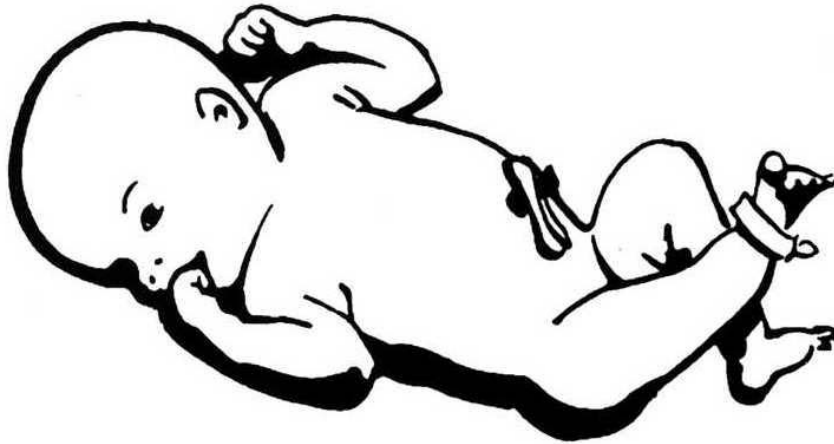


New York State

Department of Health

Newborn Screening in New York State

A Guide for Health Professionals



Newborn Screening Program
Wadsworth Center
New York State Department of Health
Empire State Plaza
Albany, NY 12201-0509

DOB: May 2003

New York State Newborn Screening Implementation Task Force

Georgianne Arnold, M.D.
Strong Memorial Hospital
Rochester, NY

Katharine B. Harris, M.B.A.
NYS Department of Health
Albany, NY

Kenneth A. Pass, Ph.D.
NYS Department of Health
Albany, NY

Marlene Belfort, Ph.D.
NYS Department of Health
Albany, NY

Robert Kaslofsky, M.D.
Albany Medical Center
Albany, NY

Jean Quarrier, J.D.
NYS Department of Health
Albany, NY

Ronald Bellisario, Ph.D.
NYS Department of Health
Albany, NY

David Kronn, M.D.
Regional Medical Genetics
Center
Valhalla, NY

Lewis Schedlbauer
NYS Department of Health
Albany, NY

Michele Caggana, Sc.D.
NYS Department of Health
Albany, NY

Christopher Kus, M.D.
NYS Department of Health
Albany, NY

Jack Sharp, M.D.
Children's Hospital of Buffalo
Buffalo, NY

David Clark, M.D.
Albany Medical Center
Albany, NY

David Martin, Ph.D.
NYS Department of Health
Albany, NY

Barbara Shepard, Ph.D.
NYS Department of Health
Albany, NY

Anne Comeau, Ph.D.
New England Regional
Newborn Screening Program
Boston, MA

Mark Morrissey, Ph.D.
NYS Department of Health
Albany, NY

Ann M. Willey, Ph.D., J.D.
NYS Department of Health
Albany, NY

Harry Hannon, Ph.D.
Centers for Disease Control
and Prevention
Atlanta, GA

Maria New, M.D.
New York Hospital Cornell
Medical Center
New York, NY

Additional editorial assistance provided by Jessica G. Davis, M.D., NY Hospital Cornell Medical Center, New York, NY; Doris L. Wethers, M.D., New York, NY; and Irene N. Sills, M.D., Albany Medical Center, Albany, NY.

Newborn Screening staff integral in the development of this guide include Katherine Cronce, John Postulka, Deborah Rodriguez, R.N., and Karen Zanni, R.N., C.N.P.

TABLE OF CONTENTS

CHAPTER 1. INTRODUCTION	1-1
References Cited	1-2
Table 1.1 New York State Newborn Screening Program Summary	1-3
 CHAPTER 2. THE COLLECTION OF SCREENING SPECIMENS	 2-1
Timing of Specimen Collection	2-1
Figure 2.1 Time Line for Specimen Collection	2-1
Table 2.1 Summary for Specimen Collection Timing	2-3
Completion of Blood Collection Form	2-3
Collection of Blood Specimen	2-4
Specimen Handling	2-5
Invalid Specimens	2-6
Table 2.2 Causes of Invalid Specimens	2-6
Declination of Services	2-7
References Cited	2-7
Related References	2-8
 CHAPTER 3. THE SCREENING LABORATORY	 3-1
Overview	3-1
Figure 3.1 Laboratory Work Flow	3-1
Table 3.1 Screening Methodology	3-2
Special Cases	3-3
Availability of Results	3-3
Where To Get Information	3-3
 CHAPTER 4. THE INHERITED METABOLIC DISORDERS (IMD):	
AMINOACIDOPATHIES AND FATTY ACID OXIDATION DISORDERS	4-1
Introduction	4-1
Testing Procedures	4-1
PKU - Phenylketonuria and Hyperphenylalanemia	4-1
Maternal PKU	4-3
MSUD – Maple Syrup Urine Disease (Branched-Chain Ketoaciduria)	4-3
Homocystinuria	4-4
MCADD - Medium-Chain Acyl-CoA Dehydrogenase Deficiency	4-6
Figure 4.1 MCADD Screening Algorithm	4-7
Additional Diseases Detectable by Tandem Mass Spectrometry	4-8
Table 4.1 Disorders Detectable by Tandem Mass Spectrometry	4-9
References Cited	4-9

CHAPTER 5: INHERITED METABOLIC DISORDERS (IMD): RED CELL	
ENZYMES.....	5-1
Introduction.....	5-1
Galactosemia.....	5-1
Biotinidase Deficiency.....	5-3
References Cited.....	5-4
CHAPTER 6. SICKLE CELL DISEASE AND OTHER HEMOGLOBINOPATHIES.....	6-1
Introduction.....	6-1
Sickle Cell Diseases.....	6-1
Medical Complications of Sickle Cell Disease in Children.....	6-2
Other Hemoglobinopathies.....	6-3
Hemoglobin Carrier Conditions.....	6-4
Follow-up for Hemoglobin Carrier Conditions.....	6-4
Transfusions.....	6-4
References Cited.....	6-5
CHAPTER 7 ENDOCRINE DISORDERS.....	7-1
Congenital Hypothyroidism.....	7-1
<i>Laboratory Detection</i>	7-1
<i>Screen Positive Results</i>	7-2
Primary Hypothyroidism.....	7-2
Secondary/Tertiary Hypothyroidism.....	7-3
Table 7.1 Symptomatology of Hypothyroidism by Organ Systems.....	7-4
Table 7.2 Recommended Diagnostic Protocol for Hypothyroidism.....	7-4
Table 7.3 Symptomatology and Physical Exam Findings of Infants with Congenital Hypothyroidism.....	7-5
TBG Deficiency.....	7-5
CAH - Congenital Adrenal Hyperplasia.....	7-5
Figure 7.1 CAH Screening Algorithm.....	7-6
CF - Cystic Fibrosis.....	7-7
Figure 7.2 Cystic Fibrosis Screening Algorithm.....	7-10
References Cited.....	7-11
CHAPTER 8. HIV ANTIBODIES.....	8-1
References Cited.....	8-1
CHAPTER 9. REPORTING TEST RESULTS.....	9-1
Computerized Telephone Access to Screening Results.....	9-1
Hospital Contacts and Designees: Responsibilities.....	9-1
Screen Negative Results.....	9-3
Invalid Specimens.....	9-4
Screen Positive Test Results - Repeat Specimen Required.....	9-4
Screen Positive Test Results - Clinical Referrals.....	9-4

CHAPTER 10. FOLLOW-UP PROTOCOLS	10-1
Obtaining Repeat Specimens	10-1
Clinical Referrals	10-3
Specialty Care Centers	10-3
References Cited	10-3
CHAPTER 11. EDUCATION	11-1
Parent Education	11-1
Educational Materials	11-2
Professional Education	11-2
CHAPTER 12. RESEARCH AND DEVELOPMENT	12-1
Annotated Bibliography of Researchers at the Division of Genetic Disorders, Wadsworth Center	12-2
CHAPTER 13. AFFILIATED PROGRAMS	13-1
Collection of Information Regarding Maternal Hepatitis B Status.	13-1
Newborn Hearing Screening	13-1
Screening for Congenital Syphilis	13-2
Table 13.1 Cases of Early Syphilis in NYS 1991 - 2000.....	13-3
References Cited	13-4
CHAPTER 14. QUESTIONS AND ANSWERS	14-1
Figure 14.1 Time Line for Specimen Collection	14-2
References Cited	14-7
CHAPTER 15. GLOSSARY	15-1
APPENDICES	

APPENDICES

Appendix A.....	Public Health Law 2500-a, NYCRR 10, Section 69-1 (02/01/1997)
Appendix B.....	2002 Newborn Screening Program Annual Report
Appendix C.....	Newborn Screening Blood Collection Form
Appendix D.....	Schleicher & Schuell Neonatal Screening Blood Specimen Collection and Handling Procedure
Appendix E.....	Schleicher & Schuell Simple Spot Check
Appendix F.....	Refusal of Newborn Screening Sample Form
Appendix G.....	Newborn Screening Program Voice Response System
Appendix H.....	Specialty Care Centers – Inherited Metabolic Diseases
Appendix I.....	Specialty Care Centers – Sickle Cell Disease and Other Hemoglobinopathies
Appendix J.....	Specialty Care Centers – Endocrine Disorders
Appendix K.....	Specialty Care Centers – Cystic Fibrosis
Appendix L.....	American Academy of Pediatrics Policy Statement on Maternal PKU
Appendix M.....	Administrative Rules and Regulations, Chapter II, Part 58.1.1
Appendix N.....	Newborn Screening Program Hospital Contact Form
Appendix O.....	Newborn Screening Program Educational Material Reorder Form
Appendix P.....	Newborn Screening: For Your Baby’s Health

Chapter 1 . INTRODUCTION

The goal of newborn screening is early identification of children at increased risk for selected metabolic or genetic diseases so that medical treatment can be promptly initiated to avert metabolic crises and prevent irreversible neurological and developmental sequelae. Early identification of these conditions is crucial, as timely intervention can lead to a significant reduction of morbidity, mortality, and associated disabilities in affected infants.¹ Today every state in the nation provides newborn screening for phenylketonuria (PKU) and congenital hypothyroidism.¹ These two disorders set the scope for the classical benefits newborn screening can achieve, whereby early identification and treatment change the potential course of the infant's life from dependent mental retardation to near full-functioning normalcy. In addition to these two disorders, many other disorders are amenable to screening using the dried blood specimen (now known as the Guthrie spot) collected at least twenty-four hours after birth. Programs across the nation have selected those conditions best suited for their populations based on pilot studies, availability of funds, the abilities and limitations of screening technology, and the availability of treatment protocols.^{2,3}

A child diagnosed with a condition included in the newborn screening panel is at increased risk for significant morbidity and mortality caused by the condition. Medical specialists in metabolic diseases, cystic fibrosis, endocrinology and hematology can provide either on-going medical care or act as consultants to primary care physicians, depending on the needs of the family and the nature and severity of the condition. In addition, since most of the conditions included in the newborn screening panel are caused by genetic mutations, families need to be referred for genetic education and counseling to best understand the particular condition, its impact on the child's health and future, and to understand the risks in future pregnancies

New York State (NYS) has played a key role in the development of newborn screening. In the 1930s George Jervis at Letchworth Village State School in Thiells, New York, identified 50 clients whose mental retardation was attributed to PKU.⁴ He pursued the study in four state institutions to identify a total of 185 PKU cases among 15,000 clients. Little could be done for these adults. However, work of Horst Bickle had suggested that early diet therapy could prevent development of the mental retardation usually seen in PKU.⁵ Early therapy depended on early detection of the affected child – before appearance of symptoms. Robert Guthrie, a microbiologist and pediatrician at State University of New York, Buffalo, devised a simple, inexpensive test which allowed screening for PKU to be done shortly after birth.⁶ In the early 1960s he coordinated a twenty-nine state pilot study of 400,000 newborns which proved so successful in identifying infants affected with PKU that many states instituted screening programs immediately.⁷ New York State's law for newborn screening, Public Health Law 2500a, went into effect in 1965 and mandated that every

newborn be screened for PKU. A copy of Public Health Law 2500a, along with the Administrative Rules and Regulations established by the Commissioner of Health to meet the requirements of this law, can be found in Appendix A of this guide.

Many modifications and amendments later, the NYS Newborn Screening program today includes eleven disorders: PKU, maple syrup urine disease (MSUD), homocystinuria, galactosemia, sickle cell disease, congenital hypothyroidism, biotinidase deficiency, HIV-1 exposure, cystic fibrosis (CF), congenital adrenal hyperplasia (CAH) and medium-chain acyl-CoA dehydrogenase deficiency (MCADD). Statistics from 2002, the most recent year of testing, indicating in more detail the results of the more than 2.9 million tests for that year, can be found in Appendix B. Copies of the annual summary, published each April, are available by contacting the NYS screening program or on the website at www.wadsworth.org/newborn/index.htm.

Since 1965, more than ten million newborns have been screened in the NYS Newborn Screening Program, with more than 12,000 infants diagnosed after initial detection by screening. Table 1.1 details when each test was incorporated into the screening profile, the number of infants screened, cases identified, and incidence in the NYS population. To be successful, the program requires the coordinated efforts of many different members of the health care profession. This guide seeks to provide the information necessary for each of these participants to function interactively and effectively. It will be updated as program changes dictate.

References Cited

1. A Report from the Newborn Screening Task Force Convened in Washington, DC. May 10-11, 1999. (2000) *Pediatrics* 106 (2): 383-427.
2. National Newborn Screening and Genetic Resource Center (NNSGRC). genes-r-us.uthscsa.edu.
3. Newborn Screening Fact Sheets. (1996) Committee on Genetics, American Academy of Pediatrics. *Pediatrics* 98 (3): 473-501.
4. Jervis G. (1937) Phenylpyruvic oligophrenia: introductory study of 50 cases of mental deficiency associated with excretion of phenylpyruvic acid. *Arch Neurol and Psychiatry* 38: 944.
5. Bickle H, Gerrard J, Hickmans EM. (1953) Influence of phenylalanine intake on phenylketonuria. *Lancet* 2: 812.
6. Guthrie R, Susi A. (1963) A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Pediatrics* 32: 338-43.
7. Guthrie R, Whitney S. (1964) Phenylketonuria detection in the newborn infant as a routine hospital procedure: a trial of a phenylalanine screening method in 400,000 infants. Children's Bureau Publication 419. Washington, D.C., U.S. DHEW.

**Table 1.1 New York State Newborn Screening Program Summary
1965-2002**

Condition/Disease	Year Testing Initiated	Live Births	Confirmed Cases	Disease Incidence Statewide
PKU	1965	10.23 million	542	1:18,872
Galactosemia	1968	8.16 million	150	1:54,391
MSUD	1968	8.16 million	34	1:239,962
Homocystinuria	1975	7.30 million	19	1:384,142
Homozygous Sickle Cell	1975	7.30 million	3,932	1:1,856
SC/CC/Other	1975	7.30 million	3,002	1:2,431
Primary Hypothyroidism	1978	6.35 million	3,086	1:2,057
Biotinidase Deficiency	1987	4.36 million	56	1:77,834
HIV-1	1997	1.54 million	Confirmed by AIDS Institute	
CF	2002	58,913	8	Data insufficient
MCADD	2002	60,479	1	to determine
CAH	2002	19,441	0	incidence
Screening Suspended in 1987				
Adenosine Deaminase Deficiency	1973	3.60 million	2	1:1.8 million
Histidinemia	1975	3.46 million	31	1:111,600

the second specimen.

Home Births It is the responsibility of the medical professional attending the birth to arrange for collection and submission of a specimen from the newborn between the third and fifth day of life.

Readmission of Newborns If a newborn is admitted to a hospital within the first thirty days of life, the admitting hospital is required to submit a specimen for screening unless proof of previous screening is provided.

Inter-Hospital Transfers The transferring hospital provides written notification to the receiving hospital indicating whether or not a newborn specimen has been submitted. Following transfer, the receiving hospital assumes responsibility for fulfilling collection requirements, including securing documentation from the transferring hospital.

Extended Hospital Stay Infants with prolonged hospitalization pose special problems for newborn screening. These infants should have a specimen taken at three to five days of life and again upon discharge, unless medical procedures prevent the collection, in which case a specimen should be obtained at the earliest possible date.

Transfusion The optimum collection time remains the period between the third and fifth day of life. If the infant is to receive a transfusion, every effort must be made to collect a specimen prior to transfusion, since even small transfusions may invalidate screening results.

Infants receiving transfusions with no prior screening test need two collections: three days or more after the most recent transfusion and three months after the final transfusion.

Total Parenteral Nutrition (TPN) The optimum collection time remains the period between the third and fifth day of life. If the infant is to receive TPN, every effort must be made to collect a specimen prior to treatment, since even small amounts of TPN may invalidate screening results.

Infants receiving TPN with no prior screening test need two collections: three days or more after the most recent TPN and three months after the final TPN.

Premature and Sick Infants Very lengthy hospital stays require a specimen to be collected between three and five days of age and again upon discharge or at one month of age, whichever comes first. Since these infants frequently receive transfusions, the recommended testing and transfusion sequence would be as follows:

- the first specimen is taken prior to the first transfusion;

- if the infant is less than 24 hours of age when transfused, a second specimen is taken 3-5 days after transfusion;
- a final specimen is taken either at the time of discharge or at one month of age - whichever occurs first.

Table 2.1 Summary for Specimen Collection Timing

<u>Infant Status</u>	<u>Time of Collection</u>
Normal, healthy	Day 1 – if to be discharged from hospital or birthing center; and repeated on day 3-5. Day 2 – Acceptable Day 3-5 – Optimum
Transfused	Prior to transfusion; or if no pretransfusion collection was taken, three days after most recent transfusion; with repeat three months after final transfusion.
TPN	Prior to initiation of TPN; or if no pre-TPN collection was taken, three days after most recent TPN; with repeat three months after final TPN.
Premature, sick, or extended stay	Prior to transfusion - any age; and on day 3-5 or three days after most recent transfusion; and at discharge or at one month of age, whichever comes first.
Transferred between hospitals	Transferring hospital provides written notification of status of specimen collection to receiving hospital. Following transfer, receiving hospital assumes responsibility for collection.
Needed repeat specimens	Requests from the screening laboratory for a repeat specimen due to screen positive results will supersede these guidelines.

Completion of Blood Collection Form

Provide all the information requested on the blood collection form, using a blue or black ballpoint pen only. This information is vital for identification and location of infants for referral or other follow-up purposes, and **must be accurate and legible.**

Note the expiration date on the back of the form – **the form must be current.** An image of the current form at the time of this printing will be found in Appendix C.

The SUBMITTER COPY (green) of the specimen form remains in the infant's hospital file. Give the PARENT COPY (pink) to the mother along with the brochure, "Newborn Screening: For Your Baby's Health."

Do not touch the areas within the circles on the filter paper with gloved or ungloved hands, before or after specimen collection since skin oils, latex and powder may affect test results. Avoid contamination of the filter paper with feeding formulas, antiseptic solutions, water, lotions, powder, etc. These may invalidate the blood specimen.

Collection of Blood Specimen

NCCLS, National Committee for Clinical Laboratory Standards, has produced a video, *Making a Difference Through Newborn Screening: Blood Collection on Filter Paper*, demonstrating proper heel stick techniques.⁸ A copy is available to loan from the Newborn Screening Program. An image of the poster "Neonatal Screening Blood Specimen Collection and Handling Procedure" is in Appendix D. It illustrates proper technique. The poster "Simple Spot Check" in Appendix E illustrates examples of improper specimen collection. Full color versions of the posters are available from the screening program. Refer also to the instructions for specimen collection on the back of the specimen collection form.

Equipment

Sterile lancet*, with tip less than 2.4mm long.

Sterile 70% alcohol pads.

Sterile gauze pads.

Warm moist towel or compress.

Current filter paper blood collection form. (Outdated blood collection forms may affect test results and are unacceptable.)

Sterile Gloves

*several collection devices made exclusively for heel stick specimens are now available. For information, contact the screening program.

Skin Puncture Site

The heel puncture should be made on the plantar (sole) surface of the foot. The safest area for heel puncture is medial to a line drawn posteriorly from the middle of the great toe to the heel, or lateral to a line drawn from between the fourth and fifth toe to the heel. Do not puncture on the posterior curvature of the heel, or on previous puncture sites.⁸

Procedure

Since laboratory analysis of the specimen depends on an assumed amount of blood in

the filter paper circle, it is imperative to carefully follow these procedures. Numerous studies have shown the variability occurring due to improper technique in specimen collection.^{9,10,11,12}

1. Place infant's leg in a position that will increase venous pressure.
2. Warm the heel site to increase blood supply to the area by covering the puncture site for three to five minutes with a warm, moist towel which has been run under tap water at a temperature of not more than 42°C.
3. Cleanse the puncture site with a sterile alcohol pad. Wipe dry with a sterile gauze pad, as residual alcohol may cause hemolysis of the blood specimen resulting in an invalid specimen.
4. With lancet or specialty device, puncture the heel skin with one continuous, deliberate motion at a slight angle (a little less than 90°). Wipe away the first drop of blood with a dry sterile gauze pad, as it is likely to contain tissue fluids that contaminate the specimen.
5. Allow a second, large drop of blood to form.
6. Lightly touch filter paper to this large drop of blood. Allow blood to soak through and completely fill the preprinted circle with a single application to this large blood drop. (To enhance blood flow, very gentle intermittent pressure may be applied to area surrounding puncture site.) **Do not use capillary tubes.** Do not touch the same circle to blood several times, as this causes a "layering effect." Do not "milk" area surrounding puncture site. Milking may cause an admixture of tissue fluids with blood specimen, resulting in an invalid specimen. Apply blood to one side of filter paper only. (Either side may be chosen for this procedure.)
7. Fill remaining circles in same manner as step 6, with successive drops of blood. If blood flow is diminished, repeat steps three through six with sterile equipment.
8. Care of skin puncture site should be consistent with your institution's procedures.

Specimen Handling

Allow blood spots to air-dry thoroughly for at least four hours in a horizontal position on a flat, non-absorbent surface away from direct heat and sunlight. Do not refrigerate specimens after collection. Mail thoroughly dried blood collection forms to the Wadsworth Center within 24 hours of collection. The mailing address is printed on the back of the form. Do not retain specimens longer than 24 hours in order to accumulate a "batch," since this may result in specimens too old to test. When placing more than one specimen in an

envelope, alternate collection forms so that blood spots on adjacent forms are not in contact.

The perfect specimen

- Has all information legibly recorded on the blood collection form.
- Is collected from an infant between 48 and 120 hours of age (third to fifth day of life).
- No foreign substances contaminate the filter paper.
- The blood completely fills all printed circles and is applied evenly on one side of the filter paper, free of layering and clots.
- Is dried for at least four hours on a flat, non-absorbent surface, away from direct heat and sunlight.
- Within 24 hours of collection, is sent to the Newborn Screening Program by first class mail or its equivalent.

Invalid Specimens

The Newborn Screening Program receives many blood spot specimens in a condition unacceptable for testing. Certain types of specimens are known to give erroneous laboratory results. In accordance with laboratory regulations, these cannot be tested and are termed invalid. This delays the screening of the newborn, and requires that the submitter repeat the collection procedure. See Appendix E for examples of unacceptable specimens.

Table 2.2 Causes of Invalid Specimens

The testing laboratory classifies unsuitable specimens into eight categories based on the following criteria:

<u>Invalid Specimens</u>	<u>Possible Causes</u>
1. Quantity of blood insufficient	Filter paper circle incompletely filled or not saturated. Blood applied with needle or capillary tube. Contamination of surface of filter paper circle. Failure to obtain any blood.
2. Blood spots appear scratched or abraded	Blood applied improperly with capillary tube or by other means.
3. Blood spots wet or discolored	Specimen not properly dried before mailing.
3. Blood spots appear supersaturated	Excess blood applied (usually with capillary tube or needle). Blood applied to both sides of filter.

