. For those patients with more attenuated forms of MPSI, enzyme replacement therapy is the recommended treatment. An FDA-approved form of alpha-L-iduronidase (Aldurazyme®) is given weekly via IV infusion. The enzyme is taken up by tissues and targeted to the lysosomes where it breaks down GAGs. Resolution of the hepatosplenomegaly occurs over time, although some features such as bony abnormalities and cardiac valvular thickening persist. Clinical outcomes when enzyme replacement therapy is initiated before onset of symptoms, for example in newborn infants detected at birth, will require further study.

ABNORMAL TEST RESULT	LIKELY CAUSES	Health Care Provider Actions
Initial screen > 24 hours IDUA < 1.8 µmol/L/hr = Inconclusive	MPS-I (Hurler, Hurler-Scheie, Scheie, Attenuated) Pseudodeficiency Carrier Variants of unknown significance (VOUS) Specimen Enzyme degraded due to heat exposure, or extended shipping time.	Obtain repeat dried blood spot specimen within 48 hours
Repeat screen IDUA <1.8 µmol/L/hr = Inconclusive, automatically reflexes to Sequencing of IDUA gene. Reported out once complete.	False Positive	If no mutations or pseudodeficiency-only no further follow-up necessary. All other results refer to genetics / metabolism

Screening Practice Considerations

Because IDUA enzyme activity is frequently low (high false positive percentage) in premature infants whose specimens are collected at < 24 hours of life, and because the vast majority of < 24 hour specimens are from newborns admitted to the NICU, screening for MPS-I is not reported on specimens collected at < 24 hours. However, a result indicating an extremely high suspicion of MPS-I disease would prompt repeat testing.

Enzymes are prone to degradation in dried blood spots if the sample is delayed in shipping or exposed to high temperatures.

Prompt referral when recommended should occur, even if there is evidence to suggest that one of the situations associated with false-positive screens is present. These situations can include heat-damaged specimens. The suspicion of this does not exclude the possibility of disease.

Phenylketonuria (PKU)

This practitioner's manual describes one condition from each of the 3 classifications (AA's, FAO's, and OA's) of disorders screened by tandem mass spectrometry (MS/MS). PKU is an example of an Amino Acid (AA) disorder detectable by this screen. While other AA's may have some common potential outcomes, each are very distinct. For more information on each condition we recommend referencing the ACMG's ACT sheets at http://www.ACMG.net, Genetic Home Reference at http://ghr.nlm.nih.gov and tables included in "Toward a Uniform Newborn Screening Panel" Genetics in Medicine, Vol 8, Supp 1, May 2006.

Phenylketonuria, or PKU, is caused by a recessively inherited enzyme defect in which the body cannot properly use the amino acid phenylalanine. All other metabolic processes are intact but phenylalanine, which comes from all dietary protein, accumulates in the blood. Excess phenylalanine cannot be converted to tyrosine due to a lack of phenylalanine hydroxylase, the enzyme that catalyzes the conversion of phenylalanine to tyrosine. Phenylalanine accumulates in the body and causes damage. Overall, PKU occurs in about 1 in 13,500 to 1:19,000 U.S. live births. With appropriate treatment, the risk of harm due to any of these conditions is substantially reduced.

Classical phenylketonuria is a disorder in which the blood phenylalanine, or phe, rises above 20 mg/dL on a normal diet (normal blood phe is less than 2.0 mg/dL). Without treatment, nearly all affected individuals develop severe developmental disabilities. Other symptoms include severe intellectual disability, microcephaly, eczematous or oily skin, cerebral palsy, convulsions, dysphasia, hyperactivity with purposeless movements, autistic-like behavior, and an abnormal EEG. In infants, vomiting may mimic pyloric stenosis. The skin and hair are usually fair, the eyes may be blue and a "mousey" odor of the baby's urine is frequent. The smell arises from phenylacetic acid.

Hyperphenylalaninemia refers to any consistent elevation of phe levels, including classical PKU. If cases of classical PKU are excluded, this includes blood phe levels less than 20 mg/dL. These may be caused by liver damage, mutation of the phenylalanine hydroxylase gene, disorders of cofactor synthesis or regeneration, or maternal PKU. In these cases, intellectual disability may or may not be present. Blood levels may remain elevated throughout life or may gradually fall towards normal. In infancy, these patients can mimic the severe PKU condition, and even in mild cases there seems to be an increased risk of the maternal PKU syndrome, as described below.

Much rarer causes of elevated phenylalanine are caused by defects of biopterin metabolism. Blood phenylalanine levels are variable. These patients have progressive neurological damage with seizures and steady deterioration which becomes noticeable sometime between 6 and 20 months of age despite early treatment with a low phenylalanine diet. In view of the severity of this group of diseases, all infants with persistently abnormal levels of phenylalanine should be tested by special blood and urine tests for biopterin abnormalities.

The cause of hyperphenylalaninemia must be determined if proper treatment is to be provided. Definitive tests can differentiate these variant forms of PKU. Treatment by dietary restriction alone is inadequate for a tetrahydrobiopterin cofactor defect. The cofactor is necessary not only for normal activity of phenylalanine hydroxylase, but also for activity of the tyrosine and tryptophan hydroxylase which are involved in serotonin and dopamine synthesis.

Maternal PKU

Pregnancy presents a special problem with PKU and other forms of hyperphenylalaninemia, as high blood levels of phe are teratogenic to the fetus. This is important in the context of newborn screening because early testing of an infant born to a mother with PKU or hyperphenylalaninemia can reflect the mother's phe levels. A positive test on a newborn who then has a normal repeat test, especially if the baby has growth retardation, microcephaly, or malformations, should raise the possibility of maternal PKU. These infants usually have a transient elevation of phenylalanine (3-20 mg/dL) reverting to normal within 24 hours. If maternal PKU is suspected, the mother should be appropriately tested and counseled.

A medically supervised phenylalanine restricted diet begun before conception with phenylalanine levels maintained within a safe range throughout pregnancy may prevent damage to the fetus.

Laboratory Test

The screening test for PKU is done by tandem mass spectrometry to determine the molecular mass of the phenylalanine and phenylalanine: tyrosine ratios. Values will be provided on the laboratory report and classified as mild elevation, abnormal or substantially elevated.

