

US Newborn Screening System Guidelines II:  
Follow-up of Children, Diagnosis, Management, and  
Evaluation  
Statement of the Council of Regional Networks for  
Genetic Services (CORN)

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## Preface

Newborn screening is an essential and productive preventive public health program. The Maternal and Child Health Bureau has maintained close involvement with NBS since the late 1950s, helping to ensure efficient and effective public NBS systems. Initially, the Children's Bureau (the former name of MCHB) assisted in evaluating the efficacy of the ferric chloride test on wet diapers as a screening test for phenylketonuria. After Dr Robert Guthrie developed a simple, sensitive,

and inexpensive screening test for PKU, using bacterial inhibition techniques on blood collected on filter paper, the Children's Bureau supported a field trial of this screening procedure in 29 states. Thus, in 1963, the basis for our current population-based systems of screening newborns for hemoglobinopathies, endocrine disorders, metabolic diseases, and infectious diseases began with the Massachusetts PKU Newborn Screening Program. With the aid of funding from the Chil-

dren's Bureau, NBS programs were implemented nationwide. Currently, each state, the District of Columbia, Puerto Rico, and the Virgin Islands screen all newborns for PKU and congenital hypothyroidism, and most include other inherited disorders. These NBS programs were the first population-based screening programs for genetic disorders and signaled the integration of genetic knowledge into public health programs.

State NBS programs reflect the need for a coordinated system of services for follow-up, diagnosis, and treatment of children identified with a disorder requiring special health care services. Children with special health care needs are defined as those who have or are at risk for a chronic physical, developmental, behavioral, or emotional condition and who also require health and related services of a type or amount

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beyond that required by children generally. Children with chronic and disabling conditions (such as hemoglobinopathies, endocrinopathies, and metabolic disorders) and their families often require an extensive range of different services. These health activities and services are essential components of care.

|                |   |
|----------------|---|
| 17-OHP         | 17-Hydroxyprogesterone                                |
| ACMG           | American College of Medical Genetics                  |
| BCAA           | Branched-amino acids                                  |
| BCKAD          | Branched-chain $\alpha$ -ketoacid dehydrogenase       |
| CAH            | Congenital adrenal hyperplasia                        |
| CAP            | College of American Pathology                         |
| CBS            | Cystathionine- $\beta$ -synthase                      |
| CH             | Congenital hypothyroidism                             |
| CLIA           | Clinical Laboratory Improvement Amendment             |
| CORN           | Council of Regional Networks for Genetic Services     |
| DHPR           | Dihydropteridine reductase                            |
| FAH            | Fumarylacetoacetate hydrolase                         |
| GALT           | Galactose-1-P-uridyl transferase                      |
| Hb             | Hemoglobin  |
| HPLC           | High-performance liquid chromatography                |
| MCHB           | Maternal and Child Health Bureau                      |
| MSUD           | Maple syrup urine disease                             |
| NBS            | Newborn screening                                     |
| NTBC           | 2-Nitro-4-trifluoromethylbenzoyl-1,3-cyclohexanedione |
| PAH            | Phenylalanine hydroxylase apoenzyme                   |
| PHCP           | Primary health care provider                          |
| PKU            | Phenylketonuria                                       |
| RBC            | Red blood cell  |
| SCD            | Sickle cell disease                                   |
| T <sub>4</sub> | Thyroxine   |
| TAT            | Tyrosine aminotransferase                             |
| TBG            | Thyroxine-binding globulin                            |
| TSH            | Thyrotropin   |

Historically, services for children with special health care needs have been difficult for families to access and for providers to coordinate. Families have had to navigate a maze of organizations, providers, and geographic and financial barriers. Primary health care providers are not always comfortable caring for a child with special health care needs and often do not have schedules that can coordinate the variety of resources a family needs. Both families and providers are

challenged by a lack of knowledge of comprehensive needs and of the corresponding community-based resources and payment mechanisms. Moreover, the systemic and interpersonal barriers that arise because of poor reciprocal communication and cross-cultural interactions are additional concerns for both families and providers. To overcome these obstacles, the MCHB believes that services for children with special health care needs should be provided within the context of a "medical home."

Establishing a medical home necessitates building new partnerships and implementing changes in physicians' office procedures, management, and staffing. Optimally, this medical home includes a primary care pediatrician working in partnership with families and other specialists, subspecialists, and the wide range of other providers needed to care for these children. In a medical home, all providers and parents share responsibility for ensuring that a child has access to the medical and non-medical services needed to help him or her achieve maximum potential. For children with special health care needs identified through state NBS programs, meeting this goal requires that (1) we develop adequate provider networks and (2) we encourage collaborative roles between primary and subspecialist providers.

These guidelines for a state NBS system come at a critical time. As the Human Genome Project nears completion, the expansion of genetic knowledge and technology into the public health arena will continue. On one hand, the Human Genome Project presents opportunities for understanding and promoting health, lowering mortality and morbidity rates, and preventing diseases. One can envision either the expansion of NBS programs to include the use of newer DNA technology for screening or the use of the programs

for screening newborns as a paradigm for screening children, adolescents, and adults. On the other hand, the transfer of knowledge and technology from genetic research into clinical service, especially as delivered in public health programs, will require extensive and thoughtful deliberation. Genetic epidemiologic information from carefully designed studies is needed to understand how genetic factors interact with each other and with environmental factors, how disease is manifested, and how morbidity and death can be prevented. By uncovering a genetic basis for most common diseases, expanded genetic services could be integrated broadly into public health programs. Integrating genetic knowledge, services, and technology into public health programs is a formidable task.

The guidelines presented in this document provide a strong tool for structuring a system for diagnosis and clinical follow-up of infants identified by state NBS programs. We also encourage the development of provider networks and collaborative relationships within the context of a medical home, which will be necessary to coordinate the care for those children identified as having hemoglobinopathies, endocrinopathies, and metabolic disorders. Furthermore, the comprehensive system of follow-up, diagnosis, management, and program evaluation will provide a sturdy framework for discussing expanded genetic screening in a public health context. Development of these guidelines has included significant input and cooperation from many professionals and experts from around the country, and the guidelines were completed with the support and appreciation of MCHB.

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## Executive Summary

This document pertains to follow-up of infants and children with special needs who have hematologic, endocrine, or metabolic disorders that are detected by population-based NBS systems by using Guthrie blood spots collected on special filter paper. Immediate medical management of an infant with one of these disorders will prevent many, and in some disorders all, of the serious clinical sequelae. The purposes of this document are: (1) to delineate administrative, logistical, and clinical guidelines for a 5-part system; (2) to emphasize the complexity of treating patients with rare disorders who should be referred to and followed up by medical staff with expertise in these potentially devastating diseases; and (3) to anticipate the expansion of NBS systems to include additional disorders that are best managed by presymptomatic screening, diagnosis, and early treatment. This document is neither a practitioner's manual nor an exhaustive presentation of screening, follow-up, diagnostic, and management protocols. General guidelines for NBS in the United States are already available in previous Council of Regional Networks for Genetic Services publications, and screening laboratory protocols have already been defined in detail.

For nearly 40 years, NBS programs have evolved toward a 5-part (see Box) preventive health care system designed to identify and treat selected conditions that would otherwise become catastrophic health problems. Provisions for establishing diagnoses and providing treatment vary by program and by clinical condition. Organizational approaches to NBS systems also vary, depending on statutory and regulatory structures and on procedures for providing health benefits to diverse populations.

All states and the District of Columbia now screen newborns for PKU and CH. More than 40 programs screen for sickle cell disease, other hemoglo-

binopathies, and galactosemia. Some programs include congenital adrenal hyperplasia, homocystinuria, maple syrup urine disease, biotinidase deficiency, and/or tyrosinemia. A few states also include other conditions that are not discussed in this document, such as cystic fibrosis, disorders of fatty acid oxidation, organic acidemias, and some congenital infections, such as toxoplasmosis and human immunodeficiency virus. Most, but not all, of these disorders are genetic.

## FOLLOW-UP

The mission of the NBS program is to eliminate or reduce the mortality, morbidity, and disabilities that result from the disorders included in the screening panel. Rapid follow-up of all infants with a "not normal" screening test result—including "borderline," "unsatisfactory," or "screen positive"—is crucial to fulfilling this mission.

### *Responsibility and Scope of the Follow-up Component of the Newborn Screening System*

The primary function of the follow-up component is to locate infants with screening results that are screen positive and to facilitate the entry of these infants into the diagnostic and management components of the NBS system in a timely fashion. The NBS system requires a locus of responsibility for administrative oversight; system evalua-

### The Five Parts of a Newborn Screening System

1. **Screening:** Testing of newborns.
2. **Follow-up:** Rapid location, follow-up, and referral of the screen-positive infant.
3. **Diagnosis:** Evaluation of the infant with a positive screening test to make a definitive diagnosis or exclude the disorder.
4. **Management:** Rapid planning and implementation of long-term therapy.
5. **Evaluation:** Validation of testing procedures, assessment of the efficiency of follow-up and intervention, and assessment of the benefit to the patient, family, and society.

tion; and the education of professionals, families, and the public. Short-term follow-up begins when the laboratory obtains an initial result that is screen positive and ends with a definitive diagnosis and documentation that appropriate treatment has been initiated regardless of the family's ability to pay. Long-term follow-up begins with treatment and continues throughout life. Long-term tracking and outcome evaluation are the responsibilities of the NBS program. Both short- and long-term follow-up require a centralized database to track outcome and to which all components of the system must provide data.

### *Components of the Newborn Screening Follow-up Program*

- Appropriately trained follow-up personnel
- Administrative, secretarial, and computer support essential for the follow-up component
- Medical direction
- Birthing facility
- PHCP
- The family
- Specialty consultants and specialty care providers
- Screening and diagnostic laboratories

### *Follow-up and Reporting of Newborn Screening Test Results*

**SCREEN-NEGATIVE (NORMAL) TEST RESULTS.** A laboratory report on every infant should be sent to the infant's

PHCP and/or birthing facility within 7 days of receipt of the specimen. Every birthing facility should have a mechanism to ensure that a report is received for every infant, and a corrective action plan should be automatically triggered if a report is not received within a defined and appropriate time frame.

**SCREEN-POSITIVE (ABNORMAL) TEST RESULTS.** The rapid follow-up of the infant with a screen-positive test result is the highest priority and the primary responsibility of the follow-up component of the system. The follow-up component must ensure that the infant is located and brought into the diagnostic and management components of the system in time to ensure optimal outcome and to prevent irreversible damage.

All NBS programs should have a written protocol defining screen-negative and screen-positive test results and detailing the procedure to be followed for each type of test result. Appropriate portions of the protocol should be shared with PHCPs and specialty consultants in the form of guidelines or a manual. The most effective methods of locating and following up infants will depend on local conditions and resources. The time frame for following up infants will vary by disorder and by the magnitude and probable significance of the screening test abnormality. Timely follow-up is important for all disorders but is especially urgent for MSUD, galactosemia, and CAH because these disorders can be rapidly fatal.

Legislation and regulations vary, but most programs require that the infant's health care provider be notified of the test result. In this context, health care providers may include the PHCP, the submitter of specimens, the birthing facility, and the physician of record. Some programs require the parent or guardian of the infant to be notified as well. NBS follow-up personnel should explain the test result, recommend follow-up action according to the program protocol, describe the general principles of treatment, and answer any questions that arise so that follow-up action can be ini-

tiated in the appropriate time frame. The NBS program follow-up personnel must ascertain whether the recommended evaluation was actually conducted in the appropriate time frame. If not, further action must be taken to initiate the appropriate workup.

**UNSATISFACTORY SPECIMENS.** Approaches to the follow-up of unsatisfactory specimens vary and may include: (1) noting that the specimen was unsatisfactory on the laboratory report, (2) sending a letter from the laboratory to the submitter of the specimen, (3) telephoning the submitter to request another specimen for repeat testing, and/or (4) treating the unsatisfactory sample with the same intensity as a screen-positive result. Programs may assign the responsibility for the follow-up of unsatisfactory specimens to laboratory or follow-up personnel or to the specimen submitter. Regardless of the locus of responsibility for the follow-up of unsatisfactory specimens, some mechanism should be in place to ensure that a satisfactory specimen is received within a reasonable time. Additional action should be taken until a satisfactory specimen is received or the case is designated "lost to follow-up." A written protocol should outline the appropriate actions to be taken to arrive at a designation of "lost to follow-up."

**ROUTINE SCREENING OF SECOND SPECIMENS.** Some programs mandate routine screening of a second specimen. Routine screening of second specimens identifies a small number of additional infants with metabolic disorders, approximately 6% to 12% of infants with hypothyroidism requiring treatment, and some infants with non-salt-wasting CAH. Routine screening of second specimens helps the program ascertain the rate of false-negative results on the initial screen, including biologic false-negative results. Second screens are also valuable for disorders in which the indicator metabolites rise relatively slowly, such as homocystinuria, tyrosinemia, and some

forms of hyperphenylalaninemia. For infants with a very short postpartum birthing facility stay, routine second screens help ensure adequate testing. When costs are calculated for screening of second specimens, a model that assumes an existing NBS program and calculates the additional cost generated by the second specimen should be used.

### *Communication*

**WITHIN THE NEWBORN SCREENING PROGRAM.** Rapid communication among the various components of the NBS program is essential for timely follow-up and for the success of the entire NBS program. One approach to facilitating rapid communication is to electronically link all components of the NBS system. A call-in or online download capability to the screening laboratory is desirable.

**BETWEEN NEWBORN SCREENING PROGRAMS.** It is the responsibility of NBS follow-up personnel to work with colleagues in other states to ensure that all infants are adequately tested and that all screen-positive test results are appropriately followed up. Protocols should provide for information transfer between concerned state programs in which residence issues are involved. All test results must be shared so that both programs may ensure final disposition of all cases.

**WITH THE PRIMARY HEALTH CARE PROVIDER AND FAMILY.** NBS personnel should explain the test result, the disorder, the recommended diagnostic workup, and the general principles of treatment to the health care provider. A call-in or online download capability to assist PHCPs in obtaining information on their patients is desirable. The nature of screening, the difference between screening tests and diagnostic tests, and the occurrence of false-positive test results should be explained when necessary. NBS follow-up personnel should explain these issues to

parents in lay language in a compassionate and supportive manner.

Programs should have protocols in place to deal with problems that are frequently encountered in following up infants with screen-positive results. These include:

- Inability to identify or locate the PHCP
- Families without a PHCP
- Communication with families who do not have telephone service
- Communication with families whose first language is not English
- Communication with adoptive or foster families or with responsible agencies when children are placed at birth or soon thereafter
- Inability to locate the family, even when a home visit is attempted
- PHCPs who refuse to take the program's recommended action
- Refusal of the patient's insurer to cover the recommended evaluation
- Families unable to pay for diagnostic testing or long-term care

### **Documentation**

All actions taken, such as telephone calls and letters, should be cited in the infant's screening record in the program database. The record should document who performed the action, when it was taken, and to whom and where it was directed. The result of the action taken should also be recorded and, if necessary, a plan for further action stated. Such documentation should be concise, legible, and protected as confidential, privileged information. All protocols, policies, and end points should be defined clearly and widely communicated. Systematic collection of data and organized documentation is necessary for problem solving, research, and evaluation of program processes and outcomes.

### **Newborn Screening Follow-up Checklist**

Checklists can be valuable tools for NBS follow-up personnel. The types of checklists may include preparation

for effective follow-up, short-term follow-up activities, long-term follow-up activities, and evaluation activities.

## **DIAGNOSIS**

### ***Diagnostic Confirmation***

A positive screening result requires assessment of the infant by a qualified physician and laboratory in a time frame appropriate for the disorder. All diagnostic test results must be reported to the follow-up and evaluation components of the NBS system. Many disorders identified by NBS programs are complicated by their underlying heterogeneity. These variations require specialized diagnostic interpretation and individualized treatment. Diagnostic laboratories and treating physicians must collaborate with the NBS system's evaluation component by providing information about results of the diagnostic tests.

### ***Staffing***

Laboratory directors and technical supervisors must meet the training and experience requirements specified by the Clinical Laboratory Improvement Amendment of 1988 (CLIA88) or more current national rules. Laboratories must be staffed with sufficient personnel to provide rapid analysis, interpretation of results, and communication of results to the experienced management team. For certain disorders, laboratory diagnostic services must be available at all times.

### ***Laboratory Testing and Quality Control***

Diagnostic laboratories must have appropriate current protocols and equipment for the rapid analyses essential to these conditions. Procedures must be in place to verify normative data, to document accuracy and precision of each method, and to maintain acceptable preanalytical, analytical, and postanalytical performance. Such procedures must include appropriate use of internal and external standards

and analysis of positive and negative controls. The sensitivity, specificity, and positive and negative predictive values of both screening tests and diagnostic tests must be integral components of laboratory control.

### ***Proficiency Testing***

Hematologic and endocrinologic proficiency testing is accomplished through the College of American Pathology and DNA-based testing through CAP or American College of Medical Genetics-sponsored proficiency programs. For metabolic diseases, diagnostic laboratories must participate in proficiency testing programs, such as the CAP or ACMG quality assurance programs in biochemical and molecular genetics. Laboratories can establish comparison procedures with other laboratories doing the same tests and run positive samples as unknowns. All diagnostic tests should meet current CLIA and statutory standards.

### ***Interpretation***

For hematologic and endocrinologic diseases, physicians with expertise in these disorders should be responsible for selection of diagnostic tests and interpretation of results. For metabolic disorders, an MD or PhD certified in clinical and/or biochemical genetics should be responsible for the interpretation of diagnostic test results. Most of these disorders are autosomal recessive. Therefore, family testing may raise concerns about paternity and should be discussed initially with the mother.

Diagnostic criteria are unique for each individual and each disease. Specifics for the diagnosis of each hematologic, endocrinologic, and inherited metabolic disorder detected through NBS are addressed individually. *Core* tests and procedures are those that are essential to rapidly confirm a diagnosis and to initiate treatment. *Supplemental* tests and procedures may be needed to refine the diagnosis of some children and for the purpose of genetic counseling. Although all tests

and procedures may not be available at all centers, they should all be accessible to patients and families when needed. All resources should be available to all children regardless of the families' ability to pay and irrespective of location of diagnostic and treatment facilities.

## MANAGEMENT

Patients identified through NBS usually require lifelong treatment. The goal of treatment is to achieve optimal health, growth, and development. These diseases require clinical management by experts and experienced health care providers who understand their etiology, pathophysiology, clinical heterogeneity, and psychosocial issues. Close communication among the family, the PHCP, and specialists is essential for optimal outcomes. The entire system of care should be integrated to assess short- and long-term outcomes. Management principles for specific diseases are discussed individually and include the required expertise of health care providers, parental health education, health maintenance, management of acute illness, genetic counseling, and psychosocial support appropriate for each condition.

## EVALUATION

### *Evaluation of the Overall System.*

The goals of NBS programs are best realized by a comprehensive system that ensures appropriate screening, follow-up, diagnosis, and lifelong management. All components of the system must function within optimal time frames. The NBS system and each of its components must continually assess their effectiveness in achieving or contributing to the overall goal. The assessment of each component requires data that can only be collected by other components of the system; therefore, data collection and analysis must be a

cooperative effort among all system components.

The NBS system should accumulate data to measure:

- The incidence of each disorder
- The effectiveness and speed of identifying affected infants and entering them into comprehensive care
- The quality of screening and diagnostic tests
- Monitoring and outcome assessment
- The costs for operating the entire system
- The cost of each component
- The cost benefit to patients, families, and society

Some of the parameters that can be used in evaluating the system include:

- Birth prevalence of each disorder (cases identified/number of births)
- Fraction of the births screened (number of infants screened/number of births)
- Ascertainment through NBS (number of cases detected through NBS/number of known cases in patients born in the catchment area)
- Age at screening (mean and distribution)
- Screening laboratory turnaround time (time from receipt of specimen to laboratory report for screen-negative, unsatisfactory, and screen-positive results)
- Analytical validity of the screening tests (accuracy)
- Clinical validity of the screening tests (sensitivity, specificity, positive predictive value, negative predictive value)
- Age at definitive diagnosis of affected infants (mean and distribution)
- Analytical validity of the diagnostic tests
- Clinical validity of the diagnostic tests
- Age at initiation of treatment (mean and distribution)
- Demographic and clinical profiles of the patients receiving treatment for each disorder

- Mortality rates among patients with each disorder
- Measures of morbidity for the population of patients with each disorder (developmental quotient/IQ, inpatient hospital days, percent with specific complications, etc)
- Measures of compliance with the recommended treatment protocols (prophylactic penicillin prescriptions filled, blood phenylalanine levels, diet records, etc)
- Measures of long-term outcome and functionality of patients receiving treatment (schooling achieved, employment, psychosocial adaptation, reproductive success, etc)
- Analytic and clinical validity of the laboratory tests used for monitoring patients
- Validity of the outcome assessment protocols used
- Cost per patient per period (month, year, or lifetime)
- Cost of each component service
- Analyses of costs and outcomes

All data cannot be collected at this time. Rigorous determination of sensitivity, specificity, and negative predictive value requires complete ascertainment of false-negative results, which is not realistically achievable. Programs with routine screening of second specimens can estimate the false-negative rate of the initial screen. Some of the data needed to evaluate the screening and short-term follow-up components of the system can be found in the annual CORN/Association of Public Health Laboratories National Newborn Screening Data reports. Other data from comprehensive care centers and long-term care programs are essential to evaluate the efficacy of the NBS system. Existing comprehensive centers have much of the data required to evaluate the diagnosis, treatment, and management portions of the system in their internal databases. At this writing, available data are incomplete because studies are still in progress. The recognition, understanding, and treatment of these disorders are still in a phase of

rapid development, and the populations diagnosed with these disorders and the treatments provided are rapidly changing. Therefore, long-term correlation of costs with outcomes is difficult.

