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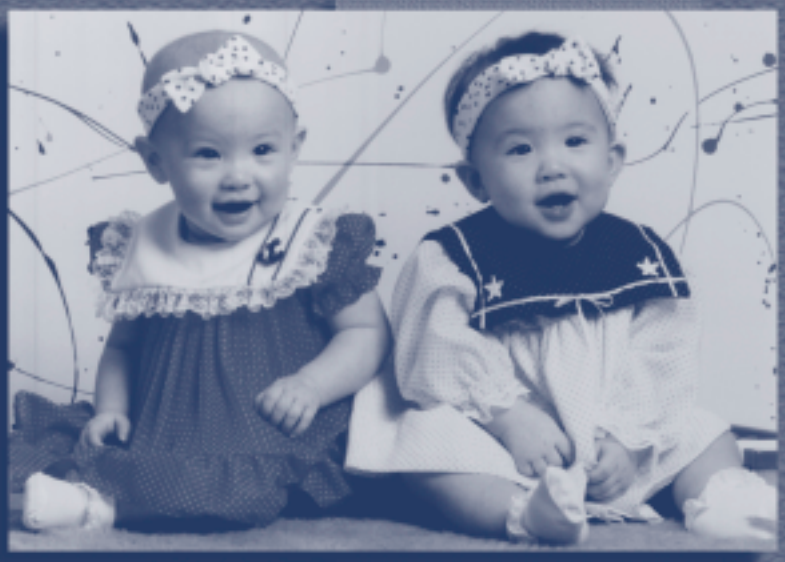

Idaho Practitioner's Manual

NEWBORN SCREENING PROGRAM

THE NORTHWEST REGIONAL NEWBORN SCREENING PROGRAM PRACTITIONER'S MANUAL

IDAHO

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Acknowledgment: *We are indebted to the Screening Coordinators in each of the regional states for their assistance and advice and to Leanne Rien, RN, for many hours dedicated to this manual.*

MEDICAL PROGRAM FOLLOW-UP
IDAHO

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 should be directed as below:

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INTRODUCTION

The disorders covered by the screening program are rare, collectively affecting about 1 in 2,000 infants, so the chance that any single infant will be affected is remote. The cost of not diagnosing one of these conditions is immense, both in human suffering and financial terms, because early diagnosis and treatment can result in normal growth and development. Babies with these conditions appear normal at birth. It is only with time that the biochemical abnormality affects the baby's health and development. By the time clinical symptoms appear, the damage may be permanent.

The goal of the Northwest Regional Newborn Screening Program (NWRNBSP) which includes Oregon, Idaho, Nevada, Alaska and Hawai`i is to identify all affected infants before damage can occur. To do so, every baby must be screened. This requires coordinated efforts from three groups of health care providers:

PRACTITIONERS: Responsible for the collection and handling of screening specimens, for providing parents with correct and current information and for prompt follow-up in the event of an abnormal result.

CENTRAL SCREENING LABORATORY: Responsible for testing, record keeping, quality control of laboratory methods, notification of results and tracking of abnormal and unresolved results.

TREATMENT AND FOLLOW-UP TEAM: Responsible for the confirmatory testing of infants with abnormal results, for the management of confirmed cases and for education of practitioners.

The practitioner's responsibilities in the program are termed **NEWBORN SCREENING PRACTICE** (see page 32). A practitioner is defined as the person(s) responsible for supervising the birth and/or the early neonatal care of an infant. Inadequate screening practices can greatly affect the quality of an infant's screening tests and increase the chances that an affected infant may be missed. At least one-third of cases missed by screening programs in the United States are caused by errors in newborn screening practice.

The central screening laboratory and the follow-up team, together with state health agencies, have developed a quality control program to assist practitioners with their screening practices. Components of this program include ongoing education for practitioners and parents, computerized monitoring of certain screening practices, and an examination of communication channels between practitioners, the laboratory, and the follow-up team.

This manual describes the disorders currently covered by the program, as well as the standards and common problems for certain screening practices, and provides general program information.

DISORDERS SCREENED BY THE PROGRAM

In the early 1960s, the most important reason for routine screening in newborns was to detect and treat phenylketonuria (PKU) in which progressive, profound mental retardation develops in most untreated individuals because the babies cannot metabolize the amino acid phenylalanine. It is now clear that neonatal hypothyroidism is three times as frequent as PKU. While it is possible to test for over 150 different analytes using a few drops of blood applied to a special filter paper, not all are practical. The conditions which are currently tested for in this program are shown in Table 1.

Newborn screening by tandem mass spectrometry (MS/MS) is an effective tool to detect serious and life threatening metabolic disorders in newborns. Using only one to two drops of blood, it is possible to screen for up to 30 separate conditions. For some disorders, like medium chain acyl-CoA dehydrogenase (MCAD) deficiency, detection by MS/MS is reliable and early treatment simple and efficacious. Unfortunately, the incidence and efficacy of early diagnosis and treatment for many of the other disorders is unavailable or unproven at this time. As with all conditions, there may be false negative results and practitioners should remain alert for signs of these conditions in infants and children regardless of screening results. Conversely, false positives are also possible.

We urge practitioners to use the term "NEWBORN SCREEN," rather than "PKU test," since other disorders besides PKU are included in the screening battery. **Babies with other disorders have sometimes been mistakenly treated for PKU because any abnormal test result was referred to as a "PKU test."**

TABLE I

SUMMARY OF DISORDERS SCREENED BY THE PROGRAM

Condition	Compound Tested for	Incidence	Symptoms if Not Treated	Treatment
Endocrine Disorders:				
Congenital Adrenal Hyperplasia (CAH) *BY REQUEST ONLY see page 42*	17-OH Progesterone	1:12,000 1:300 in Yupik Eskimos	Addisonian Crisis in all infants; salt wasting in 2/3: dehydration, shock, hyperkalemia; virilization of females	Glucocorticoid and/or mineralcorticoid (Florinef)
Congenital Hypothyroidism	Thyroid hormones (T_4 with TSH confirmation)	1:3,000	Mental retardation, other brain damage; growth delay	Thyroid hormone (L-Thyroxine)
Hemoglobin Disorders:				
Hemoglobinopathies including sickle cell anemia *BY REQUEST ONLY see page 42*	Hemoglobin patterns	1:15,000 (1:400 in African Americans)	In sickle cell disease: death by sepsis or splenic sequestration anemia, sickling crises	Penicillin & comprehensive care
Metabolic Disorders:				
Biotinidase deficiency	Biotinidase	1:60,000	Mental retardation, seizures, skin rash, alopecia, hearing loss, death	Biotin
Galactosemia	Galactosemia enzyme (GALT)	1:60,000	Severe brain damage; liver disease; cataracts; death	Galactose-restricted diet
Amino Acids: Arginase Deficiency	Arginine	1:60,000	Irritability; developmental delay; spastic tetraplegia	Low protein diet, medication
Arginosuccinate Lyase Deficiency (ASA)	Arginine/Citrulline	1:60,000	Hyperammonemia; mental retardation; seizure; death	Low protein diet, medication
Citrullinemia	Citrulline	1:60,000	Hyperammonemia; mental retardation; seizure; death	Low protein diet, medication

TABLE I (continued)

SUMMARY OF DISORDERS SCREENED BY THE PROGRAM

Condition	Compound Tested for	Incidence	Symptoms if Not Treated	Treatment
Amino Acids (continued):				
Homocystinuria	Methionine	1:100,000	Mental retardation; dislocation of lenses; marfanoid body habitus	Pyridoxine; methionine restricted, cysteine supplemented diet
Hyperphenylal-aninemia, including phenylketonuria	Phenylalanine	1:10,000	Profound mental retardation; seizures	Low phenylalanine diet
Tyrosinemia	Tyrosine	1:100,000	Vomiting, lethargy; liver disease; congulopathy renal tubular acidosis	Medication; low phenylalanine/ low tyrosine diet
Organic Acidemias: • Beta-ketothiolase deficiency • Glutaric acidemia,Type I • Isobutyryl CoA dehydro-genase deficiency • Isovaleric acidemia • Malonic aciduria • Maple Syrup Urine Disease (MSUD) • Methylmalonic acidemias (8 types) • Propionic acidemia • 3-Hydroxy-3-methylglutaryl (HMG) CoA lyase deficiency • 2-Methyl-3-hydoxybutyryl CoA dehydrogenase deficiency • 2-Methylbutyryl CoA dehydrogenase deficiency • 3-Methylcrotonyl CoA carboxylase deficiency • 3-Methylglutaconyl CoA hydratase deficiency • Multiple carboxylase deficiency	Acylcarnitines	1:53,000	Neonatal onset: irritability; lethargy; ketoacidosis; coma; death Late onset; failure to thrive; hypotonia; mental retardation Some will be asymptomatic	Dietary therapy/ Medications

TABLE I (continued)

SUMMARY OF DISORDERS SCREENED BY THE PROGRAM

Condition	Compound Tested for	Incidence	Symptoms if Not Treated	Treatment
Fatty Acid Oxidation Defects: • Carnitine uptake/ transport defects • Multiple acyl-CoA dehydrogenase deficiency (MADD) • Short chain acyl-CoA dehydrogenase deficiency (SCAD) • Medium chain acyl-CoA dehydrogenase deficiency (MCAD) • Long chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD) • Very long chain acyl-CoA dehydrogenase deficiency (VLCAD)	Acylcarnitines	1:9,300	"Reyes Like" episodes; hypoketotic hypoglycemia; lethargy; cardiomyopathy; hypotonia; mental retardation; coma; death mother may have had AFLP/HELLP syndrome; acute fatty liver of pregnancy	Dietary therapy/ Medications

