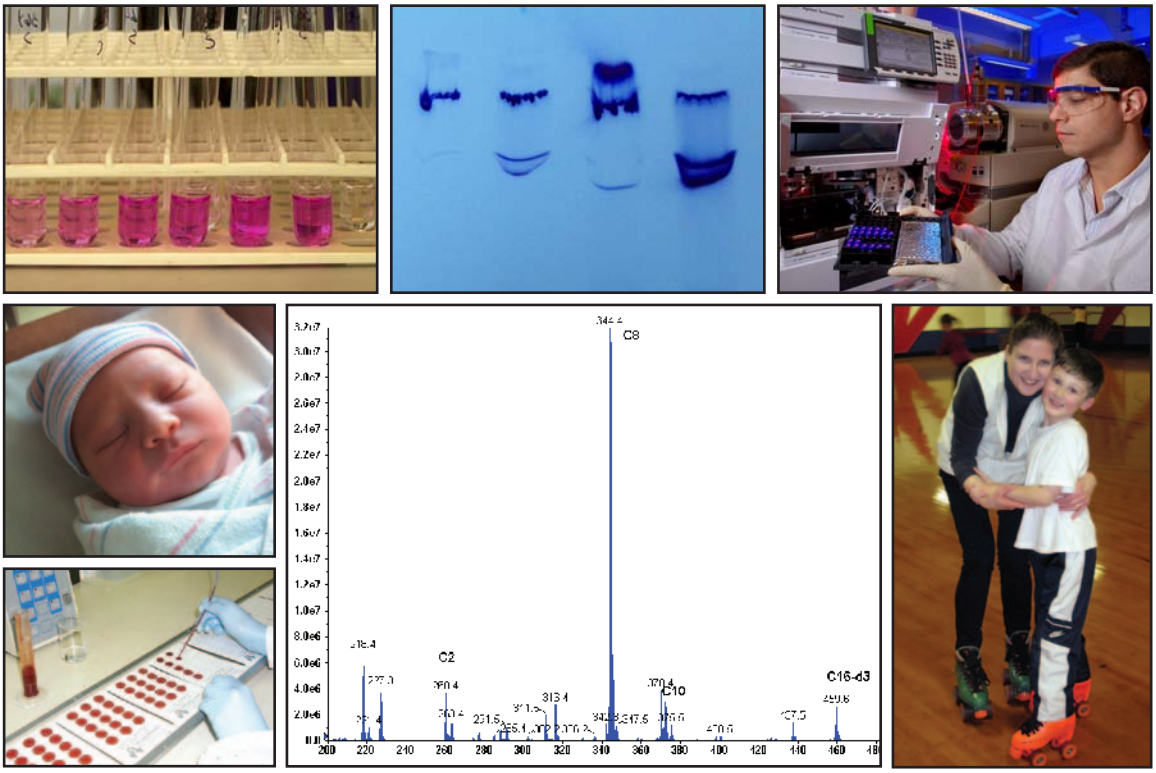
Centers for Disease Control and Prevention
MMWR
Morbidity and Mortality Weekly Report
Recommendations and Reports / Vol. 61 / No. 2
April 6, 2012

Good Laboratory Practices for Biochemical Genetic Testing and Newborn Screening for Inherited Metabolic Disorders



U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

CONTENTS

CONTENTS (*Continued*)

Disclosure of Relationship

CDC, our planners, and our presenters wish to disclose they have no financial interests or other relationships with the manufacturers of commercial products, suppliers of commercial services, or commercial supporters. Presentations will not include any discussion of the unlabeled use of a product or a product under investigational use. CDC does not accept commercial support.

Front cover photos: Top row, left to right: colorimetric biotinidase assay; thin layer chromatography analysis for mucopolysaccharidoses, a group of lysosomal disorders; laboratorian using tandem mass spectrometry. Second row, left to right: sleeping newborn; acylcarnitine profile indicating elevated C8, a biochemical marker for medium-chain acyl-CoA dehydrogenase deficiency; mother and her son, who are able to skate together because of effective laboratory monitoring and clinical management of an inherited metabolic disorder. Bottom left: dried blood spot samples for proficiency testing.

The *MMWR* series of publications is published by the Office of Surveillance, Epidemiology, and Laboratory Services, Centers for Disease Control and Prevention (CDC), U.S. Department of Health and Human Services, Atlanta, GA 30333.

Suggested Citation: Centers for Disease Control and Prevention. [Title]. MMWR 2012;61(No. RR-2):[inclusive page numbers].

Good Laboratory Practices for Biochemical Genetic Testing and Newborn Screening for Inherited Metabolic Disorders

Prepared by
Bin Chen, PhD[1]
Joanne Mei, PhD[2]
Lisa Kalman, PhD[1]
Shahram Shahangian, PhD[1]
Irene Williams, MMSc[1]
MariBeth Gagnon, MS[1]
Diane Bosse, MS[1]
Angela Ragin, PhD[1]
Carla Cuthbert, PhD[2]
Barbara Zehnbauer, PhD[1]

[1]*Division of Laboratory Science and Standards; Laboratory Science, Policy, and Practice Program Office; Office of Surveillance, Epidemiology, and Laboratory Services*
[2]*Division of Laboratory Sciences; National Center for Environmental Health/Agency for Toxic Substances and Disease Registry*

Summary

Biochemical genetic testing and newborn screening are essential laboratory services for the screening, detection, diagnosis, and monitoring of inborn errors of metabolism or inherited metabolic disorders. Under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) regulations, laboratory testing is categorized on the basis of the level of testing complexity as either waived (i.e., from routine regulatory oversight) or nonwaived testing (which includes tests of moderate and high complexity). Laboratories that perform biochemical genetic testing are required by CLIA regulations to meet the general quality systems requirements for nonwaived testing and the personnel requirements for high-complexity testing. Laboratories that perform public health newborn screening are subject to the same CLIA regulations and applicable state requirements. As the number of inherited metabolic diseases that are included in state-based newborn screening programs continues to increase, ensuring the quality of performance and delivery of testing services remains a continuous challenge not only for public health laboratories and other newborn screening facilities but also for biochemical genetic testing laboratories. To help ensure the quality of laboratory testing, CDC collaborated with the Centers for Medicare & Medicaid Services, the Food and Drug Administration, the Health Resources and Services Administration, and the National Institutes of Health to develop guidelines for laboratories to meet CLIA requirements and apply additional quality assurance measures for these areas of genetic testing. This report provides recommendations for good laboratory practices that were developed based on recommendations from the Clinical Laboratory Improvement Advisory Committee, with additional input from the Secretary's Advisory Committee on Genetics, Health, and Society; the Secretary's Advisory Committee on Heritable Disorders in Newborns and Children; and representatives of newborn screening laboratories. The recommended practices address the benefits of using a quality management system approach, factors to consider before introducing new tests, establishment and verification of test performance specifications, the total laboratory testing process (which consists of the preanalytic, analytic, and postanalytic phases), confidentiality of patient information and test results, and personnel qualifications and responsibilities for laboratory testing for inherited metabolic diseases. These recommendations are intended for laboratories that perform biochemical genetic testing to improve the quality of laboratory services and for newborn screening laboratories to ensure the quality of laboratory practices for inherited metabolic disorders. These recommendations also are intended as a resource for medical and public health professionals who evaluate laboratory practices, for users of laboratory services to facilitate their collaboration with newborn screening systems and use of biochemical genetic tests, and for standard-setting organizations and professional societies in developing future laboratory quality standards and practice recommendations. This report complements Good Laboratory Practices for Molecular Genetic Testing for Heritable Diseases and Conditions *(CDC. Good laboratory practices for molecular genetic testing for heritable diseases and conditions. MMWR 2009;58 [No. RR-6]) to provide guidance for ensuring and improving the quality of genetic laboratory services and public health outcomes. Future recommendations for additional areas of genetic testing will be considered on the basis of continued monitoring and evaluation of laboratory practices, technology advancements, and the development of laboratory standards and guidelines.*

The material in this report originated in the Laboratory Science, Policy, and Practice Program Office, May Chu, PhD, Director, and the National Center for Environmental Health/Agency for Toxic Substances and Disease Registry, Christopher J. Portier, PhD, Director.
Corresponding preparer: Bin Chen, PhD, Division of Laboratory Science and Standards, 1600 Clifton Rd., NE, MS G-23, Atlanta, GA 30329. Telephone: 404-498-2228; Fax: 404-498-2215; E-mail: bkc1@cdc.gov.

Introduction

Inherited metabolic diseases, often referred to as inborn errors of metabolism, comprise a large class of genetic diseases involving disorders of metabolism; collectively, these diseases have an incidence of at least one in 1,500 persons in the United States (*1*). Biochemical genetic testing and newborn screening tests are essential for early recognition of and timely intervention for these disorders to reduce morbidity and mortality rates and improve health outcomes. Biochemical genetic tests encompass a diverse spectrum of laboratory analyses of metabolites, enzyme activities, and functional assays for evaluation, diagnosis, treatment monitoring, disease management, and assessing a person's risk for carrying a specific disease trait (i.e., carrier status assessment), such as inborn errors of metabolism. Newborn screening is a vital state-based public health system in the United States that aims to test all newborns for an increasing number of inherited metabolic diseases and other congenital disorders, many of which require immediate treatment (*2*). The nationwide implementation of a recommended uniform screening panel of inherited metabolic diseases (Table 1) (*3*) and the consideration of additional conditions by state newborn screening programs present continuing quality assurance challenges for public health laboratories and other newborn screening facilities as well as for biochemical genetic testing laboratories that perform subsequent diagnostic testing. As advances in laboratory technology and knowledge of the genetic basis of disease increase the necessity of accurate and reliable laboratory testing in the screening, diagnosis, classification, and treatment of inherited metabolic diseases, guidelines are necessary for quality assurance and quality improvement in these areas of laboratory testing.

CDC has collaborated with the Centers for Medicare & Medicaid Services (CMS), the Food and Drug Administration (FDA), and other federal agencies; state programs; professional organizations; standard-setting institutions; and federal advisory committees to promote the quality of genetic testing and provide guidance for appropriate use of genetic tests in clinical and public health practices. In the 2009 report *Good Laboratory Practices for Molecular Genetic Testing for Heritable Diseases and Conditions*, CDC provided recommendations for good laboratory practices in molecular genetic testing and indicated the need for recommendations in other areas of genetic testing, such as biochemical genetic testing, molecular cytogenetic testing, and testing of acquired genetic variations (*4*). This report complements the 2009 CDC recommendations by providing recommendations for good laboratory practices for biochemical genetic testing and newborn screening for inherited metabolic diseases. Recommendations for additional areas of genetic testing will be considered based on continued monitoring and evaluation of laboratory practices, technology advancements, and the development of professional practice guidelines.

The purposes of this report are to 1) clarify CLIA requirements that are applicable to biochemical genetic testing and newborn screening for inherited metabolic diseases and 2) provide recommendations for additional quality assurance practices that are not specifically addressed by CLIA requirements. The recommended practices address the benefits of the quality management system (QMS) approach, factors to consider before introducing new biochemical genetic tests, establishment and verification of test performance specifications, the total laboratory testing process (which consists of the preanalytic, analytic, and postanalytic phases), confidentiality of patient information and test results, and laboratory personnel qualifications and responsibilities for laboratory testing for inherited metabolic diseases. These recommendations provide a comprehensive guide for laboratories that perform biochemical genetic testing for ensuring the quality of laboratory services and highlight laboratory practices critical for quality improvement in newborn screening for inherited metabolic diseases. This report also is intended as a resource for users of laboratory services (e.g., authorized persons under applicable state law, health-care professionals, patients, and referring laboratories) to aid in their collaboration in newborn screening systems and effective use of biochemical genetic tests. This report also might assist standard-setting organizations and professional societies with development of future laboratory quality standards and practices, federal and state agencies with strategies and policies related to genetic testing, medical and public health professionals with evaluating laboratory practices, manufacturers of in vitro diagnostics with developing new testing products, and patients and families with improving their knowledge of good laboratory practices for genetic testing. Incorporation of these recommended practices into laboratory systems can improve the quality and appropriate use of genetic testing services, leading to better health outcomes for patients and their families. Abbreviations and a glossary of terms used in this report are provided (Appendices A and B).

Background

Inborn errors of metabolism are inherited genetic disorders that affect one or more of the hundreds of biochemical pathways in the human body. Patients with these disorders are unable to properly use or synthesize certain compounds, such as fatty acids, amino acids, organic acids, or macromolecules, because of defects in the enzymes or other components of various metabolic pathways. These conditions frequently are identified in infants

TABLE 1. Recommended uniform newborn screening panel*

Condition	Core conditions†	Secondary conditions§
Inherited metabolic diseases		
Disorders of amino acid metabolism	Argininosuccinic aciduria Citrullinemia, type I Maple syrup urine disease Homocystinuria Classic phenylketonuria Tyrosinemia, type I	Argininemia Citrullinemia, type II Hypermethioninemia Benign hyperphenylalaninemia Biopterin defect in cofactor biosynthesis Biopterin defect in cofactor regeneration Tyrosinemia, type II Tyrosinemia, type III
Disorders of fatty acid oxidation	Carnitine uptake defect (carnitine transport defect) Medium-chain acyl-CoA dehydrogenase deficiency Very long-chain acyl-CoA dehydrogenase deficiency Long-chain-L-3-hydroxyacyl-CoA dehydrogenase deficiency Trifunctional protein deficiency	Short-chain acyl-CoA dehydrogenase deficiency Medium/short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency Glutaric acidemia type II Medium-chain ketoacyl-CoA thiolase deficiency 2,4-Dienoyl-CoA reductase deficiency Carnitine palmitoyltransferase type I deficiency Carnitine palmitoyltransferase type II deficiency Carnitine acylcarnitine translocase deficiency
Disorders of organic acid metabolism	Propionic acidemia Methylmalonic acidemia (methylmalonyl-CoA mutase deficiency) Methylmalonic acidemia (cobalamin disorders) Isovaleric acidemia 3-Methylcrotonyl-CoA carboxylase deficiency 3-Hydroxy-3-methylglutaric aciduria Holocarboxylase synthase deficiency ß-Ketothiolase deficiency Glutaric acidemia type I	Methylmalonic acidemia with homocystinuria Malonic acidemia Isobutyrylglycinuria 2-Methylbutyrylglycinuria 3-Methylglutaconic aciduria 2-Methyl-3-hydroxybutyric aciduria
Endocrine disorders	Primary congenital hypothyroidism Congenital adrenal hyperplasia	
Hemoglobin disorders	SS disease (sickle cell anemia) S, beta-thalassemia	Various other hemoglobinopathies
Other conditions	Biotinidase deficiency Cystic fibrosis Classic galactosemia Hearing loss Severe combined immunodeficiencies	Galactoepimerase deficiency Galactokinase deficiency T-cell related lymphocyte deficiencies

Sources: Watson MS, Mann MY, Lloyd-Puryear MA, Rinaldo P. Newborn screening: toward a uniform screening panel and system. Genet Med 2006;8(Suppl 1):1S–252S.