### *Evaluation of Laboratories*

The evaluation of NBS laboratories has been discussed in detail in a previous CORN document. (See Therrell et al in Further Reading, General References). Limited proficiency testing programs, notably the National Quality Assurance Program, are available. Laboratories should also endeavor to exchange specimens with other laboratories as a means of external verification of procedures when no formal proficiency testing programs exist. A full discussion of the issues concerning diagnostic laboratories is found in the Diagnosis section.

## **CONCLUSION**

For nearly 40 years, NBS programs in the United States have provided an important public health service by identifying newborns with congenital conditions that could be managed effectively with intervention early in life. PKU is the "index" case for these mostly genetic conditions. In previous documents, CORN committees have provided guidance on the laboratory screening system necessary for an effective NBS program, guidance on the use and retention of residual specimens after laboratory analysis of these newborn specimens, and guidance in methods to deal with

the very large number of infants detected by screening programs as carriers of hemoglobinopathies. This document continues that heritage by offering guidance in the diagnosis and treatment of infants detected by NBS programs and how best to evaluate their effectiveness.

Beyond the immediate objective of obtaining blood samples for NBS lies the challenge of realizing the benefits that can come from the early identification of rare but treatable disorders. To meet this challenge, each newborn identified through population-based screening must enter a system that rapidly results in definitive diagnosis and appropriate intervention, with prompt communication of clinical and genetic information to the family and the PHCP. Additional requirements of NBS systems include the design and implementation of expert disease-specific management programs and the evaluation of their effectiveness according to established public health principles.

This document outlines a 5-part system necessary for the effective operation of an NBS program: screening, follow-up, diagnosis, management, and evaluation. These screening programs test a small blood sample collected from the infant at the time of discharge for selected disorders. Perhaps the overriding concern regarding all parts of the system is rapid completion of each process phase to ensure that the child receives appropriate care as soon after birth as possible. Inherent in this process is open and current communication among all components of the system: the laboratory, the follow-up unit, med-

ical care personnel, and the public health team.

Each condition in current NBS profiles in the United States is discussed separately when considering diagnosis and management. For each of these, a core set of diagnostic procedures is presented. Because laboratory values vary, each laboratory must establish its own range of normal values. Importantly, the document identifies supplemental tests that may be needed for diagnosis and for counseling parents about future offspring.

Beyond diagnostic procedures, the document provides an overview for treatment of children with these conditions. For each disorder, core personnel and resources are listed.

An extensive part of the document is devoted to follow-up procedures and evaluation criteria. This was a deliberate effort on the part of the authors to emphasize that these screening programs are integral parts of a public health system in all states. Consequently, they operate under the aegis of public health law and/or regulation, with the full authority thus indicated. The programs truly are "systems" involving many components of public health and medical care delivery. Recognizing that feedback is essential for improvement of any system, the document outlines the necessary data elements generated by each unit and the value of those data to other components of the system.

Finally, the document provides a list of further readings. Several Web sites are identified so that the reader may obtain the most current information.

# Guidelines

## I. INTRODUCTION

This document relates to follow-up of infants and children with special needs who have hematologic, endocrine, or metabolic disorders that are detected by population-based NBS systems with the use of Guthrie blood spots collected on special filter paper.\*

Immediate medical treatment of an infant with one of these disorders will prevent many, and in some disorders, all of the serious clinical sequelae. Thus, NBS systems should provide for the rapid follow-up of the infant who has a positive screening test result, for confirming the presence or absence of the disorder, for referring the infant for consultation, for providing experienced medical management, and for evaluating the total system. These guidelines support a public health system based on universal NBS that accurately identifies affected children and initiates treatment before preventable, irreversible damage occurs. Laboratory testing and medical intervention should be cost-effective. The management of these rare disorders is complex. Relatively few health care professionals have collective experience in diagnosing and treating these special-needs children. The system should ensure access to quality care regardless of ability to pay.

Systems for NBS involve public health agencies, scientific staff, physicians, nurses and pediatric nurse practitioners, genetic counselors, specialized laboratory personnel, nutritionists, and social workers. This document is intended as a guide for structuring a system for the diagnosis and clinical follow-up of infants identified by NBS programs. It provides a framework for public health programs to evaluate their current effectiveness and plan for their future needs.

\*These guidelines do not apply to population-based NBS for infectious diseases, hearing impairment, or other disorders.

**Table I-1. The Five Parts of a Newborn Screening System**

1. **Screening:** Testing of newborns.
2. **Follow-up:** Rapid location, follow-up, and referral of the screen-positive infant.
3. **Diagnosis:** Evaluation of the infant with a positive screening test to make a definitive diagnosis or exclude the disorder.
4. **Management:** Rapid planning and implementation of long-term therapy.
5. **Evaluation:** Validation of testing procedures, assessment of the efficiency of follow-up and intervention, and assessment of the benefit to the patient, family, and society.

This document is neither a practitioner's manual nor an exhaustive presentation of screening, follow-up, diagnostic, and management protocols.

NBS is a 5-part preventive health care system designed to identify and treat selected conditions that otherwise would become catastrophic health problems. The 5 parts of the system are delineated in Table I-1. Although these components are discussed separately in this document, there is considerable temporal overlap of activities (Fig I-1). NBS systems utilize testing procedures that are readily available, technically feasible, economically sound, and clearly beneficial to affected newborns, their families, and society. The universal acceptance of NBS for specified disorders in the United States over the past 3 decades attests to the benefits of screening and preventive therapy. The system of screening, follow-up, and referral of the screen-positive infant, diagnostic evaluation, disease management, and program evaluation is designed to respond to federal and state mandates to provide all children with special needs, including those in traditionally underserved populations, access to effective treatment and appropriate care in a timely and cost-effective manner.

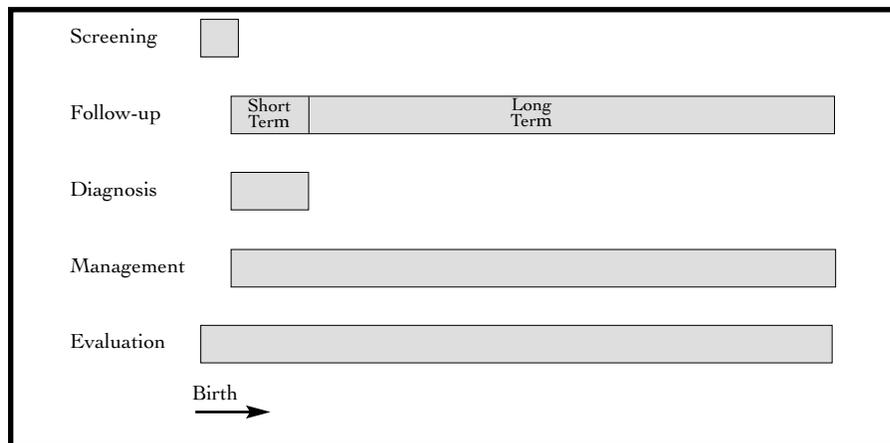
NBS systems vary with respect to the breadth and depth of the 5 components. All states and the District of Co-

lumbia now screen newborns for PKU and CH. More than 40 programs screen for SCD, other hemoglobinopathies, and galactosemia. Some programs include CAH, homocystinuria, MSUD, biotinidase deficiency, and/or tyrosinemia. A few states also include other conditions that are not discussed in this document, such as cystic fibrosis, disorders of fatty acid oxidation, organic acidemias, and some congenital infections, such as toxoplasmosis and human immunodeficiency virus. Most, but not all, of these disorders are genetic. Provisions for establishing diagnoses and providing treatment vary by program and by clinical condition.

Organizational approaches to NBS systems vary, depending on statutory and regulatory structures and on procedures for providing health care benefits to diverse populations. Some programs have well-established centralized laboratories and clinicians funded by government appropriations or by fee-for-service. Other programs contract for the facilities and capabilities of university-based laboratories and clinicians, and a few include services from the private practice sector. Screening programs in regions with low birth rates avoid wasteful duplication of effort by reaching across geographic or political borders for laboratory and/or

clinical expertise. In these areas, because of the long distances between the patient's residence and specialty consultants' offices, PHCPs are required to take a more active role as partners for follow-up case management. These varied approaches are tailored to local needs and resource availability, with the aim of providing a comprehensive system that is efficient and thorough.

The purposes of this document are: (1) to provide administrative, logistical, and clinical recommendations for a 5-part system that implements and coordinates screening tests, patient follow-up, diagnosis, management, and system evaluation; (2) to emphasize the complexity of treating patients with rare disorders who should be referred to and followed up by medical staff with expertise in these potentially devastating diseases; and (3) to anticipate the expansion of NBS systems to include additional disorders that are best managed by presymptomatic screening, diagnosis, and early treatment. General



*Figure 1-1:* Temporal Relationship of the Five Parts of the Newborn Screening System.

NBS guidelines in the United States are available in previous CORN publications, and screening laboratory protocols have already been defined in detail. Therefore, this document focuses on the remaining 4 parts of the system. It represents a consensus of professionals in medicine, genetics, and public health who are dedicated to the principle that children with rare and complex health problems need specialized, interdiscipli-

nary laboratory and clinical services. These services should be viewed as complementary and consultative to primary medical care. This document focuses on those diseases that are presently included in some or all of the mandated NBS programs in the United States. The information in this document will need to be modified as our technical and clinical knowledge of genetic testing, intervention, and prevention grows.

## II. FOLLOW-UP

### *Mission of Newborn Screening Programs*

The mission of NBS programs is to eliminate or reduce the mortality, morbidity, and disabilities that result from the disorders included in the screening panel. To accomplish this mission, NBS programs must ensure that all affected infants receive early diagnosis and optimal long-term treatment. Rapid follow-up of all infants with a “not normal” screening test result—including “borderline,” “unsatisfactory,” and “screen positive”—is crucial to fulfilling this mission.

### *Responsibility of the Follow-up Component of the Newborn Screening System*

The primary function of the follow-up component is to locate infants with positive screening results and to facilitate the entry of these infants into the diagnostic and management components of the NBS system in a timely fashion. The NBS system requires a locus of responsibility for administrative oversight, system evaluation, and the education of professionals, families, and the public. In many programs, these responsibilities are assigned to the follow-up component. In other programs, they may reside in the screening laboratory, in the diagnostic, the management, or the evaluation component of the NBS system or in other units. In some programs, a designated administrative unit oversees the entire NBS system. The ultimate responsibility for ensuring appropriate and timely follow-up, diagnosis, and management must be delineated in each program. A generalized flow diagram for the follow-up functions is presented in Fig II-1.

### *Scope*

Short-term follow-up begins when the laboratory obtains an initial result that is screen-positive and ends with definitive diagnosis and documentation

that appropriate treatment has been initiated, regardless of the family's ability to pay. Long-term follow-up begins with treatment and continues throughout life. Long-term tracking and outcome evaluation are the responsibilities of the NBS program. Both short- and long-term follow-up require a centralized database to track outcome and to which all components of the system must provide data.

The NBS system is responsible for the education of hospital staff, health care providers, parents, and the general public about NBS. PHCPs need information about the importance of NBS, the requirements and resources of the program, specimen collection techniques, the optimal timing of specimen collection, the disorders in the screening panel, the protocols for immediate follow-up to establish or rule out a diagnosis, and the short- and long-term treatment and management of each disorder. Families and PHCPs should receive educational materials about the disorder.

### *Components of the Newborn Screening Follow-up Program*

**FOLLOW-UP PERSONNEL.** NBS programs must have appropriately trained follow-up personnel. Nurses, genetic counselors, and social workers have much of the requisite education, and persons with other educational backgrounds can acquire it rapidly. Follow-up personnel must ensure that infants receive the appropriate diagnostic interventions expeditiously. Administrative, secretarial, and computer support is essential for the follow-up component. Medical direction is necessary for the interpretation of “other than normal” screening test results and in the determination of appropriate follow-up in the clinical setting.

**BIRTHING FACILITY.** Birthing facility personnel should work together with the child's PHCP and program follow-up personnel to ensure that all infants are screened and that all follow-up pro-

cedures are accomplished in a timely manner. Each NBS program should develop a policy for screening home births. The specimen requisition form should be fully and accurately completed to include the date and time of collection, the name of the ongoing PHCP when known, and other items as defined by the program. Birthing facility personnel should record the date of receipt of the report and place the report in the infant's medical record. The birthing facility receiving an unsatisfactory or screen-positive report must work with the NBS follow-up component to ensure that the physician and family are aware of the need for further follow-up action. A call-in or online download capability for screening test results is desirable.

### **PRIMARY HEALTH CARE PROVIDER.**

The PHCP is responsible for working with the NBS program to ensure that every child in his or her care is appropriately screened in a timely manner. The PHCP must follow up all screen-positive or unsatisfactory specimens in accordance with program recommendations or requirements. The PHCP must also ensure that all affected patients in the practice are enrolled and maintained in appropriate treatment programs. The PHCP should obtain and maintain a copy of the results of newborn screen testing for every child in his or her practice and be sure that the mother or guardian is apprised of the results. A call-in or online service to assist PHCPs in obtaining NBS information on their patients is desirable.

**FAMILY.** The family is a critical part of the NBS follow-up system. Educating parents about the importance of NBS should begin during the prenatal period. Understanding the purpose and importance of screening will improve compliance with follow-up. Parents should be encouraged to ask their PHCP for their infant's screening results. The parents of an infant with an unsatisfactory specimen deserve a careful explanation of the situation and ap-

appropriate reassurance and support. The parents of an infant with a positive screening result need to understand the disorder and the occurrence of false-positive results. They also need appropriate support. The PHCP should inform parents as soon as possible when the results of additional testing are received. The parents of an affected child need information regarding the nature of the disorder, required treatment, and available services and support.

**SPECIALTY CONSULTANTS.** Each NBS program should identify the specialty consultants and referral centers available to PHCPs for further evaluation of infants with screen-positive test results. These consultants should be experienced and knowledgeable about NBS and diagnosis of the disorders. Specialty care providers experienced in the long-term treatment of affected infants should also be designated. Specialty consultants should provide diagnostic and outcome data to the NBS system.

**LABORATORIES.** A number of documents about laboratory activities related to NBS and diagnosis are cited in the references section. An accessioning record of all specimens received and test results obtained must be maintained within the program, as required by CLIA88, and this information must be available for system evaluation. Each NBS program should identify laboratories suitable for further testing of infants with screen-positive test results. Laboratory issues are discussed more fully in the sections on the diagnosis and evaluation components.

### *Follow-up and Reporting of Newborn Screening Test Results*

**SCREEN-NEGATIVE (NORMAL) TEST RESULTS.** A laboratory report on every infant should be sent to the infant's PHCP and/or birthing facility within 7 days of receipt of the specimen. Every birthing facility should have a mecha-

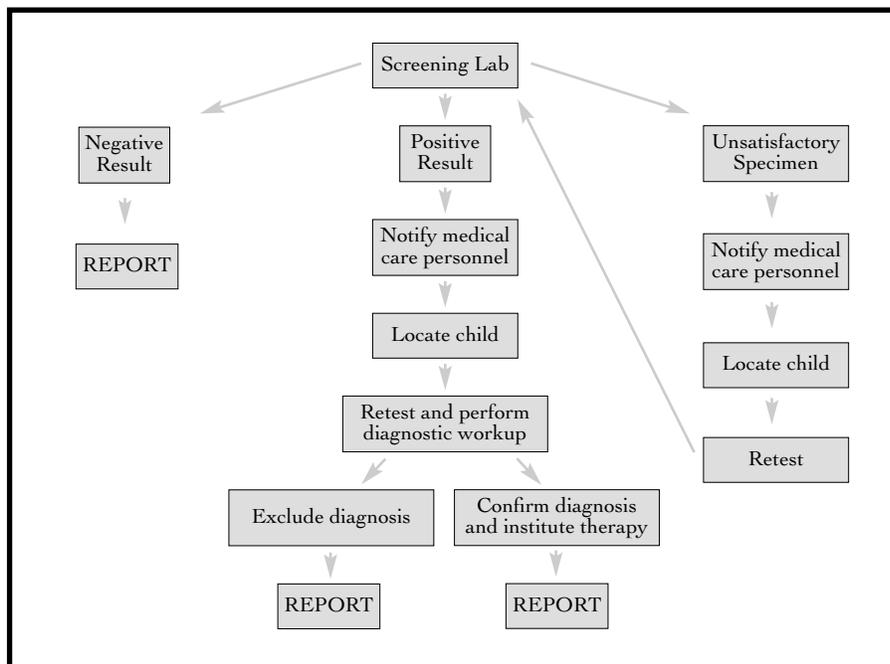


Figure II-1. Flow Diagram for Follow-up Functions.

nism to ensure that a report is received for every infant, and a corrective action plan should be triggered automatically if a report is not received within a defined and appropriate time frame.

**SCREEN-POSITIVE (ABNORMAL) TEST RESULTS.** Rapid follow-up of the infant with a screen-positive test result is the highest priority and the primary responsibility of the follow-up component of the system. The follow-up component must ensure that the infant is located and brought into the diagnostic and management components of the system in time to ensure optimal outcome and to prevent irreversible damage.

All NBS programs should have a written protocol defining screen-negative and screen-positive test results and detailing the procedure for follow-up of infants with each type of test result. The protocol should be prepared in consultation with NBS laboratory personnel and knowledgeable medical personnel. The medical personnel should include individuals with a wide range of clinical experience in the care and physiology of both healthy newborns and those with abnormalities, the detection and man-

agement of the disorders in the screening test panel, and the NBS process. Appropriate referral centers, consultants, and diagnostic laboratories should be identified in the protocols of each NBS program. Appropriate portions of the protocol should be shared with PHCPs and specialty consultants in the form of guidelines or a manual.

The most effective methods for locating and following up infants will depend on local conditions and resources. The time frame for following up infants will vary by disorder and by the magnitude and probable significance of the screening test abnormality. Timely follow-up is important for all disorders but is especially urgent for MSUD, galactosemia, and CAH, which can be rapidly fatal. This is discussed more fully in the Diagnosis and Management sections. Although every screen-positive test result must be followed up to resolution, every attempt should be made to minimize the anxiety of the family and the emotional and fiscal cost of inevitable false-positive results.

Legislation and regulations vary, but most programs require that the infant's health care provider be notified of the

test result. In this context, health care providers may include the PHCP, the submitter of specimens, the birthing facility, and the physician of record. Some programs require the parent or guardian of the infant to be notified as well. NBS programs report the most time-sensitive and significant screen-positive results by telephone and follow the call with a confirmatory fax, letter, e-mail message, or certified letter. When parents are to be notified, the PHCP should be notified first. The PHCP should be encouraged to make the initial contact with the parents immediately, and a representative of the NBS program can call, if required, after the family has already spoken with their child's PHCP.

NBS follow-up personnel should explain the test result, recommend follow-up action according to the program protocol, describe the general principles of treatment, and answer any questions that arise so that follow-up action can be initiated in the appropriate time frame.

NBS program follow-up personnel must ascertain whether the recommended evaluation was actually conducted in the appropriate time frame. If not, further action must be taken to initiate the appropriate workup. If the workup was conducted, the results must be obtained and entered into the database. Results must then be communicated to all appropriate parties involved in the infant's care. If results are normal, the case can be closed. If results are not normal and the diagnosis is confirmed, the infant must be entered into the appropriate long-term treatment and case management program, and the appropriate information must be entered into the database.

If the nature of the screening test abnormality does not require immediate referral for consultation and definitive laboratory testing, the NBS program may request an additional specimen. A mechanism should be in place to identify and test such specimens on receipt without delay. The system must be able

to ascertain when additional specimens are not received within the appropriate time frame. Further follow-up action must then be taken until a satisfactory specimen is obtained or the case is designated "lost to follow-up." Results of tests on the requested repeat specimen must be communicated to the PHCP, birthing facility, and family in a timely manner. If the result is normal, the case can be closed. If the result is again abnormal, further action must be taken until the case is resolved.

**UNSATISFACTORY SPECIMENS.** Approaches to the follow-up of unsatisfactory specimens vary and may include: (1) noting that the specimen was unsatisfactory on the laboratory report, (2) sending a letter from the laboratory to the submitter of the specimen, (3) telephoning the submitter to request a repeat specimen, and/or (4) treating the unsatisfactory sample with the same intensity as one with a screen-positive result. Programs may assign responsibility for follow-up of unsatisfactory specimens to laboratory or follow-up personnel or to the specimen submitter. Regardless of the locus of responsibility for the follow-up of unsatisfactory specimens, some mechanism should be in place to ensure that a satisfactory specimen is received within a reasonable time. Additional action should be taken until a satisfactory specimen is received or the case is designated as lost to follow-up. A written protocol should outline the appropriate actions or length of time for a case to be considered lost to follow-up.

**ROUTINE SCREENING OF SECOND SPECIMENS.** Some programs mandate routine screening of a second specimen. Routine screening of second specimens identifies a small number of additional infants with metabolic disorders, approximately 6% to 12% of infants with hypothyroidism requiring treatment, and some infants with non-salt-wasting CAH. Routine screening of second specimens helps the program ascertain

the rate of false-negative results on the initial screening test, including biologic false-negative results. Second screens are also valuable for disorders in which the indicator metabolites rise relatively slowly, as in homocystinuria, tyrosinemia, and some forms of hyperphenylalaninemia. For infants with a very short postpartum birthing facility stay, routine second screens help ensure adequate testing. When costs are calculated for screening of second specimens, a model that assumes an existing NBS program and calculates the additional cost generated by the second specimen should be used.

