Welcome to the third issue of the Heel Stick News, the newsletter for physicians, physician assistants, lab managers and staff, nurse managers, nurse practitioners, midwives, hospitals, and birthing centers. It brings you news, reports and resources from the Iowa Department of Public Health – Center for Genetics.

Our main feature in this issue is the Iowa Neonatal Metabolic Screening Program (INMSP) screening report forms to show how the INMSP results are reported to the submitting facilities. We have also included an article on phenylketonuria and its history in Iowa, which was submitted by Marcia Valbracht. In this issue you can also find information about changes in mailing dried blood spot specimens, and you will be able to test your knowledge with a quiz on newborn screening.

Since we would like to provide our readers with a wide range of information about activities of the Iowa Neonatal Metabolic Screening Program, we will be inviting you to submit ideas for future articles. If you have any comments, or would like to make a contribution, please contact Dawn Mouw, dawn.mouw@idph.state.ia.us. If you would like to view the newsletter archives, please use our website: www.idph.state.ia.us/genetics.

For Your Information.......

Dried blood spot specimens must NOT be packaged in airtight, leak-proof plastic bags! The lack of air exchange in a sealed plastic bag causes heat buildup and moisture accumulation which can damage the dried blood spot specimen.

False positive results may be due to immature endocrine or enzyme function in the newborn, the stress of birth on an infant, or the specimen being collected prior to 24 hours after birth. INMSP establishes screen cutoff values that keep the number of false positives at a minimum, yet minimize the likelihood of an affected newborn being missed.

BIOHAZARD Labeling Changes

The new federal regulations affecting the packaging standards for mailable types of infectious substances went into effect January 1, 2004. The new rules impact the mailing of dried blood spot specimens used for newborn screening (Section 8.10, Packaging for Risk Group 1 Materials). These rules are published in the Federal Register [Volume 68, No. 109/Friday, June 6, 2003/Rules and Regulations (pages 33858-33873)].

To assist facilities in meeting the new regulations, the Iowa Neonatal Metabolic Screening Program (INMSP) is providing florescent orange biohazard labels bearing the international biohazard symbol for specimen collection forms. The label must be placed at the top of the flap covering the blood spot filter paper. This flap is considered the “primary” container for the dried blood spot specimen. The biohazard label must be affixed to each newborn screening specimen form. This is the only change required under the new rules. Contact the University Hygienic Laboratory for additional labels at (515) 243-0141.

See pages 2-3 for an article on Phenylketonuria (PKU).

PKU web sites:
National PKU News
www.pkunews.org
National Coalition for PKU and Allied Disorders
www.pku-allieddisorders.org
PKU Listserv
www.listservemory.edu/archives/pku-support-l.html
Children’s PKU Network
www.pkunetwork.org/

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History of Newborn Screening for Phenylketonuria

Marcia Valbracht, MHA

Before any knowledge of enzymes or metabolism existed, Archibald Garrod published a paper titled, “The Incidence of Alkaptonuria, A Study of Chemical Individuality,” in which he showed “real insight into the inheritance of specific chemical defects in metabolism.” (Guthrie, 1986). In 1902, he coined the phrase “inborn errors of metabolism.” Though Gregor Mendel, in 1866, published his findings on heredity in peas, Versuche über Pflanzen Hybriden, his findings were largely ignored until the early 1900’s. With the resurgence of Mendel’s law of heredity, Garrod was able to piece together ideas that would form the background for newborn screening.

Dr. Asborn Folling discovered phenylketonuria, also known as PKU, almost 70 years ago in two severely retarded children. The children’s mother could smell a peculiar odor in the urine and associated this with their problem. Other doctors would not listen to her, and one referred her to psychiatric help for her “delusions.” However, Asborn Folling listened to her, smelled the urine and developed a test that would eventually demonstrate the accumulation of phenylpyruvic acid in urine and its well-known green color reaction to ferric chloride (Guthrie, 1986).

In 1953, George Jervis demonstrated the enzyme defect in phenylketonuria occurs when tyrosine forms from phenylalanine hydroxylation. That same year, Horst Bickel determined that excess phenylalanine in the diet was the cause of hyperactive and bizarre behavior in a two-year-old phenylketonuric girl. Under pressure from the mother, Bickel eventually was able to produce a diet for the little girl. New interest in the early treatment of phenylketonuria appeared.