TABLE II

NORMAL VALUES AND CRITERIA FOR REQUESTING FOLLOW-UP SPECIMENS

Analyte	Normal	Phone Follow-up*	Mail Follow-up
17-OH Progesterone	≤35 ng/mL ≤ 10 days old, BW> 2500g ≤25 ng/mL >10 days old, BW>2500 g ≤60 ng/mL ≤10 days old, BW ≤2500 g ≤40 ng/mL >10 days old, BW ≤2500 g	>70 ng/mL if ≤10 days, BW >2500 g >50 ng/mL if >10 days, BW >2500 g >150 ng/mL if ≤10 days, BW ≤2500 g >100 ng/mL if >10 days, BW ≤2500 g	>35 to ≤70 ng/mL if ≤10 days, BW >2500 g >25 to ≤50 ng/mL if >10 days, BW >2500 g >60 to ≤150 ng/mL if ≤10 days, BW ≤2500 g. >40 to ≤100ng/mL if >10 days , BW ≤2500 g
Thyroxine (T_4)	Upper 90% of T_4 determinations	T_4 in lower 10% and TSH >100 µIU/mL T_4 in lower 10% and TSH >50 in >72 hours old	T_4 in lower 3% on 2 specimens and TSH normal T_4 <5.0 µmg/dL, TSH normal (premature baby) T_4 in lower 10% and TSH 25 to 50 µIU/mL if >72 hours old and TSH 35 to 100 µIU/mL if < 72 hours old
TSH	<35 µIU/mL if ≤72 hours old <25 µIU/mL if >72 hours old or older		35.1–100 µIU/mL if ≤ 72 hours old 25.1–50.0 µIU/mL
Hemoglobin IEF	Hgb F and A	Probable homozygote	Probable homozygote
Biotinidase	Activity present	Activity absent	Partial Activity
Galactosemia Enzymes (Gal-1-PO_4 uridyl transferase, GALT)	Fluorescence present	No fluorescence and galactose ≥20 mg/dL or galactose <20 mg/dL if <48 hours old	No fluorescence and galactose, <20 mg/dL, age ≥48 hours
Arginine	< 140 µM	≥ 210 µM	≥ 140 µM but < 210 µM
Citrulline	< 90 µM	≥ 90 µM	≥ 90 µM with a normal 1st specimen
Leucine Leucine/Alanine Ratio	<300 µM <1.75 µM	≥ 300 µM, Leu/Ala ≥1.75 µM	≥ 300 µM, Leu/Ala <1.75 µM and elevation of other amino acids and on hyperalimentation (TPN)
Methionine	< 80 µM	≥ 120 µM	≥ 80 µM but <120 µM
Phenylalanine Phenylalanine/Tyrosine Ratio	<200 µM <3.0 µM	≥200 µM, Phe/Tyr ≥3.0 µM	≥200 µM, Phe/Tyr <3.0 µM
Tyrosine	< 475 µM	≥ 713 µM	≥ 475 µM but<713 µM

*All phoned results are followed by mailed confirmation. All of these tests are screening tests. Abnormal results need full evaluation/discussion with a consultant before a diagnosis is confirmed or treatment is started.
Normal and abnormal values are subject to change based on continuing statistical evaluation.

TABLE II (continued)

NORMAL VALUES AND CRITERIA FOR REQUESTING FOLLOW-UP SPECIMENS

Analyte	Normal	Phone Follow-up*	Mail Follow-up
Organic Acidemias:			
C3 (Propionyl) Propionic acidemia, Methylmalonic acidemias (8 types), Multiple carboxylase deficiency	<8.0 µM	All abnormal results will be phoned until the significance of minor abnormalities is determined	
C3-DC (Dicarboxyl Propionyl) Malonic Aciduria	<1.9 µM		
C4 (Butyryl/Isobutyryl) Isobutyryl CoA dehydrogenase deficiency,	<1.6 µM		
C4-DC (Methylmalonyl) Methylmalonic acidemias (8 types)	<1.9 µM		
C5 (Isovaleryl/2-Methyl-butyryl) Isovaleric acidemia, 2-Methyl-3-hydoxybutryl CoA dehydrogenase deficiency	<1.6 µM		
C5:1 (Tiglyl/3-Methylcrotonyl) 3-Methylcrotonyl CoA carboxylase deficiency (3-MCC), Beta-ketothiolase deficiency	<1.0 µM		
C5-OH (3-Hydroxyisovaleryl) 3-MCC, 3-Hydroxy-3-methylglutaryl (HMG- CoA) lyase deficiency, Malonic aciduria, Multiple carboxy-lase deficiency	<2.0 µM		
C5-DC (Glutaryl) Glutaric acidemia,Type I,	<1.7 µM		
C6-DC (Methylglutaryl) HMG- CoA	<3.3 µM		
Fatty Acid Oxidation Defects:			
C0 (free carnitine) Carnitine uptake/ transport defects	<7.0 µM	All abnormal results will be phoned until the significance of minor abnormalities is determined	
C4 (Butyryl/Isobutyryl) SCAD Deficiency, Multiple acyl-CoA dehydrogenase deficiency (MADD)	<1.6 µM		
C5 (Isovaleryl/2-Methyl-butyryl) MADD	<1.6 µM		
C5-DC (Glutaryl) MADD	<1.7 µM		
C6 (Hexanoyl) MCAD Deficiency, MADD	<0.9 µM		
C8 (Octanoyl) MCAD Deficiency, MADD	<0.9 µM		
C10 (Decanoyl) MCAD Deficiency, MADD	<1.0 µM		
C10:1 (Decenoyl) MCAD Defi-ciency	<0.8 µM		
C14 (Tetradecanoyl) VLCAD Deficiency, MADD	<1.0 µM		
C14:1 (Tetradecenoyl) VLCAD Deficiency	<1.4 µM		
C16 (Palmitoyl) VLCAD Deficiency, LCHAD Deficiency, MADD	<9.7 µM		
C16-OH (Hydroxy Palmitoyl) LCHAD Deficiency	<1.1 µM		
C18 (Octadecanoyl) VLCAD Deficiency	<3.2 µM		
C18:1 (Oleyl) MADD	<6.4 µM		

*All phoned results are followed by mailed confirmation. All of these tests are screening tests. Abnormal results need full evaluation/discussion with a consultant before a diagnosis is confirmed or treatment is started.
Normal and abnormal values are subject to change based on continuing statistical evaluation.

ENDOCRINE DISORDERS

CONGENITAL ADRENAL HYPERPLASIA (CAH)* *(must be requested)*

Congenital adrenal hyperplasia is an inherited defect of cortisol synthesis. The adrenal gland cannot make cortisol and overproduces male hormones. Without cortisol, infants are at risk for adrenal crisis and may be unable to regulate salt and fluids, and can die. The incidence of 21-hydroxylase deficiency is 1:12,000 live births. The incidence is 1:300 in certain Yupik Eskimo populations.

Clinical Features

Unlike some other disorders in newborn screening, infants may be symptomatic at birth. By four to five months gestation, diminished cortisol production stimulates the fetal pituitary gland to produce ACTH and excessive adrenal androgens. The androgens virilize female external genitalia, but ovaries and uterus are unaffected. Male infants may have increased scrotal pigmentation or may be asymptomatic.

In two-thirds of cases, the 21-hydroxylase deficiency causes reduced production of mineralocorticoids. This leads to a hypotensive, hyperkalemic, salt-losing crisis with rapid onset of adrenocortical failure within 7-28 days of birth. This can be fatal. In one-third of cases, the infant has a "non-salt losing" or "simple virilizing form." If untreated, children have mild postnatal virilization, rapid growth with advanced skeletal age, early puberty, and short stature as adults. In adulthood, there is hirsutism and acne. Women have irregular menses and infertility.

Causes of CAH

The term "congenital adrenal hyperplasia" or "adrenogenital syndrome" covers a group of disorders. All are due to an inborn error of steroid hormone synthesis, which blocks the production of cortisol. The low level of cortisol stimulates ACTH, causing adrenal hyperplasia and increased secretion of steroid precursors. Different enzyme defects block the metabolic pathway at different sites and result in different clinical features. The most common disorder is 21-hydroxylase deficiency, which is the disorder tested for on newborn screening. There are variants to this disorder, which have later onset. All forms of CAH are inherited as autosomal recessive disorders.

Laboratory Tests

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

Confirmation

Confirmation is by measurement of serum 17-OHP and if salt wasting is suspected, sodium, potassium and plasma renin.

Treatment

Infants should be treated with hydrocortisone and mineralocorticoids in consultation with a pediatric endocrinologist. Infants with ambiguous genitalia need chromosome analysis to confirm gender.

Screening Practice Considerations

Female infants who are virilized or infants with ambiguous genitalia should be considered at risk for this condition, tested at birth, and monitored for electrolyte abnormalities until the diagnosis is excluded. Male infants are not usually recognized at birth. In both sexes, salt wasting and shock may develop rapidly within 7–28 days of birth. Approximately five to ten percent of infants will be detected only on the second screen. This disease kills quickly. Transport all specimens four to six hours after collection and no later than 24 hours.

RESULT	LIKELY CAUSES	ACTIONS
17-OHP >70 ng/mL, ≤10 days old, BW >2500 g 17-OHP >150 ng/mL, ≤10 days old, BW <2500 g 17-OHP>50 ng/mL, >10 days old, (any birth weight)	* CAH probable * False positive	**Neonatal emergency**; Oregon medical consultant contacts infant's physician by telephone and facsimile.
17-OHP >35-≤70 ng/mL, ≤10 days old, BW >2500 g 17-OHP >25-≤50 ng/mL, >10 days old, BW >2500 g 17-OHP >60-≤150 ng/mL, ≤10 days old, BW ≤2500 g 17-OHP >40-≤100 ng/mL, >10 days old, BW≤2500 g	* Mild CAH * False positive	Oregon laboratory notifies practitioner by letter with request to repeat filter paper sample.

*This test may be added to the screening battery by special request only. Please refer to page 42 for instructions on how to request test be added to the screening battery.

CONGENITAL HYPOTHYROIDISM

Congenital hypothyroidism occurs in babies who are born without the ability to produce adequate amounts of thyroid hormone. Thyroid hormone is important for normal function of all of the body's organs and is essential for normal brain development. The incidence of congenital hypothyroidism is 1:3,000. Newborn screening for congenital hypothyroidism is done in all states in our regional program.

Clinical Features

Deficiency of thyroid hormone in an infant may result in mental retardation and other signs of brain damage if it is not diagnosed and treated early in life. Many infants with congenital hypothyroidism may appear clinically normal before three months of age, by which time some brain damage has usually occurred. Only five percent of our cases were suspected by their doctor before the results of the screening were known. Laboratory test results are the only reliable means of diagnosing congenital hypothyroidism in the newborn.

When symptoms or signs are present, they may include prolonged neonatal jaundice, constipation, lethargy and poor muscle tone, feeding problems, a large tongue, puffy face, large fontanelle, distended abdomen and umbilical hernia. Since thyroid deficiency can occur at any age, normal tests in the newborn period do not exclude deficiency in an older infant or child.