### *Communication*

**WITHIN THE NEWBORN SCREENING PROGRAM.** Rapid communication among the various components of the NBS program is essential for timely follow-up and for the entire NBS program to be successful. One approach to facilitating rapid communication is to electronically link all NBS system components. The laboratory is responsible for promptly notifying follow-up personnel of laboratory results that are not normal. Follow-up personnel should share outcome information with laboratory personnel as soon as it becomes available. NBS follow-up personnel should be able to use protocols flexibly, contacting consultants as appropriate for unusual and emergency situations.

**BETWEEN NEWBORN SCREENING PROGRAMS.** Generally, infants are screened initially by the state in which they are born. However, a significant number of babies born in some states are from families that live in a neighboring state. NBS follow-up personnel are responsible for working with colleagues in other states to ensure that all infants are adequately tested and that all screen-positive test results are followed up appropriately. Protocols should provide for information transfer between concerned state programs in which residence issues are involved. All test results must be

shared so that both programs may ensure final disposition of all cases.

**WITH THE PRIMARY HEALTH CARE PROVIDER AND FAMILY.** NBS personnel should explain the test result, the disorder, the recommended diagnostic workup, and the general principles of treatment to the health care provider. The nature of screening, the difference between screening tests and diagnostic tests, and the occurrence of false-positive test results should be explained when necessary. NBS follow-up personnel should explain these issues to parents in lay language. A compassionate and supportive manner is essential when speaking with parents. As noted previously, the PHCP generally should be notified first. PHCPs should be encouraged to make the initial contact with the parents in the event of a screen-positive test result. Contact by the PHCP may be less alarming for the family than a call from an unknown person associated with the NBS program. PHCPs should be made aware of the possibility of false-negative results, the limitations of the screening panel, and the design of the NBS system through general educational materials and programs provided by the system. The PHCP is responsible for notifying the NBS program of any child with a positive diagnosis who was not detected by the NBS program.

Programs should have protocols in place to deal with problems that frequently occur in following up infants with screen-positive results, which include:

- Inability to identify or locate the PHCP
- Families without a PHCP
- Communication with families who do not have telephone service
- Communication with families whose first language is not English
- Communication with adoptive or foster families or with responsible agencies when children are placed at birth or soon thereafter
- Inability to locate the family, even when a home visit is attempted

- PHCPs who refuse to take the program's recommended action
- Refusal of the patient's insurer to cover the recommended evaluation
- Families unable to pay for diagnostic testing or long-term care

### *Documentation*

Documentation is an essential function of follow-up staff and personnel in all NBS program components. Documentation reflects the decisions made during the follow-up process and provides evidence of internal and external actions. All actions taken, such as telephone calls and letters, should be cited in the infant's screening record in the program database. The record should document who performed the action, when it was taken, and to whom and where it was directed. The result of the action taken should also be recorded and, if necessary, a plan for further action stated. Such documentation should be concise, legible, and protected as confidential, privileged information. All protocols, policies, and end points should be defined clearly and communicated widely. Systematic collection of data and organized documentation are necessary for problem solving, research, and evaluation of program processes and outcomes.

### *Newborn Screening Follow-up Checklist*

Checklists can be valuable tools for NBS follow-up personnel and might include the following items.

#### **PREPARATION FOR EFFECTIVE FOLLOW-UP**

- Prepare and continually update a contact list of sample submitters, PHCPs, and consultants
- Include useful follow-up information on the laboratory submission form, including the name of the patient's PHCP and his/her phone number
- Provide appropriate program information (eg, pamphlets, prac-

itioner's manual) to all professionals concerned with follow-up

- Prepare a detailed protocol of follow-up procedures for each disorder included in the screening program and ensure that all backup personnel are familiar with the protocols
- Make the program visible to professionals through participation in medical meetings and through local seminars
- Establish a working link with the screening laboratory such that results are received in the most timely and effective way

#### **SHORT-TERM FOLLOW-UP ACTIVITIES**

- Regularly monitor for phone calls to be made for new cases
- Check and send follow-up letters with appropriate documentation daily
- Update case records and pursue other appropriate actions
- Close cases that meet closure criteria
- Send appropriate materials to all persons concerned with new cases

#### **LONG-TERM FOLLOW-UP ACTIVITIES**

- Document that each patient has an appropriate medical home
- Maintain accurate case management records for program evaluation
- Make periodic contact with the patient's family or physician to ascertain long-term outcome
- Facilitate access to parent education activities (eg, parent support groups, newsletters, camps)

#### **EVALUATION ACTIVITIES**

- Review records regularly for efficient operation, including time from receipt of screen-positive test result to initiation of treatment and "lost to follow-up" cases
- Contribute data to the evaluation component
- Use information to improve the overall program

### III. DIAGNOSIS

#### *Diagnostic Confirmation*

Confirmation of the diagnosis is the third component of the NBS system. A positive screening result requires assessment of the infant by a qualified physician and laboratory in a time frame appropriate for the disorder. Accurate and rapid diagnostic confirmation and treatment of patients detected by NBS systems are essential for optimal patient outcomes. All diagnostic test results, normal and abnormal, must be reported to the follow-up and evaluation components of the NBS system. Many disorders identified by NBS programs are complicated by their underlying heterogeneity. These variations require specialized diagnostic interpretation and individualized treatment. Confirmatory diagnostic testing requires prompt interpretation and communication of the results to the PHCP and to specialty consultants.

It is imperative to determine whether a child with any positive screening test result received a blood transfusion(s), and if so, the date(s) of the transfusion(s). This information is critical to properly interpret the results of screening and diagnostic studies.

Diagnostic laboratories and treating physicians must collaborate with the NBS system's evaluation component by providing information about results of the diagnostic tests. It is essential that the diagnostic laboratories meet all pertinent regulations regarding laboratory testing, including level of training of personnel, quality control, and testing proficiency. Validation of screening and diagnostic tests requires a system for evaluating NBS data. Specific issues for particular disorders are discussed individually.

#### *Staffing*

Laboratory directors must be experienced in the analysis and interpretation of appropriate analytes and in the training and supervision of laboratory personnel. Laboratory directors and tech-

nical supervisors must meet the training and experience requirements specified by the Clinical Laboratory Improvement Amendment of 1988 (CLIA88) or more current national rules. For the diagnosis of metabolic disorders, certification in clinical biochemical genetics by the American Board of Medical Genetics or its equivalent is highly recommended. Laboratories must be staffed with sufficient personnel to provide rapid analysis, interpretation of the results, and communication of results to the experienced management team. For certain disorders, laboratory diagnostic services must be available at all times.

#### *Laboratory Testing and Quality Control*

Diagnostic laboratories must have appropriate, current protocols and equipment for the rapid analyses essential to these conditions. Procedures must be in place to verify normative data, to document accuracy and precision of each method, and to maintain acceptable preanalytical, analytical, and postanalytical performance. Such procedures must include appropriate use of internal and external standards and analysis of positive and negative controls. The sensitivity, specificity, and positive and negative predicative values of both screening tests and diagnostic tests must be integral components of laboratory control.

#### *Proficiency Testing*

Hematologic and endocrinologic proficiency testing is accomplished through the College of American Pathology, and DNA-based testing through CAP- or ACMG-sponsored proficiency programs. For metabolic diseases, diagnostic laboratories must participate in proficiency testing programs, such as the CAP or ACMG quality assurance programs in biochemical and molecular genetics. Laboratories can establish comparison procedures with other laboratories doing the same tests and run positive samples as unknowns. All diagnostic

tests should meet current CLIA and statutory standards.

#### *Interpretation*

For hematologic and endocrinologic diseases, physicians with expertise in these disorders should be responsible for selection of diagnostic tests and interpretation of results. For metabolic disorders, an MD or PhD certified in Clinical and/or Biochemical Genetics should be responsible for the interpretation of diagnostic metabolic test results. Diagnostic testing includes biochemical and, increasingly, molecular analysis. Collaboration between biochemical laboratories and molecular diagnostic laboratories is recommended. This collaboration is important for providing accurate, complete diagnosis and genetic counseling. Most of these disorders are autosomal recessive. Therefore, family testing may raise concerns about paternity, which should be discussed initially with the mother.

Diagnostic criteria are unique for each individual and each disease. Specifics for the diagnosis of each hematologic, endocrine, and metabolic disorder detected through NBS are addressed on the following pages. *Core* tests and procedures are those that are essential to rapidly confirm a diagnosis and to initiate treatment. *Supplemental* tests and procedures may be needed to refine the diagnosis of some children and for the purpose of genetic counseling. Although all tests and procedures may not be available at all centers, they should all be accessible to patients and families when needed. All resources should be available to all children regardless of the families' ability to pay and irrespective of location of diagnostic and treatment facilities.

#### *Sickle Cell Disease and Other Hemoglobinopathies*

The primary purpose of neonatal screening for hemoglobinopathies is the identification of infants with SCD. Neonatal screening also identifies infants with other hemoglobin variants,

some thalassemia syndromes, and carriers. The majority of NBS programs use isoelectric focusing of an eluate from a sample punched from dried blood spots. A few programs use high-performance liquid chromatography or cellulose acetate electrophoresis as the initial screening technique. A 2-tiered system is recommended, in which all initially abnormal screens are retested by using a second sample punched from the same dried blood specimen and another complementary electrophoretic technique, HPLC, immunologic test, or DNA-based assay.

Infants with screening results that suggest SCD or other clinically significant hemoglobinopathies require confirmatory testing based on analysis of a second specimen, in part as a check against sample misidentification. Some programs also recommend additional testing of infants with Bart's hemoglobin, indicative of an  $\alpha$ -thalassemia syndrome. Confirmatory testing may be carried out by the initial screening laboratory or by other laboratories that specialize in such procedures. As shown in Table III-1, some NBS test results, such as FS and FE, may indicate a number of different genotypes, with varying clinical manifestations and severity. Confirmatory testing, generally carried out by 6 weeks of age, may be diagnostic or may only confirm the hemoglobin phenotype obtained from the initial NBS laboratory. In many cases, additional diagnostic testing and/or family studies may be required to establish the correct diagnosis. The selection and timing of diagnostic testing and interpretation of results should be individualized and must be supervised by an expert in the diagnosis of hemoglobin disorders in childhood.

**SICKLE CELL DISEASE.** Hemoglobin FS in infancy is associated with a variety of genotypes with a wide range of clinical severity. Most infants with FS screening results have homozygous SCD (SS). All infants with FS should

**Table III-1.** Clinical Interpretation of Initial Hemoglobinopathy Newborn Screening Results

| SCREEN RESULTS  | POSSIBLE CONDITION   | CLINICAL MANIFESTATIONS  | SPECIALIZED CARE REQUIRED |
|---|--|--|---------------------------|
| FS  | SCD – SS   | Severe   | Yes                       |
|   | SCD – S $\beta^0$ -thal  | Severe   | Yes                       |
|   | SCD – S $\beta^+$ -thal <sup>a</sup>                                 | Mild to moderate   | Yes                       |
|   | SCD – S $\delta\beta$ -thal  | Mild   | Yes                       |
|   | S HPFH   | Usually asymptomatic   | No                        |
| FSC   | SCD – SC   | Moderate to severe   | Yes                       |
| FSA   | SCD – S $\beta^+$ -thal  | Mild to moderate   | Yes                       |
| FSo <sup>b</sup> ther   | SCD – SD, SO <sup>Arab</sup> , SC <sup>Harlem</sup> , S Lepore, etc. | Mild to severe   | Yes                       |
| FC  | CC   | Mild hemolytic anemia  | Varies                    |
|   | C $\beta^0$ -thal  | Mild hemolytic anemia  | Varies                    |
| FCA   | C $\beta^+$ -thal  | Asymptomatic   | No                        |
| FE  | EE   | Asymptomatic   | No                        |
|   | E $\beta^0$ -thal  | Mild to severe   | Yes                       |
| F only  | Homozygous $\beta^0$ -thal   | Severe thalassemia   | Yes                       |
|   | Premature infant   | Repeat screening necessary   | No                        |
| FA+Bart's   | $\alpha$ thal silent carrier   | Asymptomatic   | No                        |
|   | $\alpha$ thal minor  | Asymptomatic   | No                        |
|   | Hb H disease   | Mild hemolytic anemia  | Yes                       |
|   | Hb H Constant Spring   | Moderate hemolytic anemia  | Yes                       |
| FAS+Bart's, FAC+Bart's, FAE+Bart's, FE+Bart's, FAOther+Bart's | $\alpha$ thal with structural Hb variant                             | Clinical course depends on the structural variant (e.g., Hb E) and number of functional alpha globin genes | Varies                    |
| FAS   | Sickle cell carrier  | Asymptomatic   | No                        |
| FAC   | Hb C carrier   | Asymptomatic   | No                        |
| FAE   | Hb E carrier   | Asymptomatic   | No                        |
| FAOther   | Other Hb variant carrier   | Usually asymptomatic   | No                        |

<sup>a</sup>Quantity of Hb A at birth sometimes insufficient for detection.  
SCD = Sickle Cell Disease, thal = thalassemia.  
HPFH = Hereditary Persistence of Fetal Hemoglobin.  
Other = Other hemoglobin variant (e.g., Hb D, O<sup>Arab</sup>).  
Hb = Hemoglobin.

begin receiving prophylactic penicillin by 2 to 3 months of age. Penicillin is continued until at least 5 years of age for those with SS and S  $\beta^0$ -thalassemia but may be discontinued in selected infants after diagnostic confirmation of other genotypes. Diagnostic testing generally begins with the core tests and procedures shown in Table III-2, which should be carried out by 6 weeks of age. Supplemental tests and procedures are indicated in many cases. DNA analysis and/or family studies are informative early in infancy, whereas the mean corpuscular volume, quantitation of Hb A<sub>2</sub> and F, and

assessment of the cellular distribution of Hb F are generally less informative until sometime between 6 months and 2 years of age. The differentiation of genotypes is further complicated by the co-inheritance of  $\alpha$ -thalassemia in some infants. Typical results are shown in Table III-3, but exceptions occur. In all cases, specialized expertise is required for accurate diagnosis.

**OTHER HEMOGLOBINOPATHIES.** Non-sickle hemoglobinopathies comprise a heterogeneous group of disorders that require specialized expertise for accurate diagnosis. Table III-4 shows the

Table III-2. Diagnostic Evaluation for Sickle Cell Disease

| SICKLE CELL DISEASE (FS, FSC, FSA, FSOther) | DIAGNOSTIC EVALUATION  |
|---|--|
| Core Tests and Procedures                   | Hemoglobin separation (by at least 2 complementary methods)<br>Isoelectric focusing<br>HPLC<br>Cellulose acetate electrophoresis<br>Citrate agar electrophoresis<br>Serial CBC, MCV, reticulocyte counts   |
| Supplemental Tests and Procedures           | Examination of peripheral blood smear<br>Family studies: Hb separation, CBC, MCV, Hb A <sub>2</sub> , Hb F and/or DNA analysis on parents<br>DNA analysis of $\beta$ globin genes<br>Hb A <sub>2</sub> (column chromatography or HPLC)<br>Hb F (alkali denaturation, RID, or HPLC)<br>Cellular distribution of Hb F (e.g, Kleihauer-Betke or immunofluorescence) |

HPLC = High Performance Liquid Chromatography, CBC = Complete Blood Count, MCV = Mean Corpuscular Volume, RID = Radial Immune Diffusion.

appropriate diagnostic evaluation for most infants with NBS results that suggest clinically significant non-sickle hemoglobinopathies. Infants with FE require family studies, DNA analysis, or repeated hematologic evaluation during the first 1 to 2 years of life to differentiate EE, which is asymptomatic, from E  $\beta^0$ -thalassemia, which may be severe and require chronic blood transfusions. Infants with Hb F only may be healthy infants who do not yet show Hb A because of prematurity or may have homozygous  $\beta^0$ -thalassemia (thalassemia major) or another thalassemia syndrome. Such infants need follow-up because premature infants without Hb A need repeat screening to identify those with SCD and other hemoglobinopathies and because homozygous  $\beta$ -thalassemia may be a severe transfusion-dependent disorder. It is important to note that most infants with  $\beta$ -thalassemia syndromes are not identified by present NBS techniques. CORN's *Practical Guide to the Diagnosis of Thalassemia* has been published previously and is included in the bibliography.

**$\alpha$ -THALASSEMIA SYNDROMES.** The follow-up of infants with Bart's Hb is guided by the goals of the individual NBS program. Infants with Bart's Hb at birth may be silent carriers or have  $\alpha$ -thalassemia minor, Hb H disease, or Hb H Constant Spring (Table III-1). Further testing is indicated for infants who develop microcytic anemia. Family studies should be considered in selected populations at high risk for Hb H disease and Hb H Constant Spring (see discussion in the Management section). Silent carriers, the largest group with Bart's Hb at birth, have a normal complete blood count, whereas persons with  $\alpha$ -thalassemia minor generally show a decreased mean corpuscular volume with mild or no anemia. Infants with >10% Bart's Hb as determined by hemoglobin electrophoresis at birth and/or those who develop more severe anemia may have Hb H disease or Hb H Constant Spring. Such infants will require more extensive diagnostic testing, including some of the supplemental tests shown in Table III-5. Clinical observation over time and family studies may be informative.

### CARRIERS OF HEMOGLOBIN VARIANTS (FAS, FAC, FAE, FAOTHER).

Approximately 50 infants who are carriers of hemoglobin variants are identified for every one identified with SCD. The initial confirmation by the screening laboratory by means of HPLC, isoelectric focusing, other electrophoretic techniques, immunologic assays, or DNA-based assays, with the use of a second sample obtained from the initial filter paper specimen usually can confirm the carrier status accurately. Some programs routinely obtain a second specimen from the infant for confirmation, in part to exclude the possibility of a mislabeled specimen.

Carriers are generally asymptomatic, and thus identification is of no immediate benefit to the infant. However, parents can benefit from knowing the child's carrier status because the information may influence their reproductive decision making. Therefore, a mechanism should be in place so that all results of hemoglobinopathy NBS can be made available to the parents. Parents of infants who are detected to be carriers of hemoglobin variants should be offered education and testing for themselves and their family. Such testing may raise concerns about paternity and should not be performed without prior discussion with the mother. Carrier screening may require complete blood counts with red blood cell indices in addition to hemoglobin separation. Defining the parent's genotype may also require quantifying hemoglobin variants, Hb A<sub>2</sub>, and Hb F or DNA-based testing. Genetic counseling of the parents is based on their test results. CORN's "Guidelines for Follow-up of Carriers of Hemoglobin Variants Detected by Newborn Screening" have been published previously and are reproduced in the Appendix.

Table III-3. Sickle Hemoglobinopathies: Diagnostic Test Results

| Disorder                     | Hb Separation by 6 weeks of age <sup>1</sup> | Serial CBC, Reticulocytes           | Hematologic Studies by 2 Years |                                    |          |                   | DNA dot blot                  | Parents' Usual Phenotypes <sup>4</sup>     |
|------------------------------|--|-------------------------------------|--------------------------------|------------------------------------|----------|-------------------|-------------------------------|--|
|                              |  |                                     | MCV <sup>2</sup>               | Hb A <sub>2</sub> (%) <sup>5</sup> | Hb F (%) | Hb F Distribution |                               |  |
| SCD – SS                     | FS   | Hemolysis and anemia by 6-12 months | N or ↑ <sup>5</sup>            | <3.6 <sup>5</sup>                  | <25      | Heterocellular    | β <sup>s</sup>                | AS AS                                      |
| SCD – S β <sup>o</sup> -thal | FS   | Hemolysis and anemia by 6-12 months | ↓ <sup>5</sup>                 | >3.6 <sup>5</sup>                  | <25      | Heterocellular    | β <sup>A</sup> β <sup>s</sup> | AS A<br>↓MCV<br>↑A <sub>2</sub><br>N or ↑F |
| SCD – S δβ-thal              | FS   | Mild anemia by 2 years              | ↓                              | <2.5                               | <25      | Heterocellular    | β <sup>s</sup>                | AS AF<br>↓MCV<br>↓A <sub>2</sub> , ↑F      |
| S HPFH                       | FS   | No hemolysis or anemia              | N or ↓                         | <2.5                               | >25      | Pancellular       | β <sup>s</sup>                | AS AF<br>↑F                                |
| SCD – S β <sup>r</sup> -thal | FSA  | Mild or no anemia by 2 years        | N or ↓                         | >3.6                               | <25      | NA <sup>6</sup>   | β <sup>A</sup> β <sup>s</sup> | AS A<br>↓MCV<br>↑A <sub>2</sub><br>N or ↑F |
| SCD – SC                     | FSC  | Mild or no anemia by 2 years        | N or ↓                         | NA <sup>7</sup>                    | <15      | NA <sup>6</sup>   | β <sup>s</sup> β <sup>c</sup> | AS AC                                      |

Table shows typical results – exceptions occur. Some rare genotypes (e.g. SD, SO<sup>Arab</sup>, SC<sup>Harlem</sup>, S Lepore, SE) not included. SCD = Sickle Cell Disease, thal = thalassemia, N = Normal, ↑ = increased, ↓ = decreased.

1. Hemoglobins reported in order of quantity (e.g., FSA = F>S>A).
2. Normal MCV: ≥ 70 at 6 to 12 months, ≥ 72 at 1 to 2 years.
3. Hb A<sub>2</sub> results vary somewhat depending on laboratory methodology.
4. Table assumes the absence of uniparental disomy and that both parents are heterozygous. In some cases parents may be homozygous or compound heterozygous.
5. Hb SS with co-existent α thalassemia may cause ↓MCV and Hb A<sub>2</sub> >3.6%; however, the newborn screening sample from such infants may show Hb Bart's.
6. NA = not applicable – test not indicated.
7. NA = not applicable – quantity of Hb A<sub>2</sub> is usually not measured in presence of Hb C.