- | | |
|--|--|
| 5. Blood spots appear diluted | Puncture site squeezed or "milked" to expel blood.
Exposure of blood spots to direct heat.
Contamination of filter paper before or after blood collection by gloved or ungloved hands or by substances such as alcohol, feeding or antiseptic solutions, hand lotion or powder. |
| 6. Blood spots exhibit "serum rings" | Alcohol not wiped off puncture site before skin puncture is made.
Allowing filter paper to come in contact with alcohol, water, hand lotion, etc.
Squeezing the area around the puncture site excessively.
Drying the specimen improperly.
Applying blood to filter paper with a capillary tube. |
| 7. Blood spots appear clotted or layered | Touching the same filter paper circle to a blood drop several times. Filling the circle from both sides of the filter paper. |
| 8. Specimen delivered to the laboratory more than 14 days after collection | Specimen held at the hospital before mailing.
Delivery delayed by the post office or other delivery service. |

Declination of Services

Newborn screening (as mandated by Section 2500a(b) of the Public Health Law, 10 NYCRR Section, Part 69-1.4) may not apply when the parent or guardian of the infant is a member of a recognized religious organization whose teachings are contrary to the testing requirement. If a parent or guardian objects to testing based on religious grounds, the hospital administrator, or other person designated by Section 4130 of the Public Health Law to register the birth of a child, is to fully inform the parent or guardian of the consequences of refusal and to inform the testing laboratory in writing of such parental refusal by submission of a signed declination of services form or similar document. (See Appendix F for sample form.) This form must include a statement to the effect that the parent or guardian is a member of a recognized religious organization, has been fully informed of the possible consequences of not having the newborn tested, and that the parent or guardian is aware of and understands the possible consequences of refusal. A specimen form with all information provided, but with no blood, should be submitted to the screening program. "Declined testing" should be written across the top, the green copy retained by the hospital, and the pink copy given to the parents.

References Cited

1. Newborn screening fact sheets. (1996) Committee on Genetics, American Academy of Pediatrics. *Pediatrics* 98 (3): 473-501.
2. Hanley WB, Demshar H, Preston MA, Borczyk A, Schoonheydt WE, Clarke JT, Feigenbaum A. (1997) Newborn phenylketonuria (PKU) Guthrie (BIA) screening and early hospital discharge. *Early Hum Dev* 47(1): 87-96.
3. Doherty LB, Rohr FJ, Levy HL. (1991) Detection of phenylketonuria in the very early newborn blood specimen. *Pediatrics* 87: 240-4.
4. McCabe ERB, McCabe L, Mosher GA, Allen RJ, Berman JL. (1983) Newborn screening for phenylketonuria: predictive validity as a function of age. *Pediatrics* 72(3): 390-8.
5. Koch R, Twelmeyer D and Berlow S. (Letters to the editor). McCabe ERB, McCabe L (Reply) (1984) Re: Newborn screening for phenylketonuria: predictive validity as a function of age. *Pediatrics* 73: 737-40.
6. *Healthy Children: Investing in the Future*. (1988) Office of Technology Assessment, Congress of the United States.
7. Holtzman C, Slazyk WE, Cordero JF, Hannon WH. (1986) Descriptive epidemiology of missed cases of phenylketonuria and congenital hypothyroidism. *Pediatrics* 78(4): 553-8.
8. *Making a difference through newborn screening: blood collection on filter paper*. The National Committee of Clinical Laboratory Standards videotape. NCCLS publication LA4-A3-V. Villanova, PA.
9. Mei JV, Alexander JR, Adam BW, Hannon WH. (2001) Use of filter paper for the collection and analysis of human whole blood specimens. *J Nutr* 131: 1631S-1636S.
10. Pappaioanou M, Kashamuka M, Behets F, et al. (1993) Accurate detection of maternal antibodies to HIV in newborn whole blood dried on filter paper. *AIDS* 7(4): 483-8.
11. Meites S, Glassco KM. (1985) Studies on the quality of specimens obtained by skin-puncture of children. 2. An analysis of blood collecting practices in a pediatric hospital. *Clin Chem* 31(10):1669-72.
12. Blood collection on filter paper for neonatal screening program. Approved standard. Third Edition. NCCLS publication LA4-A3. Villanova, PA.

Related References

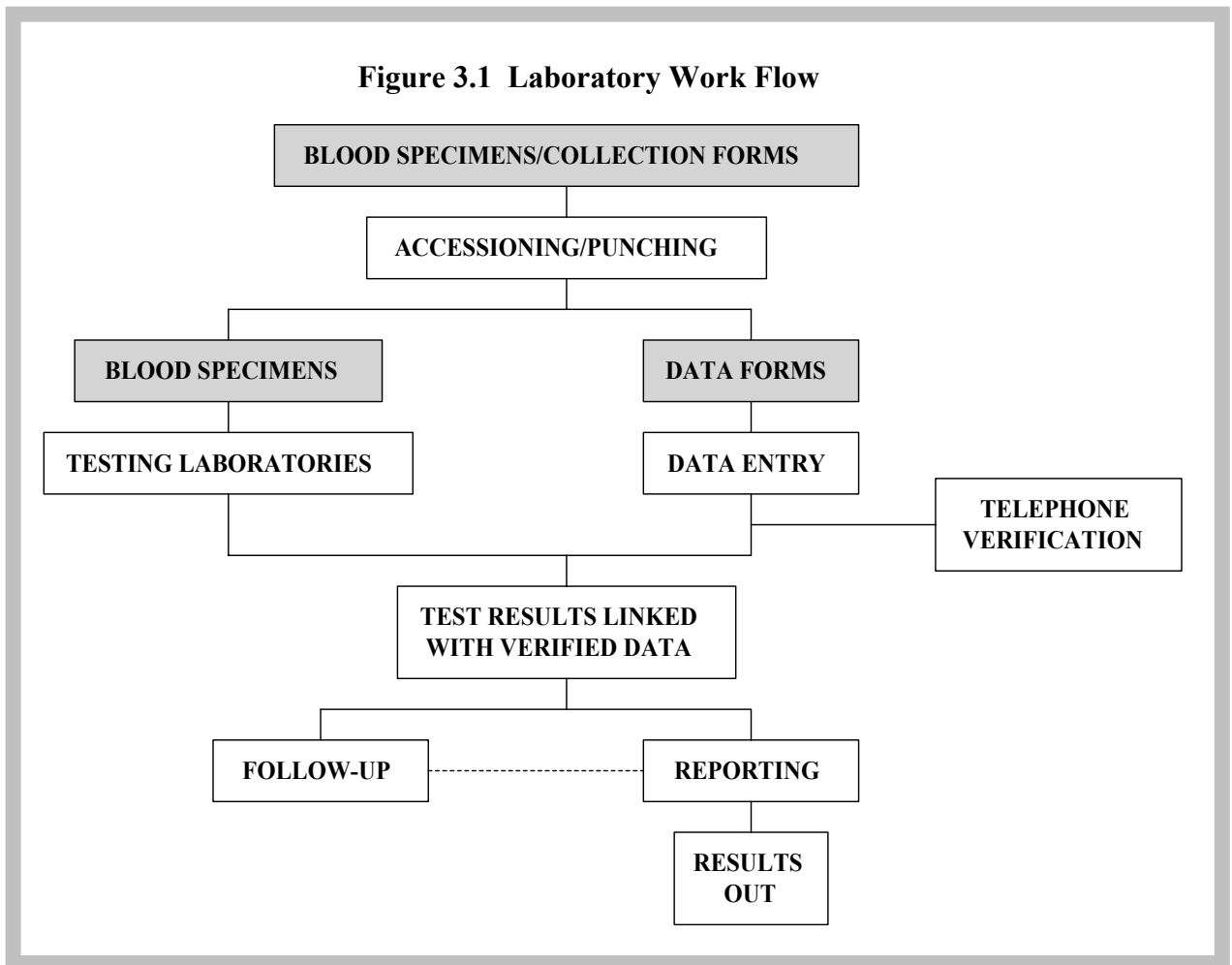
1. A Report from the Newborn Screening Task Force Convened in Washington DC, May 10-11, 1999. *Pediatrics* 106(2): 383-427.
2. Blumenfeld TA, Turi GK, Blanc WA. (1979) Recommended site and depth of newborn heel skin punctures based on anatomical measurements and histopathology. *Lancet* 1(8110): 230-3.
3. George R. (1980) Specimen collection for PKU and hypothyroidism screening. U.S. DHHS, PHS, Centers for Disease Control, Laboratory Training and Consultation Division, Atlanta. Lecture tape, slides and handbook. CDC-80-110.

4. Gray L, Miller LW, Phillip BL, Blass EM. (2002) Breast-feeding is analgesic in healthy newborns. *Pediatrics* 109(4): 590-593.
5. Hammond KB. (1980) Blood specimen collection from infants by skin puncture. *Lab Med* II (1): 9-13.
6. Meites S, Levitt MJ. (1979) Skin-puncture and blood collecting techniques for infants. *Clin Chem* 25(1): 183-9.
7. Meites S. (1988) Skin-puncture and blood-collecting technique for infants: update and problems. *Clin Chem* 34(9): 1890-4.
8. Pass KA, Levy H, ed. (1995) Early hospital discharge: Impact on newborn screening. Proceedings from a conference held in Washington, D.C. on March 31-April 1, 1995.

Chapter 3 . THE SCREENING LABORATORY

Overview

The screening laboratory is divided into work units that perform the blood testing and process the data on over 275,000 specimens annually; i.e., an average of 1,100 specimens daily. The flow of specimens and data through the various units is controlled in the following way to maximize accuracy and efficiency.



Specimens are received in the accessioning/punching unit three times daily, Monday through Friday (except holidays). Specimens received at 11:00 a.m. and 2:00 p.m. are organized and prepared for the next day's testing. The 8:00 a.m. mail, the largest of the batches received, is combined with the previous day's late mail for testing that day. Specimens are considered to be initial specimens unless the "Repeat" box is checked on the

form. All newly received specimens are matched electronically with the computer data base each night to identify repeat specimens.

After imprinting both parts of the collection form with a laboratory accession number unique for that day, the data form is separated from the blood collection card and sent to the data entry unit; the Guthrie spot portion of the card is retained for processing in the laboratories.

Information on all specimens is entered into a computer database by the data entry unit. If information is missing on the blood collection form, the telephone unit calls the hospital to obtain needed data within seven workdays.

Table 3.1 Screening Methodology

Disorder	Marker Analyte	Method	Verification Test
PKU (Phenylketonuria)	Phenylalanine	MS/MS	MS/MS
MSUD (Maple Syrup Urine Disease)	Leucine	MS/MS	MS/MS
Homocystinuria	Methionine	MS/MS	MS/MS
Galactosemia	Gal-1-P/galactose	Beutler-enzyme	Hill-metabolite
Biotinidase Deficiency	Biotinidase	Wolf-enzyme	Wolf-enzyme
Sickle Cell Disease	Hemoglobin	Alkaline electrophoresis	Acid electrophoresis/ Isoelectric focusing
Congenital Hypothyroidism	Thyroxine	ELISA	TSH (Time-resolved fluoroimmunoassay)
Cystic Fibrosis	Immunoreactive trypsin	ELISA	DNA
CAH (Congenital Adrenal Hyperplasia)	17-hydroxy-progesterone	ELISA	ELISA
HIV (Human Immunodeficiency Virus)	HIV-antibodies	ELISA	Western blot
MCADD (Medium-chain Acyl-CoA Dehydrogenase Deficiency)	Octanoylcarnitine	MS/MS	DNA

In the accessioning unit, specimen discs are punched out of the blood spots into testing vessels appropriate for each test procedure and sent to four specialized laboratories which perform the screening tests: inherited metabolic disorders (IMD), red cell enzymes, endocrine, HIV-1, and hemoglobin laboratories.

Special Cases

Each unit of the Newborn Screening Program is capable of handling special cases, such as those with a known family history of the disorder, or those infants who develop early clinical signs indicative of any of the disorders screened. Testing of specimens identified as special cases can usually be expedited with results telephoned immediately upon completion. This is particularly important in life-threatening conditions such as galactosemia, CAH, MCADD and MSUD, when rapid availability of results is essential. If an infant is symptomatic, it is recommended that a specimen be collected and shipped by overnight carrier even if an initial specimen has been mailed, but not yet received in the laboratory. Staff of the Newborn Screening Program will assist in arranging rapid processing when notified by telephone.

Availability of Results

Preliminary screening results for all tests are generally available the day after the samples have been accessioned. Confirmation of results depends on the specific verification test, with the longest procedures taking seven days.

Where To Get Information

The Newborn Screening Program can be reached during normal business hours at (518) 473-7552. Physicians registered with the screening program can obtain test results at any time via a computerized telephone answering system by calling (800) 535-3079. (See Appendix G for instructions.) Messages can be left at (518) 473-7552 during non-business hours. Emergency contact with program staff can be made by calling the NYS Department of Health (DOH) at (518) 465-9720.

Specimens and inquiries should be addressed to:

Newborn Screening Program
New York State Department of Health
Wadsworth Center
Empire State Plaza
P.O. Box 509
Albany, NY 12201-0509

Chapter 4 . THE INHERITED METABOLIC DISORDERS (IMD): AMINOACIDOPATHIES AND FATTY ACID OXIDATION DISORDERS

Introduction

PKU, MSUD, and homocystinuria are hereditary aminoacidopathies. MCADD is one of a family of fatty acid oxidation disorders. Preliminary test results for all specimens are available within one day of receipt; those highly indicative of a disorder are telephoned to the physician of record and the Inherited Metabolic Diseases (IMD) Specialty Care Center closest to the address of the mother. Reports of specimens with borderline screen positive results are mailed to the physician of record or the hospital of birth, either of which are required to collect repeat specimens.

Experienced care providers for patients with inherited metabolic disorders are available throughout New York State at the IMD Specialty Care Centers. See Appendix H for the current list of these centers. They provide the medical care and referral services required by the patient and family.¹

Testing Procedures

Tandem mass spectrometry (MS/MS) is used to test newborns for elevations in phenylalanine (Phe), methionine, leucine and octanoylcarnitine. Abnormal levels of these analytes are suggestive of PKU, homocystinuria, MSUD and MCADD, respectively. Guidelines for diagnosis and treatment of all conditions tested by newborn screening have recently been published¹ and should be consulted.

PKU – Phenylketonuria and Hyperphenylalaninemia

Newborn screening can identify individuals at risk for hyperphenylalaninemia (i.e., elevated blood Phe). This may be indicative of any of three disorders: classical PKU, hyperphenylalaninemia, or co-factor variant defect. In rare instances the elevation may be unexplained and transient. The intent of screening is to identify individuals with clinically significant Phe elevations for prompt initiation of treatment to prevent mental retardation and the development of other clinical sequelae.

Classical PKU was first described in 1934 by Asborn Fölling.² Effective treatment by dietary restriction of Phe was demonstrated by Horst Bickle 25 years later.³ In the

1960s, a simple screening method – the bacterial inhibition assay – was devised by Robert Guthrie.⁴ PKU is an inherited autosomal recessive disorder mapped to chromosome 12 that results in the inactivity or absence of the enzyme phenylalanine hydroxylase.⁵ This enzyme is responsible for converting the amino acid phenylalanine to tyrosine. Without it, serum Phe levels will quickly rise from a normal level of less than 3mg/dl (180 µmol/l) to greater than 20mg/dl (1200 µmol/l). A false-negative screening result may occur if, at the time of specimen collection, levels in an affected infant have not yet had time to rise to levels greater than the laboratory cut-off level of 6mg/dl.

Asymptomatic in the immediate newborn period, infants with untreated PKU will present with the onset of developmental delay. After four to six weeks a musty odor may be noted in the urine. By one year of age, global developmental delay, microcephaly, decreased growth rate, seizures or tremors will be evident. Most untreated PKU patients (96 - 98%) will have an IQ less than fifty.⁶ The high level of Phe also inhibits tyrosine hydroxylase so that the child often has lighter hair and skin than other family members. Treatment, which must begin early for optimal effect, involves selective restriction of Phe in the diet allowing only the precise amount needed for growth and development. This is achieved by use of a semisynthetic diet low in Phe but adequate in other nutrients. The amount of Phe from other foods is regulated. Growth and development are followed closely, in conjunction with monitoring of the diet by a trained nutritionist and periodic determinations of blood levels of Phe. IMD Specialty Care Centers specialize in the care of these children by combining the skills and knowledge of physicians, nutritionists, developmental specialists, laboratory staff and social workers to provide long-term support to the children and families. Successful treatment can result in normal or near-normal physical and mental development.

The prevalence of classical PKU (Phe concentration greater than 20mg/dl in infants on standard feedings) in NYS is about 1 in 19,000 newborns, with 12 to 18 new cases identified each year for a total of 542 cases since 1965.⁷ Newborns with initial Phe levels greater than 20 mg/dl are referred directly to the nearest treatment center for evaluation. In addition, an equal number of newborns are identified with lesser elevations (less than 20 mg/dl) of Phe. Recent evidence suggests even these lesser elevations of Phe (hyperphenylalaninemia or “hyperphe”) can have clinical and developmental significance. Thus all children with persistent mild hyperphe are referred to specialty care centers if three consecutive filter paper specimens demonstrate Phe levels greater than 3-5 mg/dl. Those patients with lower elevations of Phe (usually below 6 mg/dl) are often not treated. **It is very important that none of the infants referred to specialty care centers be placed on any kind of protein restriction prior to medical work-up regardless of the initial Phe level.** Proper diagnosis and classification currently depend upon the serum Phe and tyrosine concentrations being determined while the infant ingests some form of standard infant feeding.

Cofactor variants are very rare (less than 2% of the patient population) and involve enzyme deficiencies in the cofactor pathway rather than a deficiency of Phe hydroxylase.⁸

Tetrahydrobiopterin (the cofactor) is also a cofactor in neurotransmitter pathways. Dihydropteridine reductase deficiency and biopterin biosynthesis enzyme deficiency thus can result in defective production of serotonin and catecholamines.⁹ In addition to restricting Phe in the diet, precursor replacement therapy (l-dopa and 5-hydroxytryptophan) may be necessary. Diagnosis can be made by further testing of any Phe elevation, no matter how slight, at the specialty care centers.¹⁰

Most of the infants identified with slight elevations of Phe in the initial specimen are screen negative upon testing of the repeat specimen. No further testing is required of these infants. The initial elevation may be due to a transient Phe elevation in the baby or a heavy specimen collection resulting in excess blood in the screening assay. Transient elevations are occasionally found in low birth weight infants receiving TPN at the time of specimen collection. Testing of a second specimen collected after hyperalimentation has been suspended shows a return to a normal Phe level. Prompt collection of requested repeats will assist in identifying those infants who are in need of clinical evaluation and treatment.

Maternal PKU

Elevated Phe levels during pregnancy are teratogenic. In women with uncontrolled PKU, there is a significantly increased risk for spontaneous abortion. In the fetus of these women, risk is increased for intrauterine growth retardation, microcephaly, psychomotor retardation, and congenital heart defects. Even in women with relatively mild elevations of Phe, the risks for fetal abnormalities are increased because of a placental gradient favoring higher concentrations of Phe in the fetus. The best outcomes occur when Phe levels are maintained at or below 4-6 mg/dl prior to conception and throughout the pregnancy. Women of reproductive age with elevated Phe levels, including those with PKU and milder forms of hyperphe, must be counseled regarding these risks and encouraged to maintain low levels of Phe if a pregnancy is at all possible.¹¹ For the complete text of the policy statement developed by the American Academy of Pediatrics and used with permission from the AA P, see Appendix L.

MSUD – Maple Syrup Urine Disease (Branched-Chain Ketoaciduria)

Maple syrup urine disease, named for the peculiar odor of the urine, was first described by J.H. Menkes in 1954.¹² Also known as branched-chain ketoaciduria, MSUD is an autosomal recessive disorder which results in high serum levels of leucine, isoleucine, valine, alloisoleucine and their corresponding ketoacids. The urine takes on the characteristic burnt maple syrup odor as a result of the accumulated ketoacids. Five distinct clinical phenotypes of MSUD have been described. These include, in decreasing severity: classical, intermittent, intermediate, thiamin-responsive, and E3 (dihydrolipoyl dehydrogenase deficiency).¹³ All types are due to deficient or reduced branched-chain ketoacid dehydrogenase (BCKD) activity. The mutation for classical MSUD is on

chromosome 19.

The prevalence of MSUD in New York is about 1 in 240,000 births. Thirty-four cases have been detected since screening began in 1968. Infants with classical MSUD appear normal at birth, then may rapidly develop feeding difficulties, ketoacidosis, hypertonicity, convulsions, hypoglycemia, coma and then death. Without prompt diagnosis or treatment of the classic form, death usually occurs during the first year but may occur as early as the first week of life. Untreated infants who survive a severe metabolic assault will generally have profound mental and motor retardation.¹³ Not all infants with MSUD demonstrate this rapid downhill course, however. Some cases when identified by the screening laboratory at seven to ten days of age, have had only mild, if any, clinical signs. It has been observed that the most important factor determining long term outcome of MSUD patients is the time taken to make the diagnosis after the first symptoms appear.¹⁴ Screening results in most cases expedites diagnosis. When accompanied by a high-pitched cry, irritability, seizures, spasticity and coma, the infant should be taken off all protein sources immediately, taking care that fluid and calories are replaced. Blood analysis of the branched-chain amino acids will diagnose MSUD.¹

Quantitative measurement of the branched-chain amino acids leucine, isoleucine and valine, the presence of alloisoleucine in serum, and enzymatic assays for BCKD are all methods of confirming diagnosis used by the IMD Specialty Care Centers. The aim of treatment is to keep the concentration of toxic metabolites below pathologic concentrations while maintaining normal growth and development. The primary approach is by use of a diet restricting branched-chain amino acids. This treatment is difficult, as over-restriction must be avoided since these branched-chain amino acids are essential for normal growth.¹³ Even with close dietary control, crises requiring medical intervention may be precipitated by infection or injury. MSUD is sometimes effectively treated with thiamine.¹⁵

In classical MSUD, leucine levels, normal at birth, will be detectably elevated by four hours of age even without feedings.¹⁶ The other more moderate forms cannot be consistently detected by screening since leucine levels may be elevated only in a stressed environment such as after a high protein feeding or while in metabolic crisis.

Because of the severity of classical MSUD, reports of even slight elevations of leucine are telephoned promptly by the laboratory to the physician of record and the regional IMD Specialty Care Center.

Transient elevations are occasionally found in low birth weight infants receiving hyperalimentation at the time of specimen collection. A heavy specimen collection, i.e. a super-saturated blood spot, may yield an erroneously elevated leucine level in conjunction with one or both of the other amino acids. Testing of a second specimen collected after TPN has been suspended, shows a return to a normal leucine level.

Homocystinuria

The aminoacidopathy, homocystinuria, is seen in several disease entities of the metabolic pathways of sulfur-containing amino acids. The most frequent disorder, first described in 1962, is cystathionine β -synthase deficiency.^{17,18} Homocystinuria has a worldwide distribution, with higher prevalence in Welsh, Irish and English populations. It is an autosomal recessively inherited disease, mapped to chromosome 21.¹⁹ The enzyme deficiency blocks the metabolic conversion of homocysteine to cystathionine. Since newborn screening for homocystinuria began in New York in 1975, nineteen infants have been identified yielding a prevalence of 1:384,000.