ABNORMAL TEST RESULT	LIKELY CAUSES	Health Care Provider Actions
Mild elevation of Phenylalanine	(for all abnormal findings) -PKU -Mild hyperphenylalanemia -False positive -Maternal PKU	-Obtain repeat dried blood spot specimen testing within 48 hours
Abnormal level of phenylalanine		-Obtain repeat dried blood spot specimen testing immediately
Substantial elevation of phenylalanine possibly with elevated phe/tyr ratio		-Obtain quantitative plasma amino acids -Consult with and refer to pediatric metabolic specialist -Report confirmatory lab results, diagnosis and treatment to NNSP

Confirmation and Treatment

Consultation with a pediatric metabolic specialist should be made for confirmation and diagnosis (see Medical Consultants). Upon notification of a positive screening result (substantial elevation), the physician should collect or cause to be collected 0.5 ml heparinized plasma for quantitative testing of phenylalanine and tyrosine. Serum is also acceptable. With early and proper treatment, intellectual disability is usually preventable. Treatment should be started as soon after birth as possible in any infant with phenylalanine levels over 8 mg/dL and should be continued for life.

Frequent monitoring and diet adjustment is required, especially in the first weeks, because infants with variant forms of hyperphenylalaninemia may be indistinguishable from true PKU and improper nutritional therapy can produce brain damage or be fatal. Specific formulas for the diet management and expert nutritional supervision are provided through University of Nebraska Medical Center and Children's Hospital Metabolic Clinics.

If treatment is not started for some weeks, the results are more variable and the I.Q. tends to be lower. Patients who are not treated until after six months of age may show some improvement in I.Q., although they are likely to remain affected. Older patients usually show little change in I.Q. with treatment, but a low phenylalanine diet may help to control serious behavior problems and other manifestations of untreated PKU.

Screening Practice Considerations

Plasma phenylalanine is not detectably elevated in cord blood. It starts rising within 24 hours after birth and reaches 20 mg/dL or more within a few days. The screening test is often abnormal within 24 hours after birth and almost uniformly abnormal beyond 24 hours of birth in those infants with PKU.

The phe level of affected infants rises gradually after birth with little, if any, effect of the amount of protein ingested by the infant. The infant must be at least 24 hours of age to reliably detect PKU. Those with milder forms of hyperphenylalaninemia may need to be older to develop abnormal tests.
If an infant is tested "early" (less than 24 hours of age) a repeat test must be performed by seven (7) days of age (preferably at 24-48 hours), regardless of prior test results, as treatment started after 1 month of age may be less than optimal. If an infant is tested at less than 24 hours of age because of NICU admission, the repeat screen should be done at 48-72 hours of life.

The reliability of the screening result does NOT depend on the amount of feedings the newborn has had.

Dialysis or transfusion may temporarily lower phe levels. ***ALWAYS OBTAIN A NEWBORN SCREENING SPECIMEN PRIOR TO A TRANSFUSION.*** Children who develop cognitive disabilities before one year of age should undergo testing for PKU even if a negative screening test is documented.

Prompt confirmatory testing is required even if there is evidence to suggest that one of the situations associated with false positive screens is present. These situations can include inadequate specimen collection, or hyperalimentation. The presence of any of these does not exclude the possibility of disease.

While infants receiving hyperalimentation may have false positive results for PKU, it is generally believed the tandem mass spectrometry interpretation can distinguish those infants at higher risk of having PKU from those on hyperalimentation. This does not apply to other amino acid conditions, that are less likely to be able to be distinguished from elevations due to hyperalimentation.

Pompe disease

PD is a progressive neuromuscular disorder that is caused by deficient activity of lysosomal acid alpha-glucosidase (GAA). The disease is caused by mutations in the *GAA* gene and is inherited in an autosomal recessive manner. It has an overall incidence of approximately 1 in 40,000 in the United States, but is more common in the African-American population. Deficient GAA enzyme activity results in accumulation of lysosomal glycogen in heart and skeletal muscle. Lysosomes become swollen with glycogen and destroy muscle cells through an ill-defined mechanism.

Clinical Features

Patients display two major phenotypes. Those with classical infantile onset PD (IOPD) develop hypotonia and severe cardiomyopathy before 12 months of age. They often have poor feeding, develop early failure-to-thrive, and become respirator dependent due to diaphragm weakness. Cardiomyopathy is increasingly refractory to medications and most patients do not survive beyond 2 years of age. In contrast, late onset PD (LOPD) patients have a wider age of onset of symptoms beginning after 1 year of age (juvenile PD) to >50 years of age. In LOPD, muscle weakness and respiratory problems consisting of shortness of breath with exertion are predominant. Cardiomyopathy is not usually seen. LOPD slowly progresses over the course of many years. Nevertheless, all forms of PD are progressive and life-threatening diseases. The onset and severity of PD is related to the *GAA* mutation and amount of residual enzyme activity. Many different mutations cause PD and certain mutations predict an IOPD phenotype. In general, IOPD patients have a profound enzyme deficiency (0-2% of normal), whereas LOPD is associated with 2-10% of residual enzyme activity. IOPD infants usually have elevated blood CK levels, but CK in LOPD patients may be normal or only mildly elevated.

Laboratory Tests

Newborn screening for PD detects deficient activity of the GAA enzyme in the dried blood spot. An initial newborn screen result with low GAA activity will be designated as inconclusive and a repeat screen is recommended. If the repeat screen continues to have low GAA activity, the newborn's healthcare provider should immediately assess cardiac status via chest x-ray, ECG and ECHO; also baby should be assessed for hypotonia and order a blood test for Creatine Kinase. Consult cardiology immediately if cardiomegaly is present. Consult with and refer to genetics/metabolism for full evaluation including GAA enzyme test. The newborn dried blood spot will automatically reflex to sequencing which may take 7-10 days. Further confirmatory testing will be directed by the specialist and involve measuring the enzyme activity in leukocytes and sequencing the *GAA* gene to detect mutations. The importance of *GAA* sequencing is highlighted by the presence of a pseudo-deficiency allele in the general population that contributes to a false positive result on newborn screening using measurement of GAA enzyme activity alone.

Confirmation and Treatment

Additional confirmatory testing will include a blood draw for transaminases (Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Lactate Dehydrogenase (LDH). Therapy for PD is directed at replacing the GAA enzyme to deplete glycogen accumulation. The FDA-approved enzyme (Lumizyme®) is given via weekly or biweekly IV infusions. The enzyme is taken up by tissues and directed to the lysosome where it breaks down glycogen. If started early in life, enzyme infusions in infantile onset PD patients can reverse the cardiomyopathy or prevent it from developing. Since enzyme replacement therapy has only been available for a few years, its long-term efficacy is still under investigation. Its benefit for LOPD patients is less dramatic.