National Newborn Screening and Genetics Resource Center. National newborn screening status report. Austin, TX: National Newborn Screening and Genetics Resource Center; 2011. Available at http://genes-r-us.uthscsa.edu/nbsdisorders.pdf. Accessed February 2, 2012.

Response of the Secretary of Health and Human Services to the Secretary's Advisory Committee on Heritable Disorders in Newborns and Children, May 21, 2010. Available at http://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/recommendations/correspondence/uniformpanelsecre052110.pdf. Accessed February 2, 2012.

* Adopted by the U.S. Department of Health and Human Services as a national standard for newborn screening programs on May 21, 2010.

† Core conditions are the conditions that newborn screening is specifically designed to identify. A core condition for newborn screening should have the following features: a specific and sensitive test is available to detect the condition, the health outcomes are well understood, treatment is available and effective, and identification of the condition could affect the future reproductive decisions of the family.

§ Secondary conditions are the genetic conditions that can be identified when screening for one of the core conditions or as a consequence of confirmatory testing for an out-of-range result of a core condition.

and young children with acute or chronic symptoms. When possible, early diagnoses with timely and effective interventions are essential for preventing permanent neurologic sequelae, disabilities, and other severe adverse outcomes.

Biochemical genetic testing is a critical discipline in laboratory medicine for the evaluation, diagnosis, treatment monitoring, clinical management, and in some cases, carrier status assessment, of inherited metabolic diseases. These tests comprise highly complex and specialized laboratory procedures performed for evaluating enzyme activity, functional status of proteins, and levels of metabolites such as amino acids, organic acids, and fatty acids using a wide variety of specimen types including urine, whole blood, plasma, serum, cerebrospinal fluid, muscle biopsy, and other tissue types. Biochemical genetic tests also are among the critical follow-up procedures for diagnosing presumptive cases detected during newborn screening.

Both the number of laboratories in the United States that perform biochemical genetic tests and the numbers of tests being performed are not certain. Although a nationwide survey identified laboratories that performed biochemical genetic testing in 2003 (*5*), more recent comprehensive data are not available, and information from voluntary laboratory directories are likely to be underestimates (*6*). However, information from the College of American Pathologists (CAP) Biochemical Genetic Testing Proficiency Survey Program indicated that the number of participating laboratories increased by 15% in 6 years, from 93 laboratories in 2002 to 107 laboratories in 2010 (*7*). Despite the limited nationwide data, biochemical genetic tests are performed for approximately 270 metabolic disorders spanning diverse disease categories (Table 2). As advances in biomedical research and laboratory technology lead to better

TABLE 2. Examples of inherited metabolic diseases for which biochemical genetic tests are performed

Disease category	Examples
Disorders of amino acid metabolism (amino acid disorders)	Phenylketonuria Maple syrup urine disease
Disorders of organic acid metabolism (organic acidemias and acidurias)	Methylmalonic aciduria Propionic aciduria Glutaric acidemia type 1
Disorders of fatty acid oxidation	Medium-chain acyl-CoA dehydrogenase deficiency
Disorders of urea cycle metabolism (urea cycle disorders)	Citrullinemia Argininemia Ornithine transcarbamylase deficiency
Disorders of cholesterol synthesis	Smith-Lemli-Opitz syndrome
Lysosomal storage disorders	Gaucher disease Fabry disease Hurler syndrome Niemann-Pick disease
Disorders of mitochondrial function (mitochondrial diseases)	Leber's hereditary optic neuropathy (LHON) Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) Myoclonic epilepsy with ragged-red fibers (MERRF)
Disorders of carbohydrate metabolism (carbohydrate metabolism disorders)	Galactosemia Fructose intolerance
Glycogen storage diseases	Glycogen storage disease type 1 Pompe disease McArdle disease
Disorders of peroxisomal function (peroxisomal diseases)	Zellweger syndrome Adrenoleukodystrophy
Disorders of purine or pyrimidine metabolism (purine and pyrimidine metabolism disorders)	Lesch-Nyhan syndrome Orotic aciduria
Neurotransmitter disorders	γ-Aminobutyric acid (GABA) transaminase deficiency Tyrosine hydroxylase deficiency
Disorders of porphyrin metabolism (porphyrias)	Acute intermittent porphyria Variegate porphyria
Metal metabolism disorders	Wilson disease Menkes syndrome
Disorders of glycosylation	Carbohydrate-deficient glycoprotein syndromes, types Ia and Ib
Disorders of connective tissue (e.g., collagen and fibrillin)	Marfan syndrome Osteogenesis imperfecta
Disorders of biotinidase	Biotinidase deficiency

Sources: Raghuveer TS, Garg U, Graf WD. Inborn errors of metabolism in infancy and early childhood: an update. Am Fam Physician 2006;73: 1981–90.

National Center for Biotechnology Information. GeneTests. Seattle, WA: National Center for Biotechnology Information; 2012. Available at http://www.ncbi.nlm.nih.gov/sites/genetests/?db=genetests. Accessed February 2, 2012.

understanding of the effects of genetic variations in biochemical pathways and metabolic diseases, the use of biochemical genetic tests in diagnosis, classification, and management of inherited metabolic diseases will likely continue to increase.

Newborn screening is a state-based public health system that tests infants shortly after birth for serious or life-threatening metabolic and other conditions that, when detected early, might be managed or treated to prevent death, disability, or other severe consequences such as mental retardation. The newborn screening programs test almost all (≥97%) of the 4 million babies born in the United States each year. These tests are conducted by public health laboratories using a few drops of blood, often collected from newborns before hospital discharge, that are spotted on filter paper cards (*2*). Most states collect a fee for newborn screening, which varies depending on the state and can be paid by third-party payers. Although newborn screening programs are primarily funded by user fees, state and federal public health system funding often is necessary to support the comprehensive programs, which include education, laboratory screening, follow-up and tracking, diagnosis, treatment and management, and evaluation. Over the last decade, the increasing use of tandem mass spectrometry in newborn screening has substantially increased the number of metabolic disorders that can be detected from dried blood spot specimens (*3,8,9*). In 2010, the Secretary of the U.S. Department of Health and Human Services (HHS) adopted the recommendation of the Secretary's Advisory Committee on Heritable Disorders in Newborns and Children (SACHDNC) for a uniform screening panel (including screening for 30 core conditions and reporting 26 secondary conditions) as a national standard for newborn screening programs together with the recommendation to facilitate the inclusion of this recommended panel into all state newborn screening programs (*10*). The expansion of inherited metabolic conditions screened by newborn screening programs has presented challenges to ensuring the quality of performance and delivery of testing services not only for public health laboratories and other newborn screening facilities but also for biochemical genetic testing laboratories that perform subsequent diagnostic testing (*11*).

CLIA Oversight of Biochemical Genetic Testing and Newborn Screening

In 1988, Congress enacted Public Law 100-578, a revision of Section 353 of the Public Health Service Act (42 U.S.C. 263a) that amended the Clinical Laboratory Improvement Act of 1967 and required HHS to establish regulations to ensure the quality and reliability of laboratory testing on human specimens for disease diagnosis, prevention or treatment, or health assessment purposes (*12*). Under the CLIA regulations, laboratory testing is categorized based on the level of testing complexity as 1) waived (from routine regulatory oversight), 2) moderate complexity, or 3) high complexity. Moderate- and high-complexity testing is nonwaived testing. For nonwaived testing, CLIA regulations include requirements for proficiency testing, facility administration, quality systems for the total testing process (which consists of the preanalytic, analytic, and postanalytic phases), personnel for moderate- and high-complexity testing, and when applicable, more specific requirements for testing specialties and subspecialties (*13*). CMS administers the CLIA laboratory certification program and collaborates with FDA and CDC in providing CLIA oversight. FDA is responsible for test categorization and waiver determinations, and CDC is responsible for quality improvement studies, convening the Clinical Laboratory Improvement Advisory Committee (CLIAC), and providing scientific and technical support. CLIAC was chartered by HHS to provide recommendations and advice to HHS, CDC, CMS, and FDA regarding CLIA regulations, the impact of CLIA regulations on medical and laboratory practices, and modifications to CLIA standards to accommodate technological advances (*14*).

Although not defined as specialties or subspecialties under CLIA, biochemical genetic tests and newborn screening tests are considered high-complexity tests. Laboratories that perform these tests must meet the applicable general CLIA requirements for nonwaived testing and the personnel requirements for high-complexity testing. These laboratories may be accredited by a deemed-status accreditation program approved by CMS to meet the CLIA certification requirements (*13*). Additional state requirements also might be applicable to newborn screening laboratories.

Concerns Related to Biochemical Genetic Testing

The test procedures used to perform biochemical genetic tests are generally complex and technically demanding. Laboratory interpretation of test results is crucial for the clinical use of test result information in specific patient contexts and should be provided by trained and qualified personnel. Although data are limited, studies and reports since 2003 have revealed various concerns related to quality assurance practices in biochemical genetic testing, including test performance establishment, quality control procedures, proficiency testing, personnel qualifications and training, and results reporting (*5,15,16*). These concerns indicate areas of biochemical genetic testing practices that are in need of improvement or will likely benefit from the development and implementation of good laboratory practices.

Establishing Test Performance

A comprehensive survey on quality assurance practices in biochemical genetic testing indicated that most laboratories

that performed these tests used laboratory-developed methods and had variable practices for establishing test performance specifications such as reference intervals (*5*). The difficulty of obtaining sufficiently large numbers of samples from apparently healthy persons has made it challenging to establish reference intervals for certain analytes, especially when sample collection requires invasive techniques. The same challenge also affects the establishment of specific reference intervals by sex, age group, and other clinically relevant parameters (*15,16*). The lack of commercially available standards and reference materials presents another major challenge in establishing test performance specifications for biochemical genetic tests (*15*).

Expanded newborn screening programs also present challenges to biochemical genetic testing laboratories, such as establishing age-specific reference intervals for infants and characterizing interfering substances to facilitate disease diagnosis primarily based on metabolic alterations and often in the absence of characteristic clinical symptoms or physical signs of disorders that are more commonly detected in older children (*11*). In addition, the establishment of clinical validity for new tests might involve a substantial literature review or research before introducing these tests into clinical use (*11*).

Quality Assurance During the Three Phases of the Testing Process

Preanalytic Phase

The preanalytic phase of the testing process generally encompasses test selection and ordering; specimen collection, processing, handling, and delivery to the testing site; and the receipt of the patient's specimens with the test request information by the laboratory (*17*). Biochemical genetic tests are associated with a wide range of preanalytic variables that might affect test performance and test results because of the diverse specimen types and conditions, patient preparation status, and highly complex test procedures (*18*). Obtaining necessary clinical, medication, nutritional status, and other patient information that is critical for effective test result interpretation also can be challenging (*5,18*).

Analytic Phase

The analytic phase of the testing process includes specimen preparation, performance of test procedures, monitoring and verification of accuracy and reliability of test results, and documentation of test findings (*17*). Significant variability in quality control practices was reported for biochemical genetic tests (*5*). For example, 14% of participating laboratories reported omission of normal controls in each test run, whereas 53% and 19% of the laboratories, respectively, included controls representing affected persons or carriers in enzyme-based assays designed to identify carriers and affected persons (*5*). The scarcity of commercially available reference materials also presents a challenge to performing quality control procedures, evaluating and verifying laboratory-prepared solutions, and standardizing calibration and calibration verification practices so that test results are comparable between laboratories (*15*).

Postanalytic Phase

The postanalytic phase of the testing process includes reporting test results and archiving records, reports, and tested specimens (*17*). Variable postanalytic practices were reported for biochemical genetic tests (*5*). For example, only 24% of the surveyed laboratories reported the inclusion of a summary of test methods in biochemical genetic test reports, and only 12% had a specific written policy about confidentiality of genetic testing results (*5*). Among laboratories that performed amino acid analysis, 37% did not include results interpretation on test reports (*5*). Although these practices are not explicitly specified in CLIA regulations, they have been recommended in professional guidelines as necessary quality assurance procedures for biochemical genetic tests (*18*).

Proficiency Testing

Proficiency testing is a well-established practice for monitoring laboratory testing performance and is a key component of the external quality assessment process. Participation in proficiency testing has been reported to help laboratories reduce analytic deficiencies, improve testing procedures, and take actions necessary to prevent future errors (*19,20*). Proficiency testing samples that simulate actual patient specimens could allow the evaluation of the total testing process (which consists of the preanalytic, analytic, and postanalytic phases) and improve the monitoring of laboratory performance (*21–23*). These samples might be derived from tissue samples or cell lines made from patients with a known condition or might be synthesized by adding known concentrations of analytes into a matrix such as serum or urine.

CLIA regulations do not include proficiency testing requirements specifically for biochemical genetic or newborn screening tests. Laboratories that perform these tests must meet the general CLIA requirement to verify, at least twice annually, the accuracy of the genetic tests they perform (§493.1236[c]) (*13*). Laboratories may participate in available proficiency testing programs for the biochemical genetic tests they perform to meet this CLIA alternative performance assessment requirement.

Proficiency testing participation helps laboratories that perform biochemical genetic testing to improve quality assurance procedures through identification of areas that need improvement, such as variability in analytic performance and the lack of standardization for reportable units of

measurement (*7,20*). Formal proficiency testing or external quality assessment programs are available only for a limited number of biochemical genetic tests, such as those included in the Biochemical Genetics survey program provided by CAP and the European Research Network for Evaluation and Improvement of Screening, Diagnosis, and Treatment of Inherited Disorders of Metabolism (ERNDIM) (Appendix C) (*24,25*). Practical and technical challenges, such as the lack of proficiency testing materials, might limit the availability of comprehensive proficiency testing programs that assess both the quantitative and qualitative test methods for each analyte and examine the entire testing process.

For many rare genetic conditions (i.e., conditions that affect <200,000 U.S. persons at any given time) for which testing is performed by one or a few laboratories, substantial barriers to developing formal proficiency testing programs have been recognized. Professional guidelines have been developed for laboratories to evaluate and monitor test performance when proficiency testing programs are not available (*26*), and online registry services have been developed to facilitate sample exchange among genetic testing laboratories (*27*).

Personnel Qualifications and Training

Qualifications of laboratory personnel, including training and experience, are critical for ensuring quality performance of genetic testing because human errors can have a substantial impact on the quality of laboratory test results (*5,28*).

The qualifications of persons directing or supervising biochemical genetic testing laboratories, including specialized training, experience, and board certification in clinical biochemical genetics, correlate significantly with laboratory adherence to voluntary quality standards and guidelines for biochemical genetic testing (*5*). The need for trained, qualified personnel to ensure the quality of biochemical genetic testing also has been recognized internationally (*29*).