The major clinical features of homocystinuria include ectopia lentis, mental retardation, osteoporosis, and thromboembolism.²⁰ Dislocation of the optic lens is the hallmark of homocystinuria. Dislocated lenses are found in up to 80% of homocystinuric patients by age fifteen and are attributed to abnormal collagen formation. Osteoporosis of vertebrae and long bone elongation are also related to defective collagen formation.²¹ Major motor seizures are frequently reported in untreated children. Mental retardation is present in about half the cases; this slowly progressive retardation may be caused by a deficiency of cystathionine, toxicity of homocysteine or vascular accident involving the brain. Treatment is very effective with normal intelligence (mean IQ = 106) reported for 13 of 13 children who were compliant with treatment.²²

Thromboembolism is the major cause of death in those with homocystinuria, half of whom die by age 20 and 75% by age 30. The sensitivity of the vasculature to homocysteine assault is so great that even persons heterozygous for this condition (i.e., 1% of the population) are at increased risk for premature thromboembolic events.^{23,24} Treatment regimens, even if unable to totally control the level of homocysteine in the blood, significantly lower cardiovascular risk.²⁵ Medical management is based on control or elimination of the biochemical abnormalities by means of a diet low in methionine, with cysteine supplementation. Fifty percent of children with residual enzyme activity are responsive to pyridoxine (vitamin B6) therapy which lowers methionine levels to within normal range and decreases the homocysteine concentration.²⁶ Those not responsive to vitamin B6 are much more difficult to control and very strict dietary management is necessary.²⁷ Research in Ireland suggests that a low methionine, cysteine-enhanced diet supplemented with pyridoxine, vitamin B12 and folate could prevent the complications of homocystinuria.²⁸ Betaine therapy is sometimes effective in decreasing homocysteine levels.²⁹ Aspirin and dipyridamole decrease the tendency of platelets to stick to altered endothelium.³⁰

There are other diseases causing homocystinuria in which methionine levels are normal or low. In addition to cystathionine beta-synthase deficiency, at least 7 causes of homocystinuria are known. These are defects in vitamin B12 metabolism;³¹ deficiency of N(5,10)-methylene tetrahydrofolate reductase;³² selective intestinal malabsorption of vitamin

B12;³³ vitamin B12-responsive homocystinuria, cobalamin E type;³⁴ methylcobalamin deficiency, cobalamin G type;³⁵ vitamin B12 metabolic defect, type 2;³⁶ and transcobalamin II deficiency.³⁷ Elevations of the amino acid, methionine, are found in other conditions in newborns which are unrelated to homocystinuria, including tyrosinemia, benign hypermethioninemia, liver immaturity or failure, and, transiently, during hyperalimentation therapy.¹⁹

Due to lack of a sensitive, rapid and inexpensive method of screening for homocysteine itself, programs screening for homocystinuria do so by measuring blood methionine by MS/MS. Detection of homocystinuria is therefore limited to the form of homocystinuria displaying a methionine elevation, i.e., cystathionine β -synthase deficiency. Detection by the newborn screening program identifies a prevalence in NYS of about 1 in 380,000. This is an underestimate because methionine elevation may not be present during the first few days of life (when specimens are routinely collected), particularly in those types of homocystinuria with residual enzyme activity responsive to vitamin B6. Cases of cystathionine synthase deficiency have been documented in newborns with normal blood methionine.³⁸

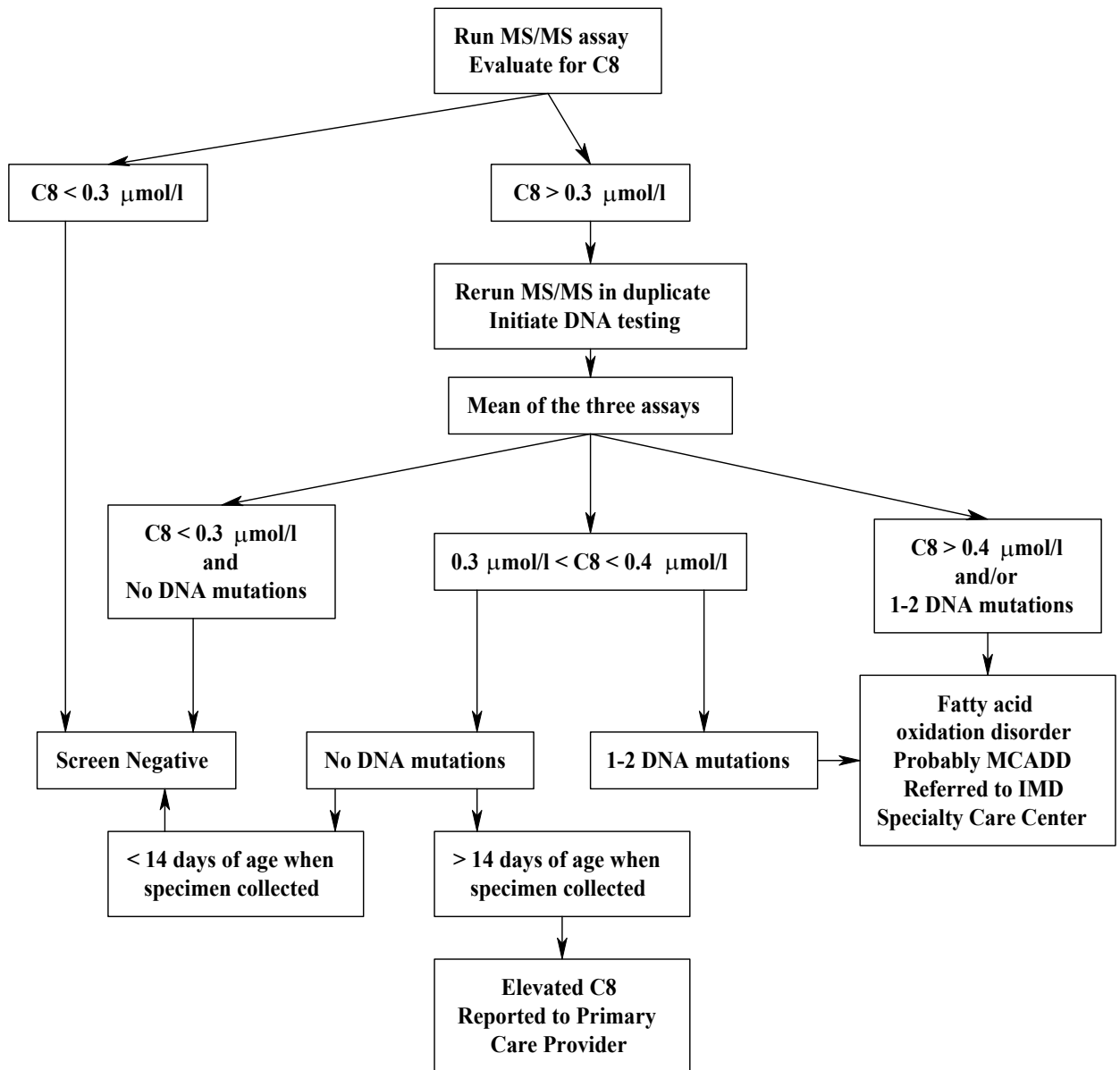
Laboratory intent to maximize the sensitivity of detection of hypermethioninemia forces the selection of as low a cutoff as seems feasible in the screening environment; even then, missed cases are inevitable as methionine levels may remain in the normal range at the time of collection. Slight elevations in methionine (1.5-2 mg %) are reported as presumptive positive, with prompt second collection required. Moderately elevated levels (4-6 mg %) with low birth weight (<2500 g), prematurity, or with health problems requiring TPN, must be repeated prior to hospital release. Persistent slight elevations in three specimens, moderate elevations without the aforementioned circumstances, and significant elevations (>6mg%), require prompt referral of the infant to an IMD Specialty Care Center. At the center, infants receive full diagnostic follow-up, and when needed, treatment and long-term case management.⁷

MCADD - Medium-Chain Acyl-CoA Dehydrogenase Deficiency

Medium-chain acyl-CoA dehydrogenase deficiency (MCADD), mapped to chromosome 1p, is an autosomal recessive condition first described in 1976.³⁹ MCADD is a fatty acid β -oxidation disorder whose expression is triggered by prolonged fasting or intercurrent illness such as infection. The incidence of MCADD in NYS is unknown, but is believed to be about 1 in 15,000 births, primarily in individuals of Northern European descent.⁴⁰

Patients with classical MCADD show elevated urinary excretion of a dicarboxylglycine, suberylglycine, as well as adipic, suberic and sebacic acids. It has been suggested that because of the defect in β -oxidation of fatty acids of medium chain length,

**Figure 4.1
MCADD SCREENING ALGORITHM**



ω -oxidation to dicarboxylic acids occurs through an alternative pathway.⁴¹

Clinical presentation is variable and may include hypoglycemia, lethargy,⁴¹ vomiting, seizures and coma.⁴² MCADD can resemble Reye's syndrome.⁴³ One-third of infants die during the first clinical episode and it may account for 1-2% of sudden infant deaths.⁴²

Although more than twenty mutations of the MCADD gene have been reported,⁴⁴ the most prevalent mutation is G985A on chromosome 1p. Approximately 80% of patients diagnosed with MCADD are homozygous for G985A and 18% are compound heterozygous for this mutation.⁴⁵ Researchers at the Centers for Disease Control and Prevention compiled a pooled analysis of published data examining the relationship between MCADD and sudden infant death syndrome. They determined that infants homozygous for G985A are at 10% greater risk for sudden death, whereas infants heterozygous for G985A are not at a significantly increased risk.⁴⁵ Half of children with MCADD will experience complications by the time they are one year of age. Diagnosis is difficult as the condition may resemble hypoglycemia, Reye's syndrome, SIDS or many metabolic disorders.⁴² Survivors of severe clinical episodes may experience muscle weakness, learning difficulties, speech delay, attention deficit disorder, failure to thrive, fatty liver, and cerebral palsy.⁴²

Newborn screening for MCADD is by MS/MS. Free carnitine and selected acylcarnitines are detected by the instrument and the blood spot octanoylcarnitine concentration is calculated. Concentrations greater than approximately 0.5 $\mu\text{mol/l}$ are suggestive of MCADD.⁴⁶ Specimens for those infants identified by MS/MS are subjected to DNA mutation analysis for the G985A mutation.

Treatment is primarily avoidance of fasting. During times of infection or anorexia the child's diet should include supplementation with a high carbohydrate drink.⁴⁶ Additional treatment includes reduction of dietary fat and L-carnitine supplementation.⁴²

Additional Disorders Detectable by Tandem Mass Spectrometry

The introduction of MS/MS technology allows screening for a large number of metabolic disorders in addition to those described above. (Table 4.1) These are rare disorders (fewer than 2 cases per year in our newborn population for each disorder) with variable presenting features. The tandem mass spectrometry allows for the simultaneous measurement of multiple metabolites. There is no incremental cost to the testing procedure to screen for these disorders. Infants with screen positive results for one of these disorders will be referred to the nearest Metabolic Specialty Care Center. Of note, MS/MS technology cannot screen for all inborn errors of metabolism. Thus, a metabolic disorder should still be considered when clinical circumstances indicate, even in the presence of a screen negative newborn screen. It is anticipated that disorders from this list will be added to the screening panel. Check with the Newborn Screening Program for a current listing of conditions in the screening panel.

Table 4.1 Disorders Detectable by Tandem Mass Spectrometry

Amino acidemias and urea cycle disorders

- 5-oxoprolinuria
- Argininemia
- Argininosuccinic lyase deficiency (ASA)
- Carbamoylphosphate synthetase deficiency (CPS)
- Citrullinemia
- Hyperammonemia, hyperornithinemia, homocitrullinuria (HHH)
- Nonketotic hyperglycinemia
- Tyrosinemia type I
- Tyrosinemia type II

Organic acidemias

- 2-Methyl butyryl-CoA dehydrogenase deficiency (2MBDH deficiency)
- 3-Methylcrotonyl-CoA carboxyl deficiency (3-MCC)
- 3-Methylglutaconyl-CoA hydratase (3MGH deficiency)
- Glutaric Aciduria Type 1/Glutaryl-CoA dehydrogenase deficiency Type 1 (GA-1)
- Isobutyryl-CoA dehydrogenase deficiency
- Isovaleric acidemia/Isovaleryl-CoA dehydrogenase deficiency (IVA)
- Malonic aciduria
- Methylmalonic acidemia (MMA)
- Mitochondrial acetoacetyl-CoA thiolase deficiency (β -KT/3-ketothiolase)
- Multiple CoA carboxylase deficiency
- Propionic acidemia/Propionyl-CoA carboxylase deficiency (PA)

Fatty acid oxidation disorders

- 2,4 Dienoyl-CoA reductase deficiency
- 3-Hydroxy 3-methylglutaryl-CoA lyase deficiency (HMG)
- Carnitine/acylcarnitine translocase deficiency (CACT)
- Carnitine palmitoyl transferase deficiency Type I (CPT-I)
- Carnitine palmitoyl transferase deficiency Type II (CPT-II)
- Long-chain acyl-CoA dehydrogenase deficiency (LCAD)
- Long-chain hydroxy acyl-CoA dehydrogenase deficiency/3-Hydroxyacyl CoA dehydrogenase deficiency (LCHAD)
- Medium-chain acyl-CoA dehydrogenase deficiency (MCADD)
- Medium-chain 3-ketoacyl-CoA thiolase (MCKAT)
- Medium-chain hydroxy acyl-CoA dehydrogenase deficiency (MCHAD)
- Multiple acyl-CoA dehydrogenase deficiency (GA-II)
- Short-chain 3-ketoacyl-CoA thiolase/3-ketothiolase (SKAT)
- Short-chain acyl-CoA dehydrogenase deficiency (SCAD)
- Short-chain hydroxy acyl-CoA dehydrogenase deficiency (SCHAD)
- Trifunctional protein deficiency (TFP)
- Very long-chain acyl-CoA dehydrogenase deficiency (VLCAD)

References Cited

1. Pass KA, Lane PA, Fernhoff PM, Hinton CF, Panny SR, Parks JS, Pelias MZ, Rhead WJ, Ross SI, Wethers DL, Elsas LJ 2nd. (2000) U.S. newborn screening system guidelines II: follow-up of children, diagnosis, management, and evaluation. Statement of the Council of Regional Networks for Genetic Services (CORN). *J Pediatr* 137(4Suppl): S1-S46.

PKU

2. Fölling A. (1934) Über Ausscheidung von Phenylbrenytraubensäure in den Harn als Stoffwechselanomalie in Verbindung mit Imbezillität. *Hoppe-Seylor's Z Physiol Chem* 227: 169-176.
3. Bickel H. (1980) Phenylketonuria: past, present, future. FP Hudson Memorial Lecture, Leeds, 1979. *J Inherit Metab Dis* 3(4): 123-132.
4. Guthrie R, Susi A. (1963) A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Pediatrics* 32: 338-343.
5. Lidsky AS, Robson KJ, Thirumalachary C, Barker PE, Ruddle FH, Woo SLC. (1984) The PKU locus in man is on chromosome 12. *Am J Hum Genet* 36: 527-533.
6. Scriver CR, Kaufman S, Eisensmith S, Woo SLC. (1995) The hyperphenylalaninemias. In *The Metabolic and Molecular Bases of Inherited Disease, 7th edition*. Scriver CR, Beaudet AL, Sly WS, Valle D (ed.) McGraw Hill: 1015-1076.
7. Amador PS, Carter TP. (1986) Historical review of newborn screening in New York State: twenty years experience. In *Genetic Disease: Screening and Management*. Proceedings of the Birth Defects Symposium, Albany, NY September 30-October 1, 1985, Carter TP, Willey AM (ed.) Alan R. Liss, Inc.: 343-357.
8. Thony B, Leimbacher W, Blau N, Harvie A, Heizmann CW. (1994) Hyperphenylalaninemia due to defects in tetrahydrobiopterin metabolism: molecular characterization of mutations in 6-pyruvoyl-tetrahydropterin synthase. *J Hum Genet* 54(5): 782-792.
9. Kaufman S. (1985) Hyperphenylalaninemia caused by defects in biopterin metabolism. *J Inherit Metab Dis* 8(Suppl 1): 20-27.
10. Naylor EW. (1986) Screening for PKU cofactor variants. In *Genetic Disease: Screening and Management*. Proceedings of the Birth Defects Symposium, Albany, NY September 30-October 1, 1985. Carter TP, Willey AM (ed.) Alan R. Liss, Inc.: 211-230.
11. AAP Committee on Genetics. (2001) Maternal Phenylketonuria. A policy statement of the American Academy of Pediatrics. *Pediatrics* 107(2): 427-428.

MSUD

12. Menkes JH, Hurst PL, Craig JM. (1954) A new syndrome: progressive familial infantile cerebral dysfunction associated with an unusual urinary substance. *Pediatrics* 14: 462-467.
13. Chuang DT, Shih VE. (1995) Disorders of branched-chain amino acid and keto acid

- metabolism. In *The Metabolic and Molecular Bases of Inherited Disease, 7th edition*. Scriver CR, Beaudet AL, Sly WS, Valle D (ed.) McGraw Hill: 1239-1278.
14. Scriver, CR, MacKenzie S, Clow CL, Delvin E. (1971) Thiamine-responsive maple-syrup-urine disease. *Lancet* 1: 310-312.
 15. Naughten ER, Jenkins J, Francis DE, Leonard JV. (1982) Outcome of maple syrup urine disease. *Arch Dis Child* 57(12): 918-921.
 16. Snyderman SE, Sansaricq C. (1985) Newborn screening for maple syrup urine disease. *J Pediatr* 107(2): 259-261.

Homocystinuria

17. Carson NAJ, Neill DW. (1962) Metabolic abnormalities detected in a survey of mentally backward individuals in Northern Ireland. *Arch Dis Child* 37: 505-515.
18. Carson NAJ, Cusworth DC, Dent CE, Field CMB, Neill DW, Westall RG. (1963) Homocystinuria: a new inborn error of metabolism associated with mental deficiency. *Arch Dis Child* 38: 425-436.
19. Mudd SH, Levy HL, Skovby F. (1995) Disorders of Transsulfuration. In: *The Metabolic and Molecular Bases of Inherited Disease, 7th edition*. Scriver CR, Beaudet AL, Sly WS, Valle, D (ed.) McGraw-Hill: 1279-1328.
20. Skovby F (1989) Inborn errors of metabolism causing homocysteinemia and related vascular involvement. *Haemostasis*, 19 (suppl 1), 4-9.
21. Grieco AJ. (1977) Homocystinuria: pathogenic mechanisms. *Am J Med Sci* 273 (2): 120-132.
22. Yap S, Rushe H, Howard PM, Naughten ER. (2001) The intellectual abilities of early-treated individuals with pyridoxine-nonresponsive homocystinuria due to cystathionine beta-synthase deficiency. *J Inherit Metab Dis* 24: 437-447.
23. Boers GH. (1989) Carriership for homocystinuria in juvenile vascular disease. *Haemostasis*, 19(Suppl 1): 29-34.
24. Brattström L, Israelsson B, Hultberg B. (1989) Plasma homocysteine and methionine tolerance in early-onset vascular disease. *Haemostasis* 19(Suppl 1): 35-44.
25. Yap S, Boers GH, Wilcken B, Wilcken DE, Brenton DP, Lee PJ, Walter JH, Howard PM, Naughten ER. (2001) Vascular outcome in patients with homocystinuria due to cystathionine beta-synthase deficiency treated chronically: a multicenter observational study. *Arterioscler Thromb Vasc Biol* 21(12):2080-2085.
26. Hooft C, Carton D and Samyn W. (1967) Pyridoxine treatment in homocystinuria. *Lancet* 1967(1): 1384.
27. Shih VE, Efron ML. (1970) Pyridoxine-unresponsive homocystinuria. *N Engl J Med* 283(22): 1206-1208.
28. Yap S, Naughten E. (1998) Homocystinuria due to cystathionine beta-synthase deficiency in Ireland: 25 years' experience of a newborn screened and treated population with reference to clinical outcome and biochemical control. *J Inherit Metab Dis* 21: 738-747.
29. Wilcken DE, Wilcken B, Dudman NP, Tyrrell PA. (1983) Homocystinuria - the effects of betaine in the treatment of patients not responsive to pyridoxine. *N Engl J Med* 309(8): 448-453.

30. Harker LA, Slichter SJ, Scott R, Ross R. (1974) Homocystinemia – vascular injury and arterial thrombosis. *N Eng J Med* 291 (11): 537-543.
31. Mudd SH, Levy HL, Abeles RH. (1969) A derangement in B12 metabolism leading to homocystinemia, cystathioninemia and methylmalonic aciduria. *Biochem Biophys Res Commun* 35: 121-126.
32. Goyette P, Sumner JS, Milos R, Duncan AMV, Rosenblatt DS, Matthews RG, Rozen R. (1994) Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. *Nature Genet* 7: 195-200.
33. Waters AH, Murphy MEB. (1963) Familial juvenile pernicious anaemia: a study of the hereditary basis of pernicious anaemia. *Brit J Haemat* 9: 1-12.
34. Schuh S, Rosenblatt DS, Cooper BA, Schroeder M-L, Bishop AJ, Seargeant LE, Haworth JC. (1984) Homocystinuria and megaloblastic anemia responsive to vitamin B-12 therapy. *New Eng J Med* 310: 686-690.
35. Watkins D, Rosenblatt DS. (1988) Genetic heterogeneity among patients with methylcobalamin deficiency: definition of two complementation groups, cblE and cblG. *J Clin Invest* 81: 1690-1694.
36. Willard HF, Mellman IS, Rosenberg LE. (1978) Genetic complementation among inherited deficiencies of methylmalonyl-CoA mutase activity: evidence for a new class of human cobalamine mutant. *Am. J. Hum. Genet.* 30: 1-13.
37. Hakami N, Neiman PE, Canellos GP, Lazerson J. (1971) Neonatal megaloblastic anemia due to inherited transcobalamin II deficiency in two siblings. *New Eng J Med* 285: 1163-1170.
38. Dhondt JL, Farriaux JP, Gaul GE, Tallent H. (1982) Diagnostic history of a case of homocystinuria without hypermethioninemia. *J Inher Metab Dis* 5(suppl 1) 8.

MCADD

39. Gregersen N, Lauritzen R, Rasmussen K. (1976) Suberylglycine excretion in the urine from a patient with dicarboxylic aciduria. *Clin Chim Acta* 70(3): 417-425.
40. Andresen BS, Dobrowolski SF, O'Reilly L, Muenzer J, McCandless SE, Frazier DM, Udvari S, Bross P, Knudsen I, Banas R, Chace DH, Engel P, Naylor EW, Gregersen, N. (2001) Medium-chain acyl-CoA dehydrogenase (MCAD) mutations identified by MS/MS-based prospective screening of newborns differ from those observed in patients with clinical symptoms: identification and characterization of a new, prevalent mutation that results in mild MCAD deficiency. *Am J Hum Genet* 68: 1408-1418.
41. Naylor EW, Mosovich LL, Guthrie R, Evans JE, Tieckelmann H. (1980) Intermittent non-ketotic dicarboxylic aciduria in two siblings with hypoglycemia: an apparent defect in beta-oxidation of fatty acids. *J Inher Metab Dis* 3(1): 19-24.
42. Iafolla AK, Thompson RJ, Jr., Roe CR. (1994) Medium-chain acyl-coenzyme A dehydrogenase deficiency: clinical course in 120 affected children. *J Pediatr* 124(3): 409-415.
43. Taubman B, Hale DE, Kelley RI. (1987) Familial Reye-like syndrome: a presentation of medium-chain acyl-coenzyme A dehydrogenase deficiency. *Pediatrics* 79(3): 382-385.
44. Andresen BS, Bross P, Udvari S, Kirk J, Gray G, Kmoch S, Chamoles N, Knudsen I,

- Winter V, Wilcken B, Yokata I, Hart K, Packman S, Harpey JP, Saudubray JM, Hale DE, Bolund L, Kolvraa S, Gregersen N. (1997) The molecular basis of medium-chain acyl-CoA dehydrogenase (MCAD) mutations identified by MS/MS-based prospective screening of newborns differ from those observed in patients with clinical symptoms: identification and characterization of a new, prevalent mutation that results in mild MCAD deficiency. *Hum Molec Genet* 6: 695-707.
45. Wang SS, Fernhoff PM, Khoury MJ. (2000) Is the G985A allelic variant of medium-chain acyl-CoA dehydrogenase a risk factor for sudden infant death syndrome? A pooled analysis. *Pediatrics* 105(5): 1175-1176.
46. Clayton PT, Doig M, Ghafari S, Meaney C, Taylor C, Leonard JV, Morris M, Johnson AW. (1998) Screening for medium-chain acyl-CoA dehydrogenase deficiency using electrospray ionization tandem mass spectrometry. *Arch Dis Child* 79(2): 109-115.