### Endocrinopathies

**CONGENITAL (PRIMARY) HYPOTHYROIDISM.** CH affects approximately 1 in 3500 newborns worldwide. In the United States, the incidence is higher in Hispanics and Native Americans and lower in African Americans. Approximately 80% of cases are accounted for by thyroid agenesis or dysgenesis. Several autosomal recessive forms of CH are associated with large thyroid glands and with avid uptake of iodine.

Programs vary in their choice of laboratory methodologies. Most US pro-

grams use measurement of thyroxine (T<sub>4</sub>) levels as the initial screening test, followed by screening a certain portion of samples exhibiting low T<sub>4</sub> levels with a test for thyrotropin (TSH). Other programs use TSH testing as the initial test and may or may not use secondary T<sub>4</sub> testing on a certain portion of the samples with elevated TSH. Still other programs use both T<sub>4</sub> and TSH testing on all samples received. Each approach has its advantages and disadvantages. Primary T<sub>4</sub> screening allows detection of additional conditions, including thyroxine-binding

globulin deficiency and secondary hypothyroidism, but generally is not considered as specific as TSH in detecting primary CH. Primary TSH screening may be more sensitive in differentiating newborns with thyroid dysgenesis and ectopy in cases of compensated hypothyroidism but may lead to higher numbers of false-positive results in samples collected before 12 hours of age because of a TSH surge at birth. A testing protocol involving determination of both T<sub>4</sub> and TSH levels in all specimens may provide the best approach to screening.

**Table III-4.** Diagnostic Evaluation for Other Hemoglobinopathies

| OTHER HEMOGLOBINOPATHIES<br>(FC, FCA, FE, F only) | DIAGNOSTIC EVALUATION   |
|---|---|
| Core Tests and Procedures                         | Hemoglobin separation (by at least 2 complementary methods)<br>Isoelectric focusing<br>HPLC<br>Cellulose acetate electrophoresis<br>Citrate agar electrophoresis<br>Serial CBC, MCV, reticulocyte counts  |
| Supplemental Tests and Procedures                 | Examination of peripheral blood smear<br>DNA analysis of $\beta$ globin genes<br>Family studies: Hb separation, CBC, MCV, Hb A <sub>2</sub> , Hb F, and/or DNA analysis on parents<br>Hb A <sub>2</sub> (column chromatography or HPLC)<br>Hb F (alkali denaturation, RID, or HPLC) |

HPLC = High Performance Liquid Chromatography, CBC = Complete Blood Count, MCV = Mean Corpuscular Volume, RID = Radial Immune Diffusion.

**Table III-5.** Diagnostic Evaluation for Alpha Thalassemia

| $\alpha$ THALASSEMIA<br>(FA+Bart's, FAS+Bart's, FAC+Bart's, FAE+Bart's, FE+Bart's, FAOther+Bart's) | DIAGNOSTIC EVALUATION  |
|--|--|
| Core Tests and Procedures  | CBC, MCV <sup>1</sup>  |
| Supplemental Tests and Procedures <sup>2</sup>   | Examination of peripheral blood smear<br>Reticulocyte count<br>Hemoglobin separation (by at least 2 complementary methods)<br>Isoelectric focusing<br>HPLC<br>Cellulose acetate electrophoresis<br>Citrate agar electrophoresis<br>DNA analysis of $\alpha$ and/or $\beta$ globin genes<br>Hb A <sub>2</sub> (column chromatography or HPLC)<br>Hb F (alkali denaturation, RID, or HPLC)<br>Hb H prep (e.g., brilliant cresyl blue)<br>Family studies: Hb separation, CBC, MCV, Hb A <sub>2</sub> , Hb F, and/or DNA analysis on parents |

1. Normal MCV  $\geq 70$  at 6 to 12 months,  $\geq 72$  at 1 to 2 years.

2. Generally indicated only for selected infants of Asian ancestry and/or those who develop anemia.

CBC = Complete Blood Count, MCV = Mean Corpuscular Volume, HPLC = High Performance Liquid Chromatography, RID = Radial Immune Diffusion.

Specimens for hypothyroid screening are best obtained after 24 hours to avoid the first-day postnatal surge in TSH. This physiologic phenomenon needs to be recognized and accounted for in both primary T<sub>4</sub> and primary

TSH screening methodologies. Infants with primary CH show a decline in T<sub>4</sub> and a rise in TSH during the first 2 weeks of life. Programs in which a routine second test is done at 1 to 2 weeks often report detecting up to 10% of

their cases on the second sample after a normal result on the first sample collected at the hospital. Although the percentage of cases detected by the second test is higher when measurement of T<sub>4</sub> is used as the primary screening test, some cases have also been detected by second testing when the initial TSH concentration was normal in a first sample collected at the hospital.

Table III-6 provides a synopsis of possible laboratory screening results and possible causes and actions. Characteristically, in primary CH, the T<sub>4</sub> concentration is low and the TSH concentration is elevated. In such cases, the PHCP of record should be notified, and the infant should be evaluated without delay. Rapid evaluation is indicated in term infants when the TSH level is extremely high regardless of the T<sub>4</sub> value. An initial evaluation should include a history and physical examination. Serum specimens are used to confirm diagnosis and to monitor thyroid hormone treatment. The laboratory evaluation done before treatment should include determination of total and/or free T<sub>4</sub>, TBG (if indicated), TSH and, optionally, antithyroid antibodies if a goiter or maternal history of autoimmune thyroid disease is present. The type of hypothyroidism can be determined through anatomic and functional assessment with 123 iodine or technetium Tc 99m uptake and scan. Perchlorate washout is used if thyroid tissue is identified and defects in thyroid hormone synthesis suspected. Ultrasound imaging can provide additional objective information about thyroid anatomy. Table III-7 indicates diagnostic tests for CH.

Prematurity complicates screening for primary hypothyroidism. The premature infant younger than 34 weeks is born with a low T<sub>4</sub> level, exhibits a minimal first-day TSH surge, and experiences a fall in total T<sub>4</sub> levels during the early weeks of life. Thus, different reference values are needed for prema-

ture infants. Most screening laboratories obtaining specimens at 2 different times recognize the fact that T<sub>4</sub> levels are normally lower in babies older than 7 days, and this must be taken into account in the normal ranges. Some premature infants with true, primary hypothyroidism have a delayed increase in serum TSH. Their disease may not be recognized unless serial specimens are obtained.

**CONGENITAL ADRENAL HYPERPLASIA.**

CAH is a family of disorders of steroid biosynthesis. Over 90% of severe classical cases result from a defect in the 21-hydroxylase gene, *CYP21*. This defect leads to low or absent 21-hydroxylase enzyme deficiency, so there is an inability to produce sufficient amounts of cortisol to inhibit release of corticotropin. The result is a buildup of cortisol precursors resulting in increased adrenal androgen production in utero. Cortisol deficiency may cause an inability to maintain adequate energy and blood glucose levels to meet the stress of injury and illness. Lethargy and coma may progress to death. Aldosterone deficiency causes dehydration by the loss of sodium and water in the urine. Serum sodium becomes low, and serum potassium becomes high. Clinical manifestations include vomiting, weakness, hypovolemic shock, and cardiac arrhythmias. Severe forms of CAH can be rapidly fatal; therefore, rapid diagnosis and intervention in the newborn are critical.

There are 3 pathophysiologic presentations: (1) genital ambiguity representing prenatal virilization of the female infant; (2) hyponatremic, hyperkalemic dehydration and failure to thrive as a result of aldosterone deficiency; and (3) progressive virilization and excessive linear growth and skeletal maturation as a result of excessive testosterone production. A fourth presentation may also exist in older children with the late-onset or cryptic form of CAH in which the symptoms are much milder and later in presenta-

**Table III-6.** Interpretations of Initial Newborn Screening Results for Congenital Hypothyroidism

| SCREEN RESULTS                         | PROBABLE CAUSE  | CLINICAL MANIFESTATIONS  | SPECIAL EVALUATION/CARE REQUIRED                        |
|--|---|--|---|
| T4 low, TSH high                       | Primary hypothyroidism<br>Maternal antibodies<br>Maternal medications (PTU, Iodine)                                     | Severe<br><br>None<br>None                                       | Diagnostic evaluation*                                  |
| T4 low, TSH normal                     | Hypothyroidism possible<br>Pituitary/hypothalamic disorder<br>Prematurity<br>TBG deficiency<br>Secondary hypothyroidism | None to severe<br><br>Severe<br><br>None<br>Variable<br>Variable | Rescreen; if confirmed, consider diagnostic evaluation* |
| T4 normal, TSH high                    | Hypothyroidism possible<br>TSH surge  | None to severe<br><br>None                                       | Rescreen; if confirmed, consider diagnostic evaluation* |
| TSH very high (with or without low T4) | Primary hypothyroidism<br>TSH surge   | Severe<br><br>None   | Diagnostic evaluation*                                  |

\* See Table III-7.

**Table III-7.** Diagnostic Tests for Congenital (Primary) Hypothyroidism

| CONGENITAL HYPOTHYROIDISM (PRIMARY)      | DIAGNOSTIC TESTS  |
|--|---|
| <b>Core Tests and Procedures</b>         | Serum total T4, T3 uptake, free T4 or T4 index, TSH   |
| <b>Supplemental Tests and Procedures</b> | Serum TBG<br>Anti-thyroid peroxidase and anti-thyroid globulin antibodies<br>Ultrasound imaging of thyroid<br><sup>125</sup> I or <sup>99</sup> Tc thyroid uptake and scan<br>Bone age<br>Molecular evaluation of thyroid dysgenesis and inborn errors of thyroid hormone synthesis |

T4 = thyroxine, T3 = triiodothyronine, TSH = thyroid stimulating hormone, TBG = thyroid-binding globulin.

tion. Milder forms of *CYP21* deficiencies, termed *non-classical CAH*, and other types of CAH not directly the result of 21-hydroxylase deficiency may not be detected by current NBS procedures. Although most programs are established to detect all cases of the most severe salt-wasting form of CAH, which usually have extremely high levels of 17-hydroxyprogesterone at birth, some cases of non-salt-wasting CAH

may go undetected because of more slowly rising or somewhat lower levels of 17-OHP. Programs in which a second test is performed at 1 to 2 weeks of age are more likely to detect non-classical cases and more cases of simple virilizing CAH.

In newborns, normal levels of 17-OHP vary with gestational age and stress, and possibly other factors. Individual screening programs vary with

**Table III-8.** Interpretations of Initial Newborn Screening Results for 17-OHP

| SCREEN RESULTS   | PROBABLE CAUSE                            | CLINICAL MANIFESTATIONS              | SPECIAL EVALUATION/CARE REQUIRED  |
|--|---|--------------------------------------|---|
| 17-OHP Extremely elevated<br>(Definition of "extremely" varies with gestational age and program)   | SW-CAH<br>SV-CAH                          | Severe<br>Severe                     | Urgent diagnostic evaluation  |
| 17-OHP Moderately elevated<br>(Definition of "moderately" varies with gestational age and program) | SW-CAH<br>SV-CAH<br>NC-CAH<br>Prematurity | Severe<br>Severe<br>Variable<br>None | Observe and rescreen;<br>2 abnormal screens,<br>move to diagnostic evaluation |

CAH = 21-hydroxylase deficient congenital adrenal hyperplasia, SW = Salt-Wasting, SV = Simple Virilizing, NC = Nonclassical, 17-OHP = 17-hydroxyprogesterone.

**Table III-9.** Diagnostic Tests for Classical 21-OH Deficiency (CAH)

| CLASSICAL 21-OH DEFICIENCY               | DIAGNOSTIC TESTS  |
|--|---|
| <b>Core Tests and Procedures</b>         | Serum sodium, potassium, chloride, CO <sub>2</sub> , and glucose<br>Serum 17-hydroxyprogesterone<br>Physical examination for dehydration, shock, lethargy, irritability<br>Examine genitalia (visualize vaginal introitus, palpate scrotum for testes)  |
| <b>Supplemental Tests and Procedures</b> | Cortisol, 11-deoxycortisol, testosterone, and androstenedione<br>BUN, renin, aldosterone, urinary sodium, and potassium<br>Pelvic ultrasound and/or genitogram<br>Karyotype and Y chromosome DNA testing<br>DNA characterization of CYP21 defect<br>ACTH stimulation study if baseline serum 17-OHP is moderately elevated, and salt-wasting and virilization not present |

respect to follow-up action levels, depending on the commercial kit in use for laboratory testing and individual experiences in case determination. Normal ranges depend on gestational age and are lower for term newborns with normal birth weight. Most programs use 2 levels of follow-up action, depending on whether the elevated 17-OHP level falls into a moderate- or high-risk category. Moderately elevated 17-OHP is usually defined as  $\geq 40$  ng/dL in term infants and  $\geq 65$  ng/dL in premature infants. Extremely elevated 17-OHP is usually defined as  $\geq 100$  ng/dL in term infants and  $\geq 200$  ng/dL in premature infants. Possible actions based on screening results are listed in Table III-8.

In cases requiring immediate action, positive screening test results for CAH should be followed by immediate notification of the PHCP of record. The PHCP should be informed of the emergency nature of this disease, and an immediate history should be obtained and a physical examination should be performed with emphasis on feeding history, vomiting, urination, weight change since birth, presence of dehydration or hypovolemic shock, and correctness of sex assignment. Table III-9 presents core procedures for diagnosing CAH, which include measurement of serum 17-OHP, electrolytes to detect low sodium and high potassium levels present in the salt-wasting form, and other metabolites

and steroids. Because of the severity of the disease, a presumptive diagnosis should initiate rapid treatment.

Supplemental diagnostic tests include those for cortisol, 11-deoxycorticosterone, testosterone, androstenedione, blood urea nitrogen, renin, aldosterone, urinary sodium, and potassium. Karyotype and Y chromosome DNA testing may be done when indicated in the differential diagnosis, as well as a pelvic sonogram and genitogram in virilized female infants. DNA testing for characterization of 21-hydroxylase alleles is optional. Corticotropin stimulation with measurement of 17-OHP levels should be done when indicated. Pediatric endocrinologists will typically be the primary subspecialists involved in the metabolic management of patients with CAH.

### Metabolic Diseases

**HYPERPHENYLALANINEMIA AND PHE-NYLKETONURIA.** *Hyperphenylalaninemia* is the term applied to the persistent elevation of plasma phenylalanine concentrations. Although PKU implies hyperphenylalaninemia in the untreated state, not all hyperphenylalaninemia is PKU. The clinical distinction between PKU and hyperphenylalaninemia is arbitrary and rests on higher plasma phenylalanine values in PKU ( $>1000$ - $1250$  mmol/L or  $>16.5$ - $20.5$  mg/dL) and a lower tolerance for dietary phenylalanine in PKU.

Hyperphenylalaninemia represents a range of disorders. The most common forms are caused by varying degrees of impairment of the phenylalanine hydroxylase apoenzyme. Absence or severe deficiency of PAH will result in rapid elevation of blood phenylalanine levels with protein ingestion or with a catabolic state, usually within the first 24 hours of life. However, infants with residual PAH activity may take longer to develop abnormal phenylalanine levels, and the elevations may be less severe. It is for this reason that the American Academy of Pediatrics recom-

mends a repeat test for all infants initially screened in the first 24 hours of life.

Approximately 2% to 5% of all infants with hyperphenylalaninemia have impaired dihydropteridine reductase or bipterin biosynthesis. Because bipterin is required in other metabolic pathways, specialized therapy is required to prevent neurologic damage or death. With the exception of mild phenylalanine hydroxylase deficiency, all of these conditions will produce mental retardation if not diagnosed and treated in early infancy.

Women with any significant degree of hyperphenylalaninemia have an increased risk of miscarriage, and their offspring, who do not necessarily have PKU, may have intrauterine growth retardation that persists after the postnatal period. If these women are not treated with a phenylalanine-restricted diet before conception and during pregnancy, more than 90% of their infants will have microcephaly, mental retardation, and/or congenital heart defects. These infants also have a transient elevation of phenylalanine that falls to normal within 24 hours of birth.

Currently, 57 NBS laboratories in the United States participate in a national NBS quality assurance program. Of these laboratories, 20 consider a blood phenylalanine value of 4 mg/dL (250  $\mu\text{mol/L}$ ) their first decision level for dividing positive test results (ie, those that are followed with further testing) from negative test results. Except for one laboratory that uses a cutoff value of 6 mg/dL (375  $\mu\text{mol/L}$ ), the remaining 36 NBS laboratories use phenylalanine cutoff values from 2 mg/dL (125  $\mu\text{mol/L}$ ) to 4.3 mg/dL (~270  $\mu\text{mol/L}$ ) phenylalanine. Clinical interpretations of abnormal screening results are outlined in Table III-10. A positive screening test result requires rapid evaluation of the newborn with respect to clinical status, age, and diet at the time of sample collection.

Core diagnostic tests for phenylalanine (Table III-11) require quantitative

**Table III-10.** Clinical Interpretation of Newborn Screening Results: Phenylalanine

| SCREEN RESULTS                           | POSSIBLE CONDITION   | CLINICAL MANIFESTATIONS  | SPECIAL EVALUATION/CARE REQUIRED     |
|--|--|--|--------------------------------------|
| Borderline<br>2-4 mg/dL<br>phenylalanine | Possible Hyperphe/PKU<br>Variant forms of PKU<br>Other metabolic disease<br>False positive                   | Usually severe<br>Mild to severe<br>Usually severe<br>None                   | Yes<br>Varies<br>Yes<br>No           |
| Abnormal<br>>4 mg/dL<br>phenylalanine    | Probable Hyperphe/PKU<br>Variant forms of PKU<br>Mother has PKU<br>Other metabolic disease<br>False positive | Usually severe<br>Mild to severe<br>Mild to severe<br>Usually severe<br>None | Yes<br>Varies<br>Varies<br>Yes<br>No |

**Table III-11:** Diagnostic Criteria for Hyperphenylalaninemia and PKU

| HYPERPHENYLALANINEMIA AND PKU            | DIAGNOSTIC CRITERIA  |  |
|--|--|--|
| <b>Core Tests and Procedures</b>         | Quantitative blood phenylalanine and tyrosine analysis                               | Phenylalanine above 250 $\mu\text{mol/L}$ or 4.0 mg/dL <sup>1</sup> and tyrosine at normal or below normal concentration |
|  | Red blood cell DHPR <sup>2</sup><br>Urine pteridine profile: bipterin plus neopterin | Reduced activity<br>Abnormal = Variant form  |
| <b>Supplemental Tests and Procedures</b> | Complete plasma amino acids <sup>3</sup>   | Elevated phenylalanine & normal or reduced tyrosine  |
|  | Quantitative organic acid analysis (urine)   | Elevated phenylketones   |
|  | DNA analysis   | Specific mutations found in the PAH gene   |
|  | Phenylalanine- <sup>13</sup> C Oxidation (Breath test)                               | Less than 10% of a dose expired as <sup>13</sup> CO <sub>2</sub>   |
|  | Parental heterozygote testing (Blood amino acids)                                    | Elevated Phe <sup>2</sup> /Tyr ratio   |

1. Some specialists consider values below this as needing further sequential evaluation.

2. DHPR = dihydropteridine reductase.

3. Considered as a core procedure by some specialists.

determination of plasma phenylalanine and tyrosine concentrations. If phenylalanine is elevated, then erythrocyte DHPR activity must be determined and urine for analysis of a pteridine profile must be obtained. Further diagnostic and management plans depend on the outcome of these diagnostic tests and the clinical evaluation of the infant. Implementation of management is urgent because the ultimate cognitive ability of the child is related to the age at which blood phenylalanine is reduced to therapeutic levels. Once the

diagnosis of hyperphenylalaninemia is confirmed, metabolic control should be achieved as rapidly as possible, ideally within the first 2 to 3 weeks of life. To diagnose hyperphenylalaninemia and PKU, the diagnostic laboratory must include ion-exchange chromatography or another accepted method for quantifying plasma phenylalanine and tyrosine, erythrocyte DHPR assay, and urinary pteridine profile.

Supplemental tests that may help in further diagnosis and nutritional management include a complete amino acid

**Table III-12.** Clinical Interpretation of Newborn Screening Results: Galactosemia

| SCREEN                                | RESULTS*               | INTERPRETATION    | CLINICAL MANIFESTATION | SPECIAL EVALUATION/CARE REQUIRED |
|---------------------------------------|------------------------|-------------------|------------------------|----------------------------------|
| Galactose Metabolites and GALT Enzyme | <u>GAL</u> <u>GALT</u> | NL                | None                   | None                             |
|                                       | NL    NL               | POSITIVE (URGENT) | SEVERE                 | URGENT                           |
|                                       | Any    Absent          | POSITIVE          | Can be severe          | Yes                              |
|                                       | Elevated    Reduced    | NL (equivocal)    | Usually none           | Varies                           |
|                                       | Elevated    NL         | NL (equivocal)    | Usually none           | Varies                           |
| GALT Enzyme only                      | NL    Reduced          | POSITIVE          | Can be severe          | Yes                              |
|                                       | –    Absent            | POSITIVE (URGENT) | SEVERE                 | URGENT                           |
|                                       | –    Reduced           | POSITIVE          | Can be severe          | Yes                              |

Absent: enzyme activity absent.  
 Any: any value of galactose metabolites.  
 Elevated: above normal range of galactose metabolites.  
 NL: normal.  
 Reduced: reduced GALT enzyme activity.  
 GAL: galactose.  
 GALT: transferase enzyme.  
 POSITIVE (URGENT): high priority follow-up. Immediate clinical evaluation, diagnostic studies, and immediate change to a lactose-free formula.  
 POSITIVE: high priority follow-up. Immediate clinical evaluation, diagnostic studies, and consider change to a lactose-free formula.  
 NL (equivocal): low-priority follow-up. Determine clinical status and consider change to a lactose-free formula.  
 \*If the infant was transfused prior to the sample collection, the measured GAL and GALT will reflect the donor red blood cells. Adjust interpretation and respond as needed.