Causes of Congenital Hypothyroidism

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

Laboratory Tests

The initial screening test is the T4 (thyroxine) assay. The 10 percent of samples with the lowest T4 results are further tested by a screening TSH assay. Different combinations of results are possible; see table below.

When the infant's physician is notified that screening results are abnormal, blood should be collected by venipuncture as soon as possible to confirm the

RESULT	LIKELY CAUSES	ACTIONS
T_4 low/TSH elevated	* Hypothyroidism probable * False positive	Lab will contact practitioner by phone and send letter requesting serum testing
T_4 < 3.0 µg/dL, TSH pending	* Prematurity * Hypothyroidism possible * False positive	Lab will contact practitioner by phone and send letter requesting further tests
T_4 low/TSH normal (on one or two specimens unless premature)	* Thyroid binding globulin (TBG) deficiency * False positive * Pituitary gland problem with secondary hypothyroidism * Prematurity - see below	Lab will contact practitioner by letter for further tests

abnormal screening results. In the case where the T4 is low and TSH is elevated, treatment can be started as soon as the serum is obtained, pending final confirmation. If the serum thyroid function tests confirm hypothyroidism, further diagnostic studies, such as a thyroid scan and bone age X-ray, may be desirable to determine the type, age of onset and severity of hypothyroidism. Generally, these studies do not change management and so are optional.

Thyroid Function in Premature Infants

In premature infants, there is a physiological reduction in blood T4 levels, TSH levels are not elevated in this situation. These cases need special observation to ensure that the low T4 levels rise into the normal range as the infant matures, but this may take several weeks.

Treatment

Treatment of congenital hypothyroidism is simple and effective. Thyroxine (e.g. Synthyroid, levoxyl or levothyroid), in pill form, is crushed, mixed with milk and administered once daily. The usual starting dose is 10-15 microgram/kg of body weight daily, usually 37.5 mcg/kg to 50 mcg/kg (11/2-2 of 25 mcg tablets). The American Academy of Pediatrics (AAP), recommends follow-up serum T4 (or free T4) and TSH as follows:

- Initiation of treatment: two and four weeks later
- First year: every 1-2 months
- Second and third year: every two to three months
- Thereafter: every 3-12 months

Treatment goals: serum T4 in the upper 1/2 of the normal range (10-16µg/dL) and TSH normalized (<10 µIU/mL). Clinical evaluations can occur less frequently. As infants grow, the dose of thyroxine is increased. Infants should also undergo periodic developmental testing. If treatment is started early and thyroid levels are monitored closely, the development remains normal.

Screening Practice Considerations

Hypothyroidism is the most common disorder covered by the program. Ninety percent of hypothyroid infants are detected on the first specimen even if it is collected a few hours after birth. In 10 percent of cases, hypothyroidism only develops in the weeks after birth and is therefore detected on the routine second screening test as production of thyroid hormone dwindles after birth. **Practitioners therefore must remain alert to clinical symptoms in older infants despite normal initial screening.**

False positive results may occur if the specimen is collected with the first few hours after birth, as the TSH rises in response to the extrauterine environment. Topical iodine may cause transient hypothyroidism in prematures.

The purpose of newborn screening is to identify infants at risk and in need of more definitive testing. As with any laboratory test, both false negative and false positive results are possible. Screening test results are insufficient information on which to base diagnosis or treatment.

HEMOGLOBIN DISORDERS

SICKLE CELL DISEASE AND OTHER HEMOGLOBINOPATHIES*

(must be requested)

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

Homozygous sickle cell disease (SCD) occurs when the recessive gene for hemoglobin S, sickle hemoglobin, is inherited from both parents. The term "clinically significant sickling syndrome" also includes conditions resulting from inheritance of one gene for hemoglobin S and certain other unusual hemoglobins, such as beta thalassemia or hemoglobin C. These doubly heterozygous conditions tend to be less severe than SCD, though all are capable of producing severe complications. The incidence of SCD in the African American population is 1:400, but also occurs at a lower frequency among all other ethnic groups. The disease incidence in a population depends on the population's ethnic composition. In Idaho, the incidence of clinically significant hemoglobinopathies is around 1:15,000.

Clinical Features

Sickle syndromes are systemic diseases and may involve any organ. They are characterized clinically by chronic hemolysis, intermittent vaso-occlusion and marked variability. Some patients experience unremitting complications, others lead a full and productive life. Early manifestations are often life-threatening and include overwhelming infection due to splenic dysfunction, splenic sequestration crisis, and aplastic crisis with profound anemia. Prior to newborn diagnosis and preventive care, mortality in the United States was 8-30 percent in the first three years of life. Some important complications include vaso-occlusive pain syndromes, osteomyelitis, acute chest syndrome, stroke, priapism, pyelonephritis, gallstones, skin ulcers, retinopathy, and decreased life expectancy.

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

Laboratory Tests

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

*This test may be added to the screening battery by special request only. Please refer to page 42 for instructions on how to request test be added to the screening battery.

RESULTS	LIKELY CAUSE	ACTION
FS (absence of A)	* Sickle cell disease or Sickle beta thalassemia	Lab will report to follow-up team who contacts practitioner by phone with instructions for diagnosis and treatment
FSC (absence of A)	* Sickle hemoglobin SC disease	Lab will report to follow-up team who contacts practitioner by phone with instructions for diagnosis and treatment
FC (absence of A)	* Homozygous C disease	Lab will report to follow-up team who contacts practitioner by phone with instructions for diagnosis and treatment
FE (absence of A)	* Homozygous hemoglobin E or hemoglobin E-beta thalassemia	Lab will report to follow-up team who contacts practitioner by phone with instructions for diagnosis and treatment
FAS	* Sickle cell carrier * Sickle beta thalassemia * Sickle cell disease in transfused infant	Lab will report by letter regarding test results and any other recommendations
FAC	* Hemoglobin C carrier * Homozygous C disease in a transfused infant	Lab will report by letter regarding test results and any other recommendations
FA+slow band	* Possible carrier for hemoglobin E, O, D, or G	Lab will report by letter regarding test results and any other recommendations
FA+fast band	* Possible alpha thalassemia * Bart's hemoglobin is a marker for alpha thalassemia	Lab will report by letter regarding test results and any other recommendations
F only	* Premature infant * Beta thalassemia major	Lab will report by letter regarding test results and any other recommendations
Predominance of A	* Transfused infant * Patient outside of neonatal age range	Lab will report by letter regarding test results and any other recommendations

Treatment

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

Carrier Detection Makes Hemoglobin Screening Different

This is the only newborn screening test which regularly identifies carriers (heterozygotes) as well as those affected by a given disease. In fact, many more carriers than disease states are identified for all hemoglobinopathies. If both parents are carriers of an autosomal recessive genetic trait, the risk of any infant of that couple being homozygous is 1/4. While the best way to handle this genetic information has yet to be agreed upon, several principles are currently operative: 1) The family is entitled to the information and it is private. 2) If both parents of a SCD carrier infant are African American, they have at least a 1/50 risk of having a subsequent child with SCD, because at least one of them is now known to be a carrier. The family should be offered testing and genetic counseling. If the family declines participation, this should be documented. 3) The abnormal screening results may need to be confirmed, using a liquid blood specimen. The Medical Consultant for hemoglobinopathy screening is available for assistance.

Screening Practice Considerations

Newborn screening for hemoglobinopathies is not done on the second specimen unless an abnormality has been identified on the first specimen. It is crucial to use the first kit for the first test; the cards are not interchangeable. Transfusion of red blood cells prior to drawing the newborn screening specimen will invalidate the hemoglobinopathy test. **Obtain a specimen before any transfusion.** Some hemoglobinopathies, particularly the thalassemias, are not reliably detected through newborn screening and a normal screening result does not eliminate the possibility that a patient has a hemoglobinopathy. Further testing or consultation should be sought if indicated by clinical suspicion.

The purpose of newborn screening is to identify infants at risk and in need of more definitive testing. As with any laboratory test, both false negative and false positive results are possible. Screening test results are insufficient information on which to base diagnosis or treatment.

METABOLIC DISORDERS

BIOTINIDASE DEFICIENCY

Detection of biotinidase deficiency requires urgent follow-up. This recessively inherited disorder affects the regeneration of the vitamin-cofactor biotin and impairs the metabolism of mitochondrial carboxylases. The incidence is estimated at 1:60,000 births. Screening for biotinidase deficiency is perfomed in all the states in our regional program.

Clinical Features

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

Laboratory Tests

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

RESULTS	LIKELY CAUSES	ACTIONS
Color change does not occur	* Biotinidase deficiency possible * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.

Treatment

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

Screening Practice Considerations

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

The purpose of newborn screening is to identify infants at risk and in need of more definitive testing. As with any laboratory test, both false negative and false positive results are possible. Screening test results are insufficient information on which to base diagnosis or treatment.

GALACTOSEMIA

Detection of galactosemia requires urgent follow-up and should be considered a potential medical emergency. Dietary galactose is most commonly ingested as lactose, the principle carbohydrate of human milk and most non-soy commercial infant formulas; it is hydrolyzed to glucose and galactose in the intestine. After absorption it is metabolized by several enzymes including galactokinase and galactose-1-phosphate uridyl transferase (GALT). When deficient, the latter causes galactosemia (1:60,000 births). Galactosemia is a recessively inherited condition and screening is performed in all the states in our regional program.

Clinical Features

The early clinical features of severe untreated galactosemia include neonatal hypoglycemia, liver damage, jaundice, failure to thrive, lethargy and sepsis. Vitreous hemorrhage has been reported in some infants. 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, renal Fanconi syndrome and mental retardation are usual.

There are several genetic variants with less severe reduction in the enzyme activity (e.g., the Duarte variant). Most of these cases are asymptomatic and are detected on newborn screening because of abnormalities. Recommendations for confirmatory testing are made by the medical consultants. The need for treatment of Duarte variant galactosomia is controversial and the consultants are available for consultation. The screening test is not designed to detect Duarte variant galactosemia and is not completely sensitive for this purpose.