Chapter 5 . THE INHERITED METABOLIC DISORDERS (IMD): RED CELL ENZYMES

Introduction

The Red Cell Enzyme Laboratory performs screening for galactosemia and biotinidase deficiency. Preliminary test results for all specimens are available within one day of receipt. Those highly indicative of a disorder are telephoned to the physician of record and the IMD Specialty Care Center closest to the address of the mother. Reports of specimens with borderline screen positive results are mailed to the physician of record or hospital of birth, which are required to collect repeat specimens. Guidelines for diagnosis and treatment of all conditions tested by newborn screening have recently been published¹ and should be consulted.

Experienced care providers for patients with red cell enzyme deficiencies are available throughout New York State at IMD Specialty Care Centers. See Appendix H for the list of IMD Specialty Care Centers. These centers provide the medical care and referral services required by the patient and family.¹

Galactosemia

An autosomal recessive inherited disorder of carbohydrate metabolism, galactosemia was first described in 1917.² One hundred thirty mutations have been identified in the GALT (galactose-1-phosphate uridyl transferase) gene on chromosome 9.³ Two common mutations associated with classic galactosemia, Q188R and K285N, account for more than 70% of galactosemia in Caucasian populations. The S135L mutation, associated with good outcomes, accounts for 65% of the alleles identified in African-Americans. Biochemically, three enzyme deficiencies in the conversion of galactose to glucose can lead to galactosemia. Galactokinase deficiency is the mildest form, the chief symptom being cataract formation as elevated galactose is reduced to galactitol in the lens. Galactose-4-epimerase deficiency, the rarest, is in most cases a biochemical abnormality with no clinical sequelae. A more severe form of this disorder has been reported presenting symptoms similar to those of classical galactosemia.⁴

Classical galactosemia, the most severe form, is caused by the absence of the GALT enzyme. GALT deficiency will result in the rapid development of clinical sequelae following lactose ingestion. Although the infant may be normal at birth, symptoms develop in a few days to two weeks after initiating milk feedings, so that the infant is often discharged from the hospital prior to onset of illness. Symptoms include vomiting, diarrhea, lethargy, jaundice, failure to thrive, and hepatomegaly.⁴ Without rapid diagnosis, the

disease may result in death, frequently associated with *E. coli* septicemia⁵ or a coagulopathy.⁶ Those infants who do not present with early symptoms may develop motor retardation, hepatomegaly and cataracts, or in early childhood, mental retardation, growth failure and cirrhosis by six months of age.

Diagnosis in the newborn period with restriction of dietary galactose is effective in reducing the clinical severity of the disease. Unfortunately, total elimination of dietary galactose does not assure full normalcy, since galactose is produced in the body from glucose.⁷ Satisfactory general health may be accompanied by some mental decline, with speech and language defects documented in those children treated from an early age. A high incidence of ovarian dysfunction due to continued galactose toxicity is observed in some women, even after successful pregnancies.^{7,1}

The newborn screening program identifies classical galactosemia by testing for the transferase enzyme in red cells.⁸ Galactosemia screening is accomplished using the Beutler enzymatic assay⁸ testing for the presence of the red cell enzyme GALT. The assay requires a 3-hour incubation time with results available on the day of receipt. Any specimen lacking the enzyme is verified immediately using a faster, modified version of the assay that measures total galactose by the Hill metabolite assay.⁹ These confirmatory tests take approximately 45 minutes. The treatment center and newborn's physician are immediately informed by telephone of the screen positive results. Kinase and epimerase deficiencies are not detectable by this method.

Screening was initiated in New York State in 1968, but not mandated until 1975. The prevalence of the disorder is 1:54,000 with 150 infants diagnosed. Since these babies can become ill very early in life they are more likely to receive a transfusion, thereby providing red cells with active enzyme and invalidating test results for up to three months.¹⁰ Therefore it is of utmost importance to collect a blood specimen prior to any anticipated transfusion. An assay to measure the serum metabolites, galactose and galactose-1-phosphate, is employed to test any baby with a screen positive enzyme test or who may have received a transfusion.¹⁰ Transfusion information is obtained from the blood collection form and the hemoglobin laboratory test results (see Chapter 6).

Some screening programs utilize a metabolite assay for galactose and galactose-1-phosphate rather than an enzyme assay. The assay will allow detection not only after transfusions, but also detection of kinase and epimerase disorders. However, this method of screening will miss patients who have not been on lactose-containing foods. In New York, many infants are on soy formulas from birth because of an older sibling with milk allergies. Metabolite assays also are associated with more false positives from infants with unrelated liver dysfunction or from premature infants. Other unexplained elevations in initial blood galactose levels have also been reported.¹¹

Identification of galactosemia must be rapid since death may result in seven to ten

days if the disorder is untreated. The screening laboratory performs this test the same day the specimens are accessioned, with results available by 3:30 p.m. Telephone calls to the nearest IMD Specialty Care Center, hospital, and private physician when identified, are made immediately. Arrangements are made between the physician and specialty center as to the course of action depending on the health of the infant. Immediate medical evaluation is necessary as parents usually are unable to accurately assess the baby's health status and may report only poor feedings. The specialty center arranges to see the infant for diagnostic testing and evaluation. Those infants diagnosed with classical galactosemia need life-long nutritional guidance, genetic counseling, and medical follow-up.

There are two variants of galactosemia which are frequently identified in screening. The African-American variant (so called because it accounts for 65% of alleles causing galactosemia in the black population⁴) shows no detectable enzyme activity in peripheral blood and normal liver enzyme levels. Clinically, this is a very mild condition.¹² The second variant is the Duarte variant. In these infants, half the normal enzyme activity is present and therefore no effects are noted. When a Duarte gene and classical galactosemia gene (one from each parent) combine to yield a double heterozygote or genetic compound heterozygote, the infant will have only 25% of normal enzyme activity.⁴

Biotinidase Deficiency

Biotinidase deficiency is an autosomal recessive disease with a prevalence in New York of about 1 in 78,000.¹³ Fifty-six infants have been diagnosed since it was added to the newborn screening profile in 1987. The biotinidase gene is found on chromosome 3 at the p25 band.¹⁴ The gene is highly polymorphic with sixty-two mutations causing profound biotinidase deficiency.¹⁵

The enzyme biotinidase is responsible for recycling the vitamin biotin in the degradation of carboxylases and in the liberation of protein-bound biotin in digestion. Biotinidase deficiency has been shown to be the primary defect in most cases formerly identified as late-onset multiple carboxylase deficiency.¹⁶ Since biotin cannot be recycled from the carboxylases, or absorbed in the protein-bound dietary form, the affected infant becomes biotin deficient. The symptoms of the disorder result from the deficiency of biotin and the biotin-dependent carboxylases. Symptoms are not present at birth, but may begin to develop in three to six months. The symptoms include cutaneous lesions such as skin rash, alopecia, conjunctivitis and fungal infections; neurological involvement such as seizures, ataxia, hypotonia, hearing loss, visual impairment and delayed development; and biochemical abnormalities presenting as metabolic acidosis and organic aciduria. Untreated, manifestations of the condition vary from mild neurologic and behavior abnormalities to metabolic crises that may be fatal.¹⁷

Pharmacologic doses of biotin administered orally are effective in the alleviation of biochemical and cutaneous symptoms and can prevent, but not reverse, neurological dysfunction. Many vitamin supplements contain biotin in a protein-bound form, which is ineffective for treatment purposes.¹⁷

A semi-quantitative colorimetric screening test for biotinidase activity was developed by Heard and Wolf.¹⁸ The simple five-step reaction requires an overnight incubation with test results available the next workday. If screen positive, the test is repeated on the same specimen to verify accuracy. The physician of record or hospital of birth is notified by letter of the screen positive result and the need for retesting. If the second specimen is screen positive, the case is referred to a IMD Specialty Care Center for diagnostic testing, clinical evaluation and treatment. In the event results on the initial specimen are significantly screen positive, the infant is referred to the IMD Specialty Care Center without a second specimen.

The false positive rate with this test is very low, and generally associated with low birth weight or prematurity. Second specimens from these infants screen negative.

References Cited

1. Pass KA, Lane PA, Fernhoff PM, Hinton CF, Panny SR, Parks JS, Pelias MZ, Rhead WJ, Ross SI, Wethers DL, Elsas LJ 2nd. (2000) U.S. newborn screening system guidelines II: follow-up of children, diagnosis, management, and evaluation. Statement of the Council of Regional Networks for Genetic Services (CORN). *J Pediatr* 137(4Suppl): S1-S46.

Galactosemia

2. Goppert F. (1917) Galaktosurie nach Milchzuckergabe bei angeborenem, familiaerem chronischem Leberleiden. *Klin. Wschr* 54: 473-477.
3. Elsas LJ, II, Lai K. (1998) The molecular biology of galactosemia. *Genet Med* 1: 40-48.
4. Segel S, Berry GT. (1995) Disorders of galactose metabolism. In *The Metabolic and Molecular Bases of Inherited Disease, 7th edition*. Scriver CR, Beaudet AL, Sly WS, Valle D (ed.) McGraw Hill: 967-1000.
5. Levy HL, Sepe SJ, Shih VE, Vawter GF, Klein JO. (1977) Sepsis due to *Escherichia coli* in neonates with galactosemia. *New Eng J Med* 297: 823-825.
6. Ruiz M, Jover S, Armas M, Duque MR, Santana C, Giros ML, Boleda M D. (1999) Galactosaemia presenting as congenital pseudoafibrinogenaemia. *J Inherit Metab Dis* 22: 943-944.
7. Gitzelmann R, Steinmann B. (1984) Galactosemia: how does long-term treatment change the outcome? *Enzyme* 32(1): 37-46.

8. Beutler E, Baluda MC. (1966) A simple spot screening test for galactosemia. *J Lab Clin Med* 68(1) 137-41.
9. Hill GN, O'Reilly D, Robertson E. (1983) A simple screening test for galactosemia based on accumulation of galactose and galactose-1-phosphate. In *Neonatal Screening*. Naruse H, Irie M (ed.) Excerpta Medica: 252-253.
10. Korson MS, Levy HL. (1990) Pitfalls in diagnosing galactosemia: false negative newborn screening following red blood cell blood transfusion (letter). *J Pediatr Gastroenterol Nutr* 10(2) 272-273.
11. Lipson MH, Russo PJ. (1982) Normal initial blood galactose levels in a newborn with galactosemia. *Am J Dis Child* 136(8): 747-748.
12. Cuatrecasas P, Segal S. (1966) Galactose conversion to D-xylulose: an alternate route of galactose metabolism. *Science* 153(735): 549-551.

Biotinidase Deficiency

13. Wolf B, Heard GS. (1989) Worldwide experience in newborn screening for biotinidase deficiency. In *Proceedings of the Seventh National Neonatal Screening Symposium*, ASTPHLD, Washington, DC.
14. Cole H, Weremowicz S, Morton CC, Wolf B. (1994) Localization of serum biotinidase (BTD) to human chromosome 3 in band p25. *Genomics* 22: 662-663.
15. Hymes J, Stanley CM, Wolf B. (2001) Mutations in BTD causing biotinidase deficiency. *Hum Mutat* 18: 375-381.
16. Wolf B, Grier RE, Allen RJ, Goodman SI, Kien CL. (1983) Biotinidase deficiency: the enzymatic defect in late-onset multiple carboxylase deficiency. *Clin Chem Acta* 131(3): 273-281.
17. Wolf B, Heard GS, Weissbecker KA, McVoy JR, Grier RE, Leshner RT. (1985) Biotinidase deficiency: Initial clinical features and rapid diagnosis. *Ann Neurol* 18(5): 614-617.
18. Heard GS, McVoy JRS, Wolf B. (1983) A screening method for biotinidase deficiency in newborns. *Clin Chem* 30(1): 125-127.

Chapter 6 . SICKLE CELL DISEASE AND OTHER HEMOGLOBINOPATHIES

Introduction

The mandate of the newborn hemoglobin testing laboratory is to identify newborns with homozygous sickle cell disease (SS). To accomplish this, screening procedures have been optimized to detect the presence of Hb S. The screening procedure used is cellulose acetate electrophoresis at pH 8.6 as developed by Garrick¹. All specimens exhibiting other than a normal newborn hemoglobin pattern, i.e., Hb F (fetal) with a lesser amount of Hb A (adult), are evaluated further by citrate agar electrophoresis² and isoelectric focusing³ to determine the presumptive hemoglobin phenotype. (The evaluation of specimens exhibiting Hb A greater than Hb F is covered in the section on transfusions.)

Sickle Cell Diseases

Sickle cell anemia is an autosomal recessive disorder resulting from synthesis of abnormal β -chains of hemoglobin.⁴ The sickle β -chain results from a single nucleotide substitution at codon 6 of chromosome 11. This leads to a replacement of glutamic acid with valine. Several conditions can exhibit the characteristic sickling of the red cell, including: sickle cell anemia (homozygous SS disease), sickle β -thalassemia, hemoglobin SC disease, hemoglobin SD disease, and hemoglobin SO (Arab) disease.^{5,6}

All infants born in New York State are tested for abnormal hemoglobins. The prevalence of all hemoglobinopathies in New York State determined by the newborn screening program is 1:1050 with 6,934 children identified since screening began in 1975. When the laboratory suspects any of the above sickle cell disease conditions in a newborn, a repeat filter paper specimen is requested from the infant's primary health care provider or hospital of birth as soon as possible for verification of the presumptive diagnosis. Currently the presence of the S and C alleles can be confirmed by allele-specific oligonucleotide hybridization. Electrophoresis, complemented with molecular testing, can help distinguish between S/thalassemias and carriers as well as confirmation of SS and SC genotypes. Eventually other variants will be incorporated into the molecular testing algorithm.

Following a positive screening result, a definitive diagnosis can be made by cellulose acetate/citrate agar electrophoretic testing of both parents for hemoglobinopathies and additional laboratory evaluation for thalassemia trait.⁵ Molecular techniques may also be utilized for a definitive diagnosis, using either liquid blood from each family member, or directly from the dried blood spot using microextraction techniques.^{7,8} If the parents are not tested, an accurate diagnosis can usually be made by molecular analysis of the infant's blood

or by retesting the infant at 4-6 months of age using citrate agar electrophoresis, quantitative cellulose acetate electrophoresis, CBC (complete blood count) and MCV (mean corpuscular volume) analysis. An accurate diagnosis must be made because each hemoglobinopathy requires specific management and carries a different prognosis. Genetic counseling for the parents will vary with their hemoglobin type.

A comprehensive care approach is recommended for individuals with a chronic illness such as sickle cell disease.⁹ The infant and his/her family requires medical intervention, genetic counseling, psychosocial services, peer support and health care education, as well as other services to assist in coping with the effects of the disease. Experienced care providers for sickle cell patients are available throughout New York State at Sickle Cell/Hematology Specialty Care Centers, hematology clinics, or through pediatric hematologists. See Appendix I for the list of Sickle Cell/Hemoglobinopathy Specialty Care Centers. These centers provide the medical care and referral services required by the patient and family.¹⁰

The clinical manifestations of sickle cell disease vary greatly among individuals with the disease. The child is at significant risk for life-threatening bacterial infections (septicemia and meningitis due to *S. pneumoniae*), painful episodes, splenic sequestration crisis, acute pulmonary events and stroke. Children with sickle cell disease should be followed at close intervals during the first two years of life. Since each case varies, the experience of the physician in dealing with sickle cell anemia becomes very important; hence, physicians who are inexperienced should not hesitate to refer their patients for care. Parental education is very important in reducing the complications of this disease. Parents should learn how to treat painful crises with analgesics and hydration, how to read thermometers, how to recognize the complications from fever, and to recognize splenic enlargement. They must fully appreciate the importance of early intervention for the febrile child and the reasons for avoidance of hot and cold temperature extremes.

The Sickle Cell Advisory Committee (SCAC) of GENES (Genetic Network of New York, Puerto Rico and the Virgin Islands) consists of physicians, social workers, nurses, genetic counselors, public health professionals, patients and their families dedicated to the well-being of people with sickle cell disease and other hemoglobinopathies. This committee has developed a book titled *Guidelines for the Treatment of People with Sickle Cell Disease*. The *Guidelines* discuss standards of care for health management and the many complications of sickle cell disease. Included are textual discussions of recommended care, simplified algorithms and references. Copies of the book and a separate booklet of the algorithms are available by contacting the NYS Genetic Services Program, Wadsworth Center, Albany, NY 12201; phone (518) 474-7148.

Medical Complications of Sickle Cell Disease in Children

Infections: Serious bacterial infections are a major cause of morbidity and mortality in patients with sickle cell anemia. Causative organisms in early childhood infections include

H. pneumoniae, *H. influenzae*, *N. meningitis*, *Salmonella*, *M. pneumoniae*, *S. aureus*, and *E. coli*. Severe overwhelming septicemia and meningitis due to *S. pneumoniae* is the most significant cause of death during childhood. Osteomyelitis and pneumonia as well as other infections can be difficult to treat and usually require in-patient hospital treatment.¹¹

Antibiotic prophylaxis has been shown to prevent life-threatening pneumococcal infections in sickle cell disease patients who are between three months and five years of age. Oral penicillin is the preferred form of treatment.^{6,9}

A program of immunization for children with sickle cell disease should include those normally recommended for children: diphtheria, pertussis, tetanus, oral polio, measles, mumps, hemophilus b conjugate, hepatitis B, and rubella. Children with sickle cell disease should also receive heptavalent conjugated pneumococcal¹² and trivalent influenza virus vaccines.⁶

Painful crises: Painful events are the most prominent manifestation of sickle cell disease. Although previous or concurrent infections may account for 30% of painful crises, other factors such as hypoxia, acidosis, dehydration, and exposure to extreme cold may serve as precipitating factors. Frequency and severity are unpredictable and vary remarkably from one patient to another.¹³ Psychological effects to patient and family resulting from the sporadic and sometimes severe painful attacks may be as profound as the physical effects and can lead to drug dependency and the breakdown of family units.¹⁴

Other Hemoglobinopathies

The screening methodologies used in newborn screening will detect hemoglobinopathies other than sickle cell disease, e.g., homozygous C disease, hemoglobin C-thalassemia disease, homozygous E disease, hemoglobin E-thalassemia disease and others. The laboratory requires a repeat specimen be collected from infants with any of these findings. The most common non-S type hemoglobinopathies the screening program detects are summarized here with brief descriptions of their clinical presentation.

Homozygous C disease (C/C) and C- β -thalassemia are characterized by a mild, chronic hemolytic anemia with splenomegaly. Numerous target cells are found in the blood smear, as well as a slightly elevated reticulocyte count. The spleen is often enlarged. In C- β -thalassemia the MCV is low. Parents should be alerted to the fact that a child with hemoglobin C disease usually has lifelong mild anemia. Treatment is usually not necessary for the anemia that is generally asymptomatic.⁵

Homozygous hemoglobin D disease (D/D) exhibits a normal or slightly reduced hemoglobin level with a slight increase in target cells.⁵

In homozygous hemoglobin E disease (E/E), the anemia is slight and individuals are generally asymptomatic.⁵

Hemoglobin E-thalassemia disease can follow a course varying from mild β -thalassemia to

a condition indistinguishable from homozygous β -thalassemia. Patients may show marked growth retardation and have difficulty maintaining hemoglobin levels above 5 g/dl. Progressive hepatosplenomegaly and frequent infections are common complications.⁵

Hemoglobin Carrier Conditions

The screening laboratory reports as a carrier condition any hemoglobin electrophoresis pattern which includes bands in addition to Hb F and Hb A, in amounts less than or equal to the amount of Hb A present. The determination is based on a visual comparison and not quantitation of band intensity. With over 450 hemoglobin variants, the most common carrier conditions identified in the laboratory, in decreasing order of frequency, are A/S (sickle trait), A/C, A/E, A/O Arab, A/D, A/G, A/fetal variant, A/Fast variant, and A/variant (variant type unknown). In NYS about 9,000 carriers are identified annually or 35 carriers for every infant identified with a hemoglobinopathy.

The hemoglobin A/Fast variant designation is used to describe a band on cellulose acetate electrophoresis that is anodal to Hb A(adult). This result can indicate three possibilities: 1) hemoglobin Barts; 2) a hemoglobin variant such as Hb J, N, or fetal; or 3) an artifact of the filter paper blood specimen. Of these possibilities, Hb Barts is the most significant, since it is an indication of α -thalassemia carrier state.⁵

Follow-up for Hemoglobin Carrier Conditions

While there may be no immediate benefit to the infant from identifying the carrier status, parents may benefit, especially if subsequent testing reveals a risk of hemoglobinopathy in a subsequent pregnancy. Prenatal care should include patient and partner screening for hemoglobin carrier status. Also, since all newborn screening results are sent to the pediatrician of record at the time of birth, parents should learn of a carrier result during the first post-birth doctor visit. In NYS hospitals of birth and sickle cell specialty care centers are required to notify parents of the carrier status of their newborn. Parents of all infants who are determined to be carriers of hemoglobin variants should be offered appropriate education, genetic counseling and testing. This counseling should be done by persons who have appropriate training and credentialing in order to assure the highest quality of services for families.¹⁵

Transfusions

Efforts should be made to collect a specimen prior to any planned transfusion. Specimens for which the electrophoresis pattern on cellulose acetate shows hemoglobin A

in a greater amount than Hb F are reported as **transfused** when the infant is less than 30 days of age at specimen collection, or when a transfusion, as recorded on the collection form, occurred less than 30 days before collection. A specimen collected prior to a transfusion is suitable for testing for hemoglobin, galactose, biotinidase, IRT, octanlylcarnitine and HIV antibodies regardless of age, birth weight or feeding status at the time of collection. If this pre-transfusion specimen was taken at less than 24 hours of age, it is then necessary to collect a repeat specimen three days after the last transfusion. This second specimen will be suitable for testing thyroxine, leucine, methionine, phenylalanine, and 17-OHP, thus completing the newborn profile.

The screening laboratory recommends that the provider follow one of three courses of action when the initial specimen is reported as transfused.

- a. If a transfusion was administered with no prior collection of a specimen, two repeat specimens are required. The **first** repeat is to be collected three days or more after the infant's most recent transfusion and a **second**, follow-up specimen is to be collected three months after the infant's final transfusion so that transfused hemoglobin will have been removed from circulating blood. Two blood collection forms will be enclosed for use in scheduling these required repeat collections. The transfusion date should be recorded on the blood collection forms.
- b. If no transfusion was administered and the infant is more than 24 hours old, all newborn screening tests performed on the initial specimen are considered to be valid and a repeat specimen is not needed. A number of infants at birth have more hemoglobin A than hemoglobin F and thus produce an erroneous transfusion pattern. Written verification that no transfusion was given should be returned to the newborn screening program upon request and a copy of the report included in the infant's permanent hospital record.
- c. If no transfusion was administered but the infant is less than 24 hours old, a second specimen must be collected three days after the last transfusion to allow the laboratory to test for those analytes that are age-dependent, i.e., thyroxine, leucine, methionine, phenylalanine, and 17-OHP.

Abnormal results other than hemoglobin testing coincident with a transfusion report will be clearly reported with appropriate requests for collection of a repeat specimen.