**Table III-13.** Diagnostic Criteria for Galactosemia (GALT Deficiency)

| GALACTOSEMIA (GALT DEFICIENCY)           | DIAGNOSTIC CRITERIA  |
|--|--|
| <b>Core Tests and Procedures</b>         | RBC gal-1-p and/or galactose    ≥ 10 mg/dL or above upper limit of normal for lab  |
|  | GALT enzyme analysis    ≤ 5% of control  |
| <b>Supplemental Tests and Procedures</b> | GALT isozymes <sup>1</sup> (Isoelectric focusing)    Abnormal biochemical phenotype                                      |
|  | DNA of GALT    Specific mutation(s) in GALT (Proband and parents, e.g., Q188R)   |
|  | Urine galactitol    ≥ 100 mmol/mol creatinine  |
|  | Total body galactose - <sup>13</sup> C oxidation (2 hr. breath test)    ≤ 15% of a dose as <sup>13</sup> CO <sub>2</sub> |

1. Some specialists consider this to be a core procedure.

profile, urine organic acid analysis, DNA mutational analysis for the specific mutations in the *PAH* gene, and quantifying carbon 13-labeled phenylalanine oxidation to <sup>13</sup>CO<sub>2</sub> in a breath test. Evaluation of parental *PAH* genotypes by biochemical or molecular methods may be useful in diagnosis, prognosis, and genetic counseling. Core and supplemental tests and procedures for the diagnosis of hyperphenylalaninemia and PKU are summarized in Table III-11.

**GALACTOSEMIA (GALACTOSE-1-PHOSPHATE URIDYL TRANSFERASE DEFICIENCY).** Galactosemia is a variable group of disorders identified by positive results of NBS tests of erythrocyte galactose-1-P-uridyl transferase with or without assessment of precursors of this blocked reaction. When the GALT fluorescent test is used, positive screening tests are classified as “absent” or “indeterminate.” In classical galactosemia, the combined level of precursors, galactose and galactose-1-phos-

phate (gal-1-P), from impaired GALT is usually greater than 10 mg/dL. When assessment of galactose and its metabolites is part of the newborn screen, interpretation of the positive screen is more complex and has implications for diagnostic testing and immediate management while results of the confirmatory studies are pending.

All newborns with positive screens should be rapidly evaluated by an experienced clinician for signs of jaundice, hepatomegaly, vomiting or feeding intolerance, and sepsis. The untreated classical disease rapidly progresses to hepatic toxicity and potential death from sepsis or bleeding. The need for rapid and correct diagnosis and immediate restriction of dietary galactose is critical. A symptomatic child should be brought in on an emergency basis for rapid evaluation and, if indicated, care in a neonatal or pediatric intensive care facility. Restriction of galactose in the diet is strongly recommended until the extent of the enzyme deficiency is confirmed. Infants with untreated classical galactosemia usually die, and infants treated late are at high risk for mental retardation, spasticity, and cataracts. Patients who are treated promptly usually have normal survival and improved neurodevelopmental outcomes.

Infants with normal GALT screens and elevated total galactose or positive GALT screens and normal total galactose require further diagnostic testing and treatment appropriate to each screening and diagnostic program. The clinical interpretations of screening results for galactosemia are shown in Table III-12. It is imperative to determine whether a child with any positive screening test result for galactosemia received a blood transfusion(s), and if so the date(s) of the transfusion(s). This information is critical for proper interpretation of the results of screening and diagnostic studies. During the course of the diagnostic evaluation, it is not uncommon to identify carriers. These families should be offered genetic counseling.

Core studies (Table III-13) require blood for quantitative analysis of the GALT enzyme and of RBC galactose and galactose-1-phosphate. Supplemental studies can be useful in management and genetic counseling. These include measurement of GALT isozymes and levels of urinary galactitol, which continue to remain significantly elevated, even during a galactose-restricted diet. There is increasing evidence that the GALT genotype may predict the phenotype. DNA analysis of the *GALT* gene is now available to determine which of the more than 130 known mutations are present. These mutations may have variable effects on GALT function and outcome of the patient with galactosemia. Some mutations in the *GALT* gene show tissue specificity, and erythrocyte GALT may not reflect total body oxidation of galactose. Therefore, the breath test of oxidation of galactose-<sup>13</sup>C to <sup>13</sup>CO<sub>2</sub> can be a valuable supplemental tool. Evaluation of parental GALT with appropriate genetic counseling may also be done as part of the diagnostic and management components. The core and supplemental studies needed for the diagnosis of galactosemia are listed in Table III-13.

**MAPLE SYRUP URINE DISEASE OR BRANCHED-CHAIN KETOACIDURIA.**

MSUD can be a rapidly fatal disease; therefore, immediate follow-up, diagnosis, and intervention are critical. Among the 16 NBS laboratories participating in a national NBS quality assurance program in 1997, the range of cutoff values for blood leucine was from 2 to 4 mg/dL. A blood leucine of 4 mg/dL (~300 μmol/L) or greater on a screening specimen requires immediate examination of the infant to determine whether abnormal feeding and signs of encephalopathy are present. These signs include a high-pitched cry, irritability, seizures, spasticity, and coma. If any of these signs or symptoms are present, all protein sources should be stopped immediately, adequate fluids and calories should be replaced, and further evalua-

**Table III-14.** Clinical Interpretation of Newborn Screening Results: Leucine

| SCREEN  | RESULT                  | POSSIBLE CONDITION | CLINICAL MANIFESTATIONS | SPECIAL CARE REQUIRED |
|---------|-------------------------|--------------------|-------------------------|-----------------------|
| Leucine | ≥ 4 mg/dL (~300 μmol/L) | MSUD               | Severe                  | URGENT                |
|         |                         | Variante MSUD      | None to severe          | Yes                   |
|         |                         | Premature infant   | Varies                  | Varies                |
|         |                         | False positive     | None                    | No                    |

tion should continue in a newborn or pediatric intensive care unit by an MD clinical or biochemical geneticist. Blood is analyzed for branched-chain amino acids (BCAA), and urine can be analyzed for presence of the characteristic organic acids (α-ketoisocaproate, α-ketoisovalerate, and α-keto-β-methylvalerate).

Some issues complicating the diagnosis of MSUD include the site and severity of the metabolic block and failure to identify allo-isoleucine. Very mild MSUD variants must be differentiated from classical MSUD. Other mild or intermittent variants of MSUD may not be detected by NBS. Timely diagnosis of the newborn with classical MSUD requires having the results of quantitative BCAA determination available within 24 hours of sampling. Clinicians skilled in the management of MSUD and access to neonatal intensive care facilities must be immediately available.

Premature or sick infants receiving hyperalimentation may have mildly elevated leucine levels caused by the amino acids in the intravenous solutions and/or immature metabolic systems. These infants must be differentiated from those with severe MSUD. Table III-14 shows the clinical interpretation of an abnormal newborn screen for leucine.

The presumptive diagnosis of MSUD requires the presence of elevated levels of the BCAAs leucine, isoleucine, and valine, including the pathognomonic amino acid, allo-isoleucine, and the demonstration of excessive amounts of their branched-chain organic acid derivatives. A presumptive diagnosis

should initiate rapid treatment. The confirmatory diagnosis requires deficient enzyme activity of the branched-chain α-ketoacid dehydrogenase in cultured fibroblasts or lymphoblasts.

Supplemental analysis of whole-body oxidation of <sup>13</sup>C leucine to <sup>13</sup>CO<sub>2</sub> by breath test to determine the degree of BCKAD impairment is available at some metabolic centers. DNA testing has not had clinical applicability, except among Mennonites, because most mutations are private and more than 6 genes are involved in the BCKAD multienzyme complex. Core and supplemental procedures are outlined in Table III-15.

**BIOTINIDASE DEFICIENCY.** Biotinidase deficiency is a potentially serious disorder that is associated with dermatitis, alopecia, neuropathy, mental retardation, seizures, deafness, and optic atrophy. Metabolic acidosis can result in coma, brain damage, or death. The enzyme defect affects the regeneration of the vitamin cofactor, biotin, and impairs the metabolism of all mitochondrial carboxylases. Affected infants may have symptoms soon after birth or, more commonly, remain asymptomatic for several months. Patients treated early are generally asymptomatic, although it is not clear whether the hearing or visual impairments are completely prevented.

Detection of enzyme activity in the screening laboratory is accomplished by a qualitative colorimetric assay. In the presence of the enzyme, a color change occurs. The clinical interpre-

**Table III-15.** Diagnostic Criteria for Maple Serum Urine Disease (MSUD)

| MSUD                                     | DIAGNOSTIC CRITERIA   |   |   |   |   |
|--|---|---|---|---|---|
| <b>Core Tests and Procedures</b>         | Blood: Quantitative amino acid analysis (µmol/L)  | Leucine<br>≥ 300 <sup>1</sup>           | Isoleucine<br>≥ 100                     | Valine<br>≥ 300                         | Alloisoleucine<br>Present   |
| <b>Supplemental Tests and Procedures</b> | Urine <sup>2</sup><br>Quantitative GC/MS (mmol/mg creatinine)<br><b>OR</b><br>Qualitative:<br>Leucine <sup>14</sup> C decarboxylation by cultured fibroblasts or lymphoblasts to <sup>14</sup> CO <sub>2</sub> <sup>2</sup><br>Leucine – <sup>15</sup> C – oxidation (breath test)<br><br>DNA analysis of the branched chain α-ketoacid dehydrogenase complex in specific ethnic groups | <u>α KIC</u><br><br>> 2<br><br>Elevated | <u>α KMV</u><br><br>> 2<br><br>Elevated | <u>α KIV</u><br><br>> 2<br><br>Elevated | ≤ 10% control<br><br>≤ 10% of a dose as <sup>15</sup> CO <sub>2</sub><br><br>Mutation(s) identified |

1. Some specialists consider values below this as needing further sequential evaluation.
2. Considered as core procedures by some specialists.
3. α KIC, α KMV, α KIV are respectively: α-ketoisocaproate, α-keto-β-methylvalerate, α-ketoisovalerate.

**Table III-16.** Clinical Interpretation of Newborn Screening Results: Biotinidase

| SCREEN RESULTS              | POSSIBLE CONDITION  | CLINICAL MANIFESTATION                     | SPECIAL EVALUATION/ CARE REQUIRED |
|-----------------------------|---|--|-----------------------------------|
| Color change does not occur | Biotinidase deficiency<br>Variant biotinidase<br>False positive | Mild to severe<br>None to moderate<br>None | Yes<br>Varies<br>No               |

**Table III-17.** Diagnostic Criteria for Biotinidase Deficiency

| BIOTINIDASE DEFICIENCY                   | DIAGNOSTIC CRITERIA   |  |
|--|-----------------------|--|
| <b>Core Tests and Procedures</b>         | Biotinidase in serum  | Less than 10% normal   |
| <b>Supplemental Tests and Procedures</b> | Urinary organic acids | Propionate and its metabolites, 3-methylcrotonylglycine, methylcitrate, lactate, and pyruvate may be present |

**Table III-18.** Clinical Interpretation of Newborn Screening Results: Methionine

| SCREEN RESULT                     | POSSIBLE CONDITION   | CLINICAL MANIFESTATIONS  | SPECIAL EVALUATION/ CARE REQUIRED                      |
|-----------------------------------|--|--|--|
| Methionine elevated (> 1-4 mg/dL) | Homocystinuria<br>Other defects of methionine metabolism<br>Other metabolic disorder<br>Liver disease<br>Prematurity<br>False positive | Usually severe<br>None to severe<br><br>Usually severe<br><br>Varies<br>Varies<br>None | Yes<br>Varies<br><br>Yes<br><br>Varies<br>Varies<br>No |

tation of an abnormal screening result for biotinidase is shown in Table III-16.

A positive screening test result is confirmed by direct measurement of biotinidase activity in serum. Affected individuals have less than 10% of normal activity; those with variants of biotinidase deficiency have 10% to 30% of normal activity. Supporting laboratory data include increased blood lactic and pyruvic acids and increased urinary 3-hydroxy isovaleric acid, 3-methylcrotonylglycine, 3-hydroxy propionic acid, and methylcitric acid. However, a significant percentage of children with clinically symptomatic biotinidase deficiency have normal urinary organic acid profiles. The core and supplemental tests and procedures are outlined in Table III-17.

**HOMOCYSTINURIA.** Failure to follow up, diagnose, and implement early therapy in infants with homocystinuria results in children with neurologic deficits, dislocated ocular lenses, glaucoma, bone mineralization abnormalities, and problems caused by vascular occlusion. Infants with homocystinuria are usually asymptomatic for the first few months of life.

Homocystinuria secondary to cystathionine-β-synthase deficiency is identified by an elevated blood methionine level on NBS. In 1997, the 13 NBS laboratories participating in a national NBS quality assurance program reported cutoff values for methionine from 0.9 to 2.0 mg/dL. However, cutoff values equal to or greater than 1 mg/dL blood methionine have been recommended by several reviewers. Screen-positive newborns should be referred to a metabolic center because the differential diagnosis of abnormalities in sulfur amino acid metabolism is complex. A significant portion of patients with classic CBS deficiency, especially those responsive to pyridoxine, will be missed by NBS because methionine rises slowly in the blood of affected infants. Screening

specimens collected in the first few days after birth may not be uniformly abnormal. Elevated methionine caused by CBS deficiency is often found on routine repeat screening. Other forms of homocystinuria do not result in elevated methionine and will not be detected by NBS.

Some screen-positive infants do not have CBS deficiency but may have a number of other problems requiring medical intervention. Infants with other metabolic conditions, such as galactosemia or tyrosinemia, may have elevated methionine levels. False-positive hypermethioninemia screening test results also occur as a result of excess infant protein intake, enzyme immaturity, liver disease, and variations in enzymes involved in the demethylation of methionine to homocysteine. It is important to differentiate among these inherited and environmental causes of hypermethioninemia. The clinical interpretation of positive screen for methionine is shown in Table III-18.

Initial core tests include measurement of plasma-free amino acids and total plasma homocysteine analysis, optimally carried out within 2 weeks of life. The diagnosis of CBS deficiency is probable if methionine is greater than 100 µmol/L, free homocystine is present in protein-free filtrates of plasma, and the total plasma homocysteine is above 25 µmol/L. Urine organic acid analysis is supplemental. Urinary excretion of methylmalonic acid is not increased in children with classic CBS deficiency. If excretion is increased, further evaluation for variant forms of homocystinuria is indicated.

Once a tentative diagnosis has been established, treatment should begin immediately. The diagnosis may be confirmed by enzyme assay in fibroblasts or other tissue. Molecular diagnosis is also available and may be useful in providing genetic counseling. Core and supplemental diagnostic studies and procedures are listed in Table III-19.

**Table III-19.** Diagnostic Criteria for Homocystinuria

| HOMOCYSTINURIA (CYSTATHIONINE-β-SYNTHASE DEFICIENCY) | DIAGNOSTIC CRITERIA  |  |
|--|--|--|
| <b>Core Tests and Procedures</b>                     | Quantitative blood amino acids<br>Total plasma homocysteine  | Methionine ≥ 100 µmol/L<br>Free homocystine: present<br>Cystine: Reduced<br>> 25 µµmol/L                 |
| <b>Supplemental Tests and Procedures</b>             | Urinary organic acids <sup>1</sup><br>Enzyme assay (fibroblasts, lymphoblasts, liver biopsy, etc.) <sup>1</sup><br>Molecular analysis of the cystathionine-β-synthase gene | No elevation in methylmalonic acid<br>Absent cystathionine-β-synthase activity<br>Mutation(s) identified |

1. Some specialists consider this a core test.

**Table III-20.** Clinical Interpretation of Newborn Screening Results: Tyrosine

| SCREEN RESULTS                    | POSSIBLE CONDITION      | CLINICAL MANIFESTATIONS | SPECIAL EVALUATION/CARE REQUIRED |
|-----------------------------------|-------------------------|-------------------------|----------------------------------|
| Tyrosine elevated (> 10-20 mg/dL) | Tyrosinemia Type 1      | Severe                  | Yes                              |
|                                   | Tyrosinemia Type 2      | Severe                  | Yes                              |
|                                   | Other metabolic disease | Usually severe          | Yes                              |
|                                   | Transient tyrosinemia   | None to mild            | No                               |
|                                   | Liver disease           | Varies                  | Varies                           |
|                                   | Prematurity             | Varies                  | No                               |
| False positive                    | None                    | None                    | No                               |

**Table III-21.** Diagnostic Criteria for Type I Tyrosinemia

| TYROSINEMIA (TYPE I AND II)              | DIAGNOSTIC CRITERIA  |   |
|--|--|---|
| <b>Core Tests and Procedures</b>         | Plasma amino acid analysis<br>Urine organic acid analysis <sup>1</sup>   | Tyr > 200 µmol/L<br>Methionine: usually elevated<br>Succinylacetone present in FAH deficiency<br>Tyrosine metabolites present in TAT deficiency                     |
| <b>Supplemental Tests and Procedures</b> | Serum α-fetoprotein<br>Urinary δALA<br>PBG synthetase in erythrocyte<br>Enzyme assay on fibroblasts or liver biopsy<br>Mutational analysis of FAH or TAT<br>cDNA from fibroblasts or liver | Elevated above 1000 IU/mL (FAH deficiency) (after 1 month of age)<br>Elevated (FAH deficiency)<br>Normal<br>FAH or TAT deficient activity<br>Mutation(s) identified |

1. GC/MS: Measuring levels of urinary succinylacetone by gas chromatography/mass spectroscopy requires specialized ion-specific monitoring or isotope dilution techniques.

FAH (fumarylacetoacetate hydrolase) deficiency = Type I tyrosinemia.

TAT (tyrosine aminotransferase) deficiency = Type II tyrosinemia.

**TYROSINEMIA TYPES I AND II.** Patients with untreated tyrosinemia Type I (fumarylacetoacetate hydrolase deficiency) develop nodular liver cirrhosis with or without splenomegaly during

infancy, generalized renal tubular disease (renal Fanconi's syndrome), and potentially fatal neurologic crises. There is a very high risk of hepatocellular carcinoma. Tyrosinemia Type II,

tyrosine aminotransferase deficiency, results in elevated levels of tyrosine and the development of palmoplantar keratosis and painful corneal erosions with photophobia. Mental retardation is present in about half of untreated patients.

Identification of infants with Type I or Type II tyrosinemia with the use of dried blood spots is problematic for several reasons: (1) there are many false-positive results, (2) transient tyrosinemia is common, and (3) true tyrosinemia may cause minimal elevation of tyrosine in the neonate.

Many causes of neonatal tyrosinemia confound the diagnosis of FAH deficiency. Transient tyrosinemia, TAT deficiency, and other causes of hypertyrosinemia, such as liver disease, are not accompanied by excretion of succinylacetone but are diagnosed clinically and by finding marked elevations in urinary para-hydroxy-phenyl acids. Premature infants or those receiving hyperalimentation may also have tyrosine elevations. Clinical information is useful for interpretation of the laboratory results. The clinical interpretation of a screen-positive result for tyrosine is shown in Table III-20.

The diagnosis of FAH deficiency is conditionally confirmed by plasma tyrosine greater than 200  $\mu\text{mol/L}$  and by detection of succinylacetone in the urine, after which treatment should begin. TAT deficiency is confirmed by elevated plasma tyrosine and increased excretion of tyrosine metabolites in the urine. The diagnosis of tyrosinemia Type I is confirmed by demonstration of deficient FAH activity in cultured fibroblasts or in a liver biopsy specimen. Core and supplemental tests and procedures are shown in Table III-21.

## IV. MANAGEMENT

Patients identified through NBS usually require lifelong treatment. The goal of treatment is to achieve optimal health, growth, and development. These diseases require clinical management by experts and experienced health care providers who understand their etiology, pathophysiology, clinical heterogeneity, and the psychosocial issues surrounding them. Close communication among the family, the PHCP, and specialists is essential for optimal outcomes. The entire system of care should be integrated to assess short- and long-term outcomes. The general principles for the management of specific diseases are outlined below.

### *Sickle Cell Disease and Other Hemoglobinopathies*

#### **SICKLE CELL DISEASE**

The term *sickle cell disease* encompasses a group of symptomatic disorders characterized by the predominance of sickle hemoglobin (Hb S) in RBCs. Four genotypes—Hb SS, Hb SC, sickle  $\beta^+$ -thalassemia, and sickle  $\beta^0$ -thalassemia—account for the majority of SCD in the United States. These disorders are characterized by chronic hemolysis, largely unpredictable acute complications that can rapidly become life-threatening, and the variable development of progressive chronic organ damage. Expert comprehensive medical care prolongs life expectancy and reduces morbidity by preventing disease-related complications or by limiting their severity and/or sequelae. The intent of this section is to provide an overview of the essential elements of appropriate medical management. Detailed guidelines regarding specific aspects of medical treatment have been published elsewhere and are included in the References section.

*Expertise of Health Care Providers.* SCD is a complex disorder with multisystem

manifestations. Appropriate treatment to ensure optimal outcome requires the active involvement of a pediatric hematologist with appropriate expertise, as well as professionals in nursing, social work, psychology, genetics, education, and counseling. Ideally, these individuals function as a multidisciplinary team that provides direct patient care. When such teams are not available locally, ongoing input from sickle cell experts should be obtained. PHCPs play an important role in the treatment of many patients with SCD. PHCPs who accept responsibility for such patients must be knowledgeable about current approaches to the medical management of SCD and arrange unimpeded access for patients to expert providers. Patients with SCD also need access to a wide variety of consultative, diagnostic, and therapeutic services, including those provided by specialists in pulmonology, gastroenterology, neurology, surgery, ophthalmology, and others.