ABNORMAL TEST RESULT	LIKELY CAUSES	Health Care Provider Actions
Initial screen > 24 hours GAA < 2.10 µmol/L/hr = Inconclusive Repeat screen GAA <2.10 µmol/L/hr =Inconclusive (an inconclusive result on the 2nd screen reflexes to GAA gene sequencing)	Pompe Disease Infantile Onset Pompe Disease Late Onset Pseudodeficiency Carrier Variant(s) of Unknown Significance (VOUS) Specimen Enzyme degraded due to heat exposure, or extended shipping time. False Positive	Obtain repeat dried blood spot specimen within 48 hours Immediately assess for cardiomegaly and hypotonia. Consult with refer to Cardiology as appropriate, and refer to genetics/ metabolism. Order blood test for Creatine Kinase. If no mutations or pseudodeficiency-only, no further follow-up recommended. All other results refer to genetics/metabolism.

Screening Practice Considerations

Because GAA enzyme activity is frequently low (high false positive percentage) in premature infants whose specimens are collected at < 24 hours of life, and because the vast majority of < 24 hour specimens are from newborns admitted to the NICU, screening for POMPE is not reported on specimens collected at < 24 hours. However, a result indicating an extremely high suspicion of Pompe disease would prompt repeat testing.

Enzymes are prone to degradation in dried blood spots if the sample is delayed in shipping or exposed to high temperatures.

Prompt confirmatory testing when recommended is required, even if there is evidence to suggest that one of the situations associated with false-positive screens is present. These situations can include heat-damaged specimen. The suspicion of this does not exclude the possibility of disease.

PROPIONIC ACIDEMIA (PA)
OR METHYLMALONIC ACIDEMIA (MMA)

*This practitioner's manual describes one condition from each of the 3 classifications (AA's, FAO's, and OA's) of disorders screened by tandem mass spectrometry (MS/MS). PA and MMA are example of Organic Acidemia's (OA's) disorder detectable by this screen. PA/MMA provides a good example of how common analyte markers may trigger differential diagnosis for more than one condition. Other OA's may have some common potential outcomes but each are very distinct. For more information on each condition we recommend referencing the ACMG's ACT sheets at http://www.ACMG.net , Genetic Home Reference at http://ghr.nlm.nih.gov and tables included in "Toward a Uniform Newborn Screening Panel" Genetics in Medicine, Vol 8, Supp 1, May 2006.

PA is caused by a defect in propionyl-CoA carboxylase which converts propionyl-CoA to methylmalonyl-CoA. MMA results from a defect in methylmalonyl-CoA mutase which converts methylmalonyl-CoA to succinyl-CoA or from lack of the required B12 cofactor for methylmalonyl- CoA mutase (cobalamin A, B, C, D, and F). The condition description below is excerpted from "Genetic Home Reference" http://ghr.nlm.nih.gov for propionic acidemia and methylmalonic acidemia.

Propionic acidemia and methylmalonic acidemia are inherited disorders in which the body is unable to process certain parts of proteins and lipids (fats) properly. They are classified as organic acid disorders, which are conditions that lead to an abnormal buildup of particular acids known as organic acids. In PA the PCCA and PCCB genes provide instructions for making two parts (subunits) of an enzyme called propionyl-CoA carboxylase. This enzyme plays a role in the normal breakdown of proteins. Certain types of fat and cholesterol in the body. Mutations in the PCCA or PCCB gene disrupt the function of the enzyme and prevent the normal breakdown of these molecules.

As a result, a substance called propionyl-CoA and other potentially harmful compounds can accumulate. This buildup damages the brain and nervous system, causing the serious health problems associated with propionic acidemia.2

In most cases, the features of propionic acidemia become apparent within a few days after birth. The initial symptoms include poor feeding, vomiting, loss of appetite, hypoitonia and lethargy. These symptoms sometimes progress to more serious medical problems, including heart abnormalities, seizures, coma, and possibly death. Less commonly, the signs and symptoms of propionic acidemia appear during childhood and may come and go over time. Some affected children experience intellectual disability or delayed development. In children with this later-onset form of the condition, episodes of more serious health problems can be triggered by prolonged periods of fasting, fever, or infections. Propionic acidemia affects about 1 in 100,000 people in the United States

In MMA the effects which usually appear in early infancy, vary from mild to life-threatening. Affected infants experience vomiting, dehydration, hypotonia, lethargy, and failure to thrive. Long-term complications can include feeding problems, intellectual disability, chronic kidney disease, and pancreatitis. Without treatment, this disorder can lead to coma and death in some cases. MMA occurs in an estimated 1in 50,000-100,000 people.

About half of methylmalonic acidemia cases are caused by mutations in the MUT gene. This gene provides instructions for making an enzyme called methylmalonyl CoA mutase.
This enzyme works with vitamin B12 to break down several amino acids, certain lipids, and cholesterol. Mutations in the MUT gene alter the enzyme's structure or reduce the amount of the enzyme, which prevents these molecules from being broken down properly.

As a result, a substance called methylmalonyl CoA and other potentially toxic compounds can accumulate in the body's organs and tissues, causing the signs and symptoms of methylmalonic acidemia. Mutations in the MUT gene that prevent the production of any functional enzyme result in a form of the condition designated mut0. Mut0 is the most severe form of methylmalonic acidemia and has the poorest outcome. Mutations that change the structure of methylmalonyl CoA mutase but do not eliminate its activity cause a form of the condition designated mut-. The mut- form is typically less severe, with more variable symptoms than the mut0 form. Other cases of methylmalonic acidemia are caused by mutations in the MMAA or MMAB genes. To function properly, methylmalonyl CoA mutase must work together with the proteins produced from these genes. Mutations that affect either of these proteins can impair the activity of methylmalonyl CoA mutase, leading to methylmalonic acidemia. The long term effects of methylmalonic acidemia depend on which gene is mutated and how the enzyme is affected by the mutation. It is likely that other unidentified gene mutations also cause methylmalonic acidemia.

Clinical Considerations

Patients with PA and severe cases of MMA typically present in the neonate with metabolic ketoacidosis, dehydration, hyperammonemia, ketonuria, vomiting, hypoglycemia, and failure to thrive. Long- term complications are common, early treatment may be lifesaving and continued treatment may be beneficial.

Lab Tests

The screening test for PA/MMA is done by tandem mass spectrometry to determine the molecular mass of the C3 and ratios of C3:C2 and C3:C16. Values will be provided on the laboratory report and classified as mildly elevated, abnormal or substantially elevated.