In the early 1960s, screening programs were created in the United States, Europe, and New Zealand using Folling’s ferric chloride reagent test. The test procedure was found inadequate due to the delay in the analysis and its inability to detect all cases of phenylketonuria. However, in 1961, Robert Guthrie invented the filter paper blood spot and the bacterial inhibition assay. Coincidentally, that same year, The National Association for Retarded Children published a poster of two siblings; the younger detected because her older sibling had the disorder. President Kennedy also launched a national program on mental retardation and increased the availability of federal funds. In the 1960’s the federal government, through the Children’s Bureau, implemented a large field trial of Guthrie’s test in 29 state health departments. The bacterial inhibition assay used “an anti-metabolite analog of phenylalanine as a growth inhibitor in an agar culture medium. Adjusting the concentration made it possible to get a very precise and sensitive change in the diameter of a growth zone surrounding a disc of dried blood, punched from a filter paper specimen card, at the normal range for phenylalanine” (Guthrie, 1986). Bacillus subtilis spores placed in a phenylalanine-free agar base are the growth medium. The inhibitor, beta-2-thienylalanine, suppressed the growth of the microbes. The presence of phenylalanine blocked the action of the inhibitor allowing the Bacillus subtilis to grow in the area around a filter paper disc containing phenylalanine. Once the growth zone surrounding the discs of the blood specimens from newborns is measured and compared to calibrators, normal specimens can be distinguished from the abnormals.

The development of other tests for inborn errors of metabolism such as leucine, methionine, and galactosemia used the same procedural application. The bacterial inhibition assay is still used to successfully screen newborns for phenylketonuria.
“The History of Newborn Screening” 
(pages 2-3)


Continued from page 2, “The History of Newborn Screening-PKU.”

years later. However, the test is not very sensitive at the low concentrations of phenylalanine found in affected infants collected at 24 hours of age, or earlier. A newborn must be old enough to metabolize food and build up detectable measures of phenylalanine in the blood. Babies should be three to four days of age before their blood is drawn when tested by the bacterial inhibition assay. This assures that no affected infants will be missed.

McCaman and Robins developed a quantitative fluorometric assay in 1961 that could use the filter paper blood spot sample. This method was later modified for use on an Autoanalyzer™. The phenylalanine eluted from the blood spots was added to ninhydrin and its fluorescence was measured against known calibrators (Jew, 1996).

In 1965, the Iowa Legislature enacted a law that recommended testing infants for PKU. In 1966 the University Hygienic Laboratory began providing PKU testing services using both the Guthrie test and the Autoanalyzer™. Testing was voluntary throughout Iowa until 1981 when testing for Galactosemia, PKU, Maple Syrup Urine Disease, and Hypothyroidism was made available to all infants.

In 1994, Isolab, Inc. developed a microplate screening kit used to quantitate phenylalanine in filter paper blood spots. The methodology was based upon the McCaman and Robins fluorometric, ninhydrin method. This method has gained acceptance in the newborn screening community. It is very sensitive to phenylalanine levels at low concentrations and has been efficacious in detecting phenylketonuric patients in collections as early as 24 hours of life (Jew, 1994). This was the method used to screen for phenylketonuria until July 1, 2002, when the newborn screening laboratory began using Tandem Mass Spectrometry (MS/MS).

MS/MS is a very sensitive and specific technique that measures molecules by their mass or how much they weigh. An added feature is the addition of the phenylketonuria to tyrosine ratio that helps distinguish hyperphenylalaninemia cases from classic phenylketonuria.

The national incidence of PKU has been estimated to be 1 in 12,000 births. From 1990 through 2000, the incidence in Iowa has been 1 in every 15,158 births.

This is an excerpt from an article published in the Hotline, Volume 41, No. 1 by the University of Iowa Hygienic Laboratory. It has been recently updated.

www.idph.state.us/genetics

Visit the Birth Defects Institute (Center for Genetics) web site. It is full of information for health professionals, parents, and consumers.

**INMSP Screening Report**

In this issue of the Heel Stick News we have placed three “mock” INMSP Screening Reports. The reports in the newsletter are to be used as a reference to assist in identifying the information that will be reported by the University Hygienic Laboratory (UHL) to the submitting birthing facility, drawing laboratory, or licensed healthcare provider. The report on page four is an example of what the submitting facility would receive from the UHL if the test results were normal. Page five shows an example of a need for a second specimen to be submitted to UHL due to the first specimen being presumptive positive for 3-Methylcrotonyl-CoA Carboxylase Deficiency (3MCC). Page six is an example of an infant who has received a transfusion prior to the collection of the specimen. A second specimen is required eight weeks after the last transfusion. As you review the report, please note that many times the submitting facility creates their own report to send to the patient’s health-care provider.