The extent to which care is provided by the PHCP versus the multidisciplinary sickle cell team will vary among patients and communities and will depend on the expertise of the PHCP, access to the sickle cell team, family preference, and the frequency and severity of disease manifestations. Some patients may receive all of their care from the pediatric hematologist, whereas other patients receive day-to-day care from PHCPs with periodic comprehensive evaluations by the sickle cell team. Because acute illnesses may prove rapidly life-threatening, it is essential that they be managed initially by providers in the patient's community with appropriate knowledge and experience.

*Parental Health Education.* Identification of infants with SCD through neonatal screening affords an opportunity to educate parents and caregivers about the child's disorder before symptoms develop. The information should be provided in a sensitive and culturally appropriate manner by health care pro-

fessionals with expertise and experience in SCD. Written, audio, video, and/or computer-based information is a valuable adjunct to face-to-face education. Initially, the focus should include the genetics and basic pathophysiology of the infant's hemoglobinopathy and the importance of regularly scheduled health maintenance visits, prophylactic administration of penicillin, routine immunizations, and special immunizations such as pneumococcal vaccine. Education about the need for urgent medical evaluation for febrile illness and for signs and symptoms of acute splenic sequestration, aplastic crisis, acute chest syndrome, stroke, and other acute complications is critical. Education about splenic sequestration includes instruction about abdominal palpation for determining spleen size. In addition, patients and families should receive information on advances, newer treatments, and experimental therapies as they become available.

*Health Maintenance.* Age-appropriate anticipatory guidance regarding disease manifestations should be provided. Patients with SCD should receive all routine immunizations, including the new 7-valent conjugated pneumococcal vaccine, recommended by the Centers for Disease Control and Prevention and the American Academy of Pediatrics. In addition, the 23-valent polysaccharide pneumococcal vaccine should be administered at age 2 years and again at age 5 years to reduce the markedly increased incidence of pneumococcal sepsis. Some programs recommend subsequent administration of polysaccharide pneumococcal vaccine in older children and adults and/or immunization with meningococcal vaccine. Yearly influenza vaccine should be provided. All infants with SS and sickle  $\beta^0$ -thalassemia (FS by newborn screen) should begin penicillin prophylaxis at 125 mg twice a day by 2 to 3 months of age. The dose is increased to 250 mg twice a day at age 3 years and should be continued at least until the fifth birth-

day. Some centers recommend prophylactic penicillin for infants and children with Hb SC and sickle  $\beta^+$ -thalassemia. Some centers also recommend folic acid supplementation.

All patients should have regularly scheduled comprehensive medical evaluations to reinforce patient and family education, to review previous disease manifestations, to document important baseline physical findings (such as presence or absence of splenomegaly) and laboratory values (such as baseline hemoglobin and reticulocyte count), to monitor growth and development, to explore psychosocial issues, and to detect organ injury. School performance should be monitored for evidence of neurodevelopmental problems. Individualized patient care plans are developed by the sickle cell team in conjunction with the PHCP. Important baseline information should be provided to parents and caregivers. The frequency of comprehensive evaluations—including schedules for laboratory testing, radiographs and other investigations, and retinal examinations for older patients—will vary depending on clinical circumstances. Some patients will develop complications or show laboratory or imaging evidence of disease manifestations that warrant more intense, specific therapy such as chronic transfusions. Selection for and implementation of chronic transfusions or newer therapies, such as hydroxyurea and bone marrow transplantation, require the expertise of the sickle cell team.

**Acute Illness.** Comprehensive care must include a predetermined plan for unhindered, 24-hour access to timely expert treatment of acute illness. This is particularly important because acute illness characterized by relatively common childhood signs and symptoms can rapidly become life-threatening. These signs and symptoms include fever, cough, abdominal pain, pallor, and a limp. Thus, such illnesses require urgent evaluation and treatment in a

setting in which knowledge and perspective about SCD are available. Examples of urgent care that is frequently required include blood cultures and parenteral administration of antibiotics for febrile illness, RBC transfusions for acute anemic events, and aggressive, appropriately monitored analgesia for severe pain. Health care providers need ready access to baseline information about the patient, including results of previous physical examinations and baseline laboratory studies. This goal is facilitated by providing such information to parents. Parental education regarding the early recognition and appropriate medical evaluation of common acute complications will help ensure that patients receive appropriate care for acute illness. These complications include fever, pain, acute chest syndrome, acute neurologic events, priapism, and acute anemia indicative of splenic sequestration and/or aplastic crisis.

**Genetic Counseling.** Testing of potential carriers requires a complete blood count and hemoglobin separation, including quantitation of Hb F and Hb A<sub>2</sub> if the mean corpuscular volume is borderline or decreased. Solubility testing is inadequate and should never be used for genetic screening for hemoglobinopathies. The availability of carrier testing for family members should be discussed initially with the mother, with sensitivity to the possibility of mistaken paternity. Parents should be counseled after results from family testing are available. Counseling should be sensitive to the cultural and social needs of the family. It should include a review of the pathophysiology of the disease, recurrence risk information, and availability of prenatal diagnosis. Genetic education should be introduced to the patient during early adolescence.

**Psychosocial Support.** Comprehensive medical care includes periodic psychosocial assessments and the availability

of services needed to optimize the patient's and family's adaptation to chronic illness. Support groups and community-based organizations are important resources. Important issues include health insurance coverage, transportation for health care, and educational issues including the education of school personnel, compensating for school absence due to illness, and vocational planning. Patients should be given age-appropriate information about their disease throughout childhood and adolescence. Transition programs help prepare them to become knowledgeable, responsible health care consumers as adolescents and adults.

#### OTHER HEMOGLOBINOPATHIES

The non-sickle hemoglobinopathies detected by NBS comprise a heterogeneous group of disorders characterized by variable degrees of anemia secondary to hemolysis and/or ineffective erythropoiesis. Some, such as homozygous E, homozygous C, and C  $\beta$ -thalassemia, are mild conditions with few or no symptoms. Individuals with homozygous E are generally asymptomatic and have microcytic hypochromic RBCs with mild or no anemia. Individuals with homozygous C have a mild microcytic hemolytic anemia, which may be complicated by aplastic crisis, splenomegaly, or cholelithiasis. Thus, patients with homozygous C may benefit from periodic evaluation by a pediatric hematologist.

Hemoglobin E  $\beta^0$ -thalassemia and homozygous  $\beta^0$ -thalassemia are the 2 most common, potentially severe non-sickle hemoglobinopathies identified by NBS. The severity of E  $\beta^0$ -thalassemia varies from asymptomatic mild anemia to severe transfusion-dependent anemia. Most infants with homozygous  $\beta^0$ -thalassemia develop profound anemia during the first year of life and require chronic transfusions for survival. Infants with either disorder may require specialized treatment

by a multidisciplinary team with expertise in thalassemia. Important therapeutic considerations during infancy and childhood include frequent blood counts, monitoring growth and development, assessment of the need for chronic transfusions, and timing of splenectomy if indicated.

Medical treatment of patients who require chronic transfusions is complex, requires specialized expertise, and must be administered or closely monitored by a multidisciplinary team. Important therapeutic issues include chronic RBC transfusions, iron chelation therapy for transfusional hemosiderosis, and periodic monitoring for evidence of chelator-associated toxicity and for organ injury secondary to iron overload. Hypersplenism typically occurs by mid-childhood and usually requires splenectomy. Intensive educational and psychosocial support is required, particularly regarding compliance with iron chelation therapy. Bone marrow transplantation should be considered for patients who have HLA-identical sibling donors.

Patients who do not require chronic transfusions require periodic medical evaluations to provide patient and family education, to document baseline laboratory findings, and to monitor development of complications, including delayed growth, bone deformities, hypersplenism, cholelithiasis, and progressive iron overload. Acute exacerbations of anemia may occur with infection, aplastic crisis, and folic acid deficiency and may require transfusion support. Thus, periodic consultation with a hematologist knowledgeable in the management of thalassemia is strongly recommended.

All of these disorders have important and potentially complex genetic implications. Testing of parents and other family members should be offered. Accurate diagnosis may require DNA-based techniques specific to the ethnic background of the family. Genetic counseling is based on these test results.

### $\alpha$ -THALASSEMIA SYNDROMES

Individuals who are silent carriers for  $\alpha$ -thalassemia have a normal complete blood count and need no specialized evaluation or medical care. Individuals who have  $\alpha$ -thalassemia minor have microcytic hypochromic RBCs with mild or no anemia.  $\alpha$ -Thalassemia minor must be differentiated from iron deficiency and  $\beta$ -thalassemia minor. Therapeutic iron should not be administered to such individuals unless coexistent iron deficiency is documented.

Infants with Hb H disease have a mild to moderate thalassemic disorder, typically with baseline hemoglobin values between 7 and 10 gm/dL. Generally, such individuals are only mildly symptomatic. Folic acid supplementation is often recommended. Patients require ongoing monitoring of growth and development and for splenomegaly, hypersplenism, and cholelithiasis. Oxidant drugs, such as sulfamethoxazole, may precipitate a hemolytic crisis and should be avoided.

Infants and children with Hb H Constant Spring disease generally have a more severe disorder that is associated with moderately severe anemia. Folate supplementation and the avoidance of oxidant drugs are recommended. Splenomegaly develops during childhood, and hypersplenism may require splenectomy. Exacerbations of anemia during viral illness or aplastic crisis or as a result of hypersplenism may require RBC transfusions. Comprehensive management by a multidisciplinary team led by a pediatric hematologist is required.

In the United States,  $\alpha$ -thalassemias are encountered most commonly in persons of African, Mediterranean, and South and Southeast Asian ancestry. In African and most Mediterranean populations,  $\alpha$ -thalassemia minor is generally caused by homozygosity for deletion of 1 of the 2  $\alpha$ -globin genes on each chromosome 16. Thus, African American and most Mediterranean individuals generally

are not at risk for having children with hydrops fetalis or Hb H disease and do not require extensive genetic counseling or prenatal diagnosis. In contrast, common  $\alpha$ -thalassemia mutations in South and Southeast Asian populations result in deletion of one or both  $\alpha$ -globin genes on one chromosome 16. Deletion of both  $\alpha$ -globin genes also occurs in Greek, Cypriot, and Turkish populations. The Hb Constant Spring mutation occurs in Asians. Thus, some Asian and Mediterranean individuals with  $\alpha$ -thalassemia minor may be at risk for pregnancies complicated by Hb H disease or by fetal hydrops, which is lethal to the infant and causes severe obstetric complications for the mother, including toxemia, hypertension, and postpartum hemorrhage. Some Asians are also at risk for Hb H Constant Spring. DNA studies to determine the  $\alpha$ -thalassemia genotype are necessary for accurate genetic counseling and prenatal diagnosis.

### *Core Personnel*

- MD with clinical expertise in hemoglobinopathies
- MD or PhD with expertise in protein- and DNA-based diagnosis of hemoglobinopathies
- Nurse coordinator
- Social worker
- Hemoglobinopathy counselor
- Ophthalmologist

### *Additional Personnel, as Needed*

- Pediatric intensivist
- Clinical psychologist
- Neurologist
- Neuropsychologist
- Neuroradiologist
- Pulmonologist
- Gastroenterologist
- Urologist
- Pediatric surgeon
- Orthopedist
- Cardiologist
- Infectious disease specialist
- Otolaryngologist
- Physical therapist

*Core Resources*

- Inpatient facility, including pediatric intensive care unit and pediatric surgical and anesthesia services
- Acute care facility that provides 24-hour access to urgent care by providers with knowledge and experience in SCD and rapid triage, blood cultures, and parenteral administration of antibiotics, intravenous fluids, analgesics, and RBC transfusions
- Laboratory services, including hemoglobin separation and DNA diagnostic services
- Audiology evaluation
- Genetic counseling and prenatal diagnostic services
- Transfusion services, including minor antigen matching, erythrocytapheresis, and outpatient administration of chronic transfusions
- Home care services including administration of intravenous fluids, parenteral antibiotics, and chelation therapy
- Multidisciplinary pain team evaluation
- Radiology services, including ultrasonography, computed tomography, magnetic resonance imaging/magnetic resonance angiography, and transcranial Doppler ultrasonography neuroimaging
- Neuropsychologic evaluations and follow-up
- Phototherapy for proliferative retinopathy
- Financial resources to ensure adequate treatment and monitoring regardless of ability to pay
- Educational materials for patients, families, and schools

*Endocrinopathies***CONGENITAL (PRIMARY) HYPOTHYROIDISM**

Severe CH, if untreated, causes growth retardation and irreversible mental retardation. The keystone of manage-

ment of CH is replacement of L-thyroxine, the primary hormone produced by the thyroid gland. Daily administered L-thyroxine is converted by peripheral tissues to the more active thyroid hormone,  $T_3$ . Serum measurements are necessary to confirm that total and/or free  $T_4$  is maintained in the normal range and TSH is suppressed. Correct dosage is important because undertreatment can lead to impaired mental development and inhibited growth, and overtreatment can lead to hyperactivity and other negative consequences. In some instances, early diagnosed hypothyroidism may, in fact, be transient in nature, and a period of discontinued treatment and re-evaluation may be appropriate approximately at the age of 2 years. This determination should be made by a pediatric endocrinologist.

*Expertise of Health Care Providers.* Treatment of CH is not particularly difficult, but the approach to its management should involve a multidisciplinary team to ensure optimal outcome for the patient. In addition to the PHCP, this team should include a pediatric endocrinologist and, as needed, professionals in psychology, health education, genetics, counseling, and nursing. If such a team is not available locally, ongoing input from hypothyroidism experts should be obtained. Periodic evaluations of biochemical markers and developmental outcome are particularly important and should be well documented.

*Health Education.* It is important that parents receive clear and appropriate information about hypothyroidism, its outcome if untreated, and appropriate treatment protocols. A structured education program should instruct the parents on thyroid function, how it is altered in their child, and what  $T_4$  replacement will and will not do. The educational program must be customized to the needs of the family, including consideration of cultural issues

and educational abilities of family members. A mechanism for transmittal of updated treatment information should be a part of the program.

*Health Maintenance.* Patients with CH should receive routine medical care from their PHCP. Consultation with a specialist will provide optimal benefit. An initial developmental, psychosocial, and social work assessment should be conducted, and treatment should be started promptly. The initial dose should be sufficient to maintain normal free  $T_4$  or TSH levels and will require periodic adjustment.

Serum  $T_4$  and TSH levels should be monitored at appropriate intervals, and  $T_4$  dose should be adjusted under the supervision of a pediatric endocrinologist. Growth, bone age, and development should be monitored closely. The thyroid gland may be monitored for the very rare appearance of nodules and precancerous lesions.

*Genetic Counseling.* The causes of CH are not clearly defined. In some cases, particularly goitrous hypothyroidism, genetic factors are likely to be a major contributor. Genetic counseling, with appropriate concern for any paternity issues, should be offered to the parents if one of the heritable causes of hypothyroidism is identified. Counseling should be sensitive to the cultural and educational characteristics of the family and should clearly explain any genetic risks.

**CORE PERSONNEL**

- MD with clinical expertise in pediatric endocrinology

**ADDITIONAL PERSONNEL, AS NEEDED**

- MD or PhD with expertise in biochemical laboratory analysis
- Neuropsychologist
- Social worker
- Nurse coordinator
- MD clinical geneticist
- Genetic counselor

### CORE RESOURCES

- Laboratories with CLIA certification for quantitative  $T_4$ ,  $T_3$ , and TSH analysis
- Hormonal therapy
- Developmental assessment protocol
- Financial resources to ensure adequate treatment and monitoring regardless of ability to pay
- Family and patient disease-specific resource information

### CONGENITAL ADRENAL HYPERPLASIA.

Unrecognized and untreated salt-wasting CAH can result in hypovolemic shock and death within days after birth. Simple virilizing CAH can result in gender misclassification and subsequent related problems in untreated females, and precocious postnatal physical and sexual development in both untreated males and females. Emergency action is required for newborns identified by the NBS program with extremely elevated 17-OHP levels. Particular attention should be given to those who have failed to regain birth weight by 7 days of life, who have a serum sodium concentration below 130 mEq/L or serum potassium concentration above 7 mEq/L, or who are hypotensive or have cardiac arrhythmias. An endocrinology specialist should be immediately involved with all such cases, and emergency treatment should be closely monitored. Confirmatory serum testing should be an integral part of the diagnosis. In cases in which the newborn is already exhibiting salt-wasting symptoms, it may be appropriate to give intravenous hydrocortisone immediately and continually along with intravenous glucose and saline solution without potassium to maintain vascular volume. Exact concentrations should be determined by a specialist knowledgeable in treating salt-wasting CAH. Cardiac status should be monitored and any arrhythmias treated appropriately.

Transfer to a pediatric intensive care unit should be done when the patient is stable.

Other elevations of 17-OHP may be handled in a less drastic manner as specified previously (see Table III-8). It is still the case that any elevation of 17-OHP may be symptomatic of salt-wasting CAH; thus, the program should not minimize this possibility and should require immediate serum confirmation of all elevations of screening 17-OHP levels. Although there is a tendency to treat elevations of 17-OHP in premature infants with less urgency because they are prone to elevations as a result of stress and other factors, they are not a protected class of newborns, and their follow-up care is equally urgent. Any signs of virilization in females should be treated suspiciously with respect to CAH. It is also the case that virilization as a result of 11-hydroxylase deficiency might exist when test results for 17-OHP are within the program's normal limits. In cases of persistently elevated levels of 17-OHP without other symptoms, it may be useful to obtain corticotropin stimulation testing as an aid to diagnosis.

#### *Expertise of Health Care Providers.*

CAH is a complex disorder that requires specialty care. Although the patient's PHCP may feel competent to handle routine health matters, the complexities of CAH, especially the salt-wasting type, are likely to be more involved than the PHCP can routinely handle. Optimal care of the patient with CAH is best approached by a multidisciplinary team that includes an endocrine specialist and professionals with experience in psychology, genetics, counseling, and nursing. When such a specialty team is not available locally, ongoing input from CAH experts should be obtained.

The treatment of CAH is replacement hormone medications. Glucocorticoids, cortisone, or hydrocortisone is

required for lifelong treatment of patients with CAH. Dexamethasone is usually not used in infants because it can interfere with normal growth. The dose is calculated on the basis of weight or body surface area. In times of vomiting, serious illness, injury, or surgery, much higher doses of glucocorticoid are required. Overtreatment with glucocorticoids produces growth failure, excessive weight gain, and other features of Cushing's syndrome. Appropriate medications, dosages, and type of administration should be determined by a pediatric endocrine specialist.

Some infants with CAH are able to maintain normal levels of sodium and potassium without the use of mineralocorticoids, but many cannot. If a mineralocorticoid is necessary, it may be given once or twice a day. Overmedication causes hypertension; therefore, blood pressure must be monitored. Some infants may also need salt supplementation.

Medications must be adjusted as the child grows. Serum adrenal hormone and renin levels may need to be monitored. Suppression of excess corticotropin secretion is assessed by monitoring plasma 17-OHP, androstenedione, and testosterone. Females who have virilization may need surgical correction. This is usually done in stages, with the first surgery before 2 years of age. In these cases, coordination among the PHCP, endocrinologist, urologist, gynecologist, and psychologist is essential.

Clinical objectives in treating CAH are prevention of further virilization, maintenance of normal growth, and normal progression of skeletal maturity. Children should be monitored every 3 months during their first year of life and appropriately thereafter, as determined by the endocrinology specialist. In cases in which the non-classical form of CAH is encountered, the decision to treat is more problematic. This is especially true in cases in which the differentiation between simple virilizing and non-classical CAH is unclear.

In these cases, corticotropin stimulation testing and genotyping may provide useful information.

**Health Education.** It is important that parents receive clear and appropriate information about CAH, its outcome if untreated, the implications of genital surgery if needed, appropriate treatment protocols, and outcome data and information. The educational program must be customized to the needs of the family, including consideration of cultural issues and educational abilities of family members. A mechanism for transmitting updated treatment information should be a part of the program. Parents should be educated about the need to increase hydrocortisone doses during stress or illness and to provide hydrocortisone by intramuscular injection if the child is vomiting or unable to take oral medication. Affected infants and children should wear medical identification bracelets emphasizing the necessity of emergency care during acute illness. Parents should also understand the need for preoperative and postoperative steroid administration for elective surgeries. These children may need more salt and water, and caretakers should understand the reasons for their water and salt craving. Monitoring growth and understanding the effects of endogenous androgen excess should be discussed when relevant, as should issues related to psychosexual development.

**Health Maintenance.** Patients with CAH should receive routine medical care from their PHCP. External consultation with a specialist will provide optimal benefit. During the first year, a specialty care provider should see the patient every 3 months. The regimen of visits should decrease with time but should continue to include appropriate biochemical, physical, and behavioral monitoring. From age 10 to 20 years, children and young adults should be seen every 6 to 12 months in the sub-

specialist treatment center. Issues of sexuality and need for further gynecologic reconstruction should be addressed as appropriate. Laboratory monitoring should be performed every 6 months in males and possibly more frequently in females because of the adverse effects of adrenal androgens on pubertal development and menstrual cyclicity. Growth rate should be monitored closely, with bone age determinations when appropriate.