ABNORMAL TEST RESULT	LIKELY CAUSES	Health Care Provider Actions
Mild elevation of C3 (propionylcarnitine)	(for all abnormal findings) -Propionic Acidemia -Methylmalonic Acidemia (mutase) -Methylmalonic Acidemia (Cbl A or B) -Maternal deficiency of B12 -False positive	-Obtain repeat dried blood spot specimen testing within 48 hours
Abnormal level of C3 and ratios of C3:C2 and or C3:C16		-Obtain repeat dried blood spot specimen testing immediately -Contact family and assess newborn for poor feeding, vomiting, lethargy, tachypnea -Obtain ketones, plasma acylcarnitines, plasma amino acids, urine organic acids for differential diagnosis -Consult with and refer to pediatric metabolic specialist -Report confirmatory lab results, dx/tx to NNSP
Substantial elevation of C3 possibly with elevations of C3:C2 and or C3:C16		

SCID

Severe Combined Immune Deficiency (SCID) is a rare, potentially fatal syndrome characterized by the absence of both T-lymphocyte and B-lymphocyte function. There are many different genetic causes of SCID. The patterns of inheritance differ based upon the type of genetic defect. Different types of SCID will also result in different laboratory findings. The result of the combined immune deficiency is an extreme susceptibility to infections. The incidence of SCID is thought to be 1/40,000 – 1/100,000 live births.

The most common type of SCID is inherited as an X-linked recessive trait and is called classical X-linked SCID (X-SCID). The mutation causing this defect is in a gene (IL2RG) on the X chromosome and causes a deficiency of the common gamma chain of the T-cell growth factor receptor and other growth factor receptors. Only males can have this type of SCID. Females who carry this mutation are not affected but can pass the mutation on to their children. This mutation results in low T-lymphocyte counts and low NK-lymphocyte counts. B-lymphocyte counts may be high, but the B-lymphocytes are not functional as they have abnormal receptors for growth factors. The phenotype is T-, B+, NK-.

Adenosine deaminase (ADA) deficiency SCID is another type of SCID. This is inherited in an autosomal recessive pattern. Mutations in the ADA gene that codes for ADA cause the deficiency. Because of the deficiency of ADA there is an accumulation of deoxyadenosine. This results in an inability of cells to divide and accumulation of other toxins that cause cell death which particularly affects lymphocytes. This type of SCID produces very low lymphocyte counts. The phenotype is T-, B-, NK-. There are other forms of SCID that are the result of various known genetic changes. Each type in some way interferes with the function of T and B lymphocytes.

Clinical Features

In those infants not diagnosed as a result of newborn screening, the common presentation of SCID is serious infection. SCID babies can be infected by organisms they come in contact with or by live virus vaccines. Pneumonia, diarrhea, fungal infections and failure to thrive are commonly seen. Without treatment for the immune system SCID is often fatal within the first year of life.

Lab Test

Screening for SCID is done by counting T cell receptor excision circles (TRECs). TRECs are pieces of DNA that are by-products generated during normal T cell maturation in the thymus. Low numbers or absence of TRECs indicates the possibility of SCID or other conditions with low T cells. The screening test for SCID involves only TREC analysis and does not include testing for gene mutations for SCID.

Using Polymerase Chain Reaction (PCR), samples are tested for the number of TRECs. If the count is low or no TRECs are detected, samples are checked for Beta Actin. Beta Actin levels should be normal even if a baby has a low number of TRECs. Low Beta Actin levels indicate a problem with DNA amplification during the testing and the test is not considered adequate for assessing the number of TRECs. The testing is considered inconclusive due to a failure to amplify (FTA). If the Beta Actin levels indicate acceptable amplification, low or absent TRECs will be reported as inconclusive or positive depending on their numbers and the gestation of the baby.

ABNORMAL TEST RESULT	LIKELY CAUSES	Health Care Provider Actions
Failure to Amplify (FTA)	Poor sample quality Sample Contaminated	Follow-up as directed – repeat collection of NBS or confirmatory testing may be requested
Inconclusive	Prematurity	Follow-up as directed - repeat collection of NBS at directed time
Positive	SCID Other conditions with T cell lymphopenia False Positive	Referral to immunologist Confirmation via flow cytometry as directed, CBC also needed Isolate baby as directed by immunologist Refrain from administering live virus vaccines Stop breast feeding until Mom's CMV status is determined If transfusion required, use only CMV negative leukoreduced irradiated products

Confirmation and Treatment

Confirmation testing for SCID will include a CBC to determine the absolute lymphocyte count and flow cytometry to enumerate the subsets of T lymphocytes. Under the direction of the Immunologist further functional tests of the lymphocytes will be undertaken when there are abnormalities in the initial confirmatory testing. Molecular testing may also be done in some cases.

Early diagnosis of SCID is important in order to initiate treatments prior to infections. Treatment early in life but especially prior to infections is known to be associated with better survival. The standard treatment for SCID is a hematopoietic stem cell transplant (HSCT). HSCT can completely reconstitute the baby's immune system. In some cases additional therapy may be necessary.

In addition, ADA deficiency may also be treated with enzyme replacement. The enzyme, polyethylene glycol (PEG)-treated ADA must be given every 1-2 weeks. Gene therapy is also being explored. It has been tried in cases of both ADA deficiency and X-linked SCID. Ongoing research is needed to refine the process. The particular treatment chosen depends upon the type of SCID the patient has and should be under the direction of a Pediatric Immunologist.

Special Considerations

When a baby is thought to possibly have SCID, certain measures must be taken in order to protect the baby from infection. In order to reduce exposure to possible infections some sort of isolation will take place at the direction of the Immunologist. Breast feeding may be stopped if Mother's CMV status needs to be determined. These babies should not be given live virus vaccines. If a transfusion is needed, only CMV negative, leukoreduced, irradiated products should be used. Under the guidance of the baby's Immunologist prophylactic medications are usually administered prior to the availability of definitive treatment. These are used to prevent infection prior to the reconstitution of the baby's immune system.

X-LINKED ADRENOLEUKODYSTROPHY (X-ALD)

X-ALD is an inherited disorder that presents with adrenal insufficiency and progressive neurologic symptoms. The disease is inherited on the X-chromosome and has an incidence of 1 in 15,000. X-ALD is caused by mutations in the *ABCD1* gene that is necessary for metabolizing very long chain fatty acids (VLCFA). Patients cannot break down VLCFA, particularly C24:0 and C26:0, and accumulate them in various lipids in brain, adrenal gland and most other tissues.