Quality Improvement for Laboratory Practices in Newborn Screening

Ensuring high-quality testing and achieving continuous quality improvement has been challenging for newborn screening laboratories as the number of inherited metabolic diseases that are included in newborn screening programs has continued to increase. For example, variability has been reported in certain newborn screening laboratory practices, including criteria for acceptance of dried blood spot specimens and cutoff values for each analyte, which might vary by state program depending on specific populations and case definitions (*30,31*). Most state programs provide training and continuing education to hospital staff members and others who submit specimens from newborns regarding appropriate collection procedures for dried blood spot specimens (*32*). Performance metrics and quality indicators have been described to meet the evaluation and improvement needs of the national newborn screening system (*33,34*).

Laboratories in the United States that test dried blood spot specimens have been voluntarily participating in the CDC Newborn Screening Quality Assurance Program (NSQAP), which enables newborn screening laboratories to meet the CLIA alternative performance assessment requirement for verifying test result accuracy at least twice per year (*35*). Laboratories gain testing proficiency through comparisons of peer performance within and among methods. Four times per year, NSQAP provides newborn screening laboratories with blind-coded dried blood spot samples that represent analytes detected for newborn screening disorders. Participating laboratories include these samples in their routine testing and test them in the same way they test dried blood spot specimens from newborns. Test performance is evaluated based on identification of test results that require additional follow-up testing (out-of-range results) compared with those that do not (in-range results) (*30*). NSQAP summarizes annual false-positive and false-negative rates for the performance assessment samples to help laboratories investigate potential sources of errors and areas of laboratory practices that need improvement. In 2008, NSQAP found that the false-positive rate for performance assessment samples was <1% for all newborn screening markers except decenoylcarnitinine (a secondary marker for medium chain acyl-CoA dehydrogenase deficiency), immunoreactive trypsinogen (a primary marker for cystic fibrosis), and succinylacetone (a specific marker for tyrosinemia type 1), whereas the false-negative rate was 1.1%–3.3% for phenylalanine (a primary marker for phenylketonuria), tyrosine (a primary marker for tyrosinemia), and immunoreactive trypsinogen (a primary marker for cystic fibrosis) (*30*). The decrease in the false-negative rate from 2002 to 2008 supports NSQAP in improving laboratory performance. NSQAP also provides quality control materials to help newborn screening laboratories monitor the quality of test performance (*30*).

Collaborative efforts by many federal agencies, advisory groups, and the private sector have led to the development of standards and mechanisms for electronic reporting of newborn screening results (*36–38*). For example, the National Library of Medicine (NLM) and Health Resources and Services Administration (HRSA) have developed guidelines for standardized terminology, coding, and electronic messaging for ordering newborn screening tests and reporting test results to facilitate complete and accurate data collection, prompt results delivery and communication, and improved patient management (*37,38*). These guidelines have called

for uniform laboratory practices in the newborn screening process, including the collection and documentation of demographic and clinical information (e.g., birth weight, gestational age, nutritional status, and transfusion information) in the preanalytic phase and the laboratory interpretation and reporting of results in the postanalytic phase (*36,38–40*).

Methods

The development and preparation of the recommendations in this report involved a multistep process that included 1) initial information collection and evaluation by CDC scientists to assess the quality assurance practices and potential areas needing improvement in biochemical genetic testing and newborn screening, 2) development of CLIAC recommendations to be considered by CDC for inclusion in a CDC guideline, 3) solicitation of input from other federal advisory committees and stakeholders that also address quality of genetic testing and newborn screening to complement the CLIAC recommendations, and 4) evaluation of all recommendations and advice received and preparation of this report by CDC scientists.

Initial Information Review and Assessment (2008–2009)

An initial information review and assessment was conducted by CDC scientists from the Division of Laboratory Science and Standards in collaboration with the CDC Newborn Screening Quality Assurance Program. The purposes of this review and assessment were to 1) identify laboratory practice issues in biochemical genetic testing and newborn screening that would benefit from recommendations for good laboratory practices; 2) define issues for consideration by a CLIAC workgroup and assess areas of expertise needed for this workgroup; 3) assess information needed to facilitate the workgroup's evaluation of current standards, guidelines, practices; and 4) help gauge the usefulness and impact of the CDC recommendations on laboratory testing quality and public health.

The information review and assessment consisted of a literature review, gathering data from existing databases and resources, and a review of regulatory and voluntary standards that are specific or applicable to biochemical genetic testing and newborn screening. A search of biomedical literature published since 2006 was conducted using the Medline and the PubMed databases with search terms including inherited metabolic diseases, inborn errors of metabolism, newborn screening, biochemical genetic testing, laboratory quality, good laboratory practice, laboratory standard, quality assurance, proficiency testing, quality assessment, and quality management. Approximately 400 English-language publications were identified, of which 18 contained information on laboratory performance or quality management practices and were specifically reviewed (*3, 5,11,15,19,20,30,33,34,41–49*). Data also were collected from state programs (*50,51*), CDC studies (*8,52,53*), and publicly available directories and databases of laboratories and laboratory testing (*6,7,24,25,29,32,54,55*). Review of these data and information sources focused on 1) assessing the scope and growth of biochemical genetic testing and newborn screening in the United States, including the number of laboratories that perform biochemical genetic testing and newborn screening, the number and type of inherited metabolic diseases for which biochemical genetic testing and newborn screening is performed, the test methods and technology used to perform these tests, test volume, availability of proficiency testing and external quality assessment programs, and the changes of these aspects over time; 2) evaluating factors in biochemical genetic testing and newborn screening that might affect testing quality; and 3) identifying concerns and deficiencies in quality assurance practices in biochemical genetic testing and newborn screening and areas that would benefit from good laboratory practice guidelines. Considering the information gaps and the small number of published studies that specifically collected information on quality assurance issues in biochemical genetic testing laboratories, this initial information gathering and review was intended to be inclusive to provide background information to the CLIAC workgroup, enable workgroup evaluation of the information gathered and issues identified, and elicit additional insights. CDC scientists also reviewed regulatory and voluntary laboratory standards that are specific or applicable to biochemical genetic testing and newborn screening, including the American College of Medical Genetics (ACMG) Standards and Guidelines for Clinical Genetic Laboratories (*56*), Clinical and Laboratory Standards Institute (CLSI) guidelines (*17,26,57–73*), CLIA regulations (*13,74*), FDA guidance documents (*75,76*), state requirements (*77,78*), accreditation checklists (*79–82*), national practice guidelines (*11,83*), and international standards and guidelines (*84–86*). To facilitate the assessment of the extent to which the identified quality assurance needs were addressed by existing standards and guidelines, CDC scientists included these regulatory and voluntary standards in comprehensive comparison documents for each of the laboratory practice areas in which quality assurance concerns or the need for specific quality assurance guidance were identified. These documents compared existing CLIA regulations with other relevant federal requirements, state regulations, accreditation standards, professional guidelines, and other voluntary national and international standards and guidelines. Sixteen comparison documents of laboratory standards and recommendations

were developed to address preanalytic practices (including the laboratory responsibility to provide test information to users of laboratory services, informed consent, test request, specimen submission and referral, and preanalytic systems assessment), analytic practices (including performance characteristics for biochemical genetic testing and newborn screening, establishment and verification of test performance specifications, calibration and calibration verification, control procedures, proficiency testing and alternative performance assessment, and equipment, instruments, and reagents), postanalytic practices (including test report and retention of records, reports, and specimens), personnel qualifications and responsibilities, and quality management practices.

Development of CLIAC Recommendations

Since 1997, CLIAC has provided HHS with recommendations on approaches and mechanisms for ensuring the quality of laboratory genetic testing (*14*). At the September 2008 CLIAC meeting, the committee provided recommendations for good laboratory practices in molecular genetic testing for heritable diseases and conditions, which were subsequently included in the 2009 CDC recommendations (*4*); CLIAC also recommended the formation of a workgroup to consider similar good laboratory practices for biochemical genetic testing (*87*).

The CLIAC Biochemical Genetic Testing Good Laboratory Practices Workgroup subsequently was formed in 2009. Workgroup members were selected by expertise needed to address the identified quality assurance issues and provide suggestions for laboratory practices, the potential impact and effectiveness of the laboratory practices to be recommended, and the representation of CLIAC as required by the Federal Advisory Committee Act (*88*). Factors for selection of workgroup members included expertise in diverse testing technology and diagnostic issues (e.g., common and rare disease testing); representation of diverse laboratory environments (e.g., large and small laboratories; laboratories in academic, private, and public health sectors; and specialized and general laboratories); representation of newborn screening and public health perspectives; expertise in laboratory performance evaluation, laboratory inspection, and laboratory accreditation; the perspective of users of laboratory services (including health-care providers, patients, and referring laboratories) and other stakeholders; experience in federal and state regulatory oversight; experience in developing accreditation standards or professional practice guidelines; experience and expertise in providing and evaluating proficiency testing and interlaboratory exchange programs; representation of in vitro diagnostic manufacturers; and representation of general laboratory services. The members of the workgroup, which included 13 nonfederal experts and representatives of CDC, CMS, and FDA, are listed at the end of this report.

The workgroup was charged with the responsibility of providing input to CLIAC for developing recommendations for good laboratory practices for biochemical genetic testing. Specific workgroup tasks included 1) suggesting the scope of the CLIAC considerations in developing good laboratory practice recommendations for biochemical genetic testing, 2) recognizing and identifying issues in biochemical genetic testing that need guidance for quality assurance, 3) identifying additional sources of data and information needed for workgroup discussion, 4) reviewing relevant practice guidelines and standards, 5) suggesting strategies for issues or areas of laboratory practices for which current standards and practice guidelines are lacking or inconsistent, and 6) formulating workgroup input for CLIAC consideration. The workgroup was advised that issues on which they could not reach consensus should also be reported to CLIAC. Through a series of meetings and teleconferences in 2009, the workgroup considered the scope of laboratory practice recommendations for biochemical genetic testing and testing for inherited metabolic diseases and suggested that recommendations be developed to apply to biochemical genetic testing as well as to newborn screening for inborn errors of metabolism. After reviewing the background information and the concerns in quality assurance practices that were identified by CDC scientists, workgroup members suggested additional information sources and issues that could affect the quality and performance of biochemical genetic testing and newborn screening. The workgroup then reviewed the 16 comprehensive comparison documents of laboratory standards and guidelines, which included federal and state regulatory requirements, professional guidelines, accreditation checklists, and international standards and guidelines that provided general or specific quality standards applicable to biochemical genetic testing and newborn screening. The workgroup also reviewed information on the HHS-approved and other certification boards for laboratory personnel, including the number of persons certified in each of the specialties for which certification is available. Suggestions and clarifications for good laboratory practices were provided by the workgroup for all issues that were recognized as needing quality assurance guidance. The outcomes of the workgroup discussions were summarized by CDC scientists into a workgroup report.

The workgroup report was presented to CLIAC at the February 2010 CLIAC meeting. CLIAC recommendations were formed from the committee discussion during the meeting by reviewing the workgroup report and making modifications and additional recommendations, as summarized in the CLIAC meeting summary (*89*). CLIAC recommended that the planned CDC recommendations include the CLIAC-recommended

good laboratory practices for biochemical genetic testing and newborn screening for inborn errors of metabolism (*89*). The CLIAC members involved in developing the recommendations are acknowledged at the end of this report.

Solicitation of Additional Input

To ensure that the recommendations provided in this report were adequately vetted with stakeholders, CDC collaborated with the National Institutes of Health (NIH) in obtaining additional input from the Secretary's Advisory Committee on Genetics, Health, and Society (SACGHS) and with HRSA in obtaining consultation from SACHDNC during 2010–2011. To complement the CLIAC recommendations, advice was solicited from both federal advisory committees regarding 1) any issue that CDC should explain or clarify for laboratories that perform biochemical genetic testing or newborn screening, 2) any additional issue pertaining to biochemical genetic testing or newborn screening laboratory practices that CDC should address in these recommendations, and 3) efforts that should be taken to encourage the implementation of the recommended practices once this report is published. Presentations regarding the CLIAC recommendations also were made at the annual conference of the Association of Public Health Laboratories (APHL), the APHL Newborn Screening and Genetics Symposium, and the ACMG annual meeting during 2010–2011. CDC scientists also convened with the APHL Newborn Screening and Genetics in Public Health Committee and its quality assurance and quality control subcommittee to discuss any recommendations needed in addition to the CLIAC recommendations and the effective approach to providing the recommendations specific for newborn screening.

Preparation of the Recommendations in this Report

CDC scientists prepared a draft of the recommended good laboratory practices based on the CLIAC recommendations and the additional input from SACGHS, SACHDNC, and APHL. In May 2011, an initial draft was provided to CLIAC, the CLIAC workgroup, CMS, FDA, CDC programs, the NIH Office of Biotechnology Activities, and HRSA for review and comment by SACHDNC and other interested groups and organizations. Comments and suggestions also were received from the Society for Inherited Metabolic Disorders, March of Dimes, and the APHL Newborn Screening and Genetics in Public Health Committee. CDC scientists reviewed all comments and suggestions. Modifications and clarifications have been incorporated in this report.

Recommended Practices for Laboratory Testing for Inherited Metabolic Disorders

The following recommended practices apply to laboratory testing for screening, detection, diagnosis, and monitoring of inherited metabolic disorders, including biochemical genetic testing and newborn screening. These recommendations are intended to provide guidelines for specific quality assurance concerns in these testing processes by addressing the following areas of laboratory practices:

- The QMS approach
- Factors to consider before introducing new biochemical genetic tests
- Establishment and verification of test performance specifications
- The preanalytic, analytic, and postanalytic testing phases
- Personnel qualifications, responsibilities, and competency assessment
- Confidentiality of patient information and test results

Many of the recommendations that follow apply generally to both biochemical genetic testing and newborn screening for inborn errors of metabolism, whereas issues that are specific for either laboratory area are discussed separately.

The QMS Approach

QMS is a systematic approach for managing and ensuring the quality and effectiveness of an organization's work operations and services (*90*). A laboratory QMS provides a framework for the implementation of policies, processes, and procedures for the quality system essentials to ensure the quality of activities throughout the laboratory's workflow (*17*). The recommended practices in this report are provided with consideration of the QMS principles and concepts and in accordance with the laboratory's workflow, including consideration and planning for introducing testing services for patient testing, establishment or verification of test performance specifications, and providing laboratory services to meet the needs in clinical and public health practices.

QMS is the basis for many international quality standards, such as the International Organization for Standardization (ISO) standards ISO 9001, ISO 17025, and ISO 15189 (*84,90,91*) and CLSI guidelines (*17,66–68,92,93*). QMS principles also have been adopted in state program requirements and accreditation standards in the United States (*77,82*). Although the QMS standards and guidelines are distinct from CLIA regulations, a QMS framework facilitates the implementation of practices to meet CLIA and other regulatory requirements, conform to professional standards, and deliver

quality laboratory services. Therefore, having a QMS in place will help laboratories that perform testing for inherited metabolic disorders apply the recommended practices in this report to improve test performance, laboratory service delivery, and the effectiveness of laboratory operations.