**Genetic Counseling.** CAH is an autosomal recessive disorder and therefore genetic counseling is appropriate. This is particularly true because prenatal treatment options are now available. Counseling should be sensitive to the cultural and educational characteristics of the family and to possible paternity issues. Genetic counseling regarding autosomal recessive inheritance should be given, including available information about prenatal diagnosis and intervention. Care should be taken to discuss the controversies regarding prenatal dexamethasone administration and its relation to prevention of virilization in females. Prenatal treatment is complex, and its discussion should carefully explain the need for collaboration among the PHCP, counselor, endocrinology specialist, and obstetrician. Potential side effects should also be carefully explained. If DNA diagnosis is available, accurate prenatal diagnosis can be made, as well as heterozygote detection provided to extended family members. These procedures should be included in any counseling discussion.

#### **CORE PERSONNEL**

- MD with clinical expertise in pediatric endocrinology
- MD or PhD with expertise in biochemical laboratory analysis
- MD clinical geneticist
- Nurse coordinator
- Genetic counselor
- Nutritionist
- Social worker

#### **ADDITIONAL PERSONNEL AS NEEDED**

- Pediatric urologist and/or gynecologist
- Psychologist or child psychiatrist

#### **CORE RESOURCES**

- Laboratories with CLIA certification for quantitative 17-OHP, cortisol, and aldosterone analysis
- Routine clinic and laboratory analysis to monitor electrolyte and nutritional parameters
- Clinical facilities to initiate and maintain ongoing medical and nutritional management and genetic counseling
- Endocrine medication and specialized laboratory analyses
- Financial resources to ensure adequate treatment and monitoring regardless of ability to pay
- Specialized development services if indicated
- Family and patient disease-specific resource information

#### **Metabolic Diseases**

The care of patients and families with the following inherited metabolic diseases is complex, because each has multisystemic manifestations and significant genetic and phenotypic heterogeneity that requires both acute and long-term (lifelong) management. Appropriate treatment to ensure optimal outcomes for each disease requires active involvement of a biochemical or clinical geneticist or a physician with appropriate expertise in inherited metabolic diseases, as well as professionals with expertise in development, nutrition, genetic counseling, nursing, social work, and psychology. Periodic evaluations of biochemical markers and developmental outcome are particularly important and should be well documented. Ideally, these physicians and paramedical professionals function as a multidisciplinary team that provides direct patient care. Such multidisciplinary centers are often designated by state public health or medical

agencies. The core personnel, consultants, and core resources for multidisciplinary teams are listed below for each metabolic disease.

When such teams are not available locally, ongoing input from inherited metabolic disease experts should be obtained. PHCPs who accept responsibility for the treatment of such patients must be knowledgeable about current approaches to the diagnosis and medical management of the respective metabolic disease and arrange unimpeded access for patients to expert providers. In addition, patients and families with these disorders also need access to a wide variety of consultative, diagnostic, and therapeutic services, including but not limited to those provided by specialists in neurology, audiology, occupational and physical therapy, and others.

The extent to which care is provided by the PHCP versus the interdisciplinary inherited metabolic disease team will vary among patients and communities and will depend on the expertise of the PHCP, access to the team of metabolic disease experts, family preference, and the frequency and severity of disease manifestations. Some patients may receive all of their care from the metabolic disease expert, whereas other patients receive day-to-day care from PHCPs with periodic comprehensive evaluations by the inherited metabolic disease team. Because acute illnesses may prove rapidly life-threatening, it is essential that patients be treated by metabolic disease centers or by providers in the community with appropriate knowledge and experience.

#### **HYPERPHENYLALANINEMIA AND PHENYLKETONURIA**

*Expertise of Health Care Providers and Management.* Treatment of hyperphenylalaninemia and PKU requires limiting dietary intake of phenylalanine to amounts sufficient for normal growth and development, but not sufficient to raise blood phenylalanine to

levels that are neurotoxic. Debate continues over what blood level of phenylalanine is acceptable in mild hyperphenylalaninemia. However, in classic PKU, the goal of therapy is to maintain blood phenylalanine between 2 and 6 mg/dL (125-375  $\mu\text{mol/L}$ ). This is accomplished by replacing a portion of phenylalanine-containing foods with synthetic phenylalanine-free medical foods. The requirements for calories are generally greater when L-amino acids are substituted for most of the required natural protein; in infancy, energy needs may exceed 120 kcal/kg/d. Natural protein intakes must be adjusted according to the patient's age and plasma levels of phenylalanine. Excess restriction of natural protein leading to phenylalanine levels less than 2 mg/dL (125  $\mu\text{mol/L}$ ) should be avoided to prevent nutritional deficiency, growth failure, and possible death. Tyrosine, the product of the blocked enzymatic reaction, is routinely added to the medical foods used in treatment of PKU. However, tyrosine may be low in those patients who do not consume all of their supplemental medical foods; therefore, blood levels of tyrosine should be monitored regularly to ensure a normal concentration.

Requirements for phenylalanine, tyrosine, and other nutrients change with age and decreasing growth velocity. Defining specific mutations in the *PAH* gene and total body oxidation of  $^{13}\text{C}$ -phenylalanine to  $^{13}\text{CO}_2$  by breath test may help predict the level of phenylalanine restriction in an individual patient. Growth parameters, development, and psycho-educational progress of each patient must be recorded, and appropriate intervention provided if needed.

All children with PKU require a lifelong phenylalanine-restricted diet, but females with PKU must be specifically informed that elevated phenylalanine levels during any pregnancy constitute a significant teratogenic risk to their offspring. In their reproductive years,

women with PKU should ideally maintain their blood phenylalanine concentrations between 2 and 4 mg/dL before and during each pregnancy to minimize the teratogenic effects of phenylalanine on their offspring, a condition termed *maternal PKU*. The dangers of untreated maternal PKU should be discussed early on with the young girl's parents and then discussed in an age-appropriate manner several times with girls ages 10 to 12 years when they assume dietary responsibility.

*Health Education.* As in any chronic lifelong condition, education of the parents and ultimately the child must be ongoing. It must include details of the condition, genetics, and basic pathophysiology, as well as practical details of dietary management throughout the individual's life. This information must be tailored to the cultural, educational, and developmental levels of parents, child, and extended family. Parents and patients must also be kept informed of new developments in the treatment of these conditions.

*Health Maintenance.* All patients with hyperphenylalaninemia or bipterin defects should receive routine pediatric care from a PHCP in their community. Intercurrent illnesses can cause temporary elevations of phenylalanine or other toxic metabolites, and dietary adjustments may be required. If possible, specialized treatment of the metabolic condition should be provided by a multidisciplinary team experienced in management of these conditions. School performance should be monitored for evidence of neurodevelopmental problems and, if present, appropriate intervention should be provided.

*Genetic Counseling.* Genetic counseling and carrier testing should be provided to parents and extended family members. Counseling should be sensitive to the cultural and social needs of the family and possible paternity issues, and in-

clude a review of the pathophysiology of the disease, recurrence risks, and availability of prenatal diagnosis. Genetic education should be introduced to the patient during early adolescence.

*Psychosocial Support.* Comprehensive medical care includes periodic psychosocial assessments and the availability of services needed to optimize adaptation by the patient and family to a chronic condition. Support groups and community-based organizations are important resources. Important issues include health insurance coverage, transportation for health care, and educational issues, including education of school personnel and assistance with vocational planning. Patients must be given age-appropriate information about their disorder throughout childhood and adolescence. Transition programs help to prepare them to become knowledgeable and responsible about their health care as adolescents and adults.

#### CORE PERSONNEL

- MD clinical geneticist
- MD and/or PhD biochemical geneticist
- Metabolic nutritionist
- Nurse coordinator
- Social worker
- Genetic counselor
- Neurodevelopmentalist

#### CORE RESOURCES

- CLIA-certified laboratories for the above biochemical, genetic, and nutritional studies
- Clinical facilities to initiate and maintain ongoing medical and nutritional management and genetic counseling
- Special medical foods, metabolic formulas, low-protein foods, tyrosine, and nutritional supplements
- Financial resources to ensure adequate treatment and monitoring regardless of the family's ability to pay
- Specialized developmental services if indicated

- Family and patient disease-specific resource information
- Family planning and adolescent services

#### GALACTOSEMIA

*Expertise of Health Care Providers and Management.* The clinical variations, that is, heterogeneity, of galactosemia require many clinical approaches to management of this disease. Treatment of a symptomatic newborn with classic transferase deficiency galactosemia may involve immediate hospitalization in a neonatal or pediatric intensive care unit to treat liver failure, renal tubular acidosis, cardiovascular shock, or *Escherichia coli* sepsis. For an asymptomatic infant, immediate conversion from breast-feeding or cow's milk formula to a lactose-free formula may be all that is required along with careful clinical and laboratory follow-up. Because death and irreversible morbidity may occur in the first week of life, it is important to remove galactose from the newborn's diet as quickly as possible and monitor the child closely while further diagnostic evaluation continues. Therapy of the newborn with classic galactosemia should be managed by clinicians and nutritionists skilled in the treatment of children with metabolic diseases. A baseline examination by a pediatric ophthalmologist should be obtained. Other professionals who may be required as the child gets older include a developmental psychologist, endocrinologist, genetic counselor, social worker, and a speech and language therapist. Laboratory services must be available to determine the biochemical phenotype, and when appropriate, the molecular genotype. Levels of galactose metabolites that should be monitored periodically include erythrocyte gal-1-P and, when available, urinary galactitol (see discussion in the Diagnosis section). Because cataracts, verbal dyspraxia, ovarian failure, ataxia, and growth and developmental delays may

be complications of galactosemia, monitoring and intervention for these problems are recommended.

The type of galactosemia is based on the GALT biochemical phenotype and/or the molecular genotype. If classic galactosemia is present, galactose is eliminated from the diet and all other sources (eg, medications, vitamins). The diet should provide the recommended dietary allowances of all essential nutrients for healthy infants, children, and adults. Galactose-free calcium and vitamin supplements are often prescribed. Dietary management for patients with variant forms of galactosemia is controversial and depends on their GALT activity and/or molecular genotype and other metabolic pathways (eg, galactose reductase, endogenous galactose production). Some metabolic centers implement a galactose-restricted diet for 6 months to 1 year for Duarte galactosemia (D/G GALT) and other phenotypes. Galactose is then reintroduced into the diet while erythrocyte gal-1-P and/or urinary galactitol levels are monitored. If metabolites remain elevated after the reintroduction of galactose, then dietary restriction of galactose should continue. Although some patients with variant forms of galactosemia may not require galactose restriction, their levels of gal-1-P and urinary galactitol should be monitored periodically.

Because of varying endogenous galactose production in different genotypes, it is difficult to predict responses to galactose in the diet of a patient with galactosemia. Research into the molecular genotype/phenotype correlation and the use of total body galactose oxidation by breath tests is helping answer some of these questions. These tools are now available at some metabolic centers. The indication for galactose restriction during pregnancy of a heterozygous mother along with prenatal diagnosis, although controversial, is recommended by several centers.

**Health Education.** As in any chronic lifelong condition, education of the parents and ultimately the child must be ongoing. This must include details of the condition, genetics, and basic pathophysiology, as well as practical details of dietary management throughout the individual's life. This information must be tailored to the cultural, educational, and developmental levels of the parents, child, and extended family. Parents and patients must also be kept informed of new developments in the treatment of these conditions.

**Health Maintenance.** All patients with classical or variant forms of galactosemia should receive routine pediatric care from a PHCP in their community. If possible, specialized treatment of the metabolic condition should be provided by a multidisciplinary team experienced in management of these conditions. School performance should be monitored for evidence of speech, language, and other neurodevelopmental problems. If such problems are present, appropriate intervention should be provided.

**Genetic Counseling.** Genetic counseling and carrier testing should be provided to parents and extended family members. Counseling should be sensitive to the cultural and social needs of the family and include a review of the pathophysiology of the disease, recurrence risks, and availability of prenatal diagnosis. As with all autosomal recessive conditions, appropriate sensitivity to paternity should be observed. Genetic education should be introduced to the patient during early adolescence.

**Psychosocial Support.** Comprehensive medical care includes periodic psychosocial assessments and the availability of services needed to optimize the adaptation of the patient and family to a chronic condition. Support groups and community-based

organizations are important resources. Important issues include health insurance coverage, transportation for health care, and educational issues including education of school personnel and assistance with vocational planning. Patients must be given age-appropriate information about their disorder throughout childhood and adolescence. Transition programs help to prepare them to become knowledgeable, responsible health care consumers as adolescents and adults.

#### **CORE PERSONNEL**

- MD clinical geneticist
- MD and/or PhD biochemical geneticist
- Metabolic nutritionist
- Nurse coordinator
- Neonatologist or pediatric intensivist
- Social worker
- Genetic counselor
- Ophthalmologist
- Neurodevelopmentalist

#### **ADDITIONAL PERSONNEL, AS NEEDED**

- Endocrinologist
- Occupational, physical, and speech and language therapists

#### **CORE RESOURCES**

- CLIA-certified laboratories for the biochemical, genetic, and nutritional studies
- Age-appropriate neonatal or pediatric intensive care facility
- Clinical facilities to initiate and maintain ongoing medical and nutritional management and genetic counseling
- Galactose-free formulas and galactose-free nutritional supplements
- Financial resources to ensure adequate treatment and monitoring regardless of the family's ability to pay
- Specialized developmental services
- Family and patient disease-specific resource information

#### **MAPLE SYRUP URINE DISEASE**

**Expertise of Health Care Providers and Management.** Treatment of MSUD mandates restricting the intake of BCAAs to amounts necessary for normal growth and development, but insufficient to raise the serum concentrations of leucine and the branched chain  $\alpha$ -ketoacids to neurotoxic levels. Plasma amino acid levels must be determined frequently to ensure that BCAA levels are neither too high nor too low. Monitoring patients' urinary dinitrophenylhydrazine and ketone testing are inexpensive techniques that can be taught to parents and older children as part of long-term management.

Although therapy must begin as early as possible, preferably within the first week of life, to minimize the likelihood of permanent brain damage, the infant with MSUD detected by NBS is almost always symptomatic and frequently already hospitalized when the screening result becomes known. If not already hospitalized, the infant should be admitted at once, usually to a neonatal or pediatric intensive care unit. In infants able to suckle formula, BCAA levels can be reduced by providing a metabolic formula free of BCAAs. Tube feedings should be tried in infants too ill to suck. Monitoring serum BCAA levels frequently, often daily, is usually required with replacement of BCAA-free formula with natural protein as the BCAA concentrations normalize. This normally takes 2 to 4 days. Depending on the plasma BCAA levels, it may be necessary to first supplement the formula with free isoleucine and valine, before leucine is added. For metabolic control of the disease, it is very important to establish anabolism by maintaining a high caloric intake, usually greater than 120 kcal/kg/d. Nasogastric tube feeding, gastrostomy feeding, and even hyperalimentation may be needed to accomplish this aim. Although hemodialysis has been used in acutely encephalo-

pathic situations to reduce neuronal accumulation of branched chain  $\alpha$ -ketoacids, it is difficult to establish an anabolic state after hemodialysis. Peritoneal dialysis is discouraged because it is too slow and always produces a catabolic state, sometimes with ileus.

It is often difficult to decide whether an encephalopathic patient with MSUD, and particularly a child who has been acutely ill and treated with BCAA-free intravenous fluids for more than 2 to 3 days, needs to have BCAA intake raised or lowered. In general, the patient who needs more BCAAs or natural protein will have low serum levels of valine and/or isoleucine, whereas the patient who needs less will have elevated concentrations of all 3 BCAAs. Similarly, urinary organic acids will identify ketones if a catabolic state persists, as well as the corresponding  $\alpha$ -ketoacids. Thiamine at a dose of 10 mg/kg/d should be given to all infants with a presumptive diagnosis of MSUD, except those children from populations known to be thiamine-unresponsive, such as Mennonites.

Where available, after the infant's condition has become stable, the evaluation by breath test of the total body oxidation of  $^{13}\text{C}$ -leucine to  $^{13}\text{CO}_2$  before and 4 weeks after starting thiamine administration may help determine thiamine responsiveness and the degree of impaired total body leucine oxidation. The frequency of serum monitoring of BCAAs is mandated by the infant's progress. Sampling can be reduced in frequency as the child's age increases and as the brain becomes less sensitive to the acute toxic effects of elevated metabolites. After hospital discharge, growth parameters and neurodevelopmental status are tracked closely with the primary care clinician and any appropriate consultant. Dietary analysis and BCAA concentrations are monitored regularly throughout infancy and childhood, especially during the times of most rapid growth rates. During acute infections or catabolic states,

close attention must be paid to the child's metabolic status. Healthy postpubertal teens and adult patients may need to be monitored only once or twice per year. The use of dinitrophenylhydrazine and/or ketone testing of urine by the patient's family is helpful for clinical monitoring and dietary adjustment by phone consultation.

**Health Education.** As in any chronic lifelong condition, education of the parents and ultimately the child must be ongoing. It must include details of the condition, genetics, and basic pathophysiology, as well as practical details of dietary management throughout the individual's life. This information must be tailored to the cultural, educational, and developmental levels of the parents, child, and extended family. Parents and patients must also be kept informed of new developments in the treatment of these conditions.

**Health Maintenance.** All patients with MSUD should receive routine pediatric care from a PHCP in their community. Intercurrent illnesses can cause rapid elevations of BCAAs and other toxic metabolites. Dietary adjustments and intravenous fluid therapy are often required. If possible, specialized treatment of the acute metabolic condition should be provided by a multidisciplinary team experienced in the management of these conditions. School performance should be monitored for evidence of neurodevelopmental problems and, if present, appropriate intervention should be provided.

**Genetic Counseling.** Genetic counseling should be provided to parents and extended family members. Counseling should be sensitive to the cultural and social needs of the family and include a review of the pathophysiology of the disease, recurrence risks, and availability of prenatal diagnosis. As with all autosomal

recessive conditions, appropriate sensitivity to paternity should be observed. Genetic education should be introduced to the patient during early adolescence.

**Psychosocial Support.** Comprehensive medical care includes periodic psychosocial assessments and the availability of services needed to optimize the adaptation of the patient and family to a chronic condition. Support groups and community-based organizations are important resources. Important issues include health insurance coverage, transportation for health care, and educational issues, including education of school personnel and assistance with vocational planning. Patients must be given age-appropriate information about their disorder throughout childhood and adolescence. Transition programs help to prepare them to become knowledgeable, responsible health care consumers as adolescents and adults.

#### CORE PERSONNEL

- MD clinical geneticist
- MD and/or PhD biochemical geneticist
- Metabolic nutritionist
- Neonatologist or pediatric intensivist
- Nurse coordinator
- Social worker
- Genetic counselor
- Neurodevelopmentalist

#### ADDITIONAL PERSONNEL, AS NEEDED

- Occupational, physical, and speech and language therapists

#### CORE RESOURCES

- CLIA-certified laboratories for the above biochemical, genetic, and nutritional studies
- Age-appropriate neonatal or pediatric intensive care facility
- Clinical facilities to initiate and maintain ongoing medical and nutritional management and genetic counseling

- BCAA-free special medical foods and metabolic formulae, low-protein foods, free BCAAs, and nutritional supplements
- Financial resources to ensure adequate treatment and monitoring regardless of the family's ability to pay
- Specialized developmental services
- Family and patient disease-specific resource information

### BIOTINIDASE DEFICIENCY

*Expertise of Health Care Providers and Management.* Management of classical biotinidase deficiency requires a supplement of oral biotin, at a dose of 5 to 20 mg/d. Intercurrent episodes of metabolic deterioration with acidosis and altered mental status caused by infection or other stresses may require acute care, including cardiorespiratory support, intravenous administration of fluids, and electrolyte management during infancy. Long-term follow-up includes psychometric, audiologic, and ophthalmologic evaluations, as well as periodic assessment of urinary organic acid excretion, which may be helpful to monitor the biotin dose and compliance. Quantitation of plasma and/or urinary biotin may also assist in the assessment of compliance in apparent biotin non-responders. Some patients with mild variants, 10% to 30% normal enzyme activity, may manifest symptoms only during episodes of infection. To date, there have been too few children with partial biotinidase deficiency who have become symptomatic to state that there are no residual problems if they are only treated when they have an infection or symptoms. Most children with partial deficiency are now treated from the time of diagnosis with biotin, usually between 1 to 5 mg/d.

*Health Education.* As in any chronic lifelong condition, education of the parents and ultimately the child must be ongoing. This must include details of the condition, genetics, and basic

pathophysiology, as well as practical details of dietary management throughout the individual's life. This information must be tailored to the cultural, educational, and developmental levels of the parents, child, and extended family. Parents and patients must also be kept informed of new developments in the treatment of these conditions.

*Health Maintenance.* All patients with biotinidase deficiency should receive routine pediatric care from a PHCP in their community. If possible, specialized treatment of the metabolic condition should be provided by a multidisciplinary team experienced in management of these conditions. School performance should be monitored for evidence of neurodevelopmental problems and, if present, appropriate intervention should be provided.

*Genetic Counseling.* Genetic counseling and carrier testing should be provided to parents and extended family members. Counseling should be sensitive to the cultural and social needs of the family and include a review of the pathophysiology of the disease, recurrence risks, and availability of prenatal diagnosis. As with all autosomal recessive conditions, appropriate sensitivity to paternity should be observed. Genetic education should be introduced to the patient during early adolescence.

*Psychosocial Support.* Comprehensive medical care includes periodic psychosocial assessments and the availability of services needed to optimize the adaptation of the patient and family to a chronic condition. Support groups and community-based organizations are important resources. Important issues include health insurance coverage, transportation for health care, and educational issues, including education of school personnel and assistance with vocational planning. Patients must be given age-appropriate information about their disorder throughout childhood

and adolescence. Transition programs help to prepare them to become knowledgeable, responsible health care consumers as adolescents and adults.