Clinical Features

Males typically show the most severe symptoms. Adrenal insufficiency (Addison disease) in X-ALD can develop at any time in the course of the disease, either before or after neurologic disease becomes apparent, and can result in fatal electrolyte disturbances. The age of onset and nature of the neurologic disease are highly variable. Approximately 30-40% of X-ALD boys have childhood cerebral ALD, which manifests as a rapidly progressive demyelinating disorder that begins at 5-10 years of age. Symptoms are initially subtle and may consist of new onset hyperactivity (ADHD), declining school performance, behavior changes, gait problems and/or visual impairment. The neurologic symptoms progress over several years to blindness, loss of speech, inability to feed and swallow, spastic quadriplegia and seizures. Affected boys survive only several years after onset of neurologic disease, but can survive longer in a severely debilitated condition. Unfortunately, the diagnosis is usually delayed until the child is seen by a neurologist and receives an MRI that reveals white matter disease in the parietal-occipital region, or less often in the frontal region of the brain. At that time, therapeutic options become limited, which underscores the importance of presymptomatic diagnosis. About 50—60% of X-ALD patients first develop neurologic symptoms of slowly progressive myelopathy with progressive spastic paraparesis (called adrenomyeloneuropathy) in the second decade of life and beyond. Heterozygous (carrier) females may develop symptoms of myelopathy in their later adult years.

Lab Test

Newborn screening detects elevated C26:0 as a component of lyso-phosphatidylcholine (i.e. C26:OLC) in the blood using tandem mass spectrometry. An initial newborn screen result with elevated C26:0 will reflex to a secondary test for C26. If this is elevated it will be reported as inconclusive and a repeat dried blood spot will need to be obtained. The repeat screen is tested only by the secondary test for C26:0 and if still elevated will reflex to sequencing of the ABCD1 gene. Results will be reported out after sequencing is completed. The screening test is very sensitive and has few false positives. Because elevated VLCFA is also a marker for certain other related peroxisomal diseases (Zellweger spectrum disorders), the screening test will also detect these ancillary diseases. Confirmatory/diagnostic testing will be directed by the specialist. Unfortunately, detection of X-ALD in newborns cannot yet predict whether the baby will later develop the severe fatal cerebral form of the disease in childhood or the later onset adrenomyeloneuropathy.

Once the diagnosis of X-ALD is confirmed, it is important to monitor boys for early appearance of demyelination. Brain MRI should be done every 6-12 months to detect involvement before the onset of clinically evident neurologic symptoms. If brain MRI detects early white matter disease, allogeneic hematopoietic stem cell transplantation will halt the neurologic disease in most cases. This life-saving therapy is thought to be mediated by migration of genetically normal transplanted stem cells into the brain where they disrupt the inflammatory process. Gene therapy that introduces the normal *ABCD1* gene into bone marrow derived stem cells is also under investigation. Monitoring for adrenal insufficiency is also critically important, since corticosteroid replacement therapy is simple and effective. Recommended screening should be done at least yearly using an 8 am cortisol and ACTH level.

ABNORMAL TEST RESULT	LIKELY CAUSES	Health Care Provider Actions
Initial screen (> 24 hours) C26:O LPC ≥ 0.25 µmol/L= Inconclusive	False positive X-ALD Childhood Cerebral ALD Adrenomyeloneuropathy Addison Disease Only Adolescent Cerebral ALD Adult Cerebral ALD	Obtain repeat dried blood spot specimen within 48 hours
Repeat screen C26:O LPC ≥ 0.25 µmol/L = Inconclusive (an inconclusive result on the 2nd screen reflexes to ABCD1 gene sequencing)	Zellweger syndrome or other diagnosis Carrier (Female) Possible adult symptoms Variant(s) of Unknown Significance (VOUS)	Refer to genetics/metabolism. Many mutations are private so prognosis may be unknown and ongoing monitoring will be necessary.

Screening Practices

Proper Time for Testing

All newborns should have their screening specimen collected between 24-48 hours of life.

Exceptions:

Newborns being discharged even if < 24 hours of age, should have an initial screen collected. A repeat is required between 24 and 48 hours of age.

Newborns being transferred to a NICU, even if < 24 hours of age. A specimen is required to be collected prior to transfer. Newborns who are to be transfused should have a NBS collected prior to transfusion even if they are less than 24 hour of age.

The trend towards early discharge from hospitals and the increase in home births complicates newborn screening.

Nebraska Requirements

Nebraska regulations (see appendix) require a newborn screening specimen to be collected between 24 and 48 hours of age, or immediately prior to transfer, transfusion or discharge, whichever occurs first.

Nebraska also requires that any infant whose newborn screening specimen was collected prior to 24 hours of age have another specimen collected by seven (7) days of age (preferably at 24-48 hours of age), regardless of prior test results. Specimens collected at less than 24 hours of age are not tested for CAH, CPH or CF, and may be unreliable for detecting aminoacidopathies. See special requirements for premature, low birth weight and sick newborns admitted to neonatal intensive care units.

Newborns Exhibiting Clinical Signs and Symptoms

The newborn screening test, like any laboratory test, may have false positives and false negatives. If signs and symptoms of one of the disorders are clinically evident, the physician should proceed to diagnostic testing, pending the results of the screen or in spite of the results of the screen. If the test results are pending for galactosemia (in which the metabolite accumulation can be very dangerous), proceed as if the infant has the condition. Contact a pediatric metabolic physician for assistance with rapid diagnosis and institution of dietary treatment (see Medical Consultants).

Blood Transfusions

If a newborn requires a blood transfusion even if prior to 24 hours of life, a specimen shall be collected before the blood transfusion. A repeat screening specimen will be required if the initial specimen is collected at < 24 hours of age.

A blood transfusion can seriously interfere with screening tests based on enzymes or other proteins intrinsic to the red blood cell, such as galactose- 1-PO4 uridyl transferase (the deficient enzyme in classical galactosemia), biotinidase, and hemoglobins. The screening panel obtained prior to the transfusion will reliably screen for biotinidase deficiency, galactosemia, and hemoglobinopathies, including sickle-cell anemia as early as birth.

Premature, Low Birth Weight and Sick Infants Admitted to the NICU

In 2011 the Nebraska Newborn Screening Program adopted via regulation, the Clinical and Laboratory Standards Institute's guidelines for screening of premature, low birth-weight and sick newborns admitted to neonatal intensive care units (I/LA-31-A).

The regulations require:

If a baby is to be transferred to another health care facility, collection of a newborn screen is required prior to transfer (regulation requirement). The NICU is required to verify that the NBS was collected or must draw an admission screen prior to providing any treatments for all premature, low birth weight and sick infants. The "admission screen" is defined as one that must be done before any treatments (with the exception of respiratory) and is targeted towards newborns who are premature, low birth weight or sick.