**INMSP Screening Reports on pages 4 - 6**
INM SP Screening Report

Patient: DOE, JOHN  
Chart Number: 12345678A 12345  
Mother’s Name: DOE, JANE  
Physician: JULIE SMITH  
Laboratory No: 1234567890  
Test: Repeat  
Date Reported: 10/17/2003

Birth Date: 10/01/2003  
Date Collected: 10/15/2003  
Date Received: 10/16/2003  
Early Collection: No  
Transfused: No  
Weight at Collection: 4049 grams

Disorder | Substance(s) Measured | Result Interpretation
--- | --- | ---
Congenital Adrenal Hyperplasia | 17-Hydroxy Progesterone | Within Normal Limits
Hypothyroidism | Thyroid Stimulating Hormone | Within Normal Limits
Galactosemia | Gal-1-Phosphatase Uridyl Transylase | Within Normal Limits
Biotinidase Deficiency | Biotinidase | Within Normal Limits
Hemoglobinopathies | Hemoglobin Phenotype | FA Within Normal Limits
Phenylketonuria | Phenylalanine | Within Normal Limits
Expanded Screening Disorders | Amino Acids and Acylcarnitines | Within Normal Limits

Expanded Screening Disorders:
- **AMINO ACID DISORDERS**: (ARG) Argininemia; (ASA) Argininosuccinic Aciduria; (ASS) Citrullinemia or ASA Synthetase Deficiency; (IHCU) Homocystinuria or Cystathionine Synthase Deficiency; (IHII) Hyperornithinemia, Hyperammonemia, Homocitrullinuria Syndrome; (HMET) Homocitrullinuria; (HORN) Hyperornithinemia or Ornithine Oxo-acid Aminotransferase Deficiency; (MSUD) Maple syrup urine disease; (NKH) Non-ketotic Hyperglycinemia; (TYR) Tyrosinemia I, II, III.
- **FATTY ACID OXIDATION DISORDERS**: (2MBCD) 2-Methylbutyryl-CoA Dehydrogenase Deficiency; (3MCC) 3-Methylcrotonyl-CoA Carboxylase Deficiency; (3 MGH) 3-Methylglutaconyl-CoA Hydratase Deficiency; (5OP) Glutathione Synthase Deficiency or 5-Oxoprolinuria; (BKT) Mitochondrial Acetoacetyl-CoA Thiolase Deficiency or 3-Ketothiolase Deficiency; (GAI) Glyceric Acidemia; (HMG) 3-Hydroxy-3-Methylglutaryl-CoA Lyase Deficiency; (IBD) Isobutyryl-CoA Dehydrogenase Deficiency; (IV-A) Isovaleric Acidemia; (MCAD) Medium-chain Acyl-CoA Dehydrogenase Deficiency; (MMA) Methylmalonic Acidemia; (PPA) Propionic Acidemia.
- **SPECIAL CODES**: (TPN) Multiple Amino Acids Elevated; (MAC) Multiple Acylcarnitines Elevated.

This is a screening test. The possibility of a false negative or a false positive result must always be considered when screening newborns for metabolic disorders. Disorder information is available in the Practitioners' Manual at www.idph.state.ia.us/genetics.
INMSP Screening Report

<table>
<thead>
<tr>
<th>Disorder</th>
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<td>Presumptive Positive (3MCC) - Resubmit</td>
</tr>
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Resubmit: Collect another complete filter paper specimen and send to INMSP. If further action is required a program medical consultant will contact you.

Expanded Screening Disorders: AMINO ACID DISORDERS: (ARG) Argininemia; (ASA) Argininosuccinic Aciduria; (ASS) Citrullinemia or ASA Synthetase Deficiency; (HCU) Homocystinuria or Cystathione Synthase Deficiency; (HINH) Hyperornithinemia, Hyperammoninemia, Homocitrullinuria Syndrome; (HMET) Hypermethioninemia; (HORN) Hyperornithinemia or Ornithine Oxo-acid Aminotransferase Deficiency; (MSUD) Maple syrup urine disease; (NKH) Non-ketotic Hyperglycinemia; (TYR) Tyrosinemia I, II, III.