### CORE PERSONNEL

- MD clinical geneticist
- MD and/or PhD biochemical geneticist
- Nurse coordinator
- Social worker
- Genetic counselor
- Neurodevelopmentalist
- Ophthalmologist
- Audiologist

### ADDITIONAL PERSONNEL, AS NEEDED

- Occupational, physical, and speech and language therapists
- Metabolic nutritionist

### CORE RESOURCES

- CLIA-certified laboratories for the biochemical, genetic, and nutritional studies
- Clinical facilities to initiate and maintain ongoing medical and nutritional management and genetic counseling
- Biotin and nutritional supplements
- Financial resources to ensure adequate treatment and monitoring regardless of the family's ability to pay
- Specialized developmental services
- Family and patient disease-specific resource information

### HOMOCYSTINURIA

*Expertise of Health Care Providers and Management.* The significance of hypermethioninemia depends on confirming a specific diagnosis. Only the management of classic CBS deficiency will be discussed here. Initial management consists of a trial of pyridoxine because about half of patients respond to this cofactor, which increases trans-sulfuration resulting in lower methionine and homocysteine concentrations. The doses of pyridoxine used have varied considerably from 25 to 1000 mg/d. There

have been reports of neonates who became apneic and unresponsive after receiving doses of pyridoxine of 500 mg per day. A maximum dose of 900 mg/1.73 m<sup>2</sup> body surface area should be administered while the respiratory status is carefully monitored. Most infants identified by NBS have been unresponsive to treatment with pyridoxine and have required dietary therapy. Dietary therapy consists of restriction of methionine and supplementation of cystine while regularly monitoring plasma methionine, homocysteine, and cysteine concentrations. The treatment goal is to reduce free plasma homocysteine to an undetectable level and, if possible, to normalize the total homocysteine level. This may be assisted by the administration of a remethylating agent in older children and adults, and by supplemental folic acid and vitamin B<sub>12</sub> to optimize remethylation of homocysteine to methionine. Long-term management includes ophthalmologic evaluation for dislocated ocular lenses, evaluation and treatment of vascular thrombotic episodes, monitoring of growth and skeletal mineralization, and neuropsychologic evaluation. Total plasma homocysteine and plasma amino acids are monitored periodically after initiation of therapy. Molecular diagnosis may help predict the severity of enzyme impairment and pyridoxine responsiveness, identify heterozygotes, and assist in genetic counseling.

*Health Education.* As in any chronic lifelong condition, education of the parents and ultimately the child must be ongoing. This must include details of the condition, genetics, and basic pathophysiology, as well as practical details of dietary management throughout the life of the individual. This information must be tailored to the cultural, educational, and developmental levels of the parents, child, and extended family. Parents and patients must also be kept in-

formed of new developments in the treatment of these conditions.

*Health Maintenance.* All patients with homocystinuria should receive routine pediatric care from a PHCP in their community. If possible, specialized treatment of the metabolic condition should be provided by a multidisciplinary team experienced in the management of these conditions. If not available locally, ongoing input from such a team should be sought. School performance should be monitored for evidence of neurodevelopmental problems and, if present, appropriate intervention should be provided.

*Genetic Counseling.* Genetic counseling and carrier testing should be provided to parents and extended family members. Counseling should be sensitive to the cultural and social needs of the family and include a review of the pathophysiology of the disease, recurrence risks, and availability of prenatal diagnosis. As with all autosomal recessive conditions, appropriate sensitivity to paternity should be observed. Genetic education should be introduced to the patient during early adolescence.

*Psychosocial Support.* Comprehensive medical care includes periodic psychosocial assessments and the availability of services needed to optimize the adaptation of the patient and family to a chronic condition. Support groups and community-based organizations are important resources. Important issues include health insurance coverage, transportation for health care, and educational issues, including education of school personnel and assistance with vocational planning. Patients must be given age-appropriate information about their disorder throughout childhood and adolescence. Transition programs help to prepare them to

become knowledgeable, responsible health care consumers as adolescents and adults.

#### CORE PERSONNEL

- MD clinical geneticist
- MD and/or PhD biochemical geneticist
- Ophthalmologist
- Metabolic nutritionist
- Nurse coordinator
- Social worker
- Genetic counselor
- Neurodevelopmentalist

#### ADDITIONAL PERSONNEL, AS NEEDED

- Occupational, physical, and speech and language therapists

#### CORE RESOURCES

- CLIA-certified laboratories for the above biochemical, genetic, and nutritional studies
- Clinical facilities to initiate and maintain ongoing medical and nutritional management and genetic counseling
- Special medical foods, metabolic formulas, low-protein foods, betaine, and nutritional supplements
- Financial resources to ensure adequate treatment and monitoring regardless of the families' ability to pay
- Specialized developmental services
- Family and patient disease-specific resource information.

#### TYROSINEMIA I AND II

*Expertise of Health Care Providers and Management.* In both FAH deficiency (tyrosinemia, type I) and TAT deficiency (tyrosinemia, type II), dietary restriction of phenylalanine and tyrosine is begun to maintain normal levels of these plasma amino acids. Other essential nutrients are maintained at normal levels, and the phenylalanine and tyrosine intake is adjusted to permit near-normal growth and development. In tyrosinemia type II, dietary therapy alone will prevent complications; however, chil-

dren with tyrosinemia type I require close supervision of growth and development with management similar to patients with PKU.

Tyrosinemia type I is a much more severe disease with multisystem involvement and complex treatments. Tyrosinemia type I produces liver failure, hypersplenism, renal tubular insufficiency, and neurologic crises, including painful paresthesias, hypertension, tachycardia, ileus, and progressive paralysis. Succinylacetone also inhibits heme biosynthesis and may produce acute porphyria-like symptoms. Organ systems must be monitored both clinically and through laboratory evaluations of liver function including serum  $\alpha$ -fetoprotein, complete blood counts including platelets, urinary organic acids for p-hydroxy-phenyl acids and succinylacetone, and electrolytes including serum potassium and phosphate. Erythrocyte porphobilinogen synthase deaminase may be normal, while urinary  $\delta$ -aminolevulinic acid is elevated, signifying impaired  $\delta$ -aminolevulinic acid dehydratase. These findings should revert to normal with adequate therapy. Electrolytes and acid base balance are maintained at normal levels.

The combination of phenylalanine and tyrosine dietary restriction and 2-nitro-4-trifluoromethylbenzoyl-1,3-cyclohexanedione (NTBC) drug therapy will reverse or stabilize these organ dysfunctions if initiated in early infancy. If therapy is delayed, the outcome will be less successful. NTBC inhibits production of succinylacetone and should be considered part of treatment for patients with FAH deficiency. At this writing, its use is investigational and requires a research protocol with informed consent from parents or guardians. The plasma tyrosine may increase during treatment with NTBC to  $>500 \mu\text{mol/L}$  such that the patients' diets need further restriction of phenylalanine and tyrosine. Children

must be closely monitored for the emergence of NTBC side effects, such as corneal clouding or thrombocytopenia.

The risk of hepatocarcinoma in patients with tyrosinemia type I remains high, even with optimal medical and nutritional therapy. If liver failure continues or recurs, or if the serum AFP rises or remains elevated while the patient is in compliance with diet and NTBC therapies, and if sonography of the liver reveals increasing nodularity, then liver transplantation is recommended to prevent metastatic hepatocarcinoma. Although liver transplantation removes the primary source of total body succinylacetone and eliminates the hepatic and neurologic complications, long-term outcome data continue to be sparse. If the liver is removed, genotyping of FAH complementary DNA is recommended to provide genetic counseling, heterozygote detection, and prenatal diagnosis, if desired, for the family. DNA-based carrier testing and prenatal diagnosis are also possible by linkage analysis of the FAH gene.

**Health Education.** As in any chronic lifelong condition, education of the parents and ultimately the child must be ongoing. This must include details of the condition, genetics, and basic pathophysiology, as well as practical details of dietary management throughout the individual's life. This information must be tailored to the cultural, educational, and developmental levels of the parents, child, and extended family. Parents and patients must also be kept informed of new developments in the treatment of these conditions.

**Health Maintenance.** All patients with tyrosinemia should receive routine pediatric care from a PHCP in their community. If possible, specialized treatment of both forms of tyrosinemia should be provided by a multidisciplinary team experienced in the

management of this condition. If not available locally, ongoing input from such a team should be sought. School performance should be monitored for evidence of neurodevelopmental problems and, if present, appropriate intervention should be provided.

**Genetic Counseling.** Genetic counseling and carrier testing should be provided to parents and extended family members. Counseling should be sensitive to the cultural and social needs of the family and include a review of the pathophysiology of the disease, recurrence risks, and availability of prenatal diagnosis. As with all autosomal-recessive conditions, appropriate sensitivity to paternity should be observed. Genetic education should be introduced to the patient during early adolescence.

**Psychosocial Support.** Comprehensive medical care includes periodic psychosocial assessments and the availability of services needed to optimize the patient's and family's adaptation to a chronic condition. Support groups and community-based organizations are important resources. Important issues include health insurance coverage, transportation for health care, and educational issues, including education of school personnel and assistance with vocational planning. Patients must be given age-appropriate information about their disorder throughout childhood and adolescence. Transition programs help to prepare them to become knowledgeable, responsible health care consumers as adolescents and adults.

#### CORE PERSONNEL

- MD clinical geneticist
- MD and/or PhD biochemical geneticist
- Neonatologist or pediatric intensivist
- Metabolic nutritionist
- Nurse coordinator

- Social worker
- Genetic counselor
- Neurodevelopmentalist

**ADDITIONAL PERSONNEL, AS NEEDED**

- Nephrologist, gastroenterologist or hepatologist, or hepatic transplant surgeon
- Occupational, physical, and speech and language therapists

**CORE RESOURCES**

- CLIA-certified laboratories for the biochemical, genetic, and nutritional studies
- Clinical facilities to initiate and maintain ongoing medical and nutritional management and genetic counseling
- Special medical foods, metabolic formulas, low-protein foods, and nutritional supplements

- Ability to initiate and monitor NTBC as an investigational drug
- Financial resources to ensure adequate treatment and monitoring regardless of the family's ability to pay
- Specialized developmental services if indicated
- Family and patient disease-specific resource information

## V. EVALUATION

### *Evaluation of the Overall System*

The goals of NBS programs are best realized by a comprehensive system that ensures appropriate screening, follow-up, diagnosis, and lifelong management. All components of the system must function within optimal time frames.

The NBS system and each of its components must continuously assess their effectiveness in achieving or contributing to the overall goal. Assessment of each component requires data that can only be collected by other components of the system; therefore, data collection and analysis must be a cooperative effort among all system components. Evaluation of the NBS system is a public health responsibility.

The NBS system should accumulate data to determine the following measurements:

- The incidence of each disorder
- The effectiveness and speed of identifying affected infants and entering them into comprehensive care
- The quality of screening and diagnostic tests
- Monitoring and outcome assessment
- The costs for operating the entire system
- The cost of each component
- The cost benefit to patients, families, and society

Some of the parameters that can be used in evaluating the system are listed below:

- Birth prevalence of each disorder (cases identified per number of births)
- Fraction of the births screened (number of infants screened per number of births)
- Ascertainment through NBS (number of cases detected through NBS per number of known cases born in the catchment area)
- Age at screening (mean and distribution)

- Screening laboratory turnaround time (time from receipt of specimen to laboratory report for screen-negative, unsatisfactory, and screen-positive results)
- Analytical validity of the screening tests (accuracy)
- Clinical validity of the screening tests (sensitivity, specificity, positive predictive value, negative predictive value)
- Age at definitive diagnosis of affected infants (mean and distribution)
- Analytical validity of the diagnostic tests
- Clinical validity of the diagnostic tests
- Age at initiation of treatment (mean and distribution)
- Demographic and clinical profiles of the patients receiving treatment for each disorder
- Mortality rates among patients with each disorder
- Measures of morbidity for the population of patients with each disorder (developmental quotient/IQ, inpatient hospital days, percentage with specific complications, etc)
- Measures of compliance with the recommended treatment protocols (prophylactic penicillin prescriptions filled, blood phenylalanine levels, diet records, etc)
- Measures of long-term outcome and functionality of patients receiving treatment (schooling achieved, employment, psychosocial adaptation, reproductive success, etc.)
- Analytical and clinical validity of the laboratory tests used for monitoring patients
- Validity of the outcome assessment protocols used
- Cost per patient per period (month, year, or lifetime)
- Cost of each component service
- Analyses of costs and outcomes

All data cannot be collected at this time. Rigorous determination of sensitivity, specificity, and negative predictive value requires complete ascertainment

of false-negative results, which is not realistically achievable. Programs that routinely screen second specimens can estimate the false-negative rate of the initial screen. Some of the data needed to evaluate the screening and short-term follow-up components of the system can be found in the annual CORN/Association of Public Health Laboratories National Newborn Screening Data reports. Data from comprehensive care centers and other long-term care programs are essential to evaluate the efficacy of the NBS system. Existing comprehensive centers have much of the data required to evaluate the diagnosis, treatment, and management portions of the system in their internal databases but lack adequate resources to collect these data. At this writing, available data are incomplete because studies are still in progress. The recognition, understanding, and treatment of these disorders are still in a phase of rapid development, and the populations diagnosed with these disorders and the treatments provided are rapidly changing. Therefore, long-term correlation of costs with outcomes is difficult.

### *Evaluation of Newborn Screening Laboratories*

All NBS testing must be done by laboratories licensed by their respective states and must meet the requirements of CLIA88. As part of these requirements, a screening laboratory must meet certain criteria for quality control and must participate in proficiency testing programs. Proficiency testing is used to evaluate the quality of the measurement process on a periodic basis, usually quarterly. Proficiency testing specimens are to be handled and analyzed in the same way as patient specimens. Laboratories must satisfactorily participate in a Health Care Financing Administration (HCFA)-approved proficiency testing program, if available, for each method used to analyze human specimens. In the absence of an HCFA-approved proficiency testing program for NBS, the National New-

born Screening Quality Assurance Program enables laboratories to meet the CLIA quality assurance requirement for verifying test accuracy.

If a proficiency testing program is not available for a specific NBS test, laboratories must have a system for verifying the accuracy and reliability of their test results at least twice a year. Laboratories can develop a self-administered profi-

ciency testing program by using available reference methods and materials. The self-administered proficiency testing program will be administered by a quality assurance officer who is not in the participating laboratory. Laboratories should also endeavor to exchange specimens with other laboratories as a means of external verification when no formal proficiency testing program exists. The labo-

ratories must then document their performance in the self-administered proficiency testing programs in a quality control manual that is available for review.

### *Evaluation of Diagnostic Laboratories*

A more complete discussion of the issues concerning diagnostic laboratories is found in the Diagnosis section.

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- The Genome Database: an international collaboration in support of the Human Genome Project: <http://gdbwww.gdb.org/>

- GeneClinicsTM: a knowledge base of expert-authored, up-to-date information relating genetic testing to the diagnosis, management, and counseling of individuals and families with inherited disorders: <http://www.geneclinics.org>
- GeneTestsTM: a directory of medical genetics laboratories: <http://www.genetests.org>
- Online Mendelian Inheritance in Man: a catalog of human genes and genetic disorders: <http://www3.ncbi.nlm.nih.gov/Omim/>
- An up-to-date listing of GALT mutations: <http://www.emory.edu:80/PEDIATRICS/medgen/research/db.htm>
- Family Village: a global community that integrates information, resources, and communication opportunities on the Internet for persons with mental retardation and other disabilities, their families, and those who provide them services and support. It includes links to government and private agencies, consumer support, treatment, and research: <http://familyvillage.wisc.edu>
- Emory University's sickle cell disease Web page: <http://www.emory.edu/PEDS/SICKLE>
- Thalassemia information: <http://www.thalassemia.com>
- National Adrenal Diseases Foundation home page with information on congenital adrenal hyperplasia, support groups, and current research: <http://medhlp.netusa.net/www/nadf.htm>
- CDC Office of Genetics and Disease Prevention: reviews, literature citations, and current activities in public health genetics with many links to other sites: <http://www.cdc.gov/GENETIC>

## APPENDIX.

### *Guidelines for Follow-up of Carriers of Hemoglobin Variants Detected by Newborn Screening*

The majority of states presently include testing for SCD in their NBS programs. The primary purpose of the screening is to detect individuals with SCD so that deaths from early infections and other complications can be prevented. Parents or guardians of infants with SCDs such as Hb SS, Hb SC, and Hb S  $\beta$ -thalassemia are actively notified so the affected infant can be promptly enrolled in medical care.

Screening for sickle cell syndromes is presently unique because both homozygotes and heterozygotes for many of the over 600 known structural hemoglobin variants may also be detected during screening. Approximately 50 infants who are carriers of hemoglobin variants are identified for every one individual who is detected with SCD. There may be no immediate benefit to the infant from identification of carrier status; however, parents may benefit from knowing the child's test result. Therefore, most agree that test results should be available to parents of infants tested in NBS programs. Because of limited public health resources and the numbers of carriers detected, a variety of approaches to follow-up of these individuals have been developed so parents may become aware of their child's test results.

The following guidelines address a number of issues that need to be considered in implementing follow-up programs for SCD and related hemoglobin variants. These guidelines are to be viewed as a broad expression of a consensus from CORN with an attempt to take into account differences of opinion, as well as local program capabilities and regulations. They endeavor to combine both ideal and practical considerations.

**1. Ideally, education about NBS, which usually includes testing**

**for SCD, should be provided to families during prenatal care—well in advance of the time of delivery.**

If all mothers are educated early in their prenatal care about the testing that will be done at birth, interested parents will know to ask their physicians about the results of NBS. Education can include verbal information, audio and video productions, and written materials. A number of community sickle cell programs can also serve as resources. Prenatal education programs need to address the cultural and ethnic diversity of the clients whose infants will be tested for hemoglobin variants.

**2. A mechanism should be in place in state NBS programs so that all results of NBS can be made available to the parents of all infants who are tested.**

All results should be reported to the submitting health care provider and/or the hospital of birth. Each screening program should develop guidelines for follow-up of carriers of all clinically significant hemoglobin variants. A mechanism for notification of families of carriers should be provided so parents can obtain test results without significant delay or inconvenience. This should involve notification of the infant's physician and/or the hospital of birth of the result so that the test result can be made a part of the medical record and so that designated individuals can notify the parents. Parents may be contacted by the state NBS program so that results are directly conveyed to the families.

**3. Parents of all infants who are detected to be carriers of hemoglobin variants should be offered appropriate education, counseling, and testing.**

These activities can be provided by the state NBS programs or

by referral to appropriate community resources. Numerous resources are already available in many communities. Private physicians, public health clinics, comprehensive sickle cell programs, and community sickle cell organizations can be utilized to enhance access to needed services. The provision of additional information, education, and counseling should be based on the parents' desire to obtain enhanced understanding of their child's test result. The information should be accurate and presented in a manner that increases understanding of the implications for the infant and other family members. Education and counseling activities should be sensitive to cultural and social needs of the family. The information should be provided in a way that prevents unnecessary concern in the parents and does not result in inappropriate care of the child. Stigmatization and discrimination must be prevented.

Information on additional resources for needed services such as individual diagnosis, prenatal diagnosis, and extended family testing should be available. Information on sources for psychological and social support should also be provided if needed by parents or other family members.

**4. Individuals who counsel should have appropriate training and credentialing in order to ensure the highest quality of services for families of carriers detected by NBS.**

Persons providing counseling to individuals with sickle and other hemoglobin variants need additional understanding of pedigree development, extended family testing, resources for individual and prenatal diagnosis, and psychologic support of individuals receiving sensitive genetic in-

formation. Educating about the potential clinical and genetic implications of carrier status requires understanding of the biochemical, clinical, and genetic implications of a test result. Training and certification must address this knowledge, as well as cultural and ethnic considerations, in providing information. A mechanism for directly establishing the individual's effectiveness and cultural competence in education and counseling is highly desirable. Counselors should be selected based on their knowledge and their cultural sensitivity and competence in dealing with the population being counseled.

Counselors certified by the American Board of Genetic Counselors are already qualified to provide this service. Appropriate training can also prepare other individuals to meet standards of quality for counseling this population about selected he-

moglobinopathies. The credentialing process should provide for a minimum background of education in health sciences, minimum standards for education curriculum, and a certification process. During its initial implementation, the certification process should allow inclusion of the large number of individuals who presently have extensive experience in providing these services. An initiative to establish a national certification program for persons who provide sickle cell counseling is under way.

**5. NBS programs should have a mechanism for monitoring and assessing the approaches to, responses to, and costs of providing carrier education and counseling services.**

Evaluation of approaches to provision of test results, education, and counseling is important. Such evaluation should determine the response to and cost of follow-up services so that differ-

ent approaches to notification can be justified and compared.

*These Guidelines for Follow-up of Carriers of Hemoglobin Variants Detected by Newborn Screening were developed by the Sickle Cell, Thalassemia, and Other Hemoglobin Variants Committee of the Council of Regional Networks (CORN) for Genetic Services. They are based on the proceedings of a one-day meeting on Counseling for Sickle Cell Trait held in conjunction with the 11th National Neonatal Screening Symposium in Corpus Christi, Texas, on September 10, 1995. They were revised and approved by the Sickle Cell, Thalassemia, and Other Hemoglobin Variants and NBS Committees of CORN with input from the National Sickle Cell Advisory Committee. The CORN Steering Committee gave its endorsement on April 12, 1997. This effort was supported in part by Project # MCJ-151006 from the Maternal and Child Health Bureau (Title V, Social Security Act), Health Resources and Services Administration, Department of Health and Human Services.*