References Cited

1. Garrick MD, Dembure P, Guthrie R. (1973) Sickle-cell anemia and other hemoglobinopathies: Procedures and strategies for screening employing spots of blood on filter paper as specimens. *N Engl J Med* 288(24): 1265-1268.
2. Schedlbauer LM, Pass KA. (1989) Cellulose acetate/citrate agar electrophoresis of

- filter paper hemolysates from heel stick. *Pediatrics* 83 (5 pt 2): 839-842.
3. Black J. (1984) Isoelectric focusing in agarose gel for detection and identification of hemoglobin variants. *Hemoglobin* 8(2): 117-127.
 4. Newborn screening fact sheets. (1996) Committee on Genetics, American Academy of Pediatrics. *Pediatrics* 98: 473-501.
 5. Weatherall DJ, Clegg JB, Higgs DR, Wood WG. (1995) The hemoglobinopathies. In: *The Metabolic and Molecular Bases of Inherited Disease*. 7th edition Scriver CR, Beauder AL, Sly WS, Valle D (eds). McGraw Hill: 3417-3484.
 6. Management and therapy of sickle cell disease. 4th edition. (2002) NIH Publication No. 02-2117.
 7. McCabe ERB, Huang SZ, Seltzer WK, Law ML. (1987) DNA microextraction from dried blood spots on filter paper blotters: potential applications to newborn screening. *Hum Genet* 75(3): 213-216.
 8. Caggana M, Conroy JM, Pass, KA. (1998) Rapid, efficient method for multiplex amplification from filter paper. *Hum Mutat* 11(5): 404-409.
 9. Wong WY. (2001) Prevention and management of infection in children with sickle cell anaemia. *Paediatr Drugs* 3(11):793-801.
 10. Pass KA, Lane PA, Fernhoff PM, Hinton CF, Panny SR, Parks JS, Pelias MZ, Rhead WJ, Ross SI, Wethers DL, Elsas LJ 2nd. (2000) U.S. newborn screening system guidelines II: follow-up of children, diagnosis, management, and evaluation. Statement of the Council of Regional Networks for Genetic Services (CORN). *J Pediatr* 137 (4 suppl): S1-S46.
 11. Gaston MH, Verter JI, Woods G, Pegelow C, Kelleher J, Presbury G, Zarkowsky H, Vichinsky E, Iyer R, Lobel JS, Diamond S, Holbrook CT, Gill FM, Ritchey K, Falletta JF. (1986) Prophylaxis with oral penicillin in children with sickle cell anemia. A randomized trial. *N Engl J Med* 314(25): 1593-1599.
 12. First pneumococcal vaccine approved for infants and toddlers. *FDA Bulletin* 2/17/2000.
 13. Powars DR. (1975) Natural history of sickle cell disease - the first ten years. *Semin Hematology* 12(3):267-285.
 14. Leavell SR, Ford CV. (1983) Psychopathology in patients with sickle cell disease. *Psychosomatics* 24(1): 23-5, 28-9, 32 passim.
 15. Wethers DL, Lane, PA, et. al. (1997) Guidelines for follow-up of carriers of hemoglobin variants detected by newborn screening. Published as an appendix to U.S. newborn screening system guidelines II: Follow-up of children, diagnosis, management, and evaluation. *J Pediatr* 137(4 suppl): S1-S46.

Chapter 7 . ENDOCRINE DISORDERS

For the purposes of this discussion, endocrine disorders in the newborn screening panel are congenital hypothyroidism, congenital adrenal hyperplasia and cystic fibrosis. Each of these are identified by serum markers: congenital hypothyroidism – T₄ and TSH; congenital adrenal hyperplasia – 17-OHP; and cystic fibrosis – immunoreactive trypsin.

Congenital Hypothyroidism

Infants with congenital hypothyroidism may appear clinically normal up to six months of age but are unable to produce adequate amounts of the thyroid hormone, thyroxine, for normal organ function and brain development. Mandated screening for congenital hypothyroidism in NYS was initiated in 1978 as radioimmunoassays for thyroxine and thyrotropin became available that were sufficiently sensitive and specific for use on dried blood specimens.¹ Since then 3,086 children have been identified with primary hypothyroidism and 2,115 with other abnormalities of thyroid function, yielding an incidence of 1:1,140 children screened.

Screening enables detection of infants with primary as well as secondary and tertiary hypothyroidism. Primary hypothyroidism, also called thyroid dysgenesis, includes hypoplasia or aplasia of the thyroid gland and enzyme deficiencies in thyroxine (T₄) synthesis (dyshormonogenesis).² Secondary hypothyroidism results from failure of the pituitary to release thyroid-stimulating hormone (TSH) or thyrotropin. Tertiary hypothyroidism results from failure of the hypothalamus to secrete thyrotropin-releasing hormone (TRH).² Ten percent of congenital hypothyroidism is inherited as dyshormonogenesis. These may be caused by mutations on many genes. In addition perhaps 2% of thyroid dysgenesis appears to be familial and may be genetic due to abnormal transcription factors important in thyroid gland formation or abnormalities in the TSH receptor.³ Congenital hypothyroidism is more than twice as common in girls as in boys.⁴

Experienced care providers for patients with endocrine disorders are available throughout New York State at Endocrine Specialty Care Centers. See Appendix J for the list of Endocrine Specialty Care Centers. These centers provide the medical care and referral services required by the patient and family.⁵

Laboratory Detection

A competitive enzyme-linked immunosorbent assay (ELISA) is used for the

quantitative measurement of T₄. TSH is quantitatively measured by time-resolved fluoroimmunoassay. Low levels of T₄, especially associated with elevated TSH values are indicative of hypothyroidism.

The hypothyroid screening procedure initially tests for T₄. Specimens with T₄ values in the lowest ten percentile are retested for T₄ and additionally for TSH. Specimens with T₄ below 2 standard deviations of the assay batch are retested for T₄ as well as tested for TSH.

Screen Positive Results

Screen positive hypothyroid test results (1% of all specimens) fall into three categories of severity; all require repeat testing.

- 1) Presumptive positive REFERRALS for hypothyroidism, which are considered medical emergencies, are as follows:
 - T₄ in the bottom tenth percentile and a TSH level equal to or greater than 30 μU/ml for infants more than 24 hours of age at the time of specimen collection.
 - T₄ in the bottom tenth percentile and a TSH level equal to or greater than 50 μU/ml for infants less than 24 hours of age at time of specimen collection.
- 2) Screen positive test results in **second** specimen REFERRALS for hypothyroidism requiring follow-up and possible treatment are as follows:
 - TSH level of a **repeat** specimen equal to or greater than 20 μU/ml.
 - T₄ level of a **repeat** specimen equal to or greater than 2 standard deviations below the mean and a TSH level less than 20 μU/ml.
- 3) Reports of presumptive positive for hypothyroidism which are not considered life-threatening or medical emergencies are as follows:
 - T₄ level equal to or greater than 2 standard deviations below the mean and a TSH level less than 20 μU/ml.
 - T₄ in the bottom tenth percentile and a TSH level equal to or greater than 20 μU/ml but less than 30 μU/ml for infants greater than 24 hours of age.
 - T₄ in the bottom tenth percentile and a TSH level equal to or greater than 20 μU/ml but less than 50 μU/ml for infants less than 24 hours of age.

Primary Hypothyroidism

The incidence of primary hypothyroidism in New York State newborns is 1:2,100 with a slightly higher frequency for Hispanics and Asians and a lower frequency for African-

Americans.⁶ 3,086 infants have been diagnosed with primary hypothyroidism since testing began. Clinical symptoms, which may appear as early as 1-3 weeks or as late as 4-12 months, include lethargy, feeding difficulty, constipation, persistent jaundice, respiratory distress, hoarse cry, dry hair and skin, enlarged tongue, abdominal distention and umbilical hernia.⁷ (Table 7.1, Table 7.3)

Analysis of venous blood, scintigraphy and radiography are necessary for diagnosing primary hypothyroidism. (Table 7.2) The role and importance of thyroid scanning in congenital hypothyroidism has been hotly debated for years. It is important in refining the etiology of the hypothyroidism but is clearly not necessary in order to initiate treatment. It is not wise to delay treatment if it is impossible to get a scan on a particular baby. Guidelines for diagnosis and treatment of all conditions tested by newborn screening have recently been published⁵ and should be consulted.

Adequate treatment for normal development is accomplished by starting daily administration of levothyroxine (L-T₄) as soon as possible. Recommended dosage is determined by age and body weight:

- 0-6 months - 10-15 µg/kg;^{8,9}
- 6-12 months - 6-8 µg/kg.

Frequent monitoring of serum T₄ and TSH levels should be used to adjust the daily dose as the infant develops.

Secondary/Tertiary Hypothyroidism

Disease characteristics:

Secondary or tertiary hypothyroidism occurs in approximately 1:40,000 NYS births with 138 diagnosed since screening began. Symptoms are similar to those of primary hypothyroidism. Since secondary or tertiary hypothyroidism is often part of a more extensive hypopituitarism, hypoglycemia may also be an indicator, due to depressed adrenocorticotrophic hormone (ACTH) and growth hormone.

Diagnosis

Confirmatory serum testing with a venous specimen is required to rule out prematurity or thyroxine binding globulin (TBG) deficiency. A low T₄ level and a low free thyroxine (FT₄) are consistent with secondary (pituitary) or tertiary (hypothalamic) hypothyroidism. (Table 7.2)

Additional testing of the TSH response to TRH stimulation

This is required to distinguish between secondary and tertiary hypothyroidism. Assessment of other endocrine functions should be considered, i.e. corticotrophins, growth hormone, etc. Therapy consists of carefully monitored doses of L-T₄ to maintain serum T₄ concentration in the upper half of normal range for age and body weight.

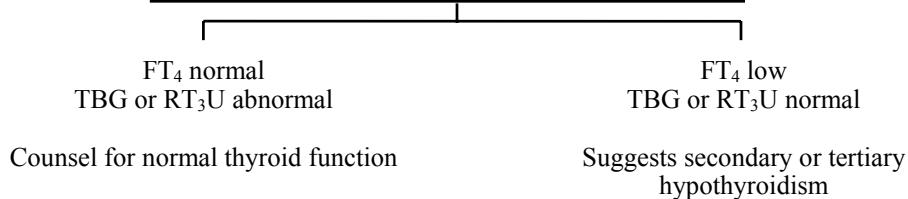
Table 7.1 Symptomatology of Hypothyroidism by Organ Systems

The manifestations of the fully developed hypothyroid state related to organ systems are the following:

- | | |
|---|---|
| <ol style="list-style-type: none"> 1. Gastrointestinal-Hepatic <ul style="list-style-type: none"> Constipation Flat glucose tolerance curve 2. Cardiovascular-Renal <ul style="list-style-type: none"> Hypotension Bradycardia Increased circulation time Anemia Depressed renal function 3. Nervous System <ul style="list-style-type: none"> Hyporeflexia Persistent infantile reflexes Evidence of organic brain damage Delayed developmental progress <ul style="list-style-type: none"> Speech disorders Short attention span Defective abstract reasoning Spasticity Tremors Convulsions EEG changes | <ol style="list-style-type: none"> 4. Musculocutaneous <ul style="list-style-type: none"> Depressed BMR and hypothermia Decreased creatine excretion Hypotonia Lethargy Thickened dry skin Hair and nail changes Myxedema accumulations 5. Skeletal <ul style="list-style-type: none"> Typical facies <ul style="list-style-type: none"> Depressed nasal bridge Puffy eyelids and cheeks Relatively narrow forehead Mandibular hypoplasia Delayed fontanel closure Depressed long bone growth Retarded dental age 6. Radiologic <ul style="list-style-type: none"> Delayed bone age <ul style="list-style-type: none"> Changes in calcification Epiphyseal dysgenesis Poor cortical bone differentiation Increased bone density |
|---|---|

Table 7.2 Recommended Diagnostic Protocol for Hypothyroidism*

1. Low T₄, Elevated TSH:
 - a. Complete physical examination
 - b. Serum studies for T₄, FT₄, TSH, TBG or RT₃U, thyroid antibodies
 - c. AP knee X-ray for bone age
 - d. Thyroid uptake and scan: ¹²³I or ⁹⁹Tc.
2. Low T₄, Normal TSH:
 - a. Serum studies for T₄, FT₄, TSH, TBG or RT₃U



- This protocol was developed with the assistance of the directors of the State approved Endocrine Specialty Care Centers.

**Table 7.3 Symptomatology and Physical Exam Findings
of Infants with Congenital Hypothyroidism**

Symptoms	Physical Exam
Poor feeding	Large posterior fontanelle
Failure to wake for feeding	Delayed closure of anterior fontanelle
Constipation	Macroglossia
Prolonged jaundice	Hoarse cry
Apnea	Umbilical hernia
Bradycardia	Goiter
Poor growth	Hypotonia
Developmental delay	

- Table prepared by Irene N. Sills, M.D., Albany Medical Center

TBG Deficiency

Thyroxine-binding globulin (TBG) deficiency, with an incidence in New York of 1:4,900 births or 1,152 infants, is most commonly ascribed to an X-linked dominant gene. It is characterized by low T₄ and low TBG serum levels and an elevated triiodothyronine (T₃) resin uptake (RT₃U). TBG deficient infants are mostly male and are clinically euthyroid because their FT₄ serum levels are usually normal. However they are usually selected for evaluation during the newborn screening process due to their persistent low T₄ levels.

A venous blood sample should be analyzed for confirmatory diagnosis. No treatment is required unless some other abnormal thyroid function is determined on blood analysis.

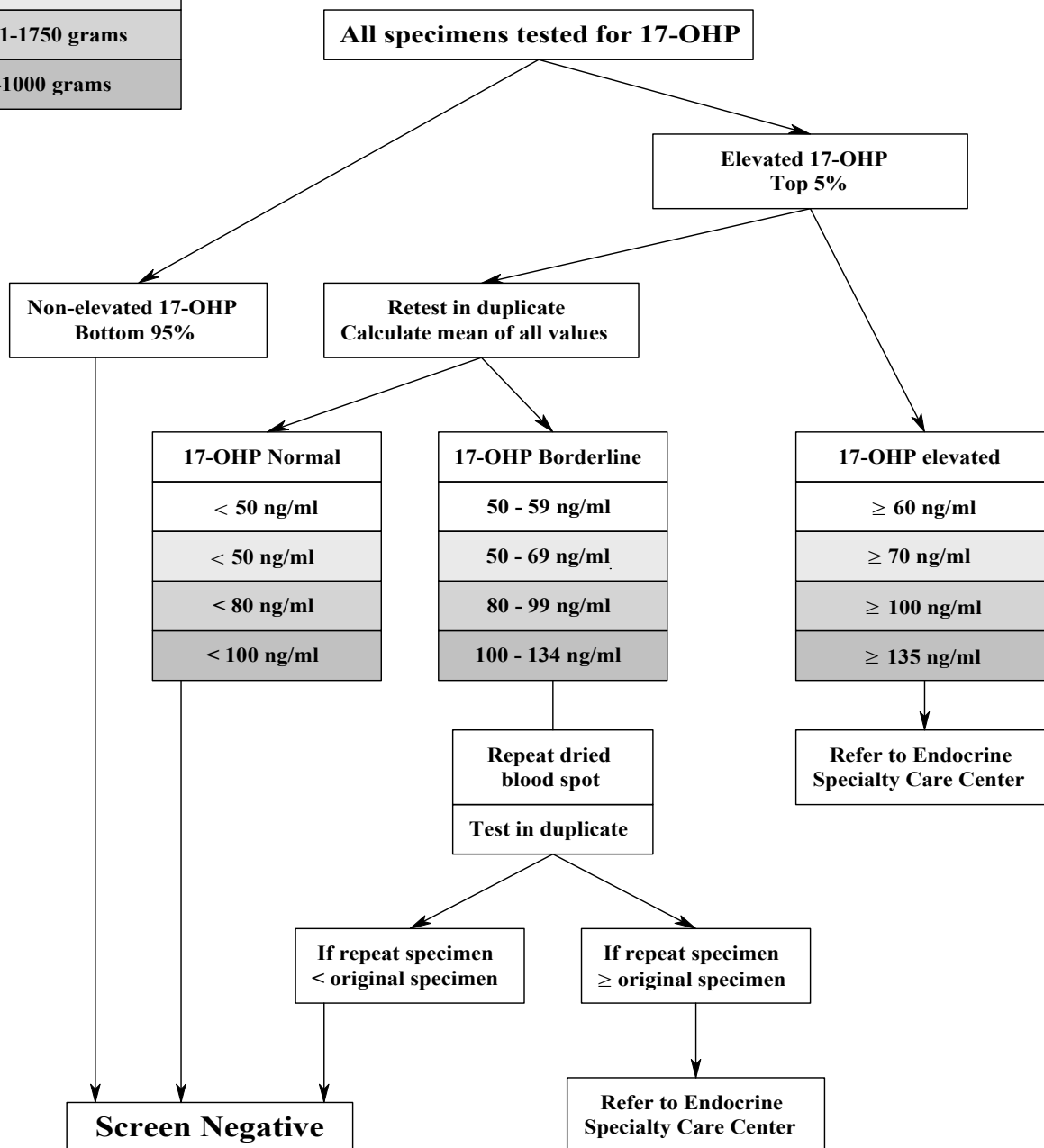
Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH) is an autosomal recessive inherited disorder caused by a deficiency of any of five of the enzymes of cortisol biosynthesis.¹⁰ The mutation site is on chromosome 6. One of the first published reports of CAH was in 1865 following the autopsy of a “male” with full female internal genitalia.¹¹

In 90-95% of CAH cases, the cytochrome P450 21-hydroxylase (CYP-21) is impaired. The enzyme 21-hydroxylase is necessary for the conversion of 17-hydroxyprogesterone to 11-deoxycortisol. Since cortisol is not synthesized, the anterior pituitary produces elevated levels of ACTH, which in turn leads to over production of testosterone, causing virilization. Hyperpigmentation also may be present.¹⁰

**Figure 7.1
CAH Screening Algorithm**

Birth weight of baby
≥ 2251 grams
1751-2250 grams
1001-1750 grams
0-1000 grams



Within the CYP-21 there are two active sites: one specific in progesterone synthesis only and a second active in either progesterone or cortisol synthesis. Both sites are defective in the salt-losing variety of CAH. Only the second is defective in the non-salt-losing form.¹² The incidence of CAH is 1:5,000 to 1:15,000 in most Caucasian populations.¹³

There are four recognized clinical forms of CAH: salt-wasting, simple virilizing, nonclassic late-onset and cryptic.¹⁰ Only the first two types are diagnosable in newborns. In female newborns, although gonads and internal genitalia are normal, the external genitalia are masculinized. This may result in the female being identified as male at birth. Postnatally, untreated males and females grow rapidly with accelerated skeletal maturation, experience penile or clitoral enlargement, and ultimately early epiphyseal closure and short stature.¹⁰ In about three-quarters of cases, an additional defect in aldosterone biosynthesis is present affecting the conversion of progesterone to deoxycorticosterone. This causes a “salt-wasting disease.” Low levels of aldosterone reduce sodium reabsorption in the kidneys. Untreated, hyponatremia and hyperkalemia can result in shock or death in the neonatal period from an inability to conserve urinary sodium. In addition, patients can experience hypoglycemia, recurrent fever and hypertension.

Newborns are screened for CAH by measuring 17-hydroxyprogesterone (17-OHP) in dried blood spots using a time-resolved fluoroimmunoassay. The serum levels of 17-OHP are elevated at birth in infants with CAH, but the rate of false positives may be higher for premature infants or those under 24 hours of age. Rapid turnaround is vital to detect boys and those nonvirilized females who may suffer early onset adrenal crisis and salt loss.¹²

Treatment with hydrocortisone suppresses excessive corticotropin production. Thus levels of 17-hydroxyprogesterone and adrenal androgens fall into the normal range. Patients with the salt-wasting form of CAH should receive mineralocorticoid replacement therapy with fluorocortisone and may need supplemental salt intake. Surgery may be considered for females to reconstruct normal female genitals.^{10,14}

Cystic Fibrosis

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene on chromosome 7. Over one thousand mutations have been identified, some of which do not cause disease. The prevalence of specific mutations and incidence among ethnic groups is variable. CF has a frequency of about 1 in 2,500 births in the Northern European population; 1 in 17,000 in African-Americans; and 1 in 9,000 in Hispanics.¹⁵ CF is rare in Asian populations. Currently there are approximately 30,000 individuals with CF in the U.S.¹⁶ and the median survival age has increased to 32 years. As many as twenty-five percent of CF patients in the U.S. are over eighteen years of age.

Twenty-five percent of children with CF remain undiagnosed by the end of the second year of life.¹⁵ After more than a decade of screening newborns for CF, the state of Wisconsin has determined that early diagnosis and initiation of therapy results in improved nutritional status. It is anticipated that this will also lead to better lung function, and prolonged survival in those diagnosed by newborn screening.¹⁷ Patients diagnosed with CF after the onset of symptoms are, on average, 72 weeks old, while those diagnosed via a positive screening test are about 12 weeks of age. When studied at ages 5 and 10 years, children diagnosed early via screening are significantly taller and heavier than those diagnosed by traditional methods. Better nutritional status is thought to be an important factor in maintaining healthy lungs in patients with CF.¹⁷

CF causes faulty transport of chloride and overabundant reabsorption of sodium within the epithelial lining of the lungs and the gastrointestinal tract. It also causes malfunction of the sweat glands, resulting in high salt content in the sweat of individuals with CF. It is this over-secretion of salt in the sweat that forms the basis for the sweat test, which, along with the identification of two known pathologic mutations, is the diagnostic standard of CF diagnosis.¹⁸

In the lung, CFTR dysfunction leads to thick viscous mucus secretion, which results in chronic bacterial bronchitis and abundant inflammation in the airways leading to more mucus secretion.¹⁹ This “vicious cycle” results in lung tissue damage, respiratory failure and death in 90% of affected patients.

About ninety percent of CF patients have pancreatic insufficiency caused by thickened secretions in the pancreatic ducts. The lack of pancreatic enzyme secretion into the duodenum results in malabsorption of fats and proteins. Despite ravenous appetites, patients experience poor weight gain and steatorrhea, which herald pancreatic insufficiency. Some CF patients with one or two “mild” alleles may not have pancreatic insufficiency.²⁰ The diagnosis of CF in these patients is often delayed. In the liver, biliary duct obstruction interferes with bile flow and can result in cirrhosis, gallstones and liver failure in a small percent of patients.

Infertility in male patients with CF occurs almost universally, due to congenital bilateral absence of the vas deferens (CBAVD). Adult male patients with CBAVD may carry one and sometimes two mutations of the CFTR gene, but most do not have any other clinical features of CF. Spermatogenesis and hormone levels are normal, but there is no means of transporting sperm.²¹ Females with CF may be fertile, but may have difficulty achieving pregnancy due to thick cervical mucus.²² However, many women with CF have successfully completed pregnancy. Of course, all their offspring are obligate CF carriers.

Treatment of CF requires a complex, multidisciplinary team approach as is found in accredited CF centers. Experienced care for patients with cystic fibrosis is available throughout New York State at Cystic Fibrosis Specialty Care Centers. See Appendix K for the list of Cystic Fibrosis Specialty Care Centers. These centers provide the medical care

and referral services required by the patient and family.⁵

Lung disease is treated with chest physiotherapy to drain secretions, antibiotics to suppress infection, and aerosols to loosen mucus or deposit antibiotics in the lungs. Pancreatic insufficiency is treated with a high calorie diet, supplemental vitamins, and supplemental pancreatic enzymes taken orally.¹⁷ Aggressive management of CF leads to improved survival.¹⁵

The newborn screening program will screen infants for CF using a two-tiered testing scheme. Immunoreactive trypsin (IRT) is measured from dried blood spots using ELISA methodology. Infants whose IRT value falls in the top 5% of values will enter the second phase of screening, CF mutation identification. Many of these high IRT values will come from conditions other than CF.