Babies admitted for short term observation who are not going to receive additional treatments are not the intended target of this requirement.

For babies whose first specimen is collected at < 24 hours of age, a repeat shall be collected at 48-72 hours of age.

For babies who are < 2000 grams at birth, another specimen should be collected at 28 days of life, or upon discharge if that occurs first.

FAQ's related to NICU admissions:

1) If a baby's first newborn screen (NBS) is collected after 24 hours of age, is it necessary to get a discharge NBS if they are in the NICU?

If the baby is > 2000 grams at birth they do not need a discharge NBS. Babies < 2000 grams at birth still need the 28 day or discharge screen.

2) What if a baby with GI obstruction or meconium ileus was in the regular nursery for close to 24 hours and then started to show signs of problems. They could be transferred to NICU and have their first screen on admission at greater than 24 hours. If this is the case, are they included in the recommendation to get a discharge newborn screen?

No, they are not, if their first screen is normal and was collected at > 24 hours. However, the GI problem still needs to be reported to the newborn screening laboratory at (412) 220-2300 as previously recommended. This is because the IRT used for screening for Cystic Fibrosis may be falsely normal in babies with gastrointestinal obstructions or meconium ileus, so the laboratory will run the 36+ mutation panel as a better screen for CF in these cases.

3) Since the requirement for serial screening for NICU admissions calls for 3 screens for babies with screens collected at < 24 hours and who weighed < 2000 grams will the lab report look different?

Yes. All reports for repeat screens collected for babies whose first specimen was collected at < 24 hours will now show results for all conditions that were screened for. This will include some babies who are discharged at < 24 hours but not transferred to NICU's. This decision was made to standardize and simplify processes in the newborn screening laboratory which helps reduce the opportunity for error.

4) Will these guidelines require more babies to get more specimens than they use to?

The idea behind the standardized practice is to be as certain as we can, that reliable screening results are available for every baby, with the least amount of specimens. In some cases this will prove to reduce the number of specimens required because it will avoid babies getting their first specimen collected post transfusion. In those cases repeat tests for hemoglobin type rather than the transfused hemoglobin type might have required multiple tests as late as 120 days past transfusion.

In other cases, it may be that the third specimen would not otherwise have been collected. The laboratory and Newborn Screening Program are looking at ways to monitor to see if this becomes a problem. Frequently however, we see babies with drawn early specimens repeated at a few days who then have abnormal screens due to hyperalimentation which is apparent with multiple amino acid elevations on screen results. When results are significantly abnormal, a confirmatory specimen will usually be recommended.

5) We have a NICU to which we transfer babies in-house. It has been our practice to collect the first newborn screening specimen at > 24 hours to avoid having to repeat. Sometimes the in-house transfers include babies who are < 2000 grams. Does this mean we will now have to collect specimens early, and then collect the repeats?

The "admission screen" is really targeting those babies who are likely to get treatments that can interfere with newborn screening results. If the baby is admitted for close observation but gets no transfusion or hyperalimination they may be good candidates to wait till > 24 hours to collect their dried blood spot specimen.

The Newborn Screening Program cannot approve, endorse or otherwise educate about any practice outside that which is required in the regulations. However, the Program and laboratory will be monitoring the data to see if this results in substantially more babies having early specimens.

6) We have a sub-set of newborns who are admitted to the NICU for evaluation and are classified as "feeders and growers". They do not receive any treatments such as blood transfusions or TPN. Many times they come directly from Labor and Delivery and were never admitted to the normal newborn nursery. Do we have to get the newborn screen upon admission to the NICU for these babies or can we wait till they are at least 24 hours of age?

The NICU may have the latitude within Nebraska's regulation to wait and collect the specimen after 24 hours. The regulation 2-005.01B states:

"Newborns transferred to neonatal intensive care units (NICU) must have a specimen collected prior to transfer, and information communicated as required at 181 NAC 2-005.01E3. The attending physician at the hospital NICU must verify and otherwise ensure a specimen is collected prior to the provision of any treatment, excluding respiratory treatment."

If the NICU finds upon admission that the newborn does not already have a specimen collected, the attending physician needs to order the screen and make sure it occurs before treatment. For the sick babies, that's probably immediately. For the well babies generally only admitted for evaluation and as "feeders and growers," you may be able to wait till 24 hours of age, if tight enough procedures are in place to ensure no treatments occur before the screen is collected.

Program Contacts

Newborn screening follow-up: 402 471-0374
Newborn screening follow-up fax: 402 471-1863
This line is connected to a pager answered after hours and on weekends.

Krystal Baumert, Follow-up Coordinator (402-471-0374)
Endocrinopathies, Metabolic Conditions, Lysosomal Storage Diseases, Home Births, Data

Karen Eveans, Follow-up Coordinator (402-471-6588)
Cystic Fibrosis, Hemoglobinopathies, Post transfusion specimens, SCID, X-ALD

Sarah Seeberger, QA/Follow-up Specialist (402 471-6759)
Drawn Early specimens, unsatisfactory/unacceptable specimens, hospital QA monitoring and QA reporting

Julie Luedtke, Program Manager (402-471-6733)
Newborn Screening systems issues, regulations, patient and professional education, quality assurance, Metabolic formula and food policy and contracts,

Staff Assistant Line, (402-471-9731)
Supplies of patient education materials, questions on metabolic foods account balances, assistance with requesting metabolic foods reimbursement and ordering foods

Nebraska Newborn Screening Program Web Site:
www.dhhs.ne.gov/publichealth/pages/nsp.aspx

Laboratory Contacts

(412) 220-2300

P.J. Borandi, Vice President and General Manager

Joseph Quashnock, PhD, Laboratory Director

Susan Felinczak, Client Services

James DiPerna, PhD, Team Lead, Tandem Mass Spectrometry

Tracy Koger, Team Lead Biochemistry

Madhuri Hegde, PhD, FACMG VP & Chief Scientific Officer

Zhili Linn, MD, Molecular

Bethany Sgroi, MS, CGC

Meredith Patik, MS, CGC

Lucy Andrews, MS, CGC

Medical Consultants

Medical consultants are available to provide consultation for the follow-up, evaluation, and long-term management of children detected in this program. Questions concerning medical aspects of the program may be directed to the following:

Cystic Fibrosis:
Accredited Cystic Fibrosis Center
University of Nebraska Medical Center CF Clinic
(402) 559-6275