Considerations Before Introducing Biochemical Genetic Testing or Offering New Biochemical Genetic Tests

Recommendations described in this report should be considered, in addition to appropriate professional guidelines and recommendations, when planning and preparing for the introduction of biochemical genetic testing or offering new biochemical genetic tests. Factors to be considered in this stage should at least include the following:

- Analytic validity, clinical validity, and usefulness of the test for patient care or public health
- Benefits of the new test (which could be a test not available elsewhere or a test that is available elsewhere but can and should be performed by the laboratory) to patient care and the laboratory's services
- Demand for the new test, which should be considered together with patient care needs if the test will not be requested frequently
- Cost-effectiveness, including the expected number of test procedures that is sufficient to maintain proficiency in test performance and results interpretation
- Reimbursement issues, including those imposed by the current procedural terminology (CPT) codes and other codes that are associated with payment and reimbursement for the new test
- Federal, state, local, and accreditation requirements that apply to the new testing
- Personnel competencies, including available technical expertise and expertise for interpreting test results and providing consultation
- Training needs of laboratory personnel who will be involved in providing the testing service, which is determined based on assessments of their responsibilities
- Selection of test methods
- Laboratory facilities, equipment, and safety considerations
- Development of technical procedures and procedure manuals
- Establishment or verification of test performance specifications, including consideration for the availability of positive and negative controls, reagents, supplies, and (although not always available) external quality assessment and proficiency testing programs
- Issues specific to newborn screening at the federal and state levels, including follow-up services, availability of confirmatory tests, state mandates, and availability of funding or appropriations to pay for the new test
- Issues specific to new test offerings, such as patient consent needs, use of previously tested patient specimens, confidentiality of test results and information pertaining to family members, and intellectual property or licensing concerns
- Consultation or collaboration needed from users of laboratory services and other laboratories, such as consulting with clinicians who might request the test to assess the needs and demands for the new test and collaborating with clinical or laboratory researchers to establish test performance specifications, especially in circumstances of rare disease testing

Using the QMS approach should facilitate the planning, evaluation, and preparation for new test implementation. Laboratories should also consider appropriate professional guidelines, recommendations, and policy statements when introducing or offering new tests.

Establishment and Verification of Test Performance Specifications

CLIA requires laboratories to establish or verify the analytic performance of each nonwaived test or test system before the test is introduced for patient testing. The calibration and control procedures also must be determined based on each test's performance specifications. Verification of test performance specifications is required when a laboratory introduces an unmodified FDA-cleared or unmodified FDA-approved test system. An FDA-cleared test system has been determined by FDA to be substantially equivalent to another legally marketed test system. A premarket notification, referred to as a 510(k), must be submitted to FDA for clearance. An FDA-approved test system is a system for which FDA has approved a premarket approval (PMA) application before marketing begins. This approval process is generally reserved for high-risk medical devices and involves a more rigorous premarket review than a premarket notification submitted to FDA for clearance.

Before reporting patient test results, the laboratory must 1) demonstrate that the manufacturer-established performance specifications for accuracy, precision, and reportable range of test results can be reproduced or verified in the laboratory setting and 2) verify that the manufacturer-provided reference intervals (or normal values) are appropriate for the laboratory's patient population (42 CFR §493.1253) (*13*). Laboratories are subject to more stringent requirements when introducing 1) FDA-cleared or FDA-approved test systems that have been modified by the laboratory, 2) laboratory-developed tests or test systems that are not subject to FDA clearance or approval (e.g., standardized methods and textbook procedures), or

3) test systems with no manufacturer-provided performance specifications. In these instances, before reporting patient test results, laboratories must conduct more extensive procedures to establish performance specifications for accuracy, precision, analytic sensitivity, analytic specificity, reportable range of test results, reference intervals or normal values, and other applicable performance characteristics (*13*).

Laboratories that perform biochemical genetic testing or newborn screening must comply with these general CLIA requirements and should adhere to the additional recommendations that follow for establishment and verification of test performance specifications. These recommendations are intended to specifically address test performance establishment for laboratory-developed biochemical genetic tests to ensure valid and reliable test performance and results interpretation. The recommendations also might be used by laboratories to verify performance specifications of unmodified FDA-cleared or FDA-approved biochemical genetic test systems to be used for patient testing.

General Principles

When establishing or verifying test performance, laboratories should review and follow professional guidelines, such as those provided by CLSI and ACMG, that are applicable and appropriate for the planned testing. Laboratories should ensure that the professional guidance is followed consistently throughout the performance establishment and verification phase and the subsequent patient testing process. Factors that should be considered include the intended use of the test, the analytes or panel of analytes to be measured, intended patient populations, test methods, and samples needed for performance establishment (*76*). For establishing performance specifications of new biochemical genetic tests, the following practices should be considered as general principles:

- Review scientific studies and pertinent references to assess the test methods and clinical usefulness of the test.
- Define the patient populations for which the test might be performed.
- Select appropriate test methods for the disease (or condition) or analyte being evaluated.
- Establish or verify test performance specifications and determine quality control parameters for the test.

Samples for Establishment of Test Performance

In general, test performance specifications should be established with an adequate number, type, and variety of samples to ensure that test results can be interpreted in the context of specific patient conditions and that the limitations of the testing and test results are known. The number and type of both positive and normal samples should be considered when selecting and determining samples needed.

The numbers of both positive and normal samples should be adequate for determining the performance specifications of the assay being established. Both disease prevalence and sample characteristics might influence sample availability, thus the availability of samples and reference materials also should be considered. For example, a large number of positive samples (and in certain circumstances, normal samples) might not be available for rare conditions; unstable samples or samples that need to be collected invasively (such as cerebrospinal fluid or muscle biopsy samples) might be limited. Laboratories should consider these factors and define test performance specifications and limitations based on the samples that are available and included in the performance establishment.

The types of samples should represent the types of patient specimens that are expected for the assay (e.g., whole blood, serum, urine, dried blood spot, fresh or frozen tissue, or prenatal specimens). For example, if the laboratory intends to perform amino acid analysis for urine, plasma, and cerebrospinal fluid specimens, test performance specifications need to be established for all three specimen types because each specimen type might be associated with a different total testing process as a result of differences in specimen collection and handling, specimen stability, interfering substances, analyte extraction, reference ranges, results interpretation, and other preanalytic, analytic, and postanalytic factors.

If the condition of the patient specimens that the laboratory anticipates to receive represent significant variance that might affect patient test results or suggest the presence of interfering substances (e.g., insufficient specimen volume or amount, specimen hemolysis, or clotting), the laboratory should include samples representing these conditions when determining test performance, specimen acceptance criteria, and the influences of the specimen variances on test results interpretation.

Analytic Performance Specifications

Performance Characteristics

For each new biochemical genetic test, laboratories should determine specifications for the following performance characteristics:

Accuracy. Accuracy is the closeness of agreement between an individual value and a true value. For each quantitative test, the laboratory is responsible for determining the ability of the test method to produce accurate results. For qualitative methods, the laboratory should establish the capacity of the test method to identify the presence or absence of the analyte (*74*). Test performance establishment also should determine trueness, or the closeness of agreement between the mean value of a measurement series and the true value. Accuracy and trueness might be assessed by testing reference materials, comparing assay results to a reference method (i.e., gold

standard), comparing split-sample results with results obtained from a method shown to provide clinically valid results, or correlating research results with the clinical presentation when establishing a test system for a new analyte, such as a newly identified disease marker (*74*).

Precision. The laboratory is responsible for determining the precision of each new test by assessing repeatability (i.e., closeness of agreement between independent test results for the same measurand and under the same conditions) and reproducibility (i.e., closeness of agreement between independent test results for the same measurand under changed conditions). Precision can be verified or established by assessing day-to-day, run-to-run, and within-run variation (as well as operator variance) by repeat testing of known patient samples, quality control materials, or calibration materials over time (*74*).

Analytical sensitivity, including limit of quantification (LOQ) and limit of detection (LOD). Laboratories should follow professional guidelines in establishing LOD and LOQ for each analyte to be measured or detected (*61*). For modified test systems, the laboratory may use the lower limit of the manufacturer's reportable range if the laboratory has demonstrated that the modification has not affected the lower limit (*74*).

Analytical specificity. Determination of analytical specificity should include the ability of the test to detect or measure the target analytes distinctly from potential interfering substances, including factors associated with specimens (e.g., specimen hemolysis, anticoagulant, lipemia, and turbidity) and factors associated with patients (e.g., clinical conditions, disease states, and medications) (*74*). Laboratories must document information regarding interfering substances using product information, literature, or the laboratory's own testing (*74*). Laboratories should adhere to professional guidelines, such as those developed by ACMG, when establishing or verifying analytical specificity for each biochemical genetic test (*18*).

Reference range or normal values. The laboratory should establish a reference range that is appropriate for the laboratory's patient population (i.e., a normal range that reflects the type of specimen and demographic variables such as age, sex, and physiologic ranges expected for the laboratory's patient population) (*74*). When possible, laboratories should establish their own reference ranges by evaluating an appropriate number of samples to verify the reference ranges provided in literature or textbooks or by manufacturers. If the samples used in these verifications are from tested patient specimens rather than from healthy controls, laboratories should systematically evaluate the reference ranges and monitor the need to make adjustments over time. If samples that represent the specimen types (or specimen matrices) expected in patient testing are not available, laboratories may use the manufacturer-suggested or published reference ranges if they are appropriate for the laboratory's patient populations. Laboratories should monitor these reference values, make adjustments when appropriate, and inform their clients of the sources of their reference values (e.g., whether they are published values or values established or verified by the laboratory).

Reportable range of test results for the test system. The laboratory is responsible for determining the reportable range of test results for each test the laboratory performs (*13*). The reportable range of patient test results can be established or verified by assaying low and high reference materials or by evaluating known samples of abnormally high and low values (*74*).

Other performance characteristics required for test performance. For example, if a laboratory performs other test procedures in conjunction with a biochemical genetic test and reports the additional test results to aid in patient care, the laboratory should document the performance characteristics of the additional test procedures. Cutoff values for analytes detected in newborn screening often need to be age adjusted with consideration of infant term and birth weight (*71*).

Multiple-Analyte or Profile Analysis

If analyses of multiple-analyte or metabolic profiles (e.g., acylcarnitine profile and organic acids profile) include pattern recognition (i.e., recognition of abnormal concentrations of specific analytes or patterns of analytes), test performance establishment or verification should include the following additional practices:

- The reference ranges for all analytes to be reported should be established or verified for the laboratory's patient population with consideration of age, sex, physiologic state, and other clinically relevant factors.
- Both normal and abnormal samples, or both samples that generate results within the expected normal or negative range of test results established for a particular condition (i.e., in-range results) and those that are associated with out-of-range results, should be analyzed and the analyte patterns verified in comparison with the reference ranges and the documented patterns of analytes in abnormal concentrations that indicate disease states.
- As many different known samples as possible should be analyzed to ensure that common elements of a diagnostic pattern are detected.
- Substances that have the potential to interfere with the analysis should be identified (*94*).

Changes to Established Performance Specifications

Laboratories should recognize that changes to a test procedure, such as using a different sample matrix (plasma vs. urine), using or promoting the test for another purpose (screening vs. diagnostic use), and changing the type of analysis

(qualitative results vs. quantitative), could affect the established test system performance specifications for accuracy, precision, analytical sensitivity, analytical specificity, and clinical use. These changes might result in a modified test system for which the performance specifications must be reestablished (*74*).

Determination of Quality Control Procedures

CLIA requires laboratories to determine the calibration and control procedures for nonwaived tests or test systems as part of the verification or establishment of performance specifications for the tests (42 CFR §493.1253[b][3]) (*13*). Laboratories must meet these requirements and should consider the recommended quality control practices for each new test before the test is introduced for patient testing.

Documentation of Information on Clinical Validity

Although CLIA regulations do not include validation of clinical performance specifications of new tests or test systems, laboratories are required to ensure that the tests being performed meet clinical expectations. For tests of high complexity such as biochemical genetic tests, laboratory directors and technical supervisors are responsible for ensuring that the testing method is appropriate for the clinical use of the test results and can provide the quality of results needed for patient care (*13*). Laboratory directors and clinical consultants must ensure laboratory consultations are available for laboratory clients regarding the appropriateness of the tests ordered and interpretation of test results (*13*). Documentation of available clinical validity information will help laboratories performing biochemical genetic testing to fulfill their responsibilities for providing consultation to health-care professionals and other users of laboratory services.

Laboratories should ensure that the tests they perform are clinically relevant and can be interpreted for specific clinical situations. Laboratory responsibilities for clinical validity include the following:

- Documentation of clinical validity parameters (including, when applicable, clinical sensitivity, clinical specificity, positive predictive value, and negative predictive value) of the genetic tests the laboratory performs from available information sources (e.g., published studies, professional practice guidelines, and communication with clinicians)
- Establishment of clinical sensitivity, clinical specificity, and predictive values (as applicable) based on internal study results obtained using previously characterized positive and normal samples if the test is completely new or data are not available from published references
- Determination of test results that suggest imminent or potentially life-threatening conditions, or of critical values or alert values that warrant immediate medical attention based on available information sources and in consultation with health-care providers and other users of the test results
- Documentation of clinical factors that might affect test results and results interpretation, such as the overall health and disease states, concomitant clinical conditions, nutritional status, medications, and variable phenotypic expression of genetic diseases (e.g., low excretor variants of glutaric aciduria type 1, intermittent findings of fatty acid oxidation disorders, or normal analyte levels in intermittent maple syrup urine disease)
- Truth in advertising, which means the claims made by the laboratory that describe the test's analytic and clinical parameters (including limitations) are valid and based on sound scientific principles
- Specific responsibilities of the laboratory director and the technical supervisor to ensure appropriate documentation and reporting of the clinical validity information for the biochemical genetic tests their laboratories perform

Documenting clinical validity is a continuous process and might require extended studies with multidisciplinary collaboration (*95*). Laboratories should monitor the progress of clinical research and advances in understanding in this area, especially for newly discovered gene-disease associations, rare disorders, and conditions with highly variable expression or uncertain clinical sensitivity. Laboratory directors and technical supervisors are responsible for using professional judgment to evaluate the results of such studies and should adhere to professional guidelines and accreditation standards to ensure that the testing performed and results interpretation are appropriate for specific clinical settings (*11,18,81,96,97*).

Additional Recommendations for Newborn Screening

Performance establishment for newborn screening tests presents special challenges because of the time-sensitive need for specimen collection so that infants with positive screening results can receive timely follow-up confirmatory testing and effective intervention. The recommendations that follow provide additional guidelines for laboratories that perform newborn screening when establishing or verifying test performance specifications.