Laboratory Tests

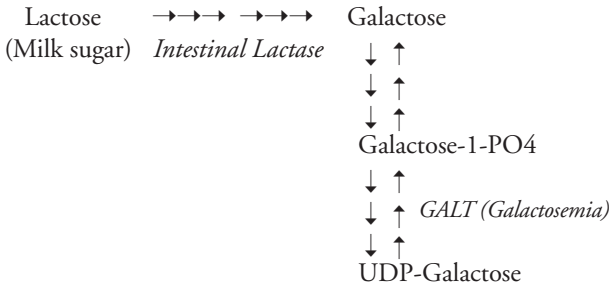
Two screening tests are used to detect galactosemia in a two tiered sequence (see diagram):

(1) GALT activity (Beutler Test) :
The enzyme test depends upon fluorescence produced by the normal galactose enzyme cascade in red blood cells. A temporarily abnormal result (diminished or absent fluorescence) is found in 1:2,000 infants. The test

RESULTS		LIKELY CAUSES	ACTIONS
GALT Test	Galactose Metabolites		
Abnormal	≥20 mg/dL	* Severe galactosemia * Variant galactosemia * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.
Abnormal	<20 mg/dL	* Severe galactosemic with little lactose intake * Variant galactosemia * Other enzyme defects in red blood cells * Improperly handled sample (heat damage or transit delay)	Contact by letter if >48 hrs old; contact by phone if < 48 hrs old or if not on lactose

may be persistently abnormal if the enzyme activity is <50% of normal. It does not differentiate milder variants from severe defects. All infants are screened with the Beutler test.

(2) Galactose (Hill Test):
Slight elevations (up to 20 mg/dL) can occur in normal neonates, but galactose metabolites are greatly elevated in infants with galactosemia if they are receiving a lactose-containing formula or breast milk. The Hill test is a fluorometric chemical spot test which measures galactose and galactose-1-phosphate. Liver disease may also cause an elevation of galactose metabolites. Only infants with an abnormal Beutler or who have been transfused will be screened with the Hill Test.



Treatment

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

Screening Practice Considerations

The GALT test should be abnormal in virtually all severe classic galactosemic infants even if the specimen is obtained before lactose is ingested, unless the infant has been transfused. **Obtain a specimen before any transfusion.** The GALT test is prone to inaccuracy if the sample is delayed in the mail or exposed to high temperature or humidity.

Galactose accumulation depends on **lactose** ingestion so that blood galactose metabolites may be normal in infants receiving soy based formula. This disease kills quickly. Transport all specimens four to six hours after collection and no later than 24 hours after collection.

The purpose of newborn screening is to identify infants at risk and in need of more definitive testing. As with any laboratory test, both false negative and false positive results are possible. Screening test results are insufficient information on which to base diagnosis or treatment.

AMINO ACID DISORDERS

UREA CYCLE DISORDERS *

The urea cycle is the metabolic pathway responsible for the detoxification of ammonia and for the synthesis of arginine and urea. There are six enzymes in the urea cycle, each of which if missing, will result in hyperammonemia. Six disorders, each with genetic and clinical variability have been reported, each representing one of the enzymes of the urea cycle. Three of these disorders can be detected in newborn screening tests*:

1. Arginase deficiency
2. Argininosuccinic aciduria
3. Citrullinemia

Estimated incidence of these conditions is 1:60,000. They are inherited as autosomal recessive traits.

Arginase Deficiency

Clinical Features

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

Citrullinemia & Argininosuccinic aciduria

Clinical Features-Neonatal Onset

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

Clinical Features-Late Onset

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

Laboratory Tests

Elevations of citrulline and arginine are detected by MS/MS. The laboratory cutoff for citrulline is <90 µM; for arginine <140 µM. Argininiosuccinic acid cannot be distinquished from citrulline using tandem mass spectrometry. Transient elevations of plasma arginine and citrulline in the newborn are unusual unless the infant is premature and/or receiving hyperalimentation.

** Screening will NOT identify all urea cycle disorders and sensitivity and specificity for Arginase deficiency is unknown. Practitioners should remain alert to the possibility of hyperammonemia in any infant with lethargy and coma in the first few days of life.*

RESULTS	LIKELY CAUSES	ACTIONS
Arginine >210 µM	* Arginase deficiency possible * Transient argininemia * Liver disease * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.
Citrulline ≥ 90 µM	* Citrullinemia, argininosuccinic aciduria possible * Transient citrullinemia * Liver disease * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.
Citrulline ≥ 90 µM on second specimen	* Mild citrullinemia, argininosuccinic aciduria possible * Transient citrullinemia * Liver disease * False positive	Lab requests repeat filter paper specimen

Treatment

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

Screening Practice Considerations

Infants with neonatal onset disease may be sick or die before screening results are known. Collect specimens before discharge and transport within four to six hours of collection and no longer than 24 hours after collection. Practitioners must remain alert to the possibility of these disorders in any newborn with lethargy or coma.

The purpose of newborn screening is to identify infants at risk and in need of more definitive testing. As with any laboratory test, both false negative and false positive results are possible. Screening test results are insufficient information on which to base diagnosis or treatment.

HYPERMETHIONINEMIA

Homocystinuria (cystathionine beta-synthase deficiency) *

The most common form of genetic homocystinuria is cystathionine beta-synthase deficiency (CBS). CBS is required for conversion of methionine to cysteine and deficiency results in the accumulation of homocystine, methionine and cysteine-homocystine disulfthides in the blood and urine. Unfortunately, methionine rises slowly in affected infants and may not be detectable on specimens obtained in the first few days after birth. Homocystinuria is inherited as an autosomal recessive trait. Occurs in approximately 1:100,000 births.

Clinical Features

Untreated patients appear normal at birth, but by the first or second year mental retardation may be apparent, most will develop dislocation of the lenses and a marfanoid body habitus, osteoporosis, and ultimately thromboembolism may develop which can result in serious and permanent disabilities or death.

Methionine Adenosyltransferase (MAT) Deficiency

Approximately 20 infants in the U.S., identified through newborn screening with persistently elevated methionine have been shown to have MAT deficiency. All but two patients have been asymptomatic, with normal growth and development. Two patients have had demyelination of the brain, but it is not clear that this is a result of MAT deficiency or other causes.

Laboratory Test

Elevation of methionine is detected by MS/MS; normal methionine levels are <80 µM. Transient elevations of plasma methionine in the newborn is unusual unless the infant is premature and/or receiving IV amino acid preparations. (i.e., TPN, hyperalimentation).

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

RESULTS	LIKELY CAUSES	ACTIONS
Methionine ≥ 80 –120 µM	* Homocystinuria/MAT deficiency possible * Tyrosinemia, Type 1 * Liver disease * Hyperalimentation * High protein diet * False Positive	Lab requests repeat filter paper specimen by mail
Methionine ≥ 120 µM	* Homocystinuria/MAT deficiency probable * Tyrosinemia, Type 1 * Liver disease * Hyperalimentation * High protein diet * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.

Treatment

Some patients will respond to pyridoxine in large doses (250-1200 mg/day). For patients unresponsive or partially responsive to pyridoxine, a methionine restricted, cysteine supplemented diet is usually effective. Betaine is usually effective. The outcome for treated patients is dependent on the age at diagnosis, adherence with therapy and severity of defect.

Screening Practice Considerations

Methionine rises slowly in affected infants, so that the first screening specimen may be normal; 80% of the homocystinuria patients detected in the NWRNSP have been found on routine second tests.

The purpose of newborn screening is to identify infants at risk and in need of more definitive testing. As with any laboratory test, both false negative and false positive results are possible. Screening test results are insufficient information on which to base diagnosis or treatment.

PHENYLKETONURIA (PKU) & HYPERPHENYLALANINEMIA

Detection of elevated phenylalanine levels requires urgent follow-up. This disorder is due to a recessively inherited enzyme defect in which the body cannot use the amino acid phenylalanine properly. All other metabolic processes are intact, but phenylalanine, which comes from all dietary protein, accumulates in the blood to toxic levels. Screening for PKU is performed in all states in our regional program.

Clinical Features

Infants with PKU seem to be normal for many months, however, without treatment, severe mental retardation, eczema and other problems usually develop. In older untreated patients the skin and hair may be fair, the eyes may be blue and a mousey odor of the skin or urine is common. Untreated blood phenylalanine level is often over 1200 µM.

Overall, PKU occurs in about 1 in 10,000-15,000 Caucasian and Hispanic births. It is less common in other races, but the racial frequency distribution is not well known. Although severe mental deficiency is the rule in untreated cases, occasional asymptomatic adults are found with normal or near normal intelligence, despite high phenylalanine levels.

Plasma phenylalanine is not detectably elevated in cord blood. It starts rising within 24 hours after birth and often reaches 1200 µM or more within a few days. The screening test is often abnormal within 24 hours and almost uniformly abnormal within 48 hours of birth.

Variant Forms of PKU (Hyperphenylalaninemia)

There are several intermediate forms of hyperphenylalaninemia in which the plasma phenylalanine levels are lower than in classic PKU (180-1200 µM). In these cases, mental retardation is variable and, in the milder variants, is completely absent. In infancy, these patients can mimic severe PKU, and for adult women the risk of the maternal PKU syndrome increases in proportion to the plasma phenylalanine.

Some forms of hyperphenylalaninemia are caused by defects of biopterin metabolism and 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. Definitive tests can differentiate these variant forms of PKU. In view of the severity of this group of diseases, all infants with persistently abnormal levels of phenylalanine will be recommended to have testing by special blood and urine tests for biopterin abnormalities. Information regarding this testing is provided through the metabolism consultants.

Maternal PKU and Hyperphenylalaninemia

Women with significant hyperphenylalaninemia have an increased risk of miscarriage and their offspring (who usually do not have PKU) may have intra-uterine growth retardation which persists postnatally. More than 90 percent of infants of untreated mothers with classical PKU have microcephaly, mental retardation, and/or congenital heart defects. They have a transient elevation of phenylalanine (240-1200 µM) which falls to normal within 24 hours. A screening test on the **mothers** of infants with transient hyper-phenylalaninemia, particularly if the infant's sample was collected in the first 24 hours after birth is recommended. Phenylalanine restricted diet begun prior to conception and during pregnancy can often prevent damage to the fetus.

The purpose of newborn screening is to identify infants at risk and in need of more definitive testing. As with any laboratory test, both false negative and false positive results are possible. Screening test results are insufficient information on which to base diagnosis or treatment.

Laboratory Tests

PKU and hyperphenylalaninemia are detected using tandem mass spectrometry (MS/MS); the normal phenylalanine level is < 200 µM.

Treatment

With proper treatment, mental retardation is totally preventable. Treatment should be started as soon after birth as possible (certainly before 3 weeks of age) in any infant recommended for treatment by the consultants and should be continued indefinitely. Frequent monitoring is required, especially in the first weeks, because variant forms of hyperphenylalaninemia may be indistinguishable from true PKU and improper nutritional therapy can be fatal.