John Colombo, MD, Director
Paul Sammut, MD
Heather Thomas, MD

Endocrinology Disorders:
University of Nebraska Medical Center
Pediatric Endocrinology Children's Hospital Endocrinology Clinic
(402) 955-3771

Monina Cabrera, MD
Melinda Chen, MD
Slaheddin Elrokshi, MBBS
Marissa Fisher, MD
Bracha Goldsweig, MD
Zoe González-García, MD

Metabolic Disorders:
Munroe-Meyer Institute for Genetics Pediatric Metabolism
University of Nebraska Medical Center
Children's Hospital Metabolic Clinic
(402) 559-6800

Richard Lutz, MD
William Rizzo, MD

Hematologic Disorders:
UNMC and Children's Hospital
(402) 955-3950

Minnie Abromowitch, MD
Jill C. Beck, MD
Peter F. Coccia, MD
Donald W. Coulter, MD
James B. Ford, DO
Bruce G. Gordon, M.D.
James L. Harper, M.D.
Christina K. Lettieri, MD
Stefanie R. Lowas, MD
Harold M. Maurer, MD

Immunologic Disorders
Children's Physicians Clinic at The Nebraska Medical Center
Children's Hospital & Medical Center
(402) 955-5570

Hana Niebur, MD
Russell Hopp, DO

Midwest Allergy and Asthma Clinic
(402) 397-7400

Ebrahim Shakir, MD

NEBRASKA TESTING LABORATORY

Where are screening tests done?

All newborns born in Nebraska have their dried blood spot screening specimens shipped to PerkinElmer Genetics Inc. laboratory in Pittsburgh, PA. This laboratory was awarded the contract since 2003 following competitive bid processes. The contract is now renewable every 2 years for up to 10 years, to avoid disruption to the Newborn Screening system. The laboratory's phone number is: (412) 220-2300.

What are the charges for testing?

The laboratory (beginning July 2018) charges $80.50 to the hospital for the 32-condition newborn screen. Hospital charges on top of that are not regulated by contract with the Newborn Screening Program. These charges are generally included in the obstetric DRG billed to insurance or the patient. $60.50 from each infant screened pays for the laboratory testing for all 32 conditions, filter paper, overnight shipping of specimens to the laboratory, and reporting of results. Twenty dollars of each screening charge is returned to the Newborn Screening Program and is used to help pay for treatment of metabolic conditions for the metabolic formula and metabolic foods as statutorily required, and for some program administrative costs.

What about repeat tests?

When repeat tests are requested because of a positive or inconclusive result, or because of a drawn early or unsatisfactory/unacceptable specimen, the laboratory does not charge for the repeat testing.

What are the requirements for confirmatory testing?

Confirmatory tests may be done at any laboratory that is CLIA certified and meets the standards for confirmatory test result reporting as specified in Nebraska's regulations. See appendix for regulations Title 181,NAC 2, 007.01E. Many confirmatory tests are sent out to reference laboratories. If the quality of information you receive from confirmatory laboratory test results does not meet these standards, you should contact the NNSP and the submitting hospital or laboratory. Due to factors such as quality of information on laboratory test result reports, quality of data to support neonatal reference ranges, rapidity of turnaround time, ability to test submitted confirmatory specimens for DNA on request if confirmatory results are positive, etc. the pediatric subspecialists have experience with high performing laboratories and may identify their preferences for your consideration.

PerkinElmer Genetics Inc.

230 Industry Drive

Pittsburgh PA 15275

1866-463-6436 or 1 412-220-2300

http://www.perkinelmer.com/
or results portal:
https://resultsportal.perkinelmergenetics.com/Search.aspx

References

181 NAC 2: REGULATIONS GOVERNING SCREENING OF INFANTS FOR METABOLIC DISEASES at www.dhhs.ne.gov/pages/reg_t181.aspx

ACMG Act sheets http://www.acmg.net

Genetic Home Reference http://ghr.nlm.nih.gov

NEBRASKA REVISED STATUTES § 71-519 TO 71-524 at www.dhhs.ne.gov/publichealth/pages/nsp.aspx

"Newborn Screening Fact Sheets", Celia I Kaye and the Committee on Genetics, PEDIATRICS 2006; 1 18;934-963 http://www.pediatrics.org/cgi/content/full/118/3/e934

"Newborn Screening of Premature, Low Birth Weight and Sick Newborns, Approved Guidline", CLSI I/LA 31-A, 2009

"Toward a Uniform Newborn Screening Panel" Genetics in Medicine, May 2006, Vol 8, Supp 1

NEBRASKA NEWBORN SCREENING PROGRAM NEWBORN TRANSFER FORM

Date of Transfer:_____ Person Completing Form: _____

Hospital of Birth:_____

Infant's Name: _____

Date of Birth:_____ Time of Birth: _____

Date of Specimen Collection:_____ Time of Specimen Collection: _____

Transferring Physician: _____

Newborn Screening Specimen Collected at Hospital of Birth: ☐ Yes ☐ No

Newborn Screening Specimen Collected Prior to 24 Hours of Age: ☐ Yes ☐ No

☐ Infant transfused? If yes, was specimen collected prior to transfusion? ☐ Yes ☐ No

If collected post-transfusion, indicate type:_____and time of transfusion_____

Receiving Hospital: _____

Receiving Physician: _____

Person Receiving Form: _____

ATTENTION RECEIVING PHYSICIAN: If the newborn screening tests have not been performed or tests need to be repeated when you take charge of the infant, you are responsible for ordering a specimen and returning the results recorded on this form to the hospital of birth.

Forward one copy of this form to the receiving hospital and fax one copy to:

Nebraska Newborn Screening Program
Department of Health & Human Services
P.O. Box 95026 Lincoln, NE 68509 Fax 402-471-1863

Department of Health & Human Services
DHHS
NEBRASKA

Newborn Blood-spot Screening Alternate Care Giver at Discharge Form

If a newborn is discharged to a caregiver other than the birth mother, please complete this form to facilitate contact and retrieval of the baby in the event he/she needs follow-up after the newborn screen. Your assistance in helping us assure timely newborn screening follow-up to prevent morbidity and mortality in newborns is greatly appreciated. Please keep a small supply of these forms where it will be easiest to incorporate into your processes.

PLEASE FAX COMPLETED FORM TO the Newborn Screening Program at:

402 471-1863.

(PLEASE PRINT)

Baby's Name:_____

Date of Birth:_____

Alternate Care Giver information:

Name:_____

Phone:_____

Address:_____

City/State:_____

Discharging Facility:_____
or (Your facility's stamp here)

Specimen Collection for Newborn Screening

Excerpt from Blood Collection on Filter Paper for NBS Programs: Approved Standard Sixth Edition (NBS01-A6)

A1 Preparation

A1.1 Wash hands vigorously.