Specimen collection time frame. Laboratories should consider the impact of the specimen collection time frame on the screening for each disorder when establishing or verifying test performance specifications. The majority of newborn screening dried blood spot specimens are initially collected from infants before age 72 hours or before hospital discharge (*98*). The specimens collected from each infant typically are used in multiple test procedures for the detection of many disorders, each of which might have a screening window (i.e.,

time interval with the greatest likelihood for disease diagnosis and effective treatment before overt symptoms or permanent damage occurs). For example, some conditions, such as maple syrup urine disease and galactosemia, warrant specimen collection in the first 24–48 hours to enable the detection of abnormal analyte levels and initiate early treatment. For other conditions such as homocystinuria, the abnormal analytes might be more readily detected on a later day. Therefore, laboratories should document the course of the abnormal analyte presentation for the diseases for which they screen and use age-adjusted reference intervals, with consideration of infant term, birth weight, and health status in test performance establishment and verification.

Number and source of samples. The number of samples included in performance establishment or verification should be sufficiently large to enable the determination of test performance specifications for population-based screening (*72*). Although samples might be available from various sources, such as tested patient specimens, reference materials, proficiency testing materials, and control materials, laboratories should consider using samples that have the same dried blood spot matrix as that used for specimen collection from newborns (*72*).

Unsatisfactory and invalid samples. If the laboratory accepts specimens that are considered unsatisfactory or invalid, such as a dried blood spot specimen that is of insufficient quantity for testing, oversaturated, scratched or abraded, or not completely dry before mailing (*69*), these specimen variances should be addressed in test performance establishment.

Cut-off and critical values. Determination of the reportable range of test results for the test system should include appropriate cut-off values and critical values that require prompt follow-up and clinical intervention. Determination of the cut-off values for each analyte should be based on considerations for statistically derived values and testing of patient samples with a confirmed diagnosis.

Continuous monitoring of test performance. Laboratories should continuously monitor the performance of their newborn screening tests and determine the need for reevaluating performance specifications as new disease information or test performance data become available (*99*).

Preanalytic Testing Phase

Test Information to Provide to Users of Laboratory Services

CLIA regulations require laboratories that perform nonwaived testing to develop and follow written policies and procedures for specimen submission and handling, specimen referral, and test requests (42 CFR §493.1241 and §1242) (*13*). Laboratories also must ensure that a qualified clinical consultant is available to assist clients with appropriate test ordering to meet clinical expectations (42 CFR §493.1457[b]) (*13*). This section describes laboratory responsibilities for ensuring appropriate test requests and specimen submission for the biochemical genetic and newborn screening tests they perform in addition to meeting these CLIA requirements.

The recommendations that follow emphasize the role of laboratories in providing specific information to users (e.g., authorized persons under applicable state law, health-care professionals, patients, referring laboratories, and payers of laboratory services) regarding the tests performed by the laboratory before the users select and order laboratory tests. For each biochemical genetic test, the following information should be provided to facilitate appropriate test selection and requests, specimen collection and handling, and submission of patient specimens together with relevant information to the laboratory:

- Information necessary for appropriate test selection
  - Intended use of the test to specify the analytes or panel of analytes to be measured, the purpose of testing and appropriate use of the test, and the recommended patient populations; for example, the intended use of an amino acid analysis being described as "analyses of amino acids in plasma, intended for diagnosis and management of amino acid disorders in newborns, infants, children, or adults" and an enzyme assay being described as "analysis of galactose-1-phosphate uridyltransferase in red blood cells, intended for the diagnosis of galactosemia in patients suspected to have the disorder and/or for carrier testing in family members"
  - Indications for testing, such as the symptoms, clinical findings, family history, or newborn screening results for which the biochemical genetic tests might be needed
  - Performance specifications for the test (when appropriate), as well as test limitations and conditions that could affect test results and result interpretation (e.g., sources of assay interference; specimen types and containers; specimen collection methods and the timing of specimen collection; the patient's conditions such as fasting, transfusion, medications, and disease states; and the disease to be evaluated)
  - Test method and testing procedures to be used, including current CPT codes when appropriate
  - Whether testing is performed with an FDA-cleared or FDA-approved test system, a laboratory-developed test, or investigational test under FDA oversight
- Information on appropriate collection, handling, and submission of specimens, including the following:
  - Any necessary patient preparation, when appropriate

  - Specimen type, amount or volume, and collection container or device
  - Specimen preparation
  - Specimen stability and transport conditions
  - Reasons for rejection of specimens (discussed in more detail in next section)
- Types of patient information required to perform and interpret the test (including, as applicable, any required patient consent information in compliance with federal, state, local, and accreditation requirements and whether preauthorization is required)
- Availability of consultation and discussion from the laboratory
- If test results might indicate genotype information, implications of test results for relatives or family members

Laboratories should review the biochemical genetic tests they perform and the procedures they use to provide and update the recommended test information. At a minimum, laboratories should ensure that the test information is available from accessible sources such as websites, service directories, information pamphlets or brochures, newsletters, instructions for specimen submission, and test request forms. Laboratories that already provide the information from these sources should continue to do so. However, laboratories also might decide to provide the information more directly to their users and should determine the situations in which such direct communication is necessary. The complexity of the language used should be appropriate for the targeted user groups (e.g., for patients, language understandable by the general public). Laboratories should also ensure the information provided in this preanalytic phase is consistent with information included on test reports.

In the United States, state newborn screening programs provide health-care providers with information on the panel of disorders screened in their states as well as educational materials for parents and the general public. The National Newborn Screening and Genetics Resource Center provides up-to-date information on the disorders for which screening is performed in each state and model brochures for providers, parents, and grandparents (*32*). Information on laboratory screening procedures often is provided as part of the state newborn screening program, together with disease information, counseling, and follow-up services for presumptive-positive infants. The number of diseases detected by newborn screening programs is expanding, thus the following information is critical for health-care professionals and others to ensure the effectiveness of the public health program:

- Appropriate collection, handling, transport, and submission of dried blood spot specimens
- The laboratory's specimen acceptance and rejection criteria
- Test performance, possible test results, and follow-up confirmatory testing when necessary
- Test limitations that could affect test results and results interpretation, such as sources of assay interference, timing of specimen collection, and infant factors (e.g., fasting or fed, receipt of transfusion, medications, or diseases)
- Types of information about the infant as well as the parents that might be needed to perform and interpret test results (including, gestational age, birth weight, and racial/ethnic background)
- Availability of consultation and discussion from the laboratory
- Information on retesting, if indicated
- Opt-out documentation, if necessary

Informed Consent

Biochemical Genetic Testing

A person who voluntarily confirms the willingness to participate in a particular test, after having been informed of all aspects of the test that are relevant to the decision to participate, is providing informed consent (*85*). Informed consent for genetic testing or specific types of genetic tests is required by law in some states; as of June 2008, a total of 12 states required informed consent before the request or performance of a genetic test (*100*). Certain states, such as Massachusetts (*101*), Michigan (*102*), Nebraska (*103*), New York (*104*), and South Dakota (*105*), specified the required components for informed consent documentation in their statutes. No professional practice guideline specifically recommends informed consent for biochemical genetic tests.

In medical practice, the persons authorized to order the tests also are responsible for obtaining the appropriate level of informed consent (*106*). Unless mandated by state or local requirements, obtaining informed consent generally is not considered a laboratory responsibility. However, when informed consent for patient testing is recommended or required by law or other applicable requirements as a method for documenting the process and outcome of informed decision making, laboratories should ensure that certain practices are followed. Laboratories should be available to assist users of laboratory services with determining the appropriate level of informed consent by providing useful and necessary information regarding the test being considered and implications of test results. Laboratories also should include appropriate methods for documenting informed consent on test request forms (e.g., check-off boxes, attestation statements, and space for signature) and evaluate whether the consent information is provided with the test request before initiating testing. Laboratories may determine the situations in which a patient specimen can be stabilized until informed consent

is obtained, following the practices for specimen retention recommended in these guidelines.

Laboratories should refer to professional guidelines and any local requirements for additional information regarding informed consent for genetic tests and should consider available models when developing the content, format, and procedures for documentation of patient consent.

Newborn Screening

Few states require explicit parental consent for participation in mandated public health newborn screening programs. Most states allow parents to opt out of the program on religious grounds, and certain state programs provide parents the option to refuse newborn screening or the retention of dried blood spot specimens after newborn screening for public health use (*107,108*). Laboratories that perform public health newborn screening should have procedures and processes in place in accordance with their state requirements. When required by state law, appropriate information about informed consent or opting out for newborn screening should be provided to the public in compliance with applicable federal, state, and local requirements.

Test Requests

CLIA requirements (42 CFR §493.1241[c]) specify that laboratories that perform nonwaived testing must ensure that the test request solicits the following information: 1) the name and address or other suitable identifiers of the authorized person requesting the test and (when appropriate) the person responsible for using the test results, or the name and address of the laboratory referring the specimen, including a contact person to facilitate reporting of imminently life-threatening laboratory results or critical values; 2) patient name or a unique patient identifier; 3) sex and either age or date of birth of the patient; 4) the tests to be performed; 5) the source of the specimen (if appropriate); 6) date and time (if appropriate) of specimen collection; and 7) any additional information relevant and necessary for a specific test to ensure accurate and timely testing and reporting of results, including interpretation (if applicable) (*13*).

Biochemical Genetic Testing

In addition to meeting the CLIA test request requirements, laboratories that perform biochemical genetic testing should solicit the following additional or more specific information on test requests:

- Patient name and any other unique identifiers needed for testing
- Date of birth
- Date and time of specimen collection (relative to symptoms and initiation of treatment when appropriate)
- The reason for referral and information on the clinical, medication, and nutritional status of the patient, including International Classification of Diseases (ICD) codes or other codes indicating the diseases or conditions to be tested for and patient preparation when indicated (e.g., how the patient was prepared)
- Patient's race/ethnicity, if applicable
- Family history or pedigree, if applicable
- When required by state law, a check-off box or other means to indicate that the appropriate level of informed consent has been obtained in compliance with federal, state, and local requirements
- Emergency contact information for the responsible clinician (for additional information or abnormal results)

Newborn Screening

In addition to meeting the CLIA test request requirements, laboratories that perform newborn screening should consider soliciting the following additional information:

- Infant's name, mother's name, and any other unique identifiers needed for testing
- Date and time of birth
- Date and time of specimen collection
- Gestational age, birth weight, and any additional information required by the state program
- Information on health, medication, and nutritional status of the infant
- Infant's race/ethnicity
- Family history relevant to newborn screening, if applicable
- Submitter's identification and address or appropriate information on the birth facility
- When required by state law, a check-off box or other means to indicate that informed consent has been obtained in compliance with federal, state, and local requirements
- Emergency contact information for the responsible clinician (for additional information or abnormal results)

For biochemical genetic testing and newborn screening, laboratory electronic information systems (both current versions and those in development) should support and ensure the collection, transmission, and retention of all test request information recommended in this report. Laboratories may specify critical information elements as required for test requisition submission and have preanalytic quality assessment procedures in place for monitoring the provision of the needed information.

Specimen Submission, Handling, and Referral

CLIA requires laboratories to establish and follow written policies and procedures for patient preparation, specimen collection, specimen labeling (including patient name or unique patient identifier and, when appropriate, specimen

source), specimen storage and preservation, conditions for specimen transportation, specimen processing, specimen acceptability and rejection, and referral of specimens to another laboratory (42 CFR §493.1242) (*13*). If a laboratory accepts a referral specimen, appropriate written instructions providing information on specimen handling and submission must be available to the referring laboratory (*13*). Laboratories that perform testing for inherited metabolic diseases must meet these general CLIA requirements and should implement all of the additional practices that follow to ensure the quality of specimen submission, handling, and referral:

- Provide specific instructions for the proper identification, collection, handling, transport, and submission of patient specimens to laboratory clients as specified in the section on the role of laboratories in providing information to users of their services.
- Provide information on any need for patient preparation before specimen collection, and specifically communicate with clinicians regarding the circumstances that might involve risk (e.g., fasting and certain challenge tests).
- Specify procedures for handling specimen submission for time-sensitive testing, testing that requires rapid or short turnaround time, or critical or labile specimens to meet the need for clinical care and patient management. These procedures also should address situations in which direct communication with the submitting clinician is needed.

Criteria for Specimen Acceptance and Rejection

Specimen acceptance criteria should be consistent with the types and conditions of the samples used to establish test performance specifications to the extent practical and feasible. Laboratories should have written criteria for acceptance and rejection of specimens, including determination and handling of situations such as

- improper handling or transport of specimen;
- mislabeling, use of inappropriate anticoagulants or media, specimen degradation, or inappropriate specimen type;
- potentially deteriorated specimen (e.g., specimens with bacterial overgrowth);
- potentially contaminated specimen that might affect results of testing procedures;
- lack of unique identifiers on the specimen or the requisition form;
- specimen not held at appropriate temperature (e.g., unfrozen specimens for urine organic acid analysis); and
- insufficient specimen quantity.

Because of the complexity and diversity of the specimens that might be encountered and the influence of specimen conditions on the quality of test results and results interpretation, the specimen acceptance and handling procedures should address common variances in specimen conditions and those that might occur in patient testing. Laboratories should have criteria for determining acceptable and unacceptable specimens, including determining whether specific variances in specimen conditions (e.g., hemolyzed whole blood specimen) still meet the specimen acceptance criteria and which tests can be performed with such specimens. If a laboratory accepts specimens that deviate substantially from the established criteria and might contain interfering substances that could affect the quality of patient test results, the laboratory should have documentation of studies based on the scientific literature or internal data to prove that the test to be performed and its performance specifications will not be compromised. If multiple tests or test panels are requested for a single specimen, determination of specimen acceptability might be made for the different test procedures. In such circumstances, appropriate terminology should be used so that a specimen can be determined unacceptable for particular tests rather than for all tests to be performed. For example, a quantity of specimen that is not large enough to allow necessary repeat testing needs to be addressed differently than a potentially compromised specimen such as a hemolyzed specimen.

In rare circumstances, when testing specimens that deviate from the laboratory's specimen acceptance criteria is critical, the laboratory should follow established procedures to note the exceptions on the test report. In specific situations, testing of nonideal specimens might be considered. For example, critical specimens that should not be rejected include those from a deceased patient from whom no additional specimen can be submitted, specimens for which a rapid response is required for management, specimens that were collected while the patient was acutely ill or as part of a timed test or challenge, or specimens that were collected using an invasive method (e.g., cerebrospinal fluid or muscle biopsy specimens). If specimens that are not ideal but still meet the laboratory criteria for acceptability are analyzed, a repeat specimen should be requested for clarification, if necessary.

Additional Considerations for Newborn Screening

Newborn screening laboratories should have policies and procedures to address the time-sensitive issues of testing and the handling of varying conditions of the infants, including specimen collection for infants who are preterm or low birth weight, too sick to be fed, or in need of special care (*71*). Written procedures addressing specimen-related issues, such as the preferred and necessary specimens and the timing of specimen collection, should be consistently applied. The laboratory should inform submitters that dried blood spot specimens should be transported or mailed to the laboratory within 24 hours after specimen collection (regardless of

weekends and holidays), and delays in specimen submission should be avoided (*69*).