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

Screening Practice Considerations

Detection may depend on the amount of protein ingested or endogenously produced by the infant, but most affected babies (90 percent) have abnormal results even in the first 24 hours of life regardless of intake. Those with milder forms of hyperphenylalaninemia require longer periods of feeding or catabolism to develop abnormal tests.

If an infant is tested < 48 hours of age, a repeat test must be done after five but before 15 days of age. (See Timing, page 32.)

Contamination of the filter paper with food, or liquids containing NutraSweet (Aspartame) may cause false positive results or inadequate specimen.

RESULTS	LIKELY CAUSES	ACTIONS
Phenylalanine ≥200 µM, Phe/Tyr ≥3.0 µM	* PKU possible * Variants forms of PKU * Mother has PKU * False positive * Transient hyperphenylalaninemia	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.

The purpose of newborn screening is to identify infants at risk and in need of more definitive testing. As with any laboratory test, both false negative and false positive results are possible. Screening test results are insufficient information on which to base diagnosis or treatment.

TYROSINEMIA

Elevated tyrosine may result from several inherited defects of tyrosine catabolism, delayed maturation of enzymes or liver disease.

Transient Tyrosinemia

Transient tyrosinemia of the newborn is common (1:1,000) and more common among Inuit and Eskimo populations in Alaska. Transient tyrosinemia is thought to arise from delayed maturation of the liver enzyme, 4-hydroxyphenylpyruvic acid dehydrogenase (4HPPD), coupled with increased protein intake and/or occult ascorbic acid deficiency. Tyrosine levels may be quite high (>500 µM) peaking at 14 days of life and resolved by 1 month. Premature infants may have prolonged hypertyrosinemia.

Clinical Features

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

Treatment

Transient tyrosinemia, while probably benign, may in some cases be treated with protein restriction to 2g/kg/day and administration of ascorbic acid (50-200 mg/day orally for 1-2 weeks) to infants found to have elevated tyrosine. If the infant is breast feeding, ascorbic acid alone may be administered.

Hepatorenal Tyrosinemia

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

Clinical Features

Tyrosinemia, Type I causes severe liver and renal disease and peripheral nerve damage. Presentation in infancy includes vomiting, lethargy, diarrhea and failure to thrive. Liver disease with hepatomegaly, hypoproteinemia, hyperbilirubinemia, hypoglycemia and coagulopathy may be present. In an international survey of 108 patients, 13% (n=14) became symptomatic in the first two weeks of life and 36% (n=39) in the first two months. Renal proximal tubular dysfunction results in aminoaciduria, hyperphosphaturia and hypophosphotemic rickets. Untreated, death in infancy or childhood from acute liver failure, neurological crises, or hepatocellular carcinoma is usual.

Treatment

Therapy with NTBC [2-(nitro-4-trifluoromethyl-benzoyl)-1-3-cyclohexanedione]blocks the formation of the toxic metabolites. NTBC is effective in preventing or halting liver and renal damage and averting acute neurological crises. Long term outcome of NTBC therapy on the development of hepatic carcinoma is yet unknown. Liver transplantation may be used in selected cases. Adjunct therapy with dietary restriction of phenylalanine and tyrosine as well as symptomatic treatment of clotting defects, rickets and proximal tubular losses may also be indicated.

Occulocutaneous Tyrosinemia

Tyrosinemia, Type II is caused by a deficiency of the enzyme tyrosine aminotransferase (TAT) and is inherited as an autosomal recessive trait. TAT deficiency is rare, with about 50 cases described worldwide.

Clinical Features

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

Treatment

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

Laboratory Tests

Tyrosinemia is detected using MS/MS; the cutoff tyrosine level is <475 µM. There is considerable overlap in tyrosine levels between normal infants, those with transient tyrosinemia and affected infants, making the tyrosine level itself not very specific. Clinical correlation, blood amino acids and urine syccinylacetone are necessary to differentiate these cases.

Screening Practice Considerations

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

RESULTS	LIKELY CAUSES	ACTIONS
Tyrosine ≥ 475 µM	* Transient Tyrosinemia * Tyrosinemia possible * Liver disease * Hyperalimentation * False Positive	Lab phones results to consultants, who phone practitioner with follow up recommendations. Notification is followed up by mail.

The purpose of newborn screening is to identify infants at risk and in need of more definitive testing. As with any laboratory test, both false negative and false positive results are possible. Screening test results are insufficient information on which to base diagnosis or treatment.

ORGANIC ACIDEMIAS

Organic acidemias (OA) result from enzyme deficiencies involved in the catabolism of multiple amino acids and other metabolites. Maple syrup urine disease is detected by an elevation of the amino acid leucine and an abnormal leucine/alanine ratio. All the other OAs are detected through elevations in acylcarnitines. All have autosomal recessive inheritance and collectively have an incidence of 1:53,000. The following OAs are screened for by MS/MS:

- Beta-ketothiolase deficiency
- Glutaric acidemia, Type 1 (glutaryl-CoA dehydrogenase deficiency)
- Isobutyryl CoA dehydrogenase deficiency
- Isovaleric acidemia, (isovaleryl-CoA dehydrogenase deficiency)
- Malonic aciduria
- Maple Syrup Urine Disease (branched chain alpha-ketoacid dehydrogenase deficiency)
- Methylmalonic acidemias, methylmalonyl CoA mutase deficiency and 5 defects of B12 metabolism
- Propionic acidemia
- 3-Hydroxy-3-methylglutaryl (HMG) CoA lyase deficiency
- 2-Methyl-3-hydroxybutryl CoA dehydrogenase deficiency
- 2-Methylbutyryl CoA dehydrogenase deficiency (mitochondrial acetoacetyl-CoA thiolase deficiency)
- 3-Methylcrotonyl CoA carboxylase (3MCC) deficiency
- 3-Methylglutaconyl CoA hydratase deficiency (3-methyl-glutaconic aciduria, Type 1)
- Multiple carboxylase deficiency

Clinical Features

Neonatal Onset: Most of these disorders have severe forms that present in the first week of life and constitute a neonatal emergency. Infants are generally well at birth, but develop irritability, lethargy, vomiting, and severe metabolic ketoacidosis, with or without hypoglycemia, in the first few days of life; this progresses to coma and death in the first month if treatment is not instituted. In methylmalonic and propionic acidemias, ammonia may also be elevated. Isovaleric acidemia is also associated with the odor of "sweaty socks." Isobutyryl CoA dehydrogenase deficiency is associated with a dilated cardiomyopathy. Even with prompt treatment, many infants with neonatal forms of organic acidemias sustain psychomotor damage and may have significant long term morbidity. These infants may be ill before the results of the screening tests are known.

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

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

Glutaric Acidemia, Type 1: Glutaric acidemia, Type 1 or GA1 is an organic acidemia with clinical features unlike those described above. In this disease, there is an accumulation of glutaric acid and 3-hydroxy glutaric acid, which are believed to be toxic to cells, particularly in the central nervous system. The classic presentation is macrocephaly at or shortly after birth. Infants have a period of apparently normal development but may have soft neurological signs, like jitteriness, irritability and truncal hypotonia. Generally between 6 and 18 months of age, patients will experience an acute encephalopathic episode resulting in damage to the basal ganglia and atrophy of the caudate and putamen. Severe dystonia, dyskinesis and other neurological findings result, either in a static or slowly progressive form. These

children are often misdiagnosed as having extrapyramidal cerebral palsy. Approximately 25% of GA1 patients will present with motor delay, hypotonia, dystonia and dyskinesis that develop gradually during the first few years of life, without any apparent acute crisis. Intellect is relatively intact. Infants with GA1 are prone to acute subdural and retinal hemorrhages, after minor head trauma. This can be misdiagnosed as child abuse. Finally, five percent of all Amish patients have been completely asymptomatic without any crises and normal development. Neurological crises and symptoms rarely occur after five years of age.

Laboratory Tests

All these disorders are detected by MS/MS. Leucine can be elevated in infants receiving hyperalimentation, usually along with other amino acid elevations. In a normal newborn, however, elevations of these compounds are unusual and require rapid follow up.

RESULT	LIKELY CAUSES	ACTIONS
Leucine > 300 µM Leu/ala >1.75	* MSUD possible * Hyperalimentation * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.
C3 (Propionyl) >8.0 µM	* Methylmalonic acidemias possible * Multiple carboxylase deficiency possible * Propionic acidemia possible * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.
C3 (DC Malonyl) >1.9 µM	* Malonic aciduria possible * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.
C4 (Butyryl) >2.3 µM	* Isobutyryl CoA dehydrogenase deficiency possible * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.
C4-DC (Methylmalonyl) >1.6 µM	* Methylmalonic acidemias possible * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.
C5 (Isovaleryl) >1.0 µM	* Isovaleric acidemia possible * 2-Methylbutyryl CoA dehydrogenase deficiency possible * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.
C5-DC (glutaryl) >1.7 µM	* Glutaric acidemia, Type 1 possible * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.

(table continued on next page)

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RESULT	LIKELY CAUSES	ACTIONS
C5-OH (methylcrotonyl) >1.7 µM	* 2-Methyl-3-hydroxy butyryl CoA dehydrogenase deficiency possible * 3-Methylcrotonyl CoA carboxylase deficiency possible * 3-Methylglutaconyl CoA hydratase deficiency possible * Multiple carboxylase deficiency possible * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.
C5:1 (Tiglyl/3-methylcrotonyl) >2.0 µM	* Beta ketothiolase deficiency possible * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.
C5-OH, C6-OH/DC Multiple elevations	* 3-hydroxy-3-methyl glutaric aciduria possible * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.
C6-DC (glutaryl) > 1.0 µM	* 3-HMG CoA lyase deficiency possible * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.

Treatment

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

Infants with GA1, in addition to diet and medications, must have aggressive supportive care during intercurrent illness through out the first 5-6 years of life. This generally entails hospitalization, IV fluid and calories during all febrile or flu like illnesses.

For individuals with MSUD, isovaleric acidemia and one or two other organic acidemias, prospective and early identification through newborn screening will be life saving and outcomes are expected to be good. For others, including those with GA1, the outcomes are less sure at this time.

Screening Practice Considerations

Affected infants must be detected early if major problems are to be prevented. Collect specimens before discharge and transport within four to six hours of collection and no later than 24 hours after collection. Practitioners must remain alert to the possibility of these diseases in any infant with lethargy, acidosis or coma.