FATTY ACID OXIDATION DISORDERS: (2DRC) 2,4-Dienoyl-CoA Reductase Deficiency; (CACT) Carnitine/Acylcarnitine Translocase Deficiency; (CPTI) Carnitine Palmitoyltransferase Deficiency-Type I; (CPT2) Carnitine Palmitoyltransferase Deficiency-Type II; (CTD) Carnitine Transport Defect; (GA2) Multiple Acyl-CoA Dehydrogenase Deficiency or Glutaric Acidemia Type B; (LCHAD) 3-Hydroxy-Long-chain Hydroxacyl-CoA Dehydrogenase Deficiency; (MCAD) Medium-chain Acyl-CoA Dehydrogenase Deficiency; (SCAD) Short-chain Acyl-CoA Dehydrogenase Deficiency; (TFP) Trifunctional Protein Deficiency; (VLCAD) Very Long-chain Acyl-CoA Dehydrogenase Deficiency.

ORGANIC ACID DISORDERS: (2MBCD) 2-Methylbutyryl-CoA Dehydrogenase Deficiency; (3MCC) 3-Methylcrotonyl-CoA Carboxylase Deficiency; (3MGH) 3-Methylglutaconyl-CoA Hydratase Deficiency; (SOP) Glutathione Synthase Deficiency or 5-Oxoprolinuria; (BKT) Mitochondrial Acetoacetyl-CoA Thiolase Deficiency or 3-Ketolithase Deficiency; (GAI) Glutaric Acidemia; (HMG) 3-Hydroxy-3-Methylglutaryl-CoA Lyase Deficiency; (IBD) Isobutyryl-CoA Dehydrogenase Deficiency; (IVA) Isovaleric Acidemia; (MCD) Multiple CoA Carboxylase Deficiency; (MMA) Methy malonic Acidemia; (PPA) Propionie Acidemia.

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## INMSP Screening Report

**Patient:** DOE, JOHN  
**Birth Date:** 10/01/2003  
**Chart Number:** 123456789012345678  
**Date Collected:** 10/15/2003  
**Mother’s Name:** DOE, JANE  
**Date Received:** 10/16/2003  
**Physician:** JULIE SMITH  
**Early Collection:** No  
**Laboratory No.:** 1234567890  
**Transfused:** Yes  
**Test:** Repeat  
**Weight at Collection:** 620 grams  
**Date Reported:** 10/17/2003

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**Transfused:** Red blood cells can interfere with the interpretation of some newborn screening results. Submit another specimen 8 weeks after the last transfusion, unless other test results require immediate recollection and follow-up.

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ORGANIC ACID DISORDERS: (2MBCD) 2-Methylbutyryl-CoA Dehydrogenase Deficiency; (3MCC) 3-Methylcrotonyl-CoA Carboxylase Deficiency; (3 MHG) 3-Methylglutacoyl-CoA Hydratase Deficiency; (SOP) Glutathione Synthase Deficiency or 5-Oxoprolinuria; (BKT) Mitochondrial Acetoacetyl-CoA Thiolase Deficiency or 3-Ketothiolase Deficiency; (GA1) Glutaric Acidemia; (HMG) 3-Hydroxy-3-Methylglutaryl-CoA Lyase Deficiency; (IBD) Isobutyryl-CoA Dehydrogenase Deficiency; (IVA) Isovaleric Acidemia; (MCD) Multiple CoA Carboxylase Deficiency; (MMA) Methymalonic Acidemia; (PPA) Propionic Acidemia.

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Newborn Screening Quiz

The quiz is an opportunity to test your knowledge about newborn screening. The answers to the quiz are found on page 8.

1. For which disorders are newborn infants in Iowa screened? Mark all that apply.
   a. Congenital Hypothyroidism  
   b. Congenital Adrenal Hyperplasia  
   c. Hearing Loss  
   d. Phenylketonuria  
   e. Hemoglobinopathies  
   f. Medium Chain Acyl-CoA Dehydrogenase Deficiency  
   g. Galactosemia  
   h. Missing Nose Disease  
   i. Expanded Screening Disorders  
   j. Cystic Fibrosis  
   k. Biotinidase Deficiency

2. Which of the following are potential sources of error when collecting newborn screening specimens? Mark all that apply.
   a. Applying blood to both sides of the filter paper.  
   b. Squeezing or milking the foot of the infant.  
   c. Using more than one drop to fill a circle.  
   d. Touching the filter paper to a baby’s heel.  
   e. Drawing the circle in round motions when using a capillary tube.  
   f. Warming the foot to enhance blood flow.  
   g. Positioning the foot so that it is in a downward position from the heart.