Terms such as unsatisfactory or invalid may be used in reference to dried blood spot specimens that are not properly collected; are of insufficient or excessive quantity; are clotted, smeared, or contaminated (*69*); or are acceptable for some but not all testing. The specimen acceptance procedures of the laboratory should address whether dried blood spot specimens that are considered unsatisfactory (e.g., a dried blood spot specimen that does not adequately fill the circle) meet the established acceptance criteria. For all unsatisfactory specimens, a second specimen should be requested.

Test Referral

Factors that should be considered when selecting laboratories for test referral might include laboratory quality, personnel expertise, turnaround time, and cost. However, cost should not be the only or the primary factor for consideration when selecting referral laboratories.

CLIA regulations at §493.1242(c) require laboratories to refer a specimen for patient testing only to a CLIA-certified laboratory or a laboratory meeting equivalent requirements as determined by CMS (*13*). Specimens should not be sent to laboratories that do not meet these requirements. The laboratory demographics lookup feature on the CMS CLIA website provides a resource to facilitate searches for laboratories that meet the CLIA certification requirements (*109*).

Preanalytic Systems Quality Assessment

Laboratories must have written policies and procedures for assessing and correcting problems identified in test requests, specimen submission and handling, test referral, and other steps of the preanalytic testing process (42 CFR §493.1249) (*13*). The preanalytic systems assessment for biochemical genetic testing and newborn screening should include the following practices:

- Laboratories must make a reasonable effort to verify or clarify test requests that are unclear or lack critical information, submitted with inappropriate specimens, or inconsistent with the intended use of test results. For rapid or time-sensitive testing, procedures for handling situations that require prompt initiation of patient testing are necessary.
- Laboratories should have policies and procedures to ensure that information necessary for selection of appropriate test methods, performance of testing procedures, and provision of test results and results interpretation is retained throughout specimen submission, results reporting, and specimen referral.
- When a laboratory recognizes that necessary information in test requests has been lost during specimen submission or test referral, the laboratory should contact the test requestor or referring laboratory to request the needed information. Effective test submission or referral procedures should then be established to prevent or minimize similar occurrences. Improving the communication between laboratories and users in the preanalytic phase also should result in improved result reporting practices.
- Laboratories should monitor and document the extent to which specimen problems occur and develop measures to reduce the frequency of these problems. Examples of preanalytic quality assessment include the following:
  - Monitoring the frequency of unacceptable specimens and specimen handling problems, such as the use of an improper blood collection tube and inadequate mixing of blood specimens with anticoagulant after collection
  - Monitoring the frequency of delays in specimen transport
  - Identifying clients who repeatedly refer unacceptable specimens or improperly complete requisition forms
  - Documenting the laboratory's efforts to reduce the recurrence of these problems

Analytic Testing Phase

Control Procedures

The analytic phase of a laboratory test typically includes the following steps: specimen processing and preparation, analyte detection or measurement, evaluation of the quality of the analytic results, and documentation of testing data and test results. Laboratories that perform testing for inherited metabolic diseases must meet the general CLIA requirements for nonwaived testing (42 CFR §493.1256), including the following quality control requirements:

- Laboratories must have control procedures in place to monitor the accuracy and precision of the entire analytic process for each test system.
- The number and type of control materials and the frequency of control procedures must be established using the applicable performance specifications verified or established by the laboratory.
- Control procedures must detect immediate errors caused by test system failure, adverse environmental conditions, or operator performance and must monitor the accuracy and precision of test performance over time.
- Each day that testing of patient specimens is performed, the laboratory must include the following:
  - At least two control materials of different concentrations for each quantitative procedure
  - A negative and positive control material for each qualitative procedure

  - A negative control material and a control material with graded or titered reactivity, respectively, for each test procedure producing graded or titered results
  - Two control materials, including one that is capable of detecting errors in the extraction process, for each test system that has an extraction phase
- If control materials are not available, the laboratory must have an alternative method for detecting immediate errors and monitoring test system performance over time; the performance of the alternative control procedures also must be documented (*13*).

Laboratories that perform testing for inherited metabolic disorders must meet these general CLIA quality control requirements and should implement the specific practices that follow to ensure the quality of laboratory test performance:

- Laboratories should validate and monitor sampling instruments to ensure there is no carryover between samples on automated instruments.
- Control procedures should be performed each time patient specimens are assayed or with each batch (i.e., group of specimens run concurrently or sequentially) of patient testing.
- Controls should be selected based on patient population, prevalence of the disease, and the purpose of the test, while being as comprehensive as possible. For example, enzyme testing for carrier status should have a normal control and a carrier control, if available. Examples of sources that have reference materials for biochemical genetic tests or newborn screening are listed (Appendix C).

Considerations for Control Materials for Rare Disease Testing and Alternative Control Procedures

When performing testing for rare diseases, if positive controls are difficult to obtain for certain test procedures, laboratories may consider using deidentified samples (i.e., samples from which individual identifiers have been removed) from interlaboratory exchange or other mechanisms. For example, if having a positive control of the same tissue type is not practical for testing procedures performed using white blood cells, laboratories may consider using a more stable tissue type, such as cultured skin fibroblasts, when available. In these circumstances, if the control materials bypass certain preparative analytic steps of the patient testing process (e.g., extraction procedures), the laboratory should have procedures for monitoring the complete analytic process including the preparative steps.

If control materials are not practical or available for rare disease testing, alternative control procedures should be developed to adequately monitor test performance. For example, spiking or enriching a normal sample with analytes to simulate abnormal samples is an acceptable alternative control procedure for certain test procedures. The CMS *Survey Procedures and Interpretive Guidelines for Laboratories and Laboratory Services* provide general guidelines for alternative control procedures, such as splitting specimens for testing by another method or in another laboratory, including previously tested patient specimens (both positive and negative) as surrogate controls, testing each patient specimen in duplicate, performing serial dilutions of positive specimens to confirm positive reactions, and conducting an additional supervisory review of results before release (*74*). Laboratories should use multiple mechanisms as applicable to their test procedures to ensure testing quality.

Special Quality Control Issues with Sequential Testing in Single-Channel Analyzers

Certain test procedures are performed with single-channel or single-column instruments (e.g., amino acid analyses) on which the run time of each specimen might take a significant portion of a working day. For these tests, acceptable control procedures include the following options, provided 1) the laboratory director is responsible for demonstrating that the control procedures are adequate for monitoring test system performance and detecting immediate errors, and 2) the laboratory has performance establishment or verification data to demonstrate that the control procedures and calibration procedures are appropriate for the laboratory's testing of patient specimens:

- Testing a mixed-level control pool (which for amino acid analysis might contain all of the amino acids to be measured at various concentrations) once during each day or 24 hours of patient specimen testing and spiking at least one internal control material (e.g., S-2-aminoethyl-1-cysteine for amino acid analysis) into each patient specimen, an approach that helps monitor the analytic process for each specimen as well as specimen-to-specimen variability.
- Testing a single-level control pool (which for amino acid analysis might contain the amino acids to be measured at the same concentration) once during each day or 24 hours of patient specimen testing and spiking an internal control material into each patient specimen
- Testing a previously tested patient specimen that showed abnormal levels of certain amino acids once each day and spiking an internal control material into each patient specimen
- If batches of patient specimens are in a test run that exceeds 24 hours, the test run may be bracketed by running a control sample at the beginning and another control sample at the end of the run. If the run time is >48 hours, a control sample should be inserted into the run within each 24-hour span. At least one internal control material should be spiked into each patient specimen. The

laboratory director should be responsible for ensuring and demonstrating that the test system has stable accuracy and precision during the defined time period for both the control samples and patient specimens. The laboratory also should consider the turnaround time needed for reporting patient results in determining the length of a test run.

Test Systems, Equipment, Instruments, Reagents, Materials, and Supplies

CLIA requires laboratories to perform patient testing by following the manufacturer's instructions and in a way that provides test results within the laboratory's stated performance specifications for each test system as determined under §493.1253 (*13*). Laboratories must meet the following requirements for the test systems, equipment, instruments, reagents, materials, and supplies that are used for performing patient testing:

- Define essential conditions for proper storage of reagents and specimens, accurate and reliable test system operation, and reporting of test results. The criteria must be consistent with the manufacturer's instructions, if provided. These conditions must be monitored and documented and, if applicable, include water quality, temperature, humidity, and protection of equipment and instruments from fluctuations and interruptions in electrical current that adversely affect patient test results and test reports.
- Label reagents, solutions, culture media, control materials, calibration materials, and other supplies, as appropriate, to indicate the identity and, when significant, titer, strength or concentration, storage requirements, preparation and expiration dates, and other pertinent information required for proper use.
- Reagents, solutions, culture media, control materials, calibration materials, and other supplies must not be used when they have exceeded their expiration date, have deteriorated, or are of substandard quality.
- Components of reagent kits of different lot numbers must not be interchanged unless otherwise specified by the manufacturer.
- Reagent, media, and supply checks must include checking each batch (if prepared in the laboratory), lot number (if commercially prepared), and shipment of reagents, disks, stains, antisera, and identification systems (i.e., systems using two or more substrates or two or more reagents, or a combination) when prepared or opened for positive and negative reactivity, as well as graded reactivity, if applicable.

Laboratories performing testing for inherited metabolic diseases must comply with these CLIA requirements and should implement the following additional practices:

- Reagents, supplies, and instruments used during routine testing should be the same as those used in test performance establishment or verification.
- New reagent lots and shipments should be tested in parallel with old lots before or concurrently with being placed in service to ensure that the new lot of reagent has maintained consistent results for patient specimens.
- Test performance specifications should be reestablished if the test system has been modified with changes that could affect its performance specifications. Such changes include using different or additional equipment or instruments, changing any critical reagent such as a conjugate or substrate, using different control or reference materials, or using a different specimen collection device.
- Equipment should be evaluated and monitored to account for basic detection or measurement drift through analysis of quality control data, function checks, and internal electronic checks.
- Laboratories are encouraged to use available mechanisms for standardizing laboratory practices for preparing and validating reagents and reference materials that are not commercially available. When available, FDA-cleared reagents should be used for patient testing. Laboratories should be aware of FDA regulations that require FDA clearance or approval for reagents and instruments (including software programs) that are developed or prepared in one laboratory and provided to another laboratory for use in patient testing (*110*). However, special issues in biochemical genetic testing are associated with the lack of availability of certain essential reagents that have received FDA clearance or approval. The lack of FDA-cleared reagents increases the responsibility of the laboratory to validate any internally developed or shared reagents, standards, or controls.

Laboratories also should consider relevant professional guidelines, such as those developed by CLSI and ACMG, that provide additional guidance for specific test methods.

Calibration and Calibration Verification Procedures

Under CLIA, calibration and calibration verification procedures are required to substantiate the continued accuracy of the test system throughout the laboratory's reportable range of test results (*13*). Calibration procedures for each test system must be performed and documented either by

- following the manufacturer's instructions using calibration materials provided or specified and with at least the manufacturer-recommended frequency or
- using criteria verified or established by the laboratory, including the acceptable limits for and the frequency of calibration and the number, type, and concentration of

calibration materials, which must be appropriate for the test system and, if possible, traceable to a reference method or reference material of known value.

Calibration verification procedures must be performed and documented either by following the manufacturer's instructions or using the criteria verified or established by the laboratory under §493.1253(b)(3), including the number, type, and concentration of the materials; acceptable limits for calibration verification; and at least a minimal (or zero) value, a midpoint value, and a maximum value near the upper limit of the range to verify the laboratory's reportable range of test results for the test system (*13*). Calibration verification procedures must be performed at least once every 6 months and when any of the following occur:

- A complete change of reagents for a procedure is introduced, unless the laboratory can demonstrate and document that changing reagent lot numbers does not affect the range used to report patient test results, and control values are not adversely affected by reagent lot number changes.
- Major preventive maintenance or replacement of critical parts occurs that might influence test performance.
- Control materials reflect an unusual trend or shift or are outside of the laboratory's acceptable limits.
- Other means of assessing and correcting unacceptable control values have failed to identify and correct the problem.
- The laboratory's established schedule for verifying the reportable range for patient test results requires more frequent calibration verification (*13*).

Laboratories performing testing for inherited metabolic diseases must comply with these CLIA calibration and calibration verification requirements and should implement the following additional practices:

- When reference materials for calibration are commercially available and stable, laboratories should consider obtaining quantities adequate for a reasonable period of testing to reduce variability in these materials (but not to exceed their expiration date).
- When reference materials for calibration are not commercially available, each laboratory preparing these materials at its own facility should ensure their validation, including verifying each new batch of the calibration or reference materials against an old batch, and ensure that appropriate calibration and calibration verification procedures are in place.
- Laboratories should refer to available professional guidelines (e.g., ACMG *Standards and Guidelines for Clinical Genetic Laboratories* [*18*] and CLSI method-specific guidelines [*65,69,72*]) for additional guidance on performing calibration and calibration verification to ensure the test accuracy and reliability.

Proficiency Testing and Alternative Performance Assessment

Proficiency testing is an important tool for assessing laboratory competence, evaluating the laboratory testing process, and providing education for laboratory personnel (*67*). CLIA regulations specifically require proficiency testing for some analytes or testing specialties, which might be provided by private-sector, nonprofit, or state-operated programs approved by HHS as meeting CLIA standards (42 CFR Part 493) (*13*). These approved programs also might provide proficiency testing for analytes and specialties for which proficiency testing is not specified, including biochemical genetic tests and other tests (*111*). Although the CLIA regulations do not include proficiency testing requirements specific for biochemical genetic tests or newborn screening tests, laboratories that perform these tests must comply with the general requirements for alternative performance assessment for any test or analyte not specified in the regulations to, at least twice annually, verify the accuracy of the tests or procedures they perform (42 CFR §493.1236[c]) (*13*). Laboratories may meet this requirement by participating in available proficiency testing programs for the biochemical genetic or newborn screening tests they perform (*112*). A list of available proficiency testing programs for biochemical genetic testing is included in this report (Appendix C).

Biochemical Genetic Testing

The following recommended practices provide more specific and stringent measures than the current CLIA requirements for test performance assessment for laboratories that perform biochemical genetic testing to monitor and evaluate the ongoing quality of the testing they perform:

- Participate in available proficiency testing at least twice per year for each biochemical genetic test the laboratory performs. Laboratories are encouraged to participate in available proficiency testing programs that examine the entire testing process encompassing the preanalytic, analytic, and postanalytic phases. Laboratories should regularly review information on the development of additional proficiency testing programs and ensure participation as new programs become available.
- Test analyte-specific or disease-specific proficiency testing challenges with the laboratory's regular patient testing workload by personnel who routinely perform the tests in the laboratory, as required by CLIA for analytes and specialties specified in the regulation.