The purpose of newborn screening is to identify infants at risk and in need of more definitive testing. As with any laboratory test, both false negative and false positive results are possible. Screening test results are insufficient information on which to base diagnosis or treatment.

FATTY ACID OXIDATION (FAO) DISORDERS

Mitochondrial beta-oxidation of fatty acids is crucially important in the body's ability to produce energy, particularly during fasting. In infants, a "fasting" state can be produced in as little as 4 hours. Fatty acids must be transported into the cytoplasm and then into the mitochondria for oxidation; carnitine is required for these transport steps. Once in the mitochondria, fatty acid chains 4-18 carbons in length must be oxidized, 2 carbons at a time, each reaction using a chain-specific enzyme, before ketogenesis can occur. There are over 20 individual steps in beta-oxidation some with multiple enzyme complexes. An enzyme block anywhere in this process or a carnitine deficiency will result in hypoketotic hypoglycemia and tissue damage related to the toxic accumulation of unoxidized fatty acids. At least 16 separate enzyme disorders have been identified, many of which may be identified by MS/MS by measuring the accumulation of various acylcarnitines.

Fatty acid oxidation disorders*

- Carnitine transport defect (enzyme unknown)
- Carnitine/acylcarnitine translocase (CT) deficiency
- Carnitine palmitoyl transferase (CPTI) deficiency
- Carnitine palmitoyl transferase II (CPTII) deficiency
- Very long chain acyl-CoA dehydrogenase (VLCAD) deficiency
- Long chain L-3 hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency
- Medium chain acyl-CoA dehydrogenase (MCAD) deficiency
- Short chain acyl-CoA dehydrogenase (SCAD) deficiency
- Multiple acyl-CoA dehyrogenase deficiency (MADD aka GA 2)
- Trifunctional protein (TFP) deficiency

MCAD is the most common, approximately 1:10,000 births; LCHAD is less frequent, but not rare. The true incidence of the other disorders is unknown. All are inherited as autosomal recessive traits.

Clinical Features

FAO disorders have overlapping symptoms and organ involvement. They fall into three major categories as described below.

Hepatic: There is no typical age of presentation, which may be on the first day of life through adulthood. Precipitating factors are fasting and/or stress associated with intercurrent illness. Patients present with "Reyes-like" symptoms including vomiting, lethargy, hypoketotic hypoglycemia, mild hyperammonemia, hyperuricemia, hypocarnitinemia and abnormal liver function tests. Liver biopsy often shows steatosis. Hepatic presentation is common in MCAD, VLCAD, LCHAD, neonatal CPT I & II and mild CT deficiency. Approximately 25 percent of individuals with MCAD will die during their first episode. In survivors, about 20 percent sustain significant neurological damage, presumably due to hypoglycemia. Patients with LCHAD also develop retinal pigmentary changes and progressive visual loss.

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

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

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

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

Treatment

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

Screening Practice Considerations

Neonatal forms of FAO disorders can present in the first few days of life. Collect specimens at discharge or between 48-72 hours. Transport within four to six hours of collection and no later than 24 hours after collection. Practitioners must remain alert to the possibility of FAO disorders in any neonate, infant or child with hypoketotic hypoglycemia or "Reyes-like" episodes.

Laboratory Tests

RESULTS	LIKELY CAUSES	ACTIONS
CO (free carnitine) <7.0 µM	* Carnitine transport defects possible * Carnitine deficiency * False	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.
C14 (Tetradecanoyl) > 1.0 µM C14:1 (Tetradecenoyl) > 1.4 µM C16 (Palmitoyl) > 9.7 µM	* VLCAD possible * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.
C16 (Palmitoyl) > 9.7 µM	* LCHAD,VLCAD, CPT II,CT deficiencies possible * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.
C16-OH (Hydroxy Palmitoyl)> 1.1 µM	* LCHAD, TFP possible * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.
C6 (Hexanoyl) > 0.9 µM C8 (Octanoyl) > 1.0 µM C10:1 (Decenoyl) > 0.8 µM	* MCAD possible * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.
C4(Butyryl/Isobutyryl) >1.6 µM	* SCAD possible * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.
C4 >1.6 µM C5 >1.0 µM C5-DC >1.7 µM C6 >1.0 µM C8 >0.9 µM C10 >0.9 µM C16 >9.7 µM	* MADD/GA II possible * False positive	Lab phones results to consultants, who phone practitioner with recommendation. Notification is followed up by mail.

The purpose of newborn screening is to identify infants at risk and in need of more definitive testing. As with any laboratory test, both false negative and false positive results are possible. Screening test results are insufficient information on which to base diagnosis or treatment.

SCREENING PRACTICES

Definition

SCREENING PRACTICES are the actions and decisions of practitioners regarding the collection, handling and follow-up of newborn screening specimens. Over 30 percent of cases of PKU and hypothyroidism missed by screening programs are due to errors in screening practice.

Who Is Responsible For Ensuring That The Screening Test Is Performed?

Idaho Department of Health and Welfare Administrative Rules and Regulations state that the administrator of the hospital where an infant is born and the person required to register the birth of a child are responsible for specimen collection.

Parent Refusal To Have The Baby Tested

The parent may refuse testing based on religious beliefs. However, it is suggested that the practitioner obtain a signed Religious Objection document and ensure that it is placed in the infant's medical record. Failure to do so may result in practitioner liability if an affected infant is not screened. Sample statements of parental religious exemption are given on page 41.

Proper Time For Testing

Specimens obtained prior to 48 hours of age are **valid** for most of the conditions covered on the newborn screening battery. There is, however, a statistical chance that aminoacidopathies may be missed. In Idaho, six percent of all infants with PKU and ten percent of those with hypothyroidism are found on the second specimen with a normal first specimen.

In Idaho, for normal, non-premature infants the specimen should be obtained between 48-72 hours and no later than five days of age. However, all infants must have a newborn screening specimen collected before hospital discharge. If an infant is tested before 48 hours of age, a second newborn screening sample must be collected after five days, but before 15 days of age.

Percentage of Affected Infants Identified by Routine First and Second Screening Tests in the NWRNBSP

DISORDER	FIRST TEST	SECOND TEST
Hemoglobinopathies	>99%	NA
Hypothyroidism	90	10%
Congenital Adrenal Hyperplasia	95	5
Phenylketonuria	>94	6
MSUD	>99	1
Other Amino Acidopathies	50-90	10-50
Galactosemia	99	1
Biotinidase Deficiency	100	0
Fatty Acid Oxidation Disorders	Unknown	Unknown

Testing Before Discharge

The law specifies that infants must be tested before discharge from the birthing unit, regardless of the age or feeding status. This is done because some infants do not return for routine postnatal care and most of

SPECIMEN TIMING		
	If 1st sample is obtained:	then 2nd sample should be obtained
"Early Testing"	≤48 hours	5-15 days
"Standard Test"	>48 hours-5 days	15 days-until collected** 2nd sample is not required if taken by these guidelines

** In any case, a specimen obtained outside the recommended guidelines is preferred over an infant having no specimen or no repeat at all.

the tests are valid at any age. Failure to collect a specimen before discharge may confer a significant liability on both the facility and responsible practitioner if an affected infant is missed as evidenced by published case law. It is best to obtain the first test as close to discharge as possible for normal newborns.

Testing Before Transfer Of Infant To Another Unit

Idaho Department of Health and Welfare Administrative Rules and Regulations state that for infants who are transferred to another hospital, the originating hospital shall assure the newborn screening specimen is drawn. If the infant is too premature or too sick to have a specimen drawn for screening prior to transfer, the originating hospital shall be responsible for clearly documenting this, and notifying the hospital to which the infant is being transferred that a newborn screening specimen has not been obtained.

Patient Demographic Information

Be sure to use the correct part of the kit: **PART 1** for the first test and **PART 2** for the second test. Failure to do so makes it difficult to track the infant's results. In addition, hemoglobin testing is only done on the first specimen. The laboratory form should be completed with the information requested; it is part of the legal record and must be legible. Infant's name, birth date, specimen date and physician's name are particularly important. It is also important to indicate what kind of food the infant has received prior to testing. Patient information is critical for rapid follow-up in the event of abnormal results. Do not use plastic imprint cards, as they often produce unreadable information and cause compression damage to the filter paper.

Special Transport

It is critically important that newborn screening specimens are received by the laboratory as soon as possible after collection. Ideally specimens should be mailed or transported as soon as they are dried (four to six hours) and no later than 24 hours after collection. Many of the conditions on the screening battery kill or maim infants in the first week of life. To prevent this, diagnosis and treatment must occur rapidly. Consideration should be given to the use of overnight courier or mail services if specimens are taking longer than two or three days to arrive at the laboratory. Significant degradation of hemoglobin and some analytes also occurs in specimens older than one week.

If couriers are used, it is advisable to establish a list of specimens sent and to document the time of specimen pickup. This protects the submitter if specimens are lost in transit.

Special Considerations

Many intensive care nurseries obtain an initial screening specimen on all infants on admission to avoid these problems:

Premature Or Sick Infants: Infants who are premature or sick should have the first test collected between five and seven days. Remember that T4 results may be quite low in normal premature infants.

Hyperalimentation And Antibiotic Therapy: These are not contraindications to testing, but samples should not be taken from the line which is used to deliver the alimentation or drugs. High levels of several amino acids can occur during hyperalimentation. Antibiotics containing pivalic acid given to mothers during labor or newborn may cause a false elevation of isovaleryl/2-methyl butyryl carnitine. Be sure to specify these therapies on the request slip.

Transfusions: A specimen should be obtained **before** the transfusion, as donor cells provide normal levels of enzymes and hemoglobins. Metabolite tests (e.g., PKU, MSUD, galactosemia) will become abnormal after a few days, regardless of the transfusion. If an infant is not screened prior to transfusion, obtain a specimen from the blood drawn for typing and cross matching if possible. Be sure to specify this on the request slip.

It may take as long as 120 days for infants to develop abnormal enzyme levels and hemoglobins after a transfusion, significantly delaying diagnosis and treatment.

Clinical Signs or Family History

There are a number of clinical situations that will modify the usual approach of obtaining a newborn screening specimen and waiting for the result. The following are suggested guidelines for particular situations that may arise in clinical practice. Regardless of any diagnostic or therapeutic interventions, a newborn screening specimen should be obtained on all infants, to test for the other conditions included in the panel.