A1.2 Wear powder-free gloves and change gloves between newborns.

A1.3 Confirm identity of newborn and ensure that all data elements on the form are complete, accurate, and consistent.

A2 Sampling Technique

A2.1 Warm heel for puncture (incision/stick) site. Use a commercially available heel-warming device containing an exothermic thermochemical composition, or warm the site with a soft cloth that has been moistened with warm water **(less than 42°C)** for three to five minutes. In some situations, warming the site may not be necessary to increase blood flow and volume.

A2.2 Position the newborn's leg lower than the heart to increase venous pressure.

*

A2.3 Wearing gloves, wipe newborn's heel with 70% isopropyl alcohol.

A2.4 Allow heel to air dry.

*

A2.5 The puncture should be made within the shaded area as illustrated in the figure above.

*

A2.6 Using a sterile lancet of recommended length, perform puncture (depth < 2.0 mm) as illustrated **or** use a retractable incision device. A retractable incision device may provide superior blood flow by making a standardized incision 1.0 mm deep by 2.5 mm long.

A2.7 Gently wipe off the first drop of blood with sterile gauze. (Initial drop contains tissue fluids, which might dilute sample.)

A2.8 Wait for formation of large blood droplet.

A2.9 Apply gentle pressure with thumb around the heel, but not near the puncture site, and ease intermittently as drops of blood form.

†

A2.10 Gently touch the filter paper card to the blood drop and fill each printed circle with a SINGLE application of blood. Do not touch the filter paper to the heel. Apply blood to one side only. Observe the saturation of each printed circle as the blood flows through the filter paper. After collection, examine both sides of the filter paper to ensure saturation.

A2.11 All used items should be disposed of in an appropriate biohazard container.

A2.12 After the specimen is collected, elevate the newborn's foot and, using sterile gauze, briefly apply gentle pressure to the puncture site until the bleeding stops. Do not apply adhesive bandages.

A2.13 Allow blood specimen to **air dry thoroughly** on a horizontally level, nonabsorbent, open surface, such as a drying rack or plastic-coated test tube rack, **for a minimum of three hours** at ambient temperature. Keep specimen away from direct sunlight. (Do not stack or heat.)

(See Appendix F.)

‡

A2.14 After the specimen has dried, place in an approved container for transport (see local regulations).

A3 Pitfalls

A3.1 (See Appendix B.) Failure to allow residual alcohol to dry might dilute the specimen and adversely affect test results.

A3.2 Puncturing the heel on posterior curvature will permit blood to flow away from puncture, making proper spotting difficult. **Do not use previous puncture sites.**

A3.3 "Milking" or squeezing the puncture site might cause hemolysis and admixture of tissue fluids with specimen.

A3.4 Do not layer successive drops of blood on the target spot. If blood flow diminishes to incompletely fill circles, **repeat** sampling technique A2.1 through A2.10.

A3.5 Avoid touching the area within the circle before and after blood collection. Do not allow water, feeding formulas, antiseptic solutions, or powder from gloves or other materials to come into contact with the specimen card before or after use.

A3.6 Do not place the specimens in the transport container until thoroughly dry. Insufficient drying adversely affects test results. Use of sealed plastic bags requires desiccation. Ideally, transport specimens within 24 hours of collection. Include tracking number and/or other identifiers on the envelope in case package is misplaced.

* Photos reprinted with permission from the New York State Department of Health, Wadsworth Center.

† Photo reprinted with permission from the Newborn Screening Reference Center, National Institutes of Health, University of the Philippines Manila.

‡ Photo reprinted with permission from Whatman, Inc.

Nebraska Newborn Screening Filter Paper Example

1) Complete the form accurately and legibly at the time of specimen collection.
2) Pull the center form prior to applying the blood spots, and;
3) Keep this in the infant's medical record.
4) Do not touch sample area.
5) Do not use if damaged.

BIOHAZARD

COLLECTION AND REPORTING (CARE) FORM - *NEBRASKA NEWBORN SCREENING PROGRAM*

Birth Date___/___/___ Time ___:___ (Military)
Collection Date___/___/___ Time ___:___ (Military)
Collector's initials_____ Gestational Age:_____ Birth Weight _____
NICU Admit ☐ Yes ☐ No
Is this specimen: ☐ Initial specimen ☐ 48-72" repeat
☐ Other repeat ☐ 28 day/ discharge repeat for a < 2000 gram birth
Has baby EVER been transfused ☐ Yes ☐ No
If Yes, Date last transfused: ___/___/___ Time ___:___ (Military)
Baby receiving TPN ☐ Yes ☐ No
If TPN yes, was it interrupted 3 hours before taking this specimen ☐ Yes ☐ No
Baby has Meconium Ileus or other bowel obstruction ☐ Yes ☐ No
Baby on Antibiotics ☐ Yes ☐ No

Baby's last name
First name *Middle*
Patient Record Number
Place of Birth
Home Birth ☐ Yes ☐ No Sex ☐ M ☐ F ☐ Unknown

Mother's last name
First name *Middle*
Address
City *State* *ZIP*
Mother's Phone (___)___-_____ Mother's DOB___/___/___

Name of Submitter / Facility
City *State (if other than NE)*
Name of Ordering Physician
(____)_____-_____
Ordering Physician's Phone
Name of Physician following baby post-discharge
(____)_____-_____
Post-discharge Physician's Phone

-Allow to air dry horizontally at least 3 hours.
-Do not let blood spots touch anything before they are dry.
-Ship within 24 hours (when transport available)

SHIP TO:
PerkinElmer
PerkinElmer Genetics
90 Emerson Lane Suite 1403
P.O. Box 219
Bridgeville, PA 15017
Phone: 412-220-2300

9735502

Date Received
Nebraska Collection and Reporting Form (Care Form)
9735502
Date Reported

Ahlstrom PerkinElmer 226 LOT 105178 / 315147 Expires 11/30/2018

NEBRASKA

Good Life. Great Mission.

DEPT. OF HEALTH AND HUMAN SERVICES

Nebraska Department of Health and Human Services

Division of Public Health
Lifespan Health Services Unit
P.O. Box 95026 Lincoln, NE 68509-5026
(402) 471-9731
(402) 471-7049 TTY
(402) 471-1863 FAX
402 742-2332 E-FAX
Dhhs.newbornscreening@nebraska.gov
Web: www.dhhs.ne.gov/nsp

PHA-PB-24 (99923) Rev. 6/13