3. Why is it important to wipe away the first drop of blood following a heel stick procedure?
   a. The first drop of blood is too concentrated so results will be high.  
   b. Tissue fluids may dilute the first drop so the results may be low.  
   c. It removes platelets which are likely to cause the blood to clot before the collection is complete.  
   d. It is not necessary to waste blood. It is more important to collect as little as possible from the infant.

4. Match the critical symptom with the associated disorder.
   2. IQ lower by 5 pts, each month if untreated.  
   3. Recurrent infections.  
   4. Severe mental retardation within 3 wks of birth.  
   5. Potential death within first week of life.  
   6. Fasting may bring metabolic crisis or coma.  
   7. Neurological damage including hearing loss & optic nerve atrophy.  
   a. Phenylketonuria  
   b. Galactosemia  
   c. Congenital Adrenal Hyperplasia  
   d. Congenital Hypothyroidism  
   e. Hemoglobinopathies  
   f. Biotinidase Deficiency  
   g. Medium Chain Acyl-CoA Dehydrogenase Deficiency

Birth Defects Prevention Month

January has been designated as Birth Defects Prevention Month to highlight the public health importance of birth defects. Public health campaigns to decrease high-risk behaviors and late (or no) prenatal care are making progress. However, there is more work to be done to support these very important prevention messages, as well as treatment and care options, including early intervention services for children and families affected by birth defects. To view the Iowa Birth Defects Registry website log onto: http://www.public-health.uiowa.edu/birthdefects/.
Resource Page

This is an opportunity for you to learn about services available in Iowa. The children diagnosed with a metabolic and congenital disorder through newborn metabolic screening will often be eligible for these services.

Dental Care for Persons with Disabilities
This program provides free dental services to Iowa children and young adults who have a disability and are from a low-income family. Without this program, needed dental services may not be received due to the financial burden with other medical and therapeutic expenses.

Target population:
Children and young adults with disabilities through 21 years of age

Criteria for eligibility: (All three criteria must be met)
1. The family income must be under 200% of the federal poverty guideline and the family cannot have Title XIX or other dental insurance.
2. The child must be age 0-20 years.
3. The child must have a special physical, health, or developmental need such as mental retardation, cerebral palsy, hemophilia, eating and psychiatric disorders, learning disabilities, visual and hearing impairments, cancer, asthma or attention deficient disorder.

Dental service available:
Comprehensive preventive and restorative dental treatment is available. No orthodontic services or cosmetic dentistry are provided.

Dental treatment under general anesthesia:
This program does not pay for medical expenses related to dental treatment under general anesthesia (for example: physician charges, operating room fees, anesthesia and recovery room fees).

Treatment sites:
Treatment is provided in Iowa City at the Center for Disabilities and Development dental clinic and in participating dental offices within the towns of Ames, Bellevue, Burlington, Carroll, Council Bluffs, Creston, Des Moines, Iowa City, Laurens, Marion, Marshalltown, Mason City, Monona, Pella, Sioux Center, Sioux City, Waukon, and Waterloo.

Quiz Answers  (Found on page7)
1. Iowa newborns are screened for (a) Congenital Hypothyroidism, (b) Congenital Adrenal Hyperplasia, (c) Hearing Loss, (d) Phenylketonuria, (e) Hemoglobinopathies, (f) Medium Chain Acyl-CoA Dehydrogenase Deficiency, (g) Galactosemia, (i) Expanded Screening Disorders, and (k) Biotinidase Deficiency.

2. Items (a) - (e) are sources of error in the collection process. Warming the foot to enhance blood flow and positioning the foot in a downward position from the heart will help to collect a good specimen.

3. (b) The first drop of blood is often diluted with tissue fluid. This could result in an affected child mistakenly being diagnosed as a false negative.

4. Although several of the disorders can cause mental retardation, it is the most significant in children with untreated Phenylketonuria. 1-c; 2-d; 3-e; 4-a; 5-b; 6-g; and 7-f.