When in doubt about the course of management for any of the conditions on the screening test, consultation with a specialist is advised.

- **Newborn Screening of an Infant Who Exhibits 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 newborn screen conditions 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. It may be necessary to **treat as if the infant has the condition**. 24-hour medical consultation is available for assistance with rapid diagnosis and institution of treatment for infants suspected to have disease.

- **If the newborn screening test result was "normal":** If clinical symptoms suggest one of the screened conditions despite a "normal" screening test, the physician should **proceed as if the patient has the condition** and immediately contact a consultant specialist for instructions on further evaluation of the patient.

- **Newborn Screening of an Infant with an Affected Sibling or Other Close Relative:** As many of the conditions tested for by newborn screening are genetic, it is possible that multiple members of a family may be affected. Prenatal diagnosis is possible for many of these conditions; if prenatal diagnosis determines that the infant is affected, appropriate treatment should be initiated immediately after birth. If prenatal diagnosis predicts an unaffected baby, practitioners should bear in mind that no prenatal diagnostic test is 100% accurate. Neonates who are siblings or close relatives of an affected individual are not part of the "general population" for whom newborn screening is designed. For any infant with a positive family history, providers should contact appropriate consultant specialists, ideally prenatally, or immediately at birth, to determine the proper diagnostic tests and proper timing of those tests.

Consultation with a specialist is usually necessary and always recommended in these situations. The directory on pages 1 and 2 offers consultants by disease for your state to assist with screening and confirmation testing.

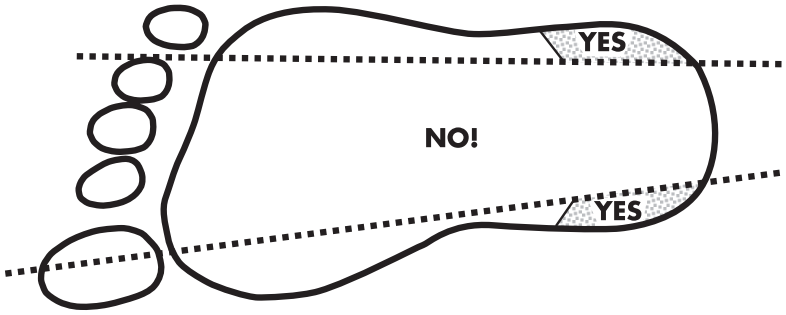
Specimen Collection[1]

1. To prevent specimen contamination, do not touch any part of the filter paper circles before, during or after collection. Disposable gloves and lactose residue from gloves or sweat/oils from fingers can contaminate the specimen.
2. Hold infant's limb lower than the heart, warm if necessary with water or a warm towel. Heat pack should not exceed 42°C and should not be left in contact with skin for a prolonged period.
3. Select a puncture site on the heel (see diagram) and cleanse with alcohol (not iodine), air dry. Cord blood is **not** a satisfactory specimen. Samples obtained from peripheral or central line are acceptable, provided that the line is not being used for hyperalimentation or for antibiotics.
4. Puncture the heel with the sterile lancet to the full length of the blade. Wipe away the first drop of blood to remove tissue fluids. Sufficient blood should collect on the heel to fill each circle by a single application of paper to a drop of blood. Apply only from one side of the filter paper. Blood should soak all the way through the paper such that the blood spots look similar on both sides. **Complete saturation of the entire circle is essential for accurate testing.**
5. It is important **not** to superimpose the blood drops on top of each other. Let each drop touch the paper about 1/8 inch away from the previous one. This prevents layering of the paper, which is one cause of false results.
6. **Collect the blood in all four circles.** A minimum of three circles is necessary to complete the test battery. If there are problems obtaining an adequate quantity of blood, it is better to fill three circles completely, than to fill four circles inadequately.
7. After blood has been collected, the foot should be elevated above the body, and a sterile gauze pad or cotton swab pressed against the puncture site until the bleeding stops.
8. Air dry specimens at room temperature for 2-4 hours in a horizontal position. Do not dry on a heater or in a microwave. Ensure that sample is completely dry before mailing.
9. Insert dried sample into envelope (do not use plastic), seal and mail. If sample(s) cannot be mailed because of a Sunday or Monday holiday, it is better to store it in a cool room and send by express mail. All specimens should be mailed first class or express. **Do not batch specimens collected on separate days. Do not put in a hot mailbox over the weekend.**

Recommendation for Heel Puncture Site in Newborns

Perform punctures on the most medial or most lateral portion of the plantar surface of the heel (see diagram). Lancets longer than 2.4 mm should not be used on infants weighing less than 2500 g.

A full-color chart illustrating proper specimen collection, "Neonatal Screening Blood Specimen Collection and Handling Procedure," may be obtained at no charge from Schleicher & Schuell, Inc., PO Box 2012, Keene, New Hampshire 03431; 800-245-4024; FAX 603-357-7700.



[1] *These instructions are consistent with the recommendations in Blood Collection on Filter Paper for Neonatal Screening Programs. National Committee for Clinical Laboratory Standards. Vol. 12 No. 13 1992 [NCCLS Document LA4-A2]*

Unsatisfactory Specimens

Newborn screening laboratories receive many specimens that are unacceptable for testing. If the specimen is improperly collected, the accuracy of the screening test results is compromised, so the laboratory must reject them. This delays the screening of the newborn and **requires that the submitter locate the infant and repeat the collection procedure**.

The following table outlines the most common errors in specimen collection.

INVALID SPECIMEN	POSSIBLE CAUSES
Quantity of blood not sufficient for testing (QNS)	Filter paper circles incompletely filled or not saturated/not all circles filled. Blood applied with needle or capillary tube. Contamination of surface of filter paper circle before or after specimen collection by gloved or ungloved hands, or by substances such as hand lotion or powder, etc.
Blood spots appear scratched or abraded	Blood applied improperly using capillary tube or other means (filter paper has been damaged or torn by device).
Blood spots wet	Specimen not properly dried before mailing.
Blood spots appear supersaturated	Excess blood applied (usually with capillary tube or needle). Blood applied to both sides of filter paper.
Blood spots appear diluted, discolored, or contaminated	Puncture site squeezed or "milked." Exposure of blood spots to direct heat. Contamination of filter paper before or after specimen collection by gloved or ungloved hands, or by substances such as alcohol, formula, water, powder, antiseptic solutions, or hand lotion. Contamination during transit.
Blood spots exhibit "serum rings"	Alcohol not wiped off puncture site before skin puncture is made. Filter paper has come into contact with alcohol, water, hand lotion, etc. Puncture site squeezed excessively. Specimen dried improperly. Blood applied to the filter paper with a capillary tube.
Blood spots appear clotted or layered	Same filter paper circle touched to a blood drop several times. Circle filled from both sides of the filter paper.
Blood will not elute from the filter paper	Blood specimen has been heat-fixed. Blood specimen is too old (more than two weeks between collection and receipt by the screening laboratory).

Consult the state screening lab for additional information and assistance with specimen collection.

A full-color chart illustrating invalid specimens and their causes, "Simple Spot Check," may be obtained at no charge from Schleicher & Schuell, Inc., PO Box 2012 Keene, New Hampshire 03431; 800-245-4024; FAX 603-357-7700.

REPORTING OF RESULTS

Practitioner Responsibility For Documentation

It is the responsibility of the practitioner to ensure every baby is tested and that a result is received and filed in the medical record. Practitioners should know the screening status of every infant in their care, regardless of age. Specific care should be paid to infants/children adopted from third world countries. Specimen collection must be documented in the infant's chart and if preferred in a separate log book. Information should include name of infant, hospital ID number, screening kit ID number, date collected, date mailed and the name of the person who collected the specimen. When screening results are returned to the submitter they should be noted in the log book and the report filed in the medical records. Private practitioners must also ensure that the second specimen is obtained at the appropriate time and that documentation is completed.

In the event that the results of an infant's screening tests are not received from the Screening Laboratory within two weeks after collection, the hospital and practitioner **must** assume responsibility for follow-up. We recommend the following procedure:

1. Contact the Oregon State Public Health Laboratory, Newborn Screening Section, Leanne Rien, RN, Coordinator, (503) 229-5466 or Idaho Department of Health and Welfare, Newborn Screening Program, (208) 334-5962, to determine if specimen was received and to request the report be mailed or faxed.
2. If the specimen was not received, it must be presumed lost. Notify the infant's private physician, local health nurse or parents by phone or letter that the specimen may have been lost and that another should be obtained without delay.
3. Document these actions in the infant's medical record.
4. Request a copy of screening results to be sent to your facility for the medical record.
5. If these steps do not result in the infant being screened, notify Idaho Newborn Screening Program.

Normal Results

Normal results are mailed daily to submitters. The complete test battery is usually completed in two to ten working days after receipt by the laboratory.

"Significant" Abnormal Results

All results considered urgent are reported immediately to the medical consultants who will phone submitting hospital or practitioners with recommendations for further action. Such action is followed by mail or fax confirmation.

Before discharge, hospital and birth unit personnel may be designated and listed on the request slip as the physician-of-record. In the event of an abnormality, the screening laboratory will refer to the physician-of-record although the infant is no longer under his or her care and may not even be known to them. **Responsibility for follow-up remains with the physician-of-record until it is actively accepted by another practitioner.**

Other Abnormal and Repeats

Lesser abnormalities or inadequate samples are reported immediately by mail or fax to the submitting hospital and/or practitioner with a request for retesting.

It is the practitioner's responsibility to ensure that all infants with abnormal results are retested, however all such infants are also tracked by the laboratory and/or the medical consultants until resolutions or diagnoses are confirmed.

The state health agency is notified for assistance when there are problems in obtaining repeat tests for infants with abnormal results.

PROBLEMS IN SCREENING PRACTICE

Infants Who Are Never Tested

In the United States, for every one percent of infants who are not tested, three cases of PKU and ten cases of hypothyroidism are likely to be missed each year. These infants represent a major medical and legal liability to the program and to practitioners involved in their care. The legal awards for missed cases have been as high as $30 million per case. Special attention should be given for older infants not born in the US (i.e., adopted and/or migrant).

Parents' Refusal To Have The Baby Tested

See page 32 for guidelines, and page 41 for an example of form to use in this situation.