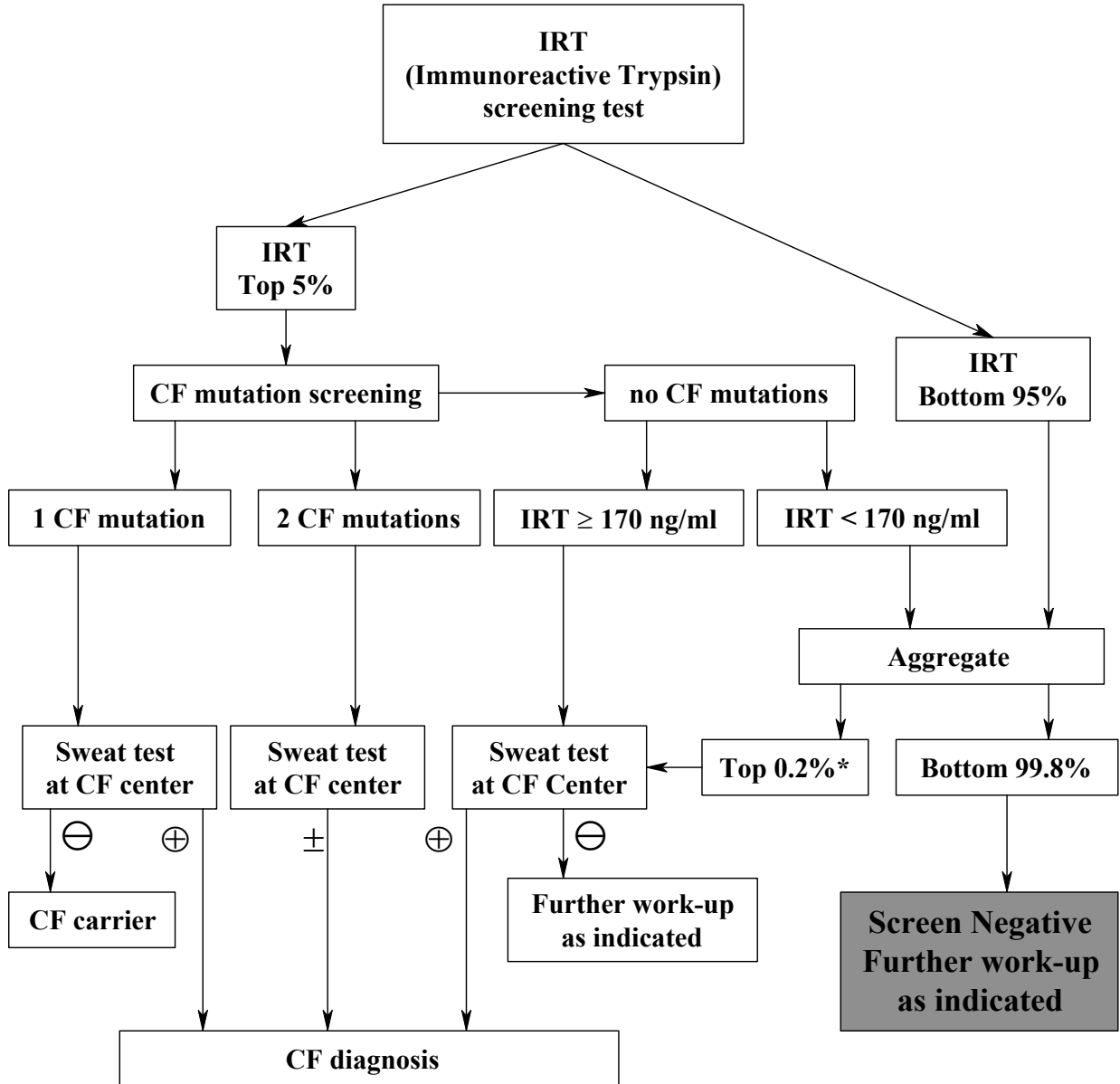
The second phase will use DNA extracted from the dried blood spots to screen for a panel of 33 mutations known to be associated with CF. The American College of Medical Genetics recently published a panel of 25 mutations, based on their frequency in the United States (> 0.1% of CF chromosomes). This panel reduces carrier risk to different levels based on ethnic background. For Ashkenazi Jewish individuals, a negative result reduces the carrier risk to 1/930 from a pretest risk of 1/29; in other Caucasians, the 1/29 a priori risk drops to 1/140. The test performs less well in African-Americans (1/65 a priori risk v. 1/207 after testing) and in Hispanic-Americans (1/46 a priori risk v. 1/105 after testing). Data from Asian-Americans are not available. Approximately 18% of Caucasians with CF will be positive for only one of the mutations in the panel and approximately 1%-4% of individuals with CF will not be positive for any mutation.

One of three possible results will be obtained following mutation analysis (see Figure 7.1):

- If two known CF mutations are detected, the infant has an extremely high likelihood of having CF, and will be referred to a CF Specialty Care Center for confirmatory sweat test and treatment.
- If one known mutation is detected, the infant may have CF or may be a carrier. Again, sweat testing at an accredited laboratory will be needed to distinguish between carriers and infants with CF. Those with a positive sweat chloride will be referred to a CF Specialty Care Center for treatment. Those with a normal sweat chloride are carriers, and their families will need genetic counseling as to the implications of having a child who carries a known CF mutation.
- Infants with no detected CF mutations will be considered screen negative, except for those with IRT values in the top 0.2% for that day (See Figure 7.2). Physicians will be reminded that a negative screening result does not rule out CF. Except for those with extremely elevated IRT values (0.2%), no further testing will be recommended.

During the initial period of screening for CF, the laboratory may modify the screening algorithm in order to increase sensitivity and specificity. False negative screening results are possible since only pancreatic insufficiency is being tested. CF infants with

Figure 7.2
Cystic Fibrosis Screening Algorithm



***Note top 0.2% calculation:**
 Total specimens screened
 less those with 1 CF mutation
 less those with 2 CF mutations
 less $\frac{\text{IRT} \geq 170 \text{ ng/ml}}{\text{Specimens in calculation}}$
 x 0.2
 Top 0.2% sent for sweat test

neonatal bowel obstruction or with pancreatic sufficiency may have normal IRT values and thus not be detected by the screening procedure. **Clinicians should remain vigilant for patients in whom signs and symptoms of CF appear, despite a screen negative result.** These children should be referred to an appropriate sweat testing laboratory. Questions concerning the possible diagnosis of CF in any patient should be referred to the closest CF specialty care center.

References Cited

Congenital Hypothyroidism

1. Bellisario R, Carter TP. (1986) Results of New York State newborn hypothyroid screening program. In: *Perinatal Genetics: Diagnosis and Treatment*. Porter IH, Hatcher NH, Willey AM (eds). Academic Press: 219-242.
2. Lash RW. (1999) Overview of Thyroid Disease in Pregnancy and Childhood. In: *Thyroid Diseases of Infancy and Childhood: Effects on Behavior and Intellectual Development*. Hauser P, Rovet J (eds). American Psychiatric Press, Inc.: 3-27.
3. Devos H, Rodd C, Gagné N, Laframboise R, Van Vliet G. (1999) A search for the possible molecular mechanisms of thyroid dysgenesis: sex ratios and associated malformations. *J Clin Endocr Metab* 84(7): 2502-2506.
4. Kopp P. (2002) Perspective: genetic defects in the etiology of congenital hypothyroidism. *Endocrinology* 143(6): 2019-2024.
5. Pass KA, Lane PA, Fernhoff PM, Hinton CF, Panny SR, Parks JS, Pelias MZ, Rhead WJ, Ross SI, Wethers DL, Elsas LJ 2nd. (2000) U.S. newborn screening system guidelines II: follow-up of children, diagnosis, management, and evaluation. Statement of the Council of Regional Networks for Genetic Services (CORN). *J Pediatr* 137(4Suppl): S1-S46.
6. Sobel EH, Saenger P. (1989) Hypothyroidism in the newborn. *Pediatr Rev* 11(1): 15-20.
7. Fisher DA. (1987) Effectiveness of newborn screening programs for congenital hypothyroidism: prevalence of missed cases. *Pediatr Clin North Am* 34(4): 881-889.
8. AAP Section on Endocrinology and Committee on Genetics (1993) Newborn screening for congenital hypothyroidism: recommended guidelines (RE9316). *Pediatrics* 91(6): 1203-1209.
9. Fisher DA. (2000) The importance of early management in optimizing IQ in infants with congenital hypothyroidism. *J Pediatr* 136: 273-274.

Congenital Adrenal Hyperplasia

10. White PC, New, MI, Dupont B. (1987) Congenital adrenal hyperplasia. *N Engl J Med* 316(24): 1519-1524.
11. Bongiovanni AM, Root AW. (1963) The adrenogenital syndrome. *N Engl J Med* 268(23): 1283-1289.

12. Orta-Flores Z, Cantu JM, Dominguez OV. (1976) Reciprocal interactions of progesterone and 17 alpha-hydroxyprogesterone as exogenous substrates of rat adrenal 21-hydroxylase. *J Steroid Biochem* 7(10): 761-767.
13. Holler W, Scholz S, Knorr D, Bidlingmaier F, Keller E, Albert ED. (1985) Genetic differences between salt-wasting, simple virilizing, and nonclassical types of congenital adrenal hyperplasia. *J Clin Endocr Metab* 60(4): 757-763.
14. Newborn Screening Fact Sheets (RE9632) (1996) *Pediatrics* 98(3): 473-501.

Cystic Fibrosis

15. Newborn Screening Fact Sheets (RE9632) (1996) *Pediatrics* 98(3): 473-501.
16. Cystic Fibrosis Foundation web page: www.cff.org.
17. Farrell PM, Kosorok MR, Rock MJ, Laxova A, Zeng L, Lai HC, Hoffman G, Laessig RH, Splaingard ML. (2001) Early diagnosis of cystic fibrosis through neonatal screening prevents severe malnutrition and improves long-term growth. Wisconsin Cystic Fibrosis Neonatal Study Group. *Pediatrics* 107(1): 1-13.
18. Knowles MR, Durie PR. (2002) What is cystic fibrosis? *N Engl J Med* 347(6): 401-407.
19. Frizzell RA. (1987) Cystic fibrosis: a disease of ion channels? *Trends Neurosci* 10: 190-193.
20. Kristidis P, Bozon D, Corey M, Markiewicz D, Rommens J, Tsui L-C, Durie P. (1992) Genetic determination of exocrine pancreatic function in cystic fibrosis. *Am J Hum Genet* 50(6): 1178-1184.
21. Kaplan E, Shwachman H, Perlmutter AD, Rule A, Khaw KT, Holsclaw DS. (1968) Reproductive failure in males with cystic fibrosis. *N Engl J Med* 279(2): 65-69.
22. Oppenheimer EA, Case AL, Esterly JR, Rothberg RM. (1970) Cervical mucus in cystic fibrosis: a possible cause of infertility. *Am J Obstet Gynecol* 108(4): 673-674.

Chapter 8 . HIV-1 ANTIBODIES

In November 1987, the NYS Department of Health began blinded testing of blood samples from newborn infants as part of a statewide epidemiological study to assess the incidence of HIV infection in women of child-bearing age. In May 1996 the Newborn Screening Program began a program of consented testing in which a mother could chose to receive her infant's HIV test results. As of February 1997, legislation was enacted that requires HIV antibody testing of all infants born in New York State. Only those parents demonstrating a religious objection to newborn screening as a whole are allowed to withdraw their infants from testing.

New York State recommends routine HIV counseling and voluntary HIV testing for all pregnant women. In addition, rapid (expedited) HIV antibody testing of the mother (with her permission) or the infant is available in delivery settings if routine HIV test results from the prenatal period are not available. Infants born to untreated HIV-positive women have a 25% chance of contracting the virus while in utero or during birth.¹ Administration of zidovudine (AZT) from the second trimester through delivery and then to the infant for six weeks reduces perinatal transmission to 8%.² Along with other interventions such as combination antiretroviral therapy and/or elective Caesarian section, this zidovudine regimen has led to dramatic declines in perinatal HIV transmission in New York State.

The goal of treatment is long-term suppression of viral replication to prevent the clinical symptoms of AIDS and preserve the immune system. Children who have been adequately treated show normal growth and maintenance of normal peripheral blood CD4 T-lymphocytes. In children less than one year of age anti-retroviral therapy should be started as soon as possible after diagnosis.³ Regimens of drug combinations have been very effective in keeping the titer of HIV-antibody low and they have been well tolerated by the infant although the child's ability to develop HIV-specific immune response is compromised. Studies are ongoing to determine the long-term toxic effect of these therapies on children.⁴

Screening is performed by enzyme-linked immunospecific assay (ELISA). A sample from the same reactive specimens is further examined by Western Blot.⁵ Positive results are reported to a designated HIV specialist in the hospital of birth. A repeat specimen is requested for viral load testing when the infant is one month of age. This follow-up testing is not performed by the newborn screening laboratory. No linkage between the two test results is made.

References Cited

1. Frank S, Esch JF, Margeson NE. (1998) Mandatory HIV testing of newborns. The impact on women. *Am J Nurs* 98(10): 49-51.
2. Recommendations of the U.S. Public Health Service Task Force on the use of zidovudine to reduce perinatal transmission of human immunodeficiency virus. (1994) *MMWR* 43(RR11): 1-20.
3. Guidelines for the use of antiretroviral agents in pediatric HIV infection. (December 14, 2001) www.hivatis.org.
4. Luzuriaga K. (1999) State-of-the-art lecture: Pediatric antiretroviral therapy. 6th Conference on Retroviruses and Opportunistic Infections. January 31-February 4, 1999 Chicago, IL.
5. Pass KA, Schedlbauer L, Berns D. (1990) Utilization of the newborn specimen for HIV seroprevalence studies. In: *Transplacental Disorders. Perinatal Infection, Treatment and Management (Including Pediatric AIDS)*. Proceedings of the 1988 Birth Defects Symposium XIX. September 26-27, 1988. Bellisario R, Mizejewski GJ (eds). Alan R. Liss, Inc.: 185-190.

Chapter 9 . REPORTING TEST RESULTS

Results of newborn screening tests, both screen negative and screen positive, are of value only if available to the physician providing primary care for the infant. To assure this, two copies of the laboratory reports are sent to the hospital of birth, one for placement in the child's permanent medical record and one for forwarding to the physician of record as identified on the blood collection form. Test results are also made available to other health care providers with the proper identification and authorization, as described in Part 58.1.8 of Chapter II of the Administrative Rules and Regulations. (See Appendix M.)

Computerized Telephone Access to Screening Results

New York physicians registered with the Newborn Screening Program can obtain screening test results through telephone access to the newborn screening computer system by calling (800) 535-3079. Written results via fax machine can also be requested through this system. In order to register and obtain instructions on the use of this system, call the screening program at (518) 473-7552. (See Appendix G for additional information.)

The Newborn Screening Program is developing a system for physician access to newborn screening results via the Internet. It is anticipated that this capability will be available in 2003.

Hospital Contacts and Designees: Responsibilities

The Newborn Screening Program annually surveys all hospitals and birthing centers to obtain the names of appropriate staff to be contacted for reporting results, processing information, and receiving supplies. The survey form, "Hospital Contacts and Designees" (DOH-1944, see sample in Appendix N), must be signed by the hospital Chief Executive Officer (CEO). It legally designates another person to meet responsibilities assigned to the CEO by NYCRR 69-1.3. Although not recommended, the CEO may choose not to identify any designee, in which case all correspondence other than screen positive test results, will be sent directly to the CEO. Designee changes during the year can be reported on an updating form available on request from the screening program. The information is maintained in computer files within the program and is used for the generation of letters, shipping labels, reports, and to access telephone numbers. The responsibilities of each contact person listed on the survey form are as follows.

Chief Executive Officer

Administrative officer responsible for overall program management within the institution submitting the specimen.

Responsibilities include:

- assure submission of a satisfactory specimen for each newborn in the hospital;
- inform parent of need and purpose of screening and give them a copy of *For Your Baby's Health*. Additional copies of this and other brochures can be ordered via the Internet at www.wadsworth.org/newborn/index.htm;
- properly store specimen collection forms;
- accurately complete all required information on forms;
- assure use of correct specimen collection technique;
- collect specimen at proper time;
- maintain record of date and time of collection;
- properly dry specimens prior to mailing;
- place test results in infant's permanent health record;
- transmit copy of test results to responsible physician;
- collect repeat specimens when first specimen is deemed unsatisfactory for testing;
- send information on the prior HIV testing and treatment history of the mother.

Director of Pediatrics, Director of Neonatology, or responsible physician

The physician named on the blood collection form, or if no physician is named, the institution's Director of Pediatrics or Neonatology is responsible for the following:

- inform parent of need and purpose of screening and interpret test results;
- conform with specimen collection and submission procedures;
- include test results in infant's health record;
- when appropriate, arrange for diagnostic evaluation and case management with an approved specialty care center;
- provide case information for tracking and follow-up reviews when requested by the screening laboratory.

Newborn Coordinator

This individual is designated by the CEO to act as a liaison between the hospital and screening program. May be called upon to resolve discrepancies relating to specimen collection or identification, incomplete information, hospital policy, or follow-up information.

Nursery Head Nurse

An alternative contact in the absence of the Newborn Coordinator so that issues may be addressed in a timely manner.

Blood Collection Form Contact

Receives, stores, and distributes the blood collection forms (DOH-1514) and educational materials. This person should also monitor the supply of these materials and request supplements when needed, using the reorder form (DOH 196) (see Appendix O) enclosed with each shipment.

Medical Records Contact

Provides information from the permanent health record of an infant or mother when requested by the screening program.

Designee to receive screen negative results

Assures that the test results report is included in the infant's permanent health record and that a copy is forwarded to the responsible physician.

Designee to receive invalid specimen notification

Arranges for repeat specimen collection or notifies the responsible physician that a repeat is necessary. In the event that no repeat is obtained, submits documentation of follow-up efforts.

Designee to receive screen positive results

This individual, who must be a physician, receives all laboratory reports of screen positive test results. Screen positive test results are subdivided into five categories to accommodate larger hospitals with specialty physicians for inherited metabolic disorders, endocrine disorders, infectious diseases, hemoglobin disorders and cystic fibrosis. If no specialist designees are named, all reports of screen positive tests will be sent to the Director of Pediatrics. When screen positive results are determined, the responsible physician named on the blood collection form is also notified. A separate copy of this physician notification letter is sent to the appropriate designee at the hospital.

If the address of the physician named on the blood collection form is that of the hospital, or if this information is incomplete, both the physician copy and hospital copy of the screen positive test report are sent to the designee, who then fulfills the duties of the responsible physician.

Screen Negative Results

Test results which are determined to be screen negative are sent in duplicate to the hospital CEO or designee for inclusion in the infant's permanent health record and for transmittal to the physician of record or current health care provider.

Invalid Specimens

Unsuitable or unsatisfactory specimens - those specimens deemed untestable by the screening laboratory - represent unscreened newborns. On receipt of such a specimen at the Wadsworth Center, notification is sent to the hospital CEO or invalid specimen designee, indicating the need for repeat collection. It is their duty to initiate efforts to obtain a satisfactory repeat specimen. Upon receipt of notification of an invalid specimen, the hospital CEO assures that the hospital copy of the original invalid specimen report is placed in the infant's permanent health record and that the physician copy is forwarded to the physician of record (the physician whose name appears on the specimen form). In the event that the physician of record is not the infant's health care provider, the hospital CEO identifies the current health care provider, sends the physician copy to the current health care provider, and notifies that provider of the need for a repeat specimen.

In the event of an invalid specimen from an out-of-hospital delivery, the birth attendant is responsible for obtaining and submitting a valid newborn screening specimen.

The hospital CEO is to work closely with the parent or guardian and the health care provider to obtain, or assure arrangements to obtain, a satisfactory repeat specimen. If necessary, the hospital CEO may consult with the Public Health Officer of the region in which the infant resides for assistance.

In the event that the hospital CEO is unable to obtain a repeat specimen, or is unable to arrange for one to be drawn, all efforts are to be documented and the laboratory notified in writing. **In the opinion of Department of Health house attorneys any legal liability pursuant to these missed screenings resides with the hospital of birth.**

Screen Positive Test Results - Repeat Specimen Required

Test results that are determined to be screen positive, but not life-threatening or medical emergencies, are sent by first class mail to the physician of record or current health care provider for appropriate action. If the physician of record or current health care provider is not known, the screen positive test results are sent to a designated hospital physician, specialty care center, or public health officer, as appropriate, for action. A copy of all screen positive test results is sent to the hospital of birth for inclusion in the infant's permanent health record. A repeat dried blood specimen is required in such instances. All repeat specimens submitted to the testing laboratory must be clearly marked "repeat."

Screen Positive Test Results - Clinical Referrals

Upon obtaining test results which are determined to be screen positive and life-threatening medical emergencies, the testing laboratory immediately telephones the

physician of record or the current health care provider and recommends referral of the infant to an approved specialty care center for confirmatory testing, diagnosis and treatment.

Following documented telephone contact, a screen positive result report is sent to the physician of record or the current health care provider. An approved specialty care center in the infant's geographical area is also notified by telephone and mail for possible treatment and follow-up activities.

If the physician of record is no longer the current health care provider and the current health care provider cannot be determined, the infant's screen positive test results are telephoned to the appropriate physician designee at the infant's hospital of birth, and/or a specialty care center or public health officer for appropriate action. All initial telephone contacts reporting screen positive test results are documented and followed with official written notification.

In addition to the physician copy of screen positive test results, the hospital copy of the results is sent to the designated physician at the hospital of birth for inclusion in the infant's permanent health record. Such a referral case is not considered complete until a written diagnosis is recorded in the newborn screening file in Wadsworth Laboratory. **It is the responsibility of the Director of Pediatrics, Director of Neonatology or responsible physician to obtain a written diagnosis and submit a copy to the newborn screening program.**

Chapter 10 . FOLLOW-UP PROTOCOLS

U.S. Newborn Screening System Guidelines II: Follow-up of Children, Diagnosis, Management, and Evaluation were published in *The Journal of Pediatrics*, October 2000. Developed by a task force assembled by CORN, the Council of Regional Networks for Genetic Services, they describe the infrastructure necessary to assure appropriate services to children identified by state newborn screening programs. With input from experts in each of the subspecialties represented by newborn screening tests, i.e. hemoglobinopathies, endocrinopathies, cystic fibrosis and metabolic disease, recommendations for diagnosis, treatment and case management are described.¹

Obtaining Repeat Specimens

Repeat collections are needed for four categories of specimen results:

- 1) unsuitable specimens which have no test results, and invalid specimens, including specimens collected from infants less than 24 hours of age;
- 2) screen positive results in the endocrine and metabolic disorders which are not within screen negative limits, but also are not highly indicative of a disorder;
- 3) suspected screen positive hemoglobin;
- 4) specimens from infants with transfusions and TPN.

Follow-up protocols to obtain needed repeats start at the source of the initial specimen. Invalid notifications are routed to the hospital CEO or designee, while presumptive hemoglobinopathy trait and transfusion reports are routed to the private physician or hospital physician designee.

If a repeat specimen is not received within three weeks of initial notification, a follow-up notice is sent to the local health officer in the infant's county of residence. The responsibilities of the local health officer are to:

- inform the parent of the importance and need for newborn screening and distribute educational materials provided by the testing laboratory;
- collect or cause to be collected a repeat specimen when notified of the need for a repeat specimen by the testing laboratory;
- submit written documentation to the testing laboratory of efforts made to secure such repeat specimen if a repeat specimen is not obtained.

In the upstate health regions and on Long Island, when a repeat specimen is not received by six weeks after initial notification, a request for assistance is sent to the NYS DOH regional health officer. When a repeat specimen is not received by thirteen weeks

after initial notification, the case is reviewed for closure. Written documentation of all follow-up efforts are included in cases closed without collecting a repeat. The CEO of the birth site is notified of the failed attempts and the resulting liability in the case of original invalid specimens.

In New York City, if a repeat is not received within four weeks after initial notification, a follow-up notice is sent to the “Invalid Designee” at the hospital of birth. The responsibilities of the hospital of birth are to :

- ensure that repeat specimens are submitted whenever the testing laboratory notifies the hospital that the initial specimen was unsatisfactory or that an additional specimen is otherwise required.
- upon notification from the responsible physician that he/she is no longer the infant’s current health provider, determine the infant’s new health care provider and cause such repeat specimen to be submitted to the testing laboratory.
- submit to the testing laboratory written documentation of all efforts made to secure such repeat specimen within ten (10) working days of cessation of specimen collection efforts if a repeat specimen is not obtained.