- When possible, laboratories performing quantitative assays should enroll in proficiency testing programs that provide feedback for specific analyte values. Qualitative proficiency testing is appropriate for tests for which quantitative technology is lacking and for certain tests, such as enzyme assays that lack consensus quantitative measurements.
- Evaluate proficiency testing results reported by the proficiency testing program and take steps to investigate the causes for disparate results, including results that might indicate bias but are within acceptable ranges. The corrective actions to be taken after disparate proficiency testing results might include reevaluation of previous patient test results and, if possible, of retained patient specimens that were previously tested, depending on the cause identified for the disparate results.

Newborn Screening

The following recommendations are for laboratories that perform newborn screening to assess test performance:

- Participate in the NSQAP and adhere to the program directions. Participation in other proficiency testing programs for monitoring test performance is encouraged.
- Include cutoff values for all analytes when reporting proficiency test results to NSQAP so that the specific cutoffs can be taken into account in the NSQAP grading algorithm to facilitate the evaluation of the laboratory results (*30,113*).

Implications for Proficiency Testing and Interlaboratory Comparison Programs

Proficiency testing and interlaboratory comparison programs should consider the need for external quality assessment for all testing for inherited metabolic diseases in improving program availability and result evaluation. To the extent possible, proficiency testing should be available for each analyte at least twice per year.

Comprehensive proficiency testing programs (e.g., NSQAP) are needed that examine the entire testing process (which consists of the preanalytic, analytic, and postanalytic phases) and are able to assess both the quantitative and qualitative test methods. Although practical and technical challenges might limit the ability of proficiency testing programs to address all testing phases for each analyte, comprehensive programs for core tests (e.g., amino acid and organic acid analyses) that combine the strengths of existing programs should be pursued (*8,24*). When possible, proficiency testing samples should simulate patient specimens; at a minimum, samples simulating patient specimens should be used for proficiency testing for the most common genetic tests. If residual patient samples are used in proficiency testing or interlaboratory comparisons, any required consent issues and deidentification procedures should be addressed according to federal, state, and local requirements and guidance.

Alternative Performance Assessment

Organized proficiency testing programs do not exist for many tests performed for inherited metabolic diseases, including biochemical genetic tests and, occasionally, new tests for newborn screening. Alternative performance assessment must be performed at least twice per year for each test for which no proficiency testing program is available and for tests for which CLIA regulations do not specify proficiency testing requirements. Laboratories should implement the following practices for alternative performance assessment:

- Although data on the effectiveness of alternative performance assessments compared with proficiency testing are unavailable, laboratories should follow professional guidelines, such as those developed by CLSI (*26*) and CAP (*82*), that provide guidance on acceptable alternative performance assessment approaches.
- Alternative assessment ideally should be performed by interlaboratory exchange or using externally derived materials.
- For circumstances in which interlaboratory exchange or externally derived materials are not practical or feasible, such as testing for rare diseases, testing performed by only one laboratory, or analysis of unstable analytes (e.g. enzymes), laboratories may consider options such as repeat testing of blinded samples, possible exchange with either a research facility or international laboratory, or interlaboratory data comparison.
- Newborn screening laboratories that consider testing for new disorders not covered by the CDC NSQAP should make the program aware of their plans to facilitate the availability of the needed proficiency testing.

Evaluation of External Quality Assessment Performance

Laboratories should document and track their performance in proficiency testing and alternative performance assessment. Quality improvement assessment should be performed periodically to evaluate performance and ensure adequate investigation of failures or concerns, implementation of corrective actions, and documentation of outcomes. Additional guidance for using proficiency testing as a quality improvement tool is available in professional guidelines (*26*).

Various resources for proficiency testing and external quality assessment and for facilitating interlaboratory sample exchanges are available to help laboratories consider approaches to meeting the proficiency testing and alternative performance assessment needs (Appendix C).

Postanalytic Testing Phase

Test Reports

Test reports must comply with the CLIA general test report requirements (42 CFR §493.1291) and should include the recommended additional information that follows to ensure accurate understanding and interpretation of test results. CLIA requires that test reports for nonwaived testing include the following information:

- Patient name and identification number or a unique patient identifier and identification number
- Name and address of the laboratory where the test was performed
- Test report date
- Test performed
- Specimen source (when appropriate)
- Test result and (if applicable) units of measurement or interpretation
- Information regarding the condition and disposition of specimens that did not meet laboratory criteria for acceptability (*13*)

For laboratory-developed tests using analyte-specific reagents, test reports must include the following statement: "This test was developed and its performance characteristics determined by (Laboratory Name). It has not been cleared or approved by the U.S. Food and Drug Administration" (21 CFR 809.30[e]) (*75*).

Biochemical Genetic Test Reports

Biochemical genetic test reports should include the following more specific information to ensure accurate results interpretation, patient management, and the ordering of any needed additional tests by persons receiving or using the test results:

- Patient's name and any other necessary unique identifiers
- Patient's date of birth
- The reason for testing that was provided on the test requisition and is needed for results interpretation
- The date and time of specimen collection and receipt by the testing laboratory, which should be distinguished, when possible, from the date and time when the test request was made and, for test referrals, the date and time when the specimen was received by the referral laboratory, or a specific indication that the "specimen collection date and/or time are unknown" if such information is not provided
- Name of the referring clinician or other authorized person who ordered the test
- When appropriate, an interpretive guide (e.g., a table or reference to literature or a website) to aid in interpretation of results
- Analytes tested, type of test method, or both
- The reference intervals or normal range appropriate for the patient (based on sex, age, and population as appropriate), other performance specifications needed for results interpretation (e.g., accuracy, LOD, LOQ, or analytic specificity), and limitations of the test (e.g., a statement on the intended use and the technical limitation of the test method) that affect the understanding and clinical use of the test results
- Test results in appropriate measurement units and current recommended standard nomenclature, including clarifications and commonly used terms if different from those currently recommended
- Interpretation of results for complex tests, profiles, testing for carrier status, and testing that involves response to challenges or multiple samples over time; should be linked to the reasons for testing and communicated in a timely and clinically relevant manner
- Test results in reference to information on family members (e.g., information regarding abnormalities previously detected in a relative used for the selection of the test method) when appropriate and necessary to ensure appropriate interpretation of the test results and understanding of their implications
- The name of the laboratory personnel providing the interpretation
- Notation to indicate whether report is preliminary, final, amended, or corrected
- If applicable, an indication that other biochemical genetic tests have been performed for the patient, or, when available, results of other relevant tests that the laboratory performed for the patient
- When appropriate, recommendations for additional testing of patient or for family members
- References to the literature, if applicable
- Recommendation for consultation with a genetics professional, when appropriate and indicated, that encompasses genetic counseling provided by trained, qualified genetic professionals such as genetic counselors, clinical geneticists, or other qualified professionals, to health-care providers, patients, or family members at risk for the conditions and also can be an educational initiative to improve understanding of genetic tests in the medical community
- The date and, when appropriate, time the test report is released

Laboratories should assess the needs of laboratory users when determining the media, format, style, and language of biochemical genetic test reports. To the extent possible, the terminology and nomenclature should be understandable by health-care professionals who are not geneticists or experts in

the specific field. This practice should be part of the laboratory quality management policies. Test reports should include all necessary information, be easy to understand, and be structured in a way that encourages users to read the entire report, rather than just a positive or negative indication. Following the format recommended in accepted practice guidelines should help ensure that the reports are structured effectively (*18*).

Newborn Screening Test Reports

Newborn screening test reports must comply with the CLIA general test report requirements (42 CFR §493.1291) and applicable state requirements. Results should be reported in a way that is consistent with the urgency of any needed intervention. For a screening result that is outside the expected range of normal test results established for a particular condition (i.e., out-of-range result) or indicates problems with the specimen or the testing process that might compromise the quality of test results according to established criteria (i.e., invalid screen), the following information should be communicated to the newborn's primary care provider without delay:

- The newborn's identifying information (name, date of birth, and time of birth), place of birth, and national or local health number
- Parent information (mother's name, home telephone number, and address if available)
- The date and time of specimen collection and arrival in the laboratory
- Analytes evaluated and type of test method, or whichever is appropriate
- Screening test results in appropriate measurement units
- The normal range and cutoff values appropriate for the newborn's conditions, including gestational age, birth weight, and health or disease status
- Notation of whether the results are out-of-range or invalid
- Required actions, including a repeat screen, confirmatory testing, clinical actions, and evaluation, as well as the timeline, steps, and instructions to complete the necessary actions
- Instructions to notify the newborn screening program when the primary care provider has been unable to contact the parents or when the primary care provider has changed
- Contact information (e.g., name, phone number, e-mail address, and fax number) for the follow-up personnel of the newborn screening program
- The date and time the test results are reported
- For all out-of-range results, the following information should be provided to the newborn's primary care provider:
  - Pediatric subspecialists resource information (including telephone numbers) for consultation or referral
  - Information about the suspected diagnosis (disease or condition) and consequences if untreated
  - Reporting requirements to the newborn screening program, including confirmatory test results, treatment date, and other information on the newborn's health outcome

For all out-of-range or invalid results, the laboratory should take actions that lead to timely additional testing and evaluation for the infant and allow the newborn screening program to evaluate the effectiveness of screening. Follow-up should occur without delay to enable timely intervention. For results requiring urgent actions, laboratories should first notify both the primary health-care provider and the designated specialist by telephone and then by paper or electronic notification.

Laboratories should monitor the development and application of guidelines and recommendations that address electronic reporting of newborn screening results, such as the HRSA/NLM guidance for sending electronic newborn screening results with Health Level Seven International (HL7) messaging (*37*) and the HL7 implementation guide developed by the Public Health Informatics Institute (*40*). When implementing the electronic reporting mechanisms, laboratories should ensure that the information systems accommodate the inclusion and delivery of the test report elements that are recommended in this report. Laboratories also should develop quality assurance procedures for the electronic reporting systems used.

Retention of Records, Reports, and Tested Specimens

Records

CLIA requires laboratories to retain records of patient testing, including test requests and authorizations, test procedures, analytic systems records, records of test system performance specifications, proficiency testing records, and quality system assessment records, for a minimum of 2 years (42 CFR §493.1105) (*13*). These requirements apply to testing for inherited metabolic diseases. Retention policies and procedures also must comply with applicable state laws and other requirements (e.g., of accrediting organizations if the laboratory is accredited). Laboratories may retain records for longer periods for quality management purposes and should consider the following recommendations when establishing record retention policies:

- Primary data from which reports are generated should be kept along with the reports, preferably electronically.
- Records of tests that generated normal results also should be retained.
- Laboratories should ensure that electronic records are accessible as electronic storage technology continues to evolve.

Reports

CLIA requires laboratories to retain or have the ability to retrieve a copy of an original test report (including final, preliminary, and corrected reports) for at least 2 years after the date of reporting and to retain pathology test reports for at least 10 years after the date of reporting (42 CFR §493.1105) (*13*).

Biochemical genetic test reports that indicate genotypic information for the disease or condition should be retained for at least 21 years after the date of reporting. This retention period is recommended as an acceptable length of time of one biological generation that is necessary to provide useful continuity of clinical history for patient management and diagnosis or treatment of family members. The laboratory policies and procedures for test report retention also must comply with applicable state laws and other requirements (e.g., of accrediting organizations if the laboratory is accredited) and should follow practice guidelines developed by recognized professional or standard-setting organizations. If state regulations require retention of biochemical genetic test reports for >21 years after the date of results reporting, laboratories must comply. Laboratories also might decide that retaining reports for >21 years is necessary for biochemical genetic test reports to accommodate patient testing needs and ongoing quality assessment activities. Because test reports may be retained using electronic methods, laboratories are encouraged to consider technology availability in addition to space and financial issues when determining solutions to test report retention.

The retention period for newborn screening test reports must be in compliance with CLIA and applicable state requirements. Certain states require different retention periods for newborn screening test reports depending on the results.

Tested Specimens

CLIA requires laboratories to establish and follow written policies and procedures that ensure positive identification and optimum integrity of patient specimens from the time of collection or receipt in the laboratory through completion of testing and reporting of test results (42 CFR §493.1232) (*13*). Depending on sample stability and integrity, technology, space, and cost, tested specimens for biochemical genetic testing should be retained as long as possible after the completion of testing and reporting of results. Patient specimens should be retained until after the final reporting of results for quality assurance and any need for additional testing of the same specimen, with adequate provisions for specimen stability. When possible, tested specimens may be retained until the next proficiency testing or the next alternative performance assessment to allow for identification of problems in patient testing and for corrective action to be taken. Laboratories also may retain tested specimens for a longer period or indefinitely for quality assurance and educational purposes (e.g., tested specimens from abnormal cases).

The laboratory director is responsible for ensuring that the laboratory policies and procedures for specimen retention comply with applicable federal, state, and local requirements (including laboratory accreditation requirements, if applicable) and are consistent with the laboratory quality assurance and quality assessment activities. In circumstances in which required patient consent is not provided with the test request, the laboratory should notify the test requestor and determine the period after which the test request might be rejected and the specimen discarded because of specimen degradation or deterioration. Laboratory specimen retention procedures should be consistent with patient decisions.

The retention of residual newborn screening specimens is subject to federal, state, and local requirements. Residual specimens are valuable for the laboratory's ongoing quality assurance, quality assessment, and personnel competency assessment activities. Certain states have established policies under state law to retain residual dried blood spot specimens without personal identifiers for public health and research uses after newborn screening testing is completed and to allow parents to request that their children's residual specimens be destroyed after newborn screening tests are completed (*107*).

Postanalytic Systems Assessment

The CLIA postanalytic systems assessment requirements apply to testing for inherited metabolic diseases. Quality assessment of the postanalytic system generally includes the following:

- Practices and other issues related to test reports: assessing, monitoring, and evaluating the accuracy and completeness of the laboratory's test reports (e.g., patient information, test results, reference ranges, and the disposition of unacceptable specimens)
- The time required by the laboratory to complete testing and report test results
- Procedures for notifying the test requestor about the test results, including routine tests, urgent testing, abnormal results, and critical values or alert values that warrant immediate medical attention
- If the laboratory uses an electronic information system, a mechanism to periodically verify the accuracy of data and calculations, the results transmitted to interfaced systems, and patient-specific information
- The record retention procedures and practices of the laboratory
- The specimen retention policies and procedures of the laboratory

Biochemical Genetic Testing

Laboratories that perform biochemical genetic testing should have procedures in place to address the following postanalytic or interpretive issues, which often are unique to biochemical genetic testing:

- When diagnostic testing shows an abnormality, testing of other analytes might be critical to clarify the diagnosis (e.g., elevated methylmalonic acid suggesting that testing of homocysteine level is needed).
- Reflex testing (i.e., follow-up testing that is automatically initiated when certain test results are observed in the laboratory) might be needed when useful and appropriate to clarify or expand primary or initial test results.
- Testing by another method or with another tissue type might clarify or confirm the diagnosis for more effective clinical management of the patient or the patient's family.
- Additional specimens might be needed when testing unstable analytes to verify that the initial specimen quality was not compromised.