Common Misconceptions

Failure to test a baby is often due to misconceptions about the program or the tests. For example, some practitioners believe the diseases are so rare they advise parents not to have the testing done. Approximately 50-100 infants per year are identified with one of the conditions described in this manual in the five state regional program. Others believe the first test is of no value if obtained before 48 hours of age. Over 85 percent of affected infants have abnormal results on the first test regardless of age. See table on page 32.

Timing Of The Tests

High rates of early discharge and home delivery make this a difficult problem in newborn screening. Timing recommendations are clear.

All infants should have a specimen collected before discharge, ideally between 48-72 hours of age. For infants screened before 48 hours, a second specimen should be collected after five days and before 15 days.

In Idaho in 2002, 64 percent of all first samples were obtained before 48 hours of age, and 70-75 percent of babies do get two samples. **Remember that 10 percent of all cases of hypothyroidism are detected from the second sample, with a normal first sample.**

Specimen Inadequacy

Less than one percent of samples submitted to the laboratory contain insufficient blood to do all the tests or are contaminated with milk, stool or urine. This generates an automatic request for a retest and delays the testing.

Samples which are heated during drying or transport may be damaged so that some tests are invalid.

Inadequate Demographic Information

In Idaho five percent of specimens are missing key patient data. For example, the specimen does not list the infant's name, or it is unreadable. In the event of an abnormal result, these specimens are difficult and time consuming to match to the correct infant, especially in large facilities. The most common cause for unreadable specimens is illegible handwriting. It is important to print clearly and neatly. Data which are critical include: name, birthdate/hour, sample date/hour, feeding status, mother's name, as well as practitioner's name.

Problems Related To Specimen Transport To The Laboratory

All specimens should be transported as soon as they are dry and no later than **24 hours after collection**. They should be received by the screening laboratory no later than five days after collection. About 35 percent of Idaho's specimens take longer than five days to reach the laboratory. While some are due to unavoidable postal delays such as holidays, most are caused by failure to mail the samples promptly. Samples may be delayed because of sluggish in-house mail, inefficient courier services or simple forgetfulness, but perhaps the most dangerous practice is "batch mailing," when samples are held up to a week so postage costs can be reduced.

Specimens collected on Sundays or Monday holidays are best stored in a cool room and sent express mail at the first opportunity.

The importance of early sample collection and prompt transit is illustrated by the fact that babies with galactosemia, organic acidemias and fatty acid oxidation disorders may die within a week or two of birth. Enzyme activity and hemoglobin may be destroyed or diminished in specimens which are older than 10 days or which have been exposed to heat and humidity.

EDUCATIONAL SERVICES

Screening Practice Surveillance Program

In an effort to assist hospitals, birth facilities, and individual practitioners, the laboratory monitors the screening practices (transit time, inadequate specimens, demographic omissions and timing errors).

Screening Practice Profiles are available from the Idaho Newborn Screening program by contacting:

Idaho Department of Health and Welfare
PO Box 83720
Boise, ID 83720-0036
(208) 334-5962
FAX (208) 332-7307

Birth Facilities, Health Departments, And Community Practitioners

Video tape demonstrations showing correct collection procedures, complaints, problems and questions are available to borrow or purchase by contacting Leanne Rien, RN at 503-229-5466.

Parents And Lay Public

Parent brochures are included with each kit order. Additional brochures are available upon request by contacting Leanne Rien, RN at 503-229-5466.

There is no charge for any of these eductional materials or services.

SCREENING KIT INFORMATION

Only a standardized, quality tested filter paper can be used for specimen submission (Schleicher and Schuell 903). Requests for kits must be made using a kit request form showing the quantity requested and must include a check for prepayment. Orders and payment should be submitted to:

Idaho Department of Health and Welfare
Newborn Screening Program
PO Box 83720
Boise, ID 83720-0036
(208) 334-5962

Please allow two-three weeks for preparation and shipping. It is the responsibility of the user to pay the postage to send specimens to the laboratory. Kit orders and specimens are not to be sent by collect mail.

Type Of Kits

Hospitals and birthing facilities receive double kits with identical kit numbers, so that the first and second specimens can be easily matched. Health departments, clinics, and private practitioners may order double or single kits. Single kits should only be used when the second part of a double kit has been lost or damaged. **Please note: all kits are precoded for the specific individual/facility; they must not be loaned to, or borrowed from, other facilities.** To obtain uncoded kits (to replace lost kits or to deal with emergency situations), contact Idaho Newborn Screening Program at 208-334-5962.

Cost Of Diagnostic Tests For Confirmation Of Abnormal Screening Results

When diagnostic quantitative tests are requested by the laboratory or by the medical consultant for full evaluation of an infant considered likely to have one of the disorders, the costs of performing these tests for indigent families will be absorbed by the program provided that the samples are properly handled and submitted to a laboratory approved by this program. Fees for tests which are not specifically requested, or which are sent to other laboratories, will not be reimbursed.

EXEMPTIONS

Religious Objections

A religious exemption can be claimed from the requirement for the newborn screening tests. In this event, the person otherwise responsible for submitting the specimen for testing is responsible for submitting a completed statement on the back of the specimen form to the State Laboratory signed by the infant's parent using the following language:

Statement of Religious Exemption

The undersigned parent of _____states that this child is exempt from testing for detection of disorders covered in the newborn screening battery in that the child is being reared as an adherent to a religion the teachings of which are opposed to such testing.

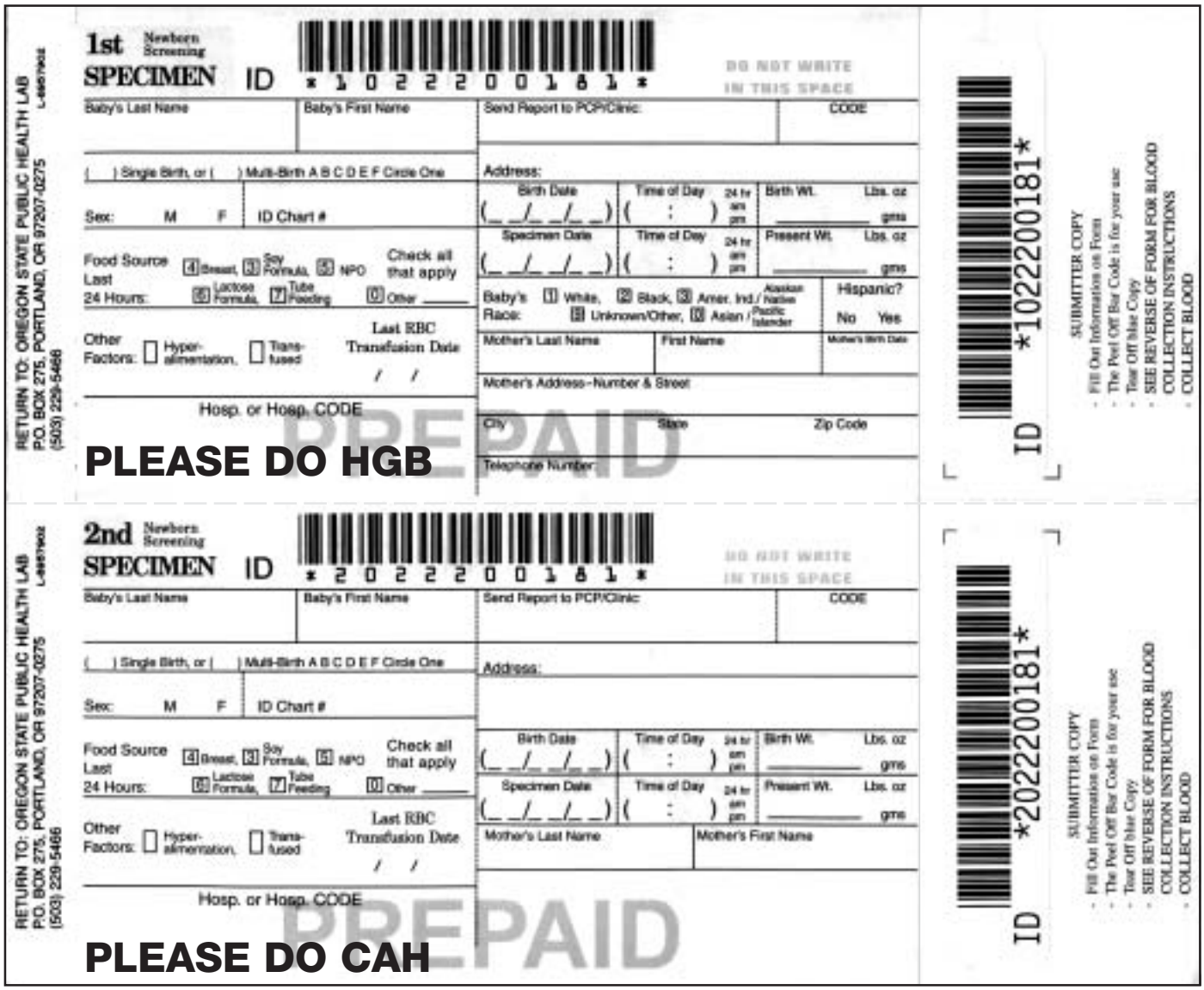
_____ _____

PARENT'S SIGNATURE DATE

INSTRUCTIONS FOR SPECIAL REQUESTS

A hospital, practitioner or midwife may request newborn screening be done for Sickle Cell Disease or Congenital Adrenal Hyperplasia on an infant. The request may be made on either the first newborn screen or the second newborn screen (although the first specimen is preferred). Using the newborn screening kit example below as a guide, very clearly write on the form at the bottom, "PLEASE DO HGB" for Sickle Cell Disease or "PLEASE DO CAH" for Congenital Adrenal Hyperplasia. Highlighting in yellow or writing in red will help the laboratory staff see the specimen request. Further questions can be directed to Idaho Newborn Screening Program (208) 334-5962.

It is recommended if a practitioner is requesting these two tests on a routine basis, they order a special stamp to use instead of writing on the form. Be sure to stamp all copies of the newborn screening form.



A COLLABORATIVE PROJECT INVOLVING:

Oregon Department of Human Services

Oregon Health & Science University

Alaska Department of Health & Social Services

State of Hawai`i Department of Health

Idaho Department of Health & Welfare

Nevada State Health Division

DHS
Oregon Department of Human Services

In compliance with the American with Disabilities Act (ADA), if you need this information in alternate format, please call: Oregon State Public Health Laboratories at (503) 229-5882.

http://www.ohd.hr.state.or.us/nbs/index.cfm
http://www.oregon.gov