If a repeat is not received within eight weeks of initial notification, a request for assistance is sent to the NYS DOH Regional Office in New York City. When a repeat is not received within thirteen weeks of initial notification the case is reviewed for closure. Direct parental contact may be attempted by letter. The parent is sent an educational brochure, collection form and letter explaining the need to have the infant brought to a physician or clinic to collect the repeat specimen. Since the Newborn Screening Laboratory operates under NYS clinical laboratory regulations, test results are not reported to the parent by the laboratory. A telephone number is provided to New York City parents so they can call follow-up staff with information or questions pertaining to the need for repeat testing. Under special circumstances, i.e. when an infant with abnormal results requiring a repeat cannot be brought in for retesting, arrangements can be made for staff from certain hospitals to go to the home to collect the needed repeat specimen. For invalid cases, the CEO is notified of the failure to test the infant and the resulting legal liability.

Thirteen weeks after the close of the calendar year, all chief executive officers are supplied with information on the number of specimens submitted from their institutions, the number that were unsuitable and the reason for unsuitability. Included is data on those unsuitable specimens for which no suitable repeat was received with the case closed as “lost to follow-up.”

Clinical Referrals

In accordance with part 69-1 of the New York Codes, Rules and Regulations, the Newborn Screening follow-up program:

- in the case of screen positive results, notifies either the physician of record, current health provider or hospital designee by telephone or mail, depending on the condition and level of the analyte;
- notifies the specialty care center if appropriate;
- records diagnoses and case follow-up information submitted by health care providers and specialty care centers;
- maintains tracking records on identified cases;
- provides educational activities and materials.

Specialty Care Centers

Specialty Care Centers are health care facilities established under Article 28 of the Public Health Law and approved by the New York State Department of Health to provide diagnosis, treatment, and medical services to children identified by the NYS Newborn Screening Program. Specialty care centers have been designated for cystic fibrosis, inherited metabolic diseases, endocrine disorders, and sickle cell disease and other hemoglobinopathies. They are listed in Appendices H, I, J, and K. The centers throughout NYS maintain life-long contact with identified cases to monitor and adjust treatment therapies as necessary and to provide necessary education and genetic counseling to affected families.

Once the screening program refers an infant to a specialty care center, a written diagnosis is obtained from that center for recording in the newborn screening file. Biweekly reports of infants identified by the screening program are provided to the NYS Infant and Child Health Assessment Program (ICHAP) so that they may be enrolled in the program.

Long-term tracking and health assessment surveys are periodically conducted by the newborn screening program to ascertain the impact of screening and treatment protocols on the affected population.

References Cited

1. Pass KA, Lane PA, Fernhoff PM, Hinton CF, Panny SR, Parks JS, Pelias MZ, Rhead WJ, Ross SI, Wethers DL, Elsas LJ 2nd. (2000) U.S. newborn screening system guidelines II: Follow-up of children, diagnosis, management, and evaluation. Statement of the Council of Regional Networks for Genetic Services (CORN). *J Pediatr* 137(4 suppl): S1-S46.

Chapter 11 . EDUCATION

Parent Education

Education of both parents and professionals is an essential component of the NYS Newborn Screening Program. Public Health Law 2500a mandates that parents be informed of the purpose and need for newborn screening. The educational brochure, "Newborn Screening: For Your Baby's Health," (Appendix P) is distributed by the newborn screening program for this purpose. This brochure also includes information on cost, the procedure for specimen collection, reasons for retests, brief descriptions of the disorders in the screening profile, and questions frequently asked by parents. It is to be given to parents along with the parental copy of the blood collection form containing the infant's name and a laboratory identification number, when a newborn screening blood test is obtained from their infant. The brochure is presently available in English, French, Spanish, Haitian Creole, Russian, Chinese and Vietnamese.

Informed parents are better prepared to follow-up on presumptive positive test results and to facilitate timely evaluation, diagnosis and treatment of affected infants. Immediate action and treatment are necessary for the prevention of mental retardation, morbidity and mortality associated with the disorders identified by the newborn screening program. Prior knowledge of the newborn screening program by parents and discussion of test results with the infant's health care provider further assure that all infants receive the benefits of this most important public health program. Prior knowledge of the screening also reduces the stress that may be associated with requests for a repeat test. The necessity for a repeat test does not necessarily mean that the infant has one of the screened disorders or may become developmentally delayed. **Thus, discussion of this public health activity by the obstetrician is strongly urged.**

The parent copy (pink) of the blood collection form received by the parent at the time of specimen collection is to be given to the infant's health care provider by the parents at the first health care visit. It provides a means of obtaining the screening results. If the health care provider has not already received a copy of the infant's test results from the hospital of birth, inquiry for results should be made to the hospital of birth or via the automated telephone system (see Chapters 3 and 9 and Appendix G). The parent copy of the collection form provides the laboratory identification number, essential for retrieval of the test results. Knowledge of the newborn screening laboratory test results by the infant's health care provider during the first few weeks of life should be a part of the neonate's routine health care and the results should be included in all infant health care records. A toll free telephone number ((800) 535-3079) provides physicians with access to a computer voice-response system in the Wadsworth Center. This system offers newborn screening results after proper identification of the caller and infant.

Educational Materials

The parent educational materials listed below are currently available free of charge through the newborn screening program. They may be ordered by telephone or mail or by utilizing the reorder form included with each shipment. Copies of the appropriate brochure for use by health professionals in discussions with the parent are included with letters reporting screen positive results.

1. *Newborn Screening: For Your Baby's Health* – this brochure, available in English, Spanish, French, Haitian Creole, Russian, Chinese and Vietnamese, is to be used by hospitals and health care providers for parent education regarding newborn screening.
2. *Testing Your Newborn Baby's "Invisible" Health* – this flyer, available in English and Spanish, is for prenatal parent education regarding newborn screening.
3. *The Family Connection - Sickle Cell Trait* – this brochure, available in English, Spanish and French, is designed to be given to parents in a genetic counseling situation.
4. *The Family Connection - Hemoglobin C Trait* – this brochure, available in English, Spanish and French, is designed to be given to parents in a genetic counseling situation.
5. There are brochures, available in English, for each of the conditions included in the NYS newborn screening panel except for HIV. The appropriate brochure is designed to be given by the physician to the parents of an infant with a positive screen test result.

Professional Education

An integral part of the newborn screening process is professional education. This includes:

- Information on the goals of the newborn screening program;
- Public Health Law 2500a (the Newborn Screening Law);
- Rules and regulations pursuant to Public Health Law 2500a; 10 NYCRR Subpart 69-1;
- Disorders screened by the newborn screening program;
- The vital importance of early diagnosis and treatment of "at risk" infants;
- The importance of obtaining and handling a valid specimen suitable for testing purposes.

Newborn screening materials used for this purpose are:

- Blood Collection Form (DOH 1514) – a set of instructions is printed on the back of the form.
- Neonatal Screening Blood Specimen Collection and Handling Procedure – an instructional poster that pictorially depicts correct specimen collection technique. It has

been distributed to all hospitals servicing newborns in New York State and is available from the Newborn Screening Program. (See Appendix D.)

- Simple Spot Check – an instructional poster that pictorially depicts valid and invalid specimens as categorized by the Newborn Screening Program. It explains possible causes of unsuitable specimens. It is available from the Newborn Screening Program. (See Appendix E.)
- The National Committee of Clinical Laboratory Standards videotape, " Making a Difference Through Newborn Screening: Blood Collection on Filter Paper." (NCCLS publication LA4-A3-V. Villanova, PA. This is available on loan basis from the Newborn Screening Program.
- Information sheets on each condition included with the laboratory test report.

Staff of the newborn screening program are available for educational presentations. Please call (518) 473-7552 to discuss and schedule presentations.

Chapter 12 . RESEARCH AND DEVELOPMENT

To be effective, a program with the size and complexity of New York's Newborn Screening Program must constantly evaluate its procedures in order to develop and implement technological improvements. Therefore, research and development become an integral part of the testing laboratory. Professional staff within the program currently manage \$480,000 in grants for program development and evaluation of new technologies relevant to newborn screening. Current projects include examining automated technologies in testing for the aminoacidopathies and hemoglobinopathies, development of multiplex assay systems, application of molecular techniques to the dried blood specimen and exploring improved methods for follow-up in positive hemoglobinopathy cases. If successful, these technologies could have significant positive impact on specimen testing time, accuracy, and reporting.

With funds provided by a federal grant, large-scale testing for biotinidase was evaluated in New York during a two-year period, leading to implementation of mandated testing in 1989. The incidence of the disorder was determined and New York State was the first program worldwide to identify biotinidase deficiency in black and Hispanic populations. These data have proven valuable to other state screening programs as they implement testing for hemoglobinopathies. Still another study involved trials of a new immunoassay for sickle hemoglobin, and showed that although the assay was dependable, it was not suited to the needs of newborn screening programs. New York State served as one of four sites enlisted as training centers for hemoglobinopathy detection in newborns. Screening staff from various states were brought to the Wadsworth Center to learn current screening techniques. Through coordinated instruction, this program provides uniformity of newborn hemoglobin testing throughout the country.

Spin-offs of the HIV seroprevalence study, in which newborn blood spots were tested for HIV antibodies, included better documentation of specimen collections within hospitals, reduced invalid rates due to poor collection techniques, expanded computer capabilities for monitoring program parameters, and better coordination with related programs within the Health Department.

Residual specimens remaining after all testing is complete are coded and stored for medical purposes of the newborn's family. Requests for use of this resource should be directed to the director of the screening program. Specimens may also be used for research purposes, but any specimen used for this purpose has all identifiers removed. No links can be made to an individual newborn.

The following section presents other research and development activities of the Division of Genetic Disorders, within which the screening program is located. As can be seen, these are vibrant, ongoing activities serving both the program in New York as well as other screening programs.

**Annotated Bibliography of Researchers in the Division of Genetic Disorders,
Wadsworth Center**

Marlene Belfort, Ph.D.

- Wood DW, Derbyshire V, Wu W, Cartrain M, Belfort M, and Belfort G. (2000) Optimized single-step affinity purification with a self-cleaving intein applied to human acidic fibroblast growth factor. *Biotechnology Progress*. 16: 1055-1063.
- Belfort M. (2001) The win-win potential for motherhood and science. *Current Biology* 11: R41-R42.
- Ichiyanagi K, Beauregard A, Lawrence S, Smith D, Cousineau B, Belfort M. (2002) Retrotransposition of the L1.LtrB group II intron proceeds predominately via reverse splicing into DNA targets. *Molecular Microbiology* 46:1259-1272.

Ronald Bellisario, Ph.D.

- Bellisario R, Colinas RJ, Pass KA. (2001) Simultaneous measurement of antibodies to three HIC-1 antigens in newborn dried blood-spot specimens using a multiplexed microsphere-based immunoassay. *Early Human Development* 64: 21-25.
- Reilly AA, Bellisario R, Pass KA. (1998) Multivariate discrimination for phenylketonuria (PKU) and non-PKU hyperphenylalaninemia after analysis of newborns' dried blood-spot specimens for six amino acids by ion-exchange chromatography. *Clin Chem* 44: 317-326.
- Bellisario R, Colinas RJ, Pass KA. (2000) Simultaneous measurement of thyroxine and thyrotrophin from newborn dried blood-spot specimens using a multiplexed fluorescent microsphere immunoassay. *Clin Chem* 46: 1422-1424.

Michele Caggana, Sc.D.

- Crawford DC, Caggana M, Harris KB, Lorey F, Nash C, Pass KA, Templeis C, Olney RS. (2002) Characterization of β -globin haplotypes using blood spots from a population-based cohort of newborns with sickle cell disease (2002 *in press*) *Genetics in Medicine*.
- Steinberg KK, Beck J, Nickerson D, Hayes R, Gallagher M, Caggana M, Reid Y, Cosentino M, Ji J, Johnson D, Early M, Lorey F, Hannon H, Sampson E (2002) DNA banking for epidemiologic studies. *Epidemiology* 13: 246-254.
- Olivarez L, Caggana M, Pass KA, Ferguson P, Brewer G. (2001) Estimate of the frequency of Wilson's disease in the U.S. Caucasian population: a mutation analysis approach. *Annals of Human Genetics* 65: 459-463.
- Conroy JM, Trivedi GT, Sovd T, Caggana M. (2000) The allele frequency of mutations in four genes that confer enhanced susceptibility to venous thromboembolism in an unselected group of New York State newborns. *Thrombosis Research* 99: 317-324.
- Caggana M, Conroy JM, Pass KA. (1998) A rapid efficient method for multiplex amplification from filter paper. *Human Mutation* 11: 404-409.

M. Joan Curcio, Ph.D.

- Scholes DT, Banerjee M, Bowen B, Curcio MJ. (2001) Multiple regulators of Ty1 transposition in *Saccharomyces cerevisiae* have conserved roles in genome maintenance. *Genetics* 159: 1449-1465.
- Bryk M, Banerjee M, Conte D, Curcio MJ. (2001) The Sgs1 helicase of *Saccharomyces cerevisiae* inhibits retrotransposition of Ty1 multimeric arrays. *Mol Cell Biol* 21: 5374-5388.
- Conte D, Curcio MJ. (2000) Fus3 controls Ty1 transpositional dormancy through the invasive growth pathway. *Mol Micro* 35: 415-427.

Victoria Derbyshire, Ph.D.

- Dean AB, Stanger MJ, Dansereau JT, Van Roey P, Derbyshire V, Belfort M. (2002) Zinc finger as distance determinant in the flexible linker of intron endonuclease I-TevI. *PNAS* 99: 8554-8561.
- VanRoey P, Waddling CA, Fox KM, Belfort M, Derbyshire V. (2001) Intertwined structure of the DNA-binding domain of intron endonuclease I-TevI with its substrate. *EMBO J.* 20, 3631-3637.
- Wood D, Wu W, Belfort G, Derbyshire V, and Belfort, M. (1999) Protein engineering of inteins: genetic system yields self-cleaving element for bioseparations. *Nature Biotechnology* 17: 889-892.

Lorraine Flaherty, Ph.D.

- Bolivar V, Cook M, Flaherty L. (2001) Mapping of quantitative trait loci with knockout/congenic strains. *Genome Research* 11: 1549-1552.
- Bolivar V, Pooler O, Flaherty L. (2001) Inbred strain variation in contextual and cued fear conditioning behavior. *Mammalian Genome* 12: 651-656.
- Bihl F, Lariviere L, Qureshi ST, Flaherty L, Malo D. (2000) LPS-hyporesponsiveness of mnd mice is associated with a mutation in Toll-like receptor 4. *Gene and Immunity* 2: 56-59.

Robert L. Glaser, Ph.D.

- Madigan JP, Chotkowski HL, Glaser RL. (2002) DNA double-strand break-induced phosphorylation of *Drosophila* histone variant H2Av helps prevent radiation-induced apoptosis. *Nucleic Acids Res* 30(17): 3698-3705.
- Leach TJ, Mazzeo M, Chotkowski HL, Madigan JP, Wotring MG, Glaser RL. (2000) Histone H2A.Z is widely but nonrandomly distributed in chromosomes of *Drosophila melanogaster*. *J Biol Chem* 275(30): 23267-23272.
- Leach TJ, Chotkowski HL, Wotring MG, Dilwith RL, Glaser RL. (2000) Replication of heterochromatin and the structure of polytene chromosomes. *Mol Cell Biol* 20(17): 6308-6316.

Todd A. Gray, Ph.D.

- Kuerbitz SJ, Pahys J, Wilson A, Compitello N, Gray TA. (2002) Hypermethylation of the imprinted NNAT locus occurs frequently in pediatric acute leukemia. *Carcinogenesis* 23(4): 559-564.

- Gray TA, Azama K, Whitmore K, Min A, Abe S, Nicholls RD. (2001) Phylogenetic conservation of the makorin-2 gene, encoding a multiple zinc-finger protein, antisense to the RAF1 proto-oncogene. *Genomics* 77(3): 119-125.
- Gray TA, Nicholls RD. (2000) Diverse splicing mechanisms fuse the evolutionarily conserved bicistronic MOCS1A and MOCS1B open reading frames. *RNA* 6(7): 928-936.
- Gray TA, Hernandez L, Carey AH, Schaldach MA, Smithwick MJ, Rus K, Marshall Graves JA, Stewart CL, Nicholls RD. (2000) The ancient source of a distinct gene family encoding proteins featuring RING and C3H zinc-finger motifs with abundant expression in developing brain and nervous system. *Genomics*, 66:76-86.

Steven D. Hanes, Ph.D.

- Gottlieb S, Hanes SD, Golden JA, Oakey RJ, Budarf ML. (1998) Goosecoid-like, a gene deleted in DiGeorge and Velocardiofacial syndromes, recognizes DNA with a Bicoid-like specificity and is expressed in the developing mouse brain. *Human Molecular Genetics* 7: 1497-1505.
- Burz DS, Hanes SD. (2001) Isolation of mutations that disrupt cooperative DNA binding by the Drosophila Bicoid protein. *J Mol Biol* 305: 219-230.
- Devasahayam G, Chaturvedi V, Hanes S D (2002) The Ess1 prolyl-isomerase is required for growth and morphogenetic switching in *Candida albicans*. *Genetics* 160: 37-48.

Anne Messer, Ph.D.

- Manley K, Shirley TL, Flaherty L, Messer A. (1999) Msh2 deficiency prevents in vivo somatic instability of the CAG repeat in Huntington transgenic mice. *Nature Genetics* 23: 471-473
- Chu-LaGraff Q, Kang X-W, Messer A. (2001) Expression of the Huntington's Disease transgene in neural stem cell cultures from R6/2 transgenic mice. *Brain Res Bull* 56: 307-312.
- Bolivar V, Ganus J, Messer A. (2002) The development of behavioral abnormalities in the motor neuron degeneration (mnd) mouse. *Brain Res* 937: 74-82.

Randall H. Morse, Ph.D.

- Stafford GA, Morse RH. (2001) GCN5 dependence of chromatin remodeling and transcriptional activation by the GAL4 and VP16 activation domains in budding yeast. *Mol Cell Biol* 21: 4568-4578.
- Yu L, Sabet N, Chambers A, Morse RH. (2001) The N-terminal and C-terminal domains of RAP1 are dispensable for chromatin opening and GCN4-mediated HIS4 activation in budding yeast. *J Biol Chem* 276: 33257-33264.

Dilip K. Nag, Ph.D.

- Jankowski C, Nag DK. (2002) Most meiotic CAG repeat tract-length alterations in yeast are SPO11 dependent. *Mol Gen and Genomics* 267: 64-70.
- Sarkar PK, Florczyk MA, McDonough KA, Nag DK. (2002) SSp2, a sporulation-specific gene necessary for outer spore wall assembly in the yeast *Saccharomyces cerevisias*. *Mol Gen and Genomics* 267: 348-358.

Kenneth A. Pass, Ph.D.

- Pass KA, Lane PA, Fernhoff PM, Hinton CF, Panny SR, Parks JS, Pelias MK, Rhead WR, Ross SI, Wethers DL, Elsas LJ^{2nd}. (2000) U.S. Newborn screening system guidelines II: Follow-up of children, diagnosis, management, and evaluation. Statement of the Council of Regional Networks for Genetic Services (CORN). J Peds 137(4Suppl): S1-S46.
- Pass KA, Harris K, Lorey F, Choi R, Kling MA. (2000) Newborn screening for sickle cell disease – California, Illinois, and New York. MMWR 49:729-731, 2000.
- Pass KA. Lessons learned from newborn screening for PKU. (2000) In: *Genetics and Public Health in the 21st Century*. M. Khuri, W Burke, EJ Thomson, eds. Oxford Univ Press.

Hadeh Payami, Ph.D.

- Payami H, Zarepari S, Montee K, Sexton G, Kaye J, Bird T, Yu C, Wijsman E, Heston L, Schellenberg G. (1996) Gender difference in Apolipoprotein E-associated risk for familial Alzheimer's disease: A clue to the higher incidence of AD in women. Am J Hum Genet, 58:803-811.
- Payami H, Schellenberg G, Zarepari S, Kaye J, Sexton G, Head, M, Jarvik L, Miller B, McManus D, Bird T, Katzman R, Heston L, Norman D, Small G. (1997) Evidence for association of HLA-A2 with onset age of Alzheimer's disease. Neurology, 49:1-7.
- Gonzales McNeal M, Zarepari S, Camicioli R, Dame A, Howison D, Quinn J, Ball M, Kaye J, Payami H. (2001) Predictors of healthy brain aging. J Gerontol, 56A:B294-B301.
- Zarepari S, Camicioli R, Sexton G, Bird T, Swanson P, Kaye J, Nutt J, Payami H. (2002) Analysis of onset age of Parkinson's disease: Apolipoprotein E genotypes. Am J Med Genet 107:156-161.

Abigail Snyder-Keller, Ph.D.

- Snyder-Keller A, Sam C, Keller RW, Jr. (2000) Enhanced susceptibility to cocaine- and pentylentetrazol-induced seizures in prenatally cocaine-treated rats. Neurotoxicology and Teratology 22: 231-236.
- Snyder-Keller A, Keller RW, Jr. (2001) Spatiotemporal analysis of Fos expression associated with cocaine- and PTZ-induced seizures in prenatally cocaine-treated rats. Experimental Neurology 170: 109-120.
- Mitchell ES, Keller RW, Jr., Snyder-Keller A. (2002) Immediate-early gene expression in concurrent prenatal ethanol- and/or cocaine-exposed rat pups: intrauterine differences in cocaine levels and Fos expression. Developmental Brain Research 133: 141-149.

James N. Turner, Ph.D.

- Kam L, Shain W, Turner JN, Bizios R. (2002) Selective Adhesion of Astrocytes to Surfaces Modified with Immobilized Peptides. Biomaterials 23: 511-515.

Kam L, Shain W, Turner JN, Bizios R. (2001) Axonal Outgrowth of Hippocampal Neurons on Micro-Scale Networks of Polylysine-Conjugated Laminin. *Biomaterials* 22: 1049-1105.

Shen H, Roysam B, Stewart C, Turner JN, Tanenbaum H. (2001) Optimal Scheduling of Tracing Computations for Real-Time Vascular Landmark Extraction from Retinal Images. *IEEE Trans Biomed* 5: 77-91.

Jonathan R. Wolpaw, M.D.

Wolpaw JR, Tennissen AM. (2001) Activity-dependent spinal cord plasticity in health and disease. *Annu Rev Neurosci* 24: 807-843.

Kubler A, Kotchoubey B, Kaiser J, Wolpaw JR. (2001) Brain-computer communication: unlocking the locked in. *Psycho Bull* 127(3): 358-375.

Wolpaw JR. (2001) Spinal cord plasticity in the acquisition of a simple motor skill. In *Spinal Cord Plasticity: Alterations in Reflex Function*. Eds. Patterson MM, Grau JW, Kluwer Academic Publishers, pp. 101-125.

Wolpaw JR, Kaas JH. (2001) Taking sides: Corticospinal tract plasticity during development (Editorial). *Neurology* 57: 1530-1531.

Wolpaw JR. (2001) Motoneurons and spinal control of movement. In *Encyclopedia of Life Sciences*, (www.els.net) Nature Publishing Group, London, 12: 325-332.

Carp JS, Chen XY, Sheikh H, Wolpaw JR. (2001) Effect of chronic nerve cuff and EMG electrodes on rat triceps surae units. *Neurosci Lett* 312:1-4.

Chen XY, Wolpaw JR. (2002) Probable corticospinal tract control of spinal cord plasticity in the rat. *J Neurophysiol* 87:645-652.

Chapter 13 . AFFILIATED PROGRAMS

Collection of Information Regarding Maternal Hepatitis B Status.

Effective May 1991, the use of the newborn screening blood collection form was enlisted to assist in identifying infants at risk for hepatitis B infection from maternal exposure at or near the time of delivery. Information regarding the hepatitis B surface antigen (HBsAg) status of the newborn's mother is requested on the specimen form (Appendix C). Screening of all pregnant women for HBsAg is required by Public Health Law 2500e, with details in Title 10 Subpart 69-3 of NYCRR.¹ This information rapidly identifies infants in need of follow-up, since newborn infants whose mothers are positive for HBsAg are at increased risk for hepatitis B infection. Nearly all infants who acquire the infection early in life become chronic carriers of the virus, and though healthy in childhood, many will develop liver cirrhosis or carcinoma leading to a premature death in early adulthood.^{2,3} Perinatal infection is largely preventable through immunoprophylaxis with hepatitis B immune globulin and hepatitis B vaccine at birth, with follow-up doses of vaccine at one and six months.⁴

Data collected on the newborn specimen form is transmitted to the Infant and Child Health Assessment Program (ICHAP) for use by ICHAP nurses or local health departments to assure follow-up medical treatment is obtained. Further information regarding this program may be obtained by calling (518) 474-6411.

Newborn Hearing Screening

Legislation requiring hospitals to screen all newborn infants for hearing deficiency became law in New York State on April 1, 2000. Chapter 585 of the Laws of 1999 directed the Commissioner of Health to develop a program to screen newborn infants for hearing impairments as early in life as possible and directed the Department to consult with health care providers and others to establish a statewide newborn hearing screening program.

According to the American Academy of Pediatrics Task Force on Newborn and Infant Hearing, "significant hearing loss is one of the most common major abnormalities present at birth, and, if undetected, will impede speech, language, and cognitive development."⁵ Approximately 1 to 3 infants per 1,000 are born with significant hearing loss. An additional 3 infants per 1,000 have moderate degrees of hearing loss.⁶ The average age that children with hearing loss are identified in the United States is 12-25 months of age. Consequences of late identification of hearing loss include delayed speech and language development and associated effects on social and emotional growth and academic achievement.⁶ Advances in technology have made it possible to detect the presence of hearing loss in the neonatal

period. Recent research has demonstrated that infants with hearing impairment have significantly better language outcomes when they are identified by six months of age versus later than age six months.⁷

The two techniques currently used for newborn hearing screening are evoked otoacoustic emissions (OAE) and the auditory brainstem response (ABR). Both techniques provide objective information about auditory system function.

New York State Newborn Hearing Screening Program regulations require facilities to report aggregate data to the Department of Health as necessary to monitor the effectiveness of the newborn hearing screening program.

For babies who fail the initial newborn hearing screening, follow-up services may include referral for re-screening or for diagnostic audiologic evaluation, keeping in mind the goals of newborn hearing screening – confirmation and/or diagnosis of permanent hearing loss by age three months and initiation of habilitation by age six months.

Education regarding newborn hearing screening should be provided for parents and health professionals including general information on the importance of early identification of hearing loss, developmental milestones for communication and signs of hearing loss in young children. When increased risk of progressive or late onset hearing loss is present, monitoring of hearing levels should be incorporated into general health surveillance. For more information call the Newborn Hearing Screening Program, Department of Health Early Intervention at (518) 473-7016.

Screening for Congenital Syphilis

Public Health Law 2500a details the disorders which must be screened in the newborn, and further gives the Commissioner of Health discretionary powers to designate screening for other diseases and conditions. This authority has been invoked to require that all newborns be screened for exposure to syphilis. Details regarding this requirement are contained in Title 10 Subpart 69-2 of NYCRR. The screening assay is not part of the screening panel of the newborn screening laboratory using the Guthrie spot. Testing must be performed by a licensed laboratory as arranged by the hospital.

Reported cases of early syphilis (< 1 year duration) have declined overall since peaking in 1990.⁸ See Table 13.1.

Although prenatal screening for syphilis is required, many women who deliver an infant with syphilis are members of groups which traditionally receive inadequate prenatal care (i.e. age 18 or under, unmarried and of low socioeconomic and educational status). National and state statistics document that as many as 87% of all women giving birth to congenitally infected infants have received little or no care for their syphilis.⁹ Therefore, it

is imperative to test all infants at the time of birth since many carry no detectable signs or symptoms of the disease. Serious consequences of syphilis in the infant may not become obvious until months or years later when bone deformities, organ destruction or central nervous system damage may be revealed.¹⁰

Table 13.1
Cases of Early Syphilis in NYS
1991 - 2000

Year	# of cases	% decline
1991	11486	
1992	8709	24%
1993	5643	35%
1994	3481	38%
1995	2548	25%
1996	1416	44%
1997	897	37%
1998	797	11%
1999	850	7% increase
2000	599	31%

Women may be negative for syphilis when tested early in pregnancy, but become infected between screening and delivery. Furthermore, infected women may receive treatment in pregnancy but become reinfected before delivery. To diagnose those infants born with syphilis, Title 10 Subpart 69-2 includes the following:

- All infants born after 22 weeks, whether alive or dead, must have a sample of blood removed from the umbilical cord and submitted to an approved laboratory for standard serological tests for syphilis.
- If, at time of birth, the peripheral blood from the mother is tested for syphilis, the cord blood test requirement is waived. The infant's peripheral blood must be tested after any positive test result of the mother's blood.
- Bloods submitted for testing to an approved laboratory must be clearly marked as cord, newborn or mother/delivery to facilitate reporting; all samples must be tested with a quantitative non-treponemal test on the *same* sample.
- All positive serologic results must be reported to the responsible physician or birth attendant and the local health department within 24 hours of receipt of the test results. The responsible physician or birth attendant must ascertain that both the mother and the infant are treated with appropriate therapy, and that follow-up is arranged. The responsible physician or birth attendant must cooperate with the designated public health investigators to assure that diagnosis, therapy and any necessary follow-up testing

meet current standards of care.

Questions regarding diagnosis, treatment or case follow-up of cases of early or congenital syphilis may be directed to the New York State Bureau of Sexually Transmitted Disease Control, (518) 474-3598, or the New York City Sexually Transmitted Control Program, (212) 788-4415.

References Cited

1. Recommendations for Universal Screening of Pregnant Women for Hepatitis B. Health Series Memorandum # 88-81, 11/1/88. NYS Department of Health, 1988.
2. Stevens CE. (1987) Perinatal hepatitis B virus infection: screening of pregnant women and protection of the infant. *Ann Intern Med* 107(3): 412-413.
3. Arevalo JA, Washington AE. (1988) Cost-effectiveness of prenatal screening and immunization for hepatitis B virus. *JAMA* 259(3): 365-369.
4. Stevens CE, Toy P, Tong MJ, Taylor PE, Vyas GN, Nair PV, Gudavalli M, Krugman S. (1988) Perinatal hepatitis B virus transmission in the United States: prevention by passive-active immunization. *JAMA* 253(12): 1740-1745.
5. Newborn and infant hearing loss: Detection and intervention. (1999) Task Force on Newborn and Infant Hearing, American Academy of Pediatrics. *Pediatrics* 103: 527-530.
6. Facts on hearing loss in children. American Speech-Language –Hearing Association (1999) www.asha.org/infant_hearing/facts.htm
7. Yoshinaga-Itano C, Sedley AL, Coulter DK, Mehl AL. (1998) Language of early- and later-identified children with hearing loss. *Pediatrics* 105: 1151-1171.
8. NYS Department of Health. Communicable Disease in NYS reports, revised 8/99. www.health.state.ny.us/nysdoh/epi/mainrpt.htm.
9. Gust DA, Levine WC, St Louis ME, Braxton J, Berman SM. (2002) Mortality associated with congenital syphilis in the United States 1992-1998. *Pediatrics Electronic Pages* 109(5): E79. <http://www.pediatrics.org/cgi/content/full/109/5/e79>
10. Axelrod D. Letter to Hospital Administrators. November 21, 1989.

Chapter 14 . QUESTIONS AND ANSWERS

What disorders are tested in Newborn Screening?

- As of 2003, the New York State newborn screening panel includes: phenylketonuria, homocystinuria, maple syrup urine disease, galactosemia, sickle cell disease, congenital hypothyroidism, cystic fibrosis, congenital adrenal hyperplasia, medium-chain acyl-CoA dehydrogenase deficiency, HIV exposure and biotinidase deficiency. From time to time, at the discretion of the NYS Commissioner of Health, the newborn specimens may also be tested for other disorders.

Who must be tested?

- Every baby born in New York State must be tested for the panel of disorders. The only legally permissible declination is for those parents who are members of a recognized religious organization whose teachings and tenets are contrary to testing.

If a New York State resident delivers a baby in another state or country, must that newborn be tested by the New York program?

- No. Each state and many countries have screening programs, but the number and type of disorders in the screening panel varies. It would be to the newborn's benefit for parents to be informed regarding the local screening policies. If testing through the New York State program is desired, arrangements can be made with the baby's pediatrician to submit a specimen.

Who is responsible for collection of the specimen for screening?

- The Chief Executive Officer of the hospital or institution of birth is responsible for assuring that a satisfactory specimen is submitted. The Birth Attendant is responsible for the initial blood specimen from all infants born outside of, and not admitted to, a responsible institution.

How is the blood collection form processed prior to blood collection?

- Assure a currently valid Blood Collection Form (DOH-1514) is used by checking expiration date on form.
- All information is recorded on the form.
- The pink Parental Copy is removed and given to the mother, along with a brochure entitled "Newborn Screening: For Your Baby's Health."
- The green Submitter Copy is removed and filed until results are received. This is an important reference when contacting the program for assistance.

How is the specimen to be taken?

- A blood specimen must be taken from the infant's heel and applied to the circles on the special paper of the form. Collection procedures are summarized on the reverse side of the form and in Chapter 2 of this manual.

How much does screening cost?

- Currently no charge is levied by the screening laboratory in New York State. Laboratory operating costs are covered by the State.

Where should the specimens be sent?

- The address is on the back of the blood collection form (DOH 1514).
- Specimens should be sent first class mail or its equivalent within 24 hours of collection to:

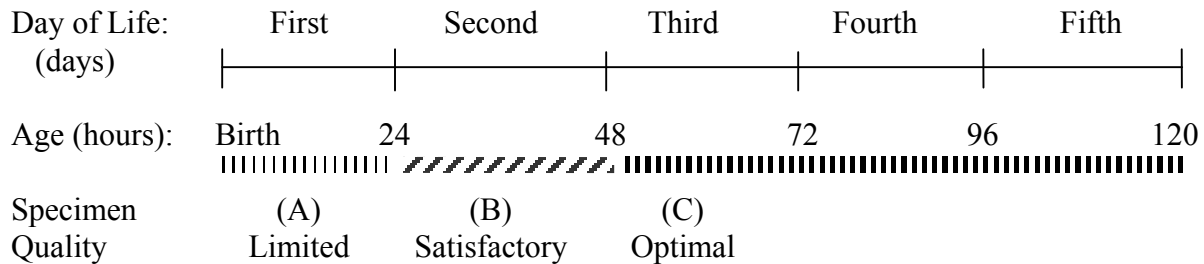
Newborn Screening Program
 New York State Department of Health
 Wadsworth Center
 Empire State Plaza
 P.O. Box 509
 Albany, NY 12201-0509

Couriers may deliver specimens to:

Wadsworth Center
 New York State Department of Health
 Dock J-P1 Level
 Empire State Plaza
 Albany, NY 12237

When should the specimen be taken?

Figure 14.1 Time Line for Specimen Collection



- Period A – Birth to 24 hours of age (first day of life or day of birth). Though a repeat specimen will be necessary, a specimen should be taken in the following instances:
 - The infant will be discharged from the hospital or institution prior to 24 hours of age.
 - A blood transfusion is to be administered.
 These specimens are valid for testing galactose-1-phosphate uridyl transferase (galactosemia), sickle hemoglobin (sickle cell disease), octanoylcarnitine (MCADD), and biotinidase (biotinidase deficiency). A repeat (second) collection should be taken

between 48 and 120 hours of age.

- Period B – 24 to 48 hours of age (second day of life). This interval is a satisfactory collection time. Statistically there may be a slightly increased risk of erroneous results in specimens taken during this interval, but after extensive review, the American Academy of Pediatrics concluded that the collection of a routine repeat blood specimen was acceptable if the initial screening was performed within this interval.
- Period C – 48 to 120 hours of age (third to fifth day of life). This interval is the optimum time for collection.

Why must a specimen be taken prior to 24 hours of age if it is invalid, and must be repeated?

- These specimens are valid for some tests, and provide a registration of the infant for use in tracking collection of the repeat specimen through the follow-up system.

What about feedings?

- Effects of feeding practices on the accuracy of screening in the first three days of life remain uncertain. However, it is generally felt they are a minor factor relative to the infant's age.

What about TPN (hyperalimentation)?

- The specimens of infants receiving TPN may give erroneous and misleading results in tests for MCADD and the aminoacidopathies. Whenever possible, a newborn screening specimen should be taken before initiation of these procedures.

Why should every baby be tested?

- The screening panel has been selected to identify those newborns at risk for disorders that may not be readily identified by physicians. The disorders are rare, initial symptoms are minimal or too generalized to lead to early diagnosis. Delayed identification could lead to irreversible damage or death. Since the genetic disorders are recessive, there may be no known family history prior to identification of a condition in an infant.

Should every baby be screened twice?

- No. The Office of Technology Assessment of the U.S. Congress has concluded that the cost of screening every infant twice is not justified by the finding of additional cases on second screen.¹ Physicians should review the newborn's record to assure that every infant has been screened.

What if there is a family history of a disorder as in a previous child?

- To expedite processing and in some cases provide confirmatory testing, the screening laboratory should be informed of a family history of one of the disorders in the panel. It is recommended that two specimens taken at least a few days apart be sent for analysis. If the family history involves galactosemia or maple syrup urine disease, the nearest IMD Specialty Care Center should be notified prior to delivery to arrange immediate

testing after birth, since these infants with these disorders, if not treated immediately, may have a rapid downhill course. A newborn screening specimen should also be submitted for complete panel analysis.

What if an infant develops symptoms of a disorder in the first weeks of life?

- The screening program should be telephoned by hospital staff or the physician to ascertain if the test results are available. Results will be reported and tests repeated for assurance of accuracy. Often calls for results come in prior to receipt of a specimen. If this occurs, an additional specimen should be collected at the hospital and sent by an overnight courier for prompt testing.

What information must be given to obtain the screening result?

- The laboratory can only report results to a physician or person acting for a physician. The more identifying information available on the infant, the more efficient matching will be against the 1100 specimens received daily. The laboratory identification number printed on the specimen form is the most accurate identifier. Information should mirror that submitted on the blood collection form including:
 - Preprinted laboratory ID from the submitted specimen collection form;
 - Infant's last name as recorded on collection form;
 - Infant's sex;
 - Date of birth;
 - Date of specimen.
 - Medical record number (if available);
 - Hospital of birth;
 - Mother's name as submitted on the collection form;
 - Other last names assigned the infant or mother (adoption, paternity or marriage).

Can screening results be reported to parents?

- No. Since the screening laboratory functions under NYS Clinical Laboratory Standards, results can only be reported to a physician or person acting for a physician who in turn can inform the parent.

To whom are the results reported?

- Screen negative results are mailed to the submitting hospital, specifically to the CEO or the designee appointed to receive screen negative results. A copy should be forwarded by the hospital to the physician of record.
- Invalid notification is mailed to the hospital CEO or the designee appointed to receive invalid notification.
- Screen positive results, requiring a repeat specimen, are mailed to two sites – the physician of record and the Director of Pediatrics or the designee appointed to receive screen positive results (must be a licensed physician). Results are mailed to only one site if the physician of record is at the hospital address, in which case both copies are mailed to the Director of Pediatrics or designee appointed to receive screen positive results (must

be a licensed physician).

- Screen positive, highly abnormal results requiring immediate clinical intervention are telephoned to the appropriate treatment center staff and the physician of record, or to the submitting hospital's Director of Pediatrics or the designee appointed to receive screen positive results.

How long does it take to obtain routine screen negative results?

- Three to seven weeks. Screen negative results are sent to the hospital of birth with a copy to be forwarded to the physician of record.

Specimen collection to laboratory receipt	4 - 10 days
Laboratory processing	4 - 5 days
Return mail to hospital	4 - 10 days
Hospital processing	5 - 10 days
Results mailed to physician	<u>4 - 10 days</u>
	21 - 45 days

How long does it take to obtain screen positive results requiring repeat collections?

- Two to four weeks. Screen positive results requiring repeats are sent by mail directly to the responsible physician.

Specimen collection to laboratory receipt	4 - 10 days
Laboratory processing	4 - 7 days
Return mail to physician	<u>4 - 10 days</u>
	12 - 27 days

How long does it take to obtain results requiring immediate clinical intervention?

- Five days to two weeks. Screen positive results highly suggestive of clinical disease are telephoned to the responsible physician and to the nearest Specialty Care Center.

Specimen collection to laboratory receipt	4 - 10 days
Laboratory processing	<u>1 - 3 days</u>
	5 - 13 days

Who is responsible for obtaining needed repeats?

- Invalid specimens - the CEO (or designee) at the submitting hospital or birth attendant if born outside a hospital.
- Screen positive - physician of record, when listed at an address other than the hospital, otherwise the Director of Pediatrics (or designee) at the submitting hospital.

When should repeats be collected?

- Invalid specimens - repeat should be taken as soon as arrangements can be made, since this infant has not been screened.
- Screen positive - unless instructed otherwise, a repeat should be taken within one week

of notification.

How should a physician obtain routine results (i.e., infant not presenting with clinical signs requiring immediate medical attention) when a parent presents a pink collection receipt?

- The physician should contact the hospital of birth for results if they have not already been received. The hospital of birth is sent two copies of the report. One copy is for filing in the infant's permanent health file, the second is to be forwarded to the physician of record. Alternatively, the physician may telephone the laboratory voice response system to obtain test results at (800) 535-3079. For information on this system and to register for its use, phone (518) 473-7552.

Does a screen negative result mean the infant is not at risk for that condition?

- **No.** Many factors can contribute to a negative result in the newborn screen. Some of these – late onset of the condition, transfusion, TPN, mislabeling of the specimen – can result in a “false-negative” result for that child. No child displaying symptoms for one of the conditions in the screening panel should be judged not-at-risk for that condition. All possibilities, including a false-negative newborn screen, should be considered when evaluating a sick infant.

Why are specific values of test results not reported?

- Screening assays are designed to identify a few abnormal test results out of large numbers of normal test results. Some of the tests are qualitative (i.e. the enzyme is present or absent), while others are quantitative. Misleading information may be obtained by interpretation of results beyond the broad categories established in the screening laboratories. Screening test results are inappropriate for basing diagnoses or treatment. The purpose of screening is to identify those infants at risk for the disorders and in need of more definitive follow-up tests. Therapy should not be initiated without confirming the screening results.

Can results be obtained from the HIV antibody screening performed on the newborn screening blood collection cards?

- Yes. The Newborn Screening Program began mandatory testing of newborns for HIV antibodies in 1997. Results of this testing are included on the newborn screening report sent to the pediatrician of record and the hospital of birth. If a person wishes to be tested for HIV antibodies, arrangements can be made with their physician, clinic, or by calling the AIDS hot line at (800) 962-5065.

Where can I obtain additional information specific to each condition in the screening profile?

- In addition to the references cited in each chapter of this publication, "Newborn Screening Fact Sheets" (Pediatrics 98[3], 1996) provide an excellent summary of all conditions in the New York screening profile. Clinical specialists in each of these conditions are available throughout New York for consultation. Contact the Newborn

Screening Program for further information.

How can I contact the screening program?

- The screening program may be reached by phone at (518) 473-7552 between 8:30 a.m. and 4:30 p.m. each workday, or by mail at the following address:

Director
Newborn Screening Program
New York State Department of Health
Wadsworth Center
Empire State Plaza, P.O. Box 509
Albany, New York 12201-0509.

For emergency contact, call the NYS Department of Health at (518) 465-9720.

References Cited

1. Healthy Children: Investing in the Future (1988) Office of Technology Assessment, Congress of the United States.

Chapter 15 . GLOSSARY

17-OHP	17-hydroxyprogesterone
3-MMC	3-Methylcrotonyl-CoA carboxylase deficiency
AAP	American Academy of Pediatrics
ABR	Auditory brainstem response
ACTH	Adrenocorticotrophic hormone
AIDS	Acquired immune deficiency syndrome
ASA	Argininosuccinic lyase deficiency
AZT	Zidovudine
BCKD	Branched-chain ketoacid dehydrogenase
BIA	Bacterial inhibition assay
β -KT	Mitochondrial acetoacetyl-CoA thiolase deficiency
C	Centigrade
CAH	Congenital adrenal hyperplasia
CAT	Carnitine/acylcarnitine translocase deficiency
CBAVD	Congenital bilateral absence of the vas deferens
CBC	Complete blood count
CC	Homozygous hemoglobin C
CEO	Chief executive officer
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane regulator
CoA	Coenzyme A
CORN	Council of Regional Networks for Genetic Services
CPS	Carbamoylphosphate synthetase deficiency
CPT-I	Carnitine palmitoyl transferase deficiency type I
CPT-II	Carnitine palmitoyl transferase deficiency type II
dl	Deciliter
DNA	Deoxyribonucleic acid
DOH	Department of Health
E3	Dihydrolipoyl dehydrogenase
ELISA	Enzyme-linked immunosorbent assay
FT ₄	Free thyroxine
G	Gram
GA-I	Glutaric aciduria type I/Glutaryl-CoA dehydrogenase deficiency type I
GA-II	Glutaric acidemia type II/Multiple acyl-CoA dehydrogenase deficiency
GALT	Galactose-1-phosphate uridylyl transferase
Gal-1-P	Galactose-1-phosphate
GENES	The Genetic Network of New York, Puerto Rico and the Virgin Islands
Hb	Hemoglobin
HBsAg	Hepatitis B surface antigen
HHH	Hyperammonemia, hyperornithinemia, homocitrullinuria

HIV	Human immunodeficiency virus
HMG	3-Hydroxy-3-methylglutaryl-CoA lyase deficiency
Hyperphe	Hyperphenylalaninemia
ICHAP	Infant and Child Health Assessment Program
ID	Identification
IMD	Inherited metabolic disorders
IQ	Intelligence quotient
IRT	Immunoreactive trypsin
IVA	Isovaleric acidemia/Isovaleryl-CoA dehydrogenase deficiency
Kg	Kilogram
L	Liter
L-T ₄	Levothyroxine
LCAD	Long-chain acyl-CoA dehydrogenase deficiency
LCHAD	Long-chain hydroxy acyl-CoA dehydrogenase deficiency/3-Hydroxyacyl
CoA	dehydrogenase deficiency
MCADD	Medium-chain acyl-CoA dehydrogenase deficiency
MCV	Mean corpuscular volume
Mg	Milligram
ml	Milliliter
MMA	Methylmalonic acidemia
MS/MS	Tandem mass spectrometry
MSUD	Maple syrup urine disease
NCCLS	National Committee for Clinical Laboratory Standards
Ng	Nanogram
NYCRR	New York Codes, Rules and Regulations
NYS	New York State
OAE	Otoacoustic emissions
PA	Propionic acidemia/Propionyl-CoA carboxylase deficiency
Phe	Phenylalanine
PKU	Phenylketonuria
RT ₃ U	Triiodothyronine resin uptake
SC	Heterozygous hemoglobin S and hemoglobin C
SCAC	Sickle Cell Advisory Committee of GENES
SCAD	Short chain acyl-CoA dehydrogenase deficiency
SCHAD	Short chain hydroxy acyl-CoA dehydrogenase deficiency
SS	Homozygous hemoglobin S/sickle cell disease
SIDS	Sudden infant death syndrome
T ₃	Triiodothyronine
T ₄	Thyroxine
TBG	Thyroxine binding globulin
TFP	Trifunctional protein deficiency
TLC	Thin layer chromatography
TPN	Total parenteral nutrition
TRH	Thyrotropin-releasing hormone

TSH	Thyroid stimulating hormone, thyrotropin
VLCAD	Very long-chain acyl-CoA dehydrogenase deficiency
μg	Microgram
μmol	Micromole
μU	Microunits