In addition, the laboratory should have a system in place to facilitate the consideration of the preanalytic information needed for adequate interpretation of test results and the inclusion of such information on the test report. For example, circumstances when more than one biochemical genetic test has been requested on a patient should be recognized and noted in test reports. Specimen collection time often is critical for accurate interpretation of test results, particularly for conditions such as intermittent maple syrup urine disease that present normal analyte (e.g., amino acids) levels during asymptomatic intervals. Inclusion of the reasons for testing in test reports, even though the specific reasons for a test might not always be provided with the test requisition, is helpful for the users of test results because test reports might be sent to health-care providers different from the test requestors. The laboratory's policies and procedures for postanalytic systems assessment should address monitoring of these recommended practices and assessment of their effectiveness.

Newborn Screening

Newborn screening laboratories should have policies and procedures for postanalytic systems assessment that address all postanalytic laboratory practices in newborn screening. In particular, an ongoing review process should be in place to monitor and assess the effectiveness of the following procedures:

- Procedures for immediate reporting of results that are considered out of range or are indicative of a clinical emergency, including notification of the newborn's primary care provider and documentation of the reporting and report receipt
- Procedures for obtaining a second, freshly collected specimen for confirmatory analysis for each abnormal screening result
- Immediate reporting of unsuitable specimens to facilitate timely repeat testing
- Laboratory responsibilities in the comprehensive system for follow-up of each positive screening result, including facilitating the reporting of the medical care decisions and actions to the newborn screening program by specifying the reporting requirements in test reports

Ensuring Confidentiality of Patient Information

CLIA requires laboratories to ensure the confidentiality of patient information throughout all phases of the testing process that are under laboratory control (42 CFR §493.1231) (*13*). Laboratories should follow more specific requirements and comply with additional guidelines (e.g., the Health Insurance Portability and Accountability Act of 1996 [HIPAA] privacy rule [*114*], state requirements, accreditation standards, and professional guidelines) to establish procedures to protect the confidentiality of patient information, including information related to genetic testing. Laboratories that perform testing for inherited metabolic diseases should establish and follow procedures that include defined responsibilities of all employees to ensure appropriate access, documentation, storage, release, and transfer of confidential information and prohibit unauthorized or unnecessary access or disclosure.

Information Regarding Family Members

In certain circumstances, information about family members is needed for selection of test methods and test performance or should be included in test reports to ensure appropriate interpretation of test results. Therefore, laboratories must have procedures and systems to ensure confidentiality of all patient information, including that of family members, in all testing procedures and reports in compliance with CLIA requirements and other applicable federal, state, and local regulations.

Requests for Test Results to Assist with Health Care for a Family Member

When a health-care provider requests the genetic test information of a patient to assist with providing care for a family member of the patient, the following practices are recommended:

- Requests should be handled following established laboratory procedures regarding release and transfer of confidential patient information.

- Laboratories may release patient test information only to the authorized person ordering the test, the persons responsible for using the test results (e.g., health-care providers of the patient designated by the authorized person to receive test results), and the laboratory that initially requested the test. If a health-care provider who provides care for a family member of the patient is authorized to request the patient's test information, the laboratory should request the patient's authorization before releasing the genetic test results to the health-care provider.
- When patient consent is required for testing, the consent form should include the laboratory confidentiality policies and procedures and should describe situations in which test results might be requested by health-care providers caring for family members of the patient.
- Laboratory directors are responsible for determining and approving circumstances in which access to confidential patient information is appropriate, including when, how, and to whom information is to be released, in compliance with federal, state, and local requirements.

The HIPAA privacy rule and CLIA regulations are federal regulations intended to provide minimum standards for ensuring confidentiality of patient information; states or localities might have more restrictive standards. Although the HIPAA privacy rule allows health-care providers that are covered entities (i.e., health-care providers that conduct certain transactions in electronic form, health-care clearinghouses, and health plans) to use or disclose protected health information for treatment purposes without patient authorization and to share protected health information to consult with other providers to treat a different patient or to refer a patient, the regulation indicates that states or institutions may implement stricter standards to protect the privacy of patients and the confidentiality of patient information (*115*). Laboratories must comply with applicable requirements and follow professional practice guidelines in establishing policies and procedures to ensure confidentiality of patient information, including information and test results on biochemical genetic testing and newborn screening.

Personnel Qualifications, Responsibilities, and Competency Assessments

Laboratory Director Qualifications and Responsibilities

Qualifications

CLIA requires directors of laboratories that perform high-complexity testing to meet at least one of the following sets of qualifications (42 CFR §493.1443) (*13*):

- Be a doctor of medicine or a doctor of osteopathy and have board certification in anatomic or clinical pathology or both
- Be a doctor of medicine, doctor of osteopathy, or doctor of podiatric medicine and have at least 1 year of laboratory training during residency or at least 2 years of experience directing or supervising high-complexity testing
- Have an earned doctoral degree in a chemical, physical, biological, or clinical laboratory science from an accredited institution and current certification by a board approved by HHS (*116*)

Directors of laboratories that perform testing for inherited metabolic diseases must meet these qualification requirements. Because CLIA requirements are minimum qualifications, laboratories that perform testing for inherited metabolic diseases should evaluate the tests they perform to determine whether additional knowledge, training, or expertise is necessary to fulfill the responsibilities of laboratory director.

Responsibilities

CLIA requires directors of laboratories that perform high-complexity testing to be responsible for the overall operation and administration of the laboratory, which includes responsibility for the following (42 CFR §493.1445):

- Ensuring the quality of all aspects of test performance and results reporting for each test performed in the laboratory
- Ensuring that the physical and environmental conditions of the laboratory are appropriate and safe
- Ensuring enrollment in HHS-approved proficiency testing programs
- Employing a sufficient number of laboratory personnel with appropriate education, experience, training, and competency required for patient testing
- Establishing policies and procedures for personnel competency assessment and monitoring
- Specifying the responsibilities and duties of each consultant, supervisor, and testing employee
- Ensuring compliance with applicable requirements and regulations

Directors of laboratories that perform testing for inherited metabolic diseases must fulfill these CLIA responsibility requirements. In addition, laboratory directors should have responsibility for the following:

- Ensuring documentation of the clinical validity of any biochemical genetic or newborn screening test the laboratory performs, following the recommended practices in this report
- Determining specific policies and procedures for assessing and ensuring the competency of all laboratory personnel, including technical supervisors, clinical consultants, general supervisors, and testing personnel

Technical Supervisor Qualifications and Responsibilities

Qualifications

CLIA regulations set forth minimum qualifications of technical supervisors for high-complexity testing in chemistry, clinical cytogenetics, and other specialties and subspecialties but do not specify qualification requirements for technical supervisors for biochemical genetic testing or newborn screening. Because CLIA requirements are intended to be minimum standards, laboratory directors should assess the tests their laboratories perform to determine whether additional qualifications are necessary for the technical supervisors to ensure quality throughout the testing process. Technical supervisors of testing for inherited metabolic diseases should have the qualifications that are appropriate for the section they are supervising, the types of testing performed, and the purpose for performing the testing. The recommended technical supervisor qualifications that follow are based on the complexity of testing for inherited metabolic diseases and the training, experience, and expertise required to provide technical supervision of laboratories performing these tests. These recommended qualifications are not regulatory requirements; rather, they should be considered part of recommended laboratory practices for ensuring the quality of testing for inherited metabolic diseases.

Biochemical genetic testing. Technical supervisors of laboratories that perform biochemical genetic testing should have either one of the following sets of qualifications:

- Qualifications equivalent to the CLIA qualification requirements for clinical cytogenetics technical supervisors (42 CFR §493.1449[p]), which include either one of the following subsets of qualifications:
  - Be a doctor of medicine, doctor of osteopathy, or doctor of podiatric medicine licensed to practice medicine, osteopathy, or podiatry in the state in which the laboratory is located and have 4 years of training or experience (or both) in genetics, 2 of which are in the area of biochemical genetic testing
  - Have an earned doctoral degree in a chemical, physical, biological, or clinical laboratory science from an accredited institution and have 4 years of training or experience (or both) in genetics, 2 of which are in the area of biochemical genetic testing
- Current certification in biochemical genetic testing by an HHS-approved board, such as the American Board of Medical Genetics

Newborn screening. Technical supervisors of public health newborn screening laboratories must meet the CLIA qualification requirements for technical supervisors of high-complexity testing that pertain to the specialties and subspecialties in which the laboratory performs testing and should have the following additional qualifications:

- Have at least 4 years of laboratory training or experience in newborn screening systems (which include dried blood spot specimen acquisition and handling; relevant biochemical, immunological, hematological, and chemical methods, including tandem mass spectrometry; data processing and quality assurance; report generation; and follow-up and referral protocols)
- Meet any additional state requirements that apply

Responsibilities

CLIA requires technical supervisors of laboratories that perform high-complexity testing to be responsible for the technical and scientific oversight of the laboratories (42 CFR §493.1451) (*13*). Technical supervisor responsibilities include the following:

- Selecting testing methods appropriate for the clinical use of the test results
- Verifying or establishing performance specifications for each test or test system
- Enrolling the laboratory in HHS-approved proficiency testing programs
- Establishing and maintaining an appropriate quality control program and ensuring the quality of test performance throughout the testing process
- Resolving technical problems
- Ensuring all necessary remedial or corrective actions are taken before patient test results are reported
- Implementing laboratory personnel competency assessment policies, including evaluating and ensuring the competency of all testing personnel, identifying training needs, ensuring testing personnel receive regular in-service training and education appropriate for the type and complexity of the laboratory services performed, and documenting performance of testing personnel regularly as required

Biochemical genetic testing. Technical supervisors of laboratories that perform biochemical genetic testing must fulfill these CLIA responsibility requirements for high-complexity testing. In addition, when deemed necessary by the laboratory director, the responsibilities of the technical supervisor might also include one or more of the following tasks:

- Assessing the suitability of test requests for the expected clinical use of the test results
- Ensuring appropriate documentation of clinical validity information before offering new testing for patients
- Reviewing test results and their interpretation before reporting test results, and if appropriate, signing test

reports or providing other documentation of the review on the test reports
- Providing explanations or clarifications to questions regarding test reports, including test results and interpretation
- Evaluating test results and the need to refer to another laboratory or seek further consultation
- Providing on-site technical supervision for biochemical genetic testing

Newborn screening. Technical supervisors for public health newborn screening must meet the CLIA responsibility requirements for high-complexity testing. In addition, these technical supervisors should follow state-specific policies and practices to determine when additional testing is needed.

Clinical Consultant Qualifications and Responsibilities

Qualifications

CLIA requires clinical consultants for high-complexity testing to have either one of the following sets of qualifications (42 CFR §493.1455) (*13*):
- Be qualified as a laboratory director for high-complexity testing as specified in the regulations
- Be a doctor of medicine, doctor of osteopathy, or doctor of podiatric medicine licensed to practice medicine, osteopathy, or podiatry in the state in which the laboratory is located

These CLIA requirements provide minimum qualifications required for persons who provide clinical consultations for high-complexity testing. For laboratory testing for inherited metabolic diseases, clinical consultants should also have relevant training or experience in the testing for which they provide clinical consultation.

Biochemical genetic testing. Clinical consultants for biochemical genetic testing should have any one of the following additional sets of qualifications, which are more specific than those required by CLIA:
- Be a doctor of medicine, doctor of osteopathy, or doctor of podiatric medicine and be either board-certified or board-eligible in clinical genetics or clinical biochemical genetics
- Be a doctor of medicine, doctor of osteopathy, or doctor of podiatric medicine and have 2 years of experience in biochemical genetic testing, diagnosis and management of inborn errors of metabolism, or both
- Have an earned doctoral degree in a relevant discipline, be currently certified by a board approved by HHS, and have 2 years of training or experience in biochemical genetic testing

Responsibilities

CLIA regulations require clinical consultants for high-complexity testing to be responsible for providing consultation to laboratory clients regarding the appropriateness of the testing ordered and the interpretation of test results (42 CFR §493.1457) (*13*). Persons providing clinical consultations for testing for inherited metabolic diseases must meet the following CLIA responsibility requirements for clinical consultants for high-complexity testing:
- Be available to provide consultation to laboratory clients, including assisting them with ordering appropriate tests to meet clinical expectations and discussing the quality and interpretation of test results
- Ensure that test reports include pertinent information required for interpretation of specific patient conditions

General Supervisor Qualifications and Responsibilities

Qualifications

CLIA requires general supervisors of laboratories that perform high-complexity testing to meet at least one of the following sets of qualifications (42 CFR §493.1461) (*13*):
- Be qualified as a laboratory director or technical supervisor
- Be a doctor of medicine, doctor of osteopathy, or doctor of podiatric medicine licensed to practice medicine, osteopathy, or podiatry in the state in which the laboratory is located
- Have a doctoral, master's, or bachelor's degree in a chemical, physical, biological, or clinical laboratory science and 1 year of training or experience in high-complexity testing
- Have an associate's degree or equivalent in a chemical, physical, biological, or clinical laboratory science and 2 years of training or experience in high-complexity testing

Biochemical genetic testing. General supervisors of laboratories that perform biochemical genetic testing must fulfill these CLIA qualification requirements for high-complexity testing. CLIA qualification requirements apply to high-complexity testing in general; therefore, laboratories that perform specialized biochemical genetic testing (i.e., not general laboratory tests such as lipids or cholesterol testing) should ensure that general supervisors have specific training or experience in the biochemical genetic tests the laboratory performs. General supervisors for laboratories performing these biochemical genetic tests should have one of the following sets of qualifications:
- Meet qualifications for laboratory director or technical supervisor as recommended in this report
- Be a doctor of medicine, doctor of osteopathy, or doctor of podiatric medicine and have 1 year training or experience in biochemical genetic testing relevant to the tests performed by the laboratory
- Have a doctoral or master's degree in a chemical, physical, biological, or clinical laboratory science and have 1 year

of training or experience in biochemical genetic testing relevant to the tests performed by the laboratory
- Have a bachelor's degree in a chemical, physical, biological or clinical laboratory science and have 2 years of training or experience in biochemical genetic testing relevant to the tests performed by the laboratory

Newborn screening. General supervisors of laboratories that perform public health newborn screening must meet the CLIA qualification requirements for general supervisor for high-complexity testing and should also meet any additional state or local qualification requirement that might be more stringent than the CLIA requirements.

Responsibilities

CLIA regulations require general supervisors for high-complexity testing to be responsible for day-to-day supervision or oversight of the laboratory operation and personnel performing testing and reporting test results (42 CFR §493.1463) (*13*). General supervisors of laboratories that perform testing for inherited metabolic diseases must meet the following CLIA responsibility requirements for general supervisors for high-complexity testing:

- Be accessible to testing personnel at all times testing is performed
- Provide day-to-day supervision and direct supervision of all testing personnel
- Monitor testing procedures to ensure the quality of analytical performance
- Fulfill the following duties when delegated by the laboratory director or technical supervisor:
  - Ensure that remedial actions are taken when test systems deviate from the established performance specifications.
  - Ensure that patient test results are not reported until all corrective actions have been taken and the test system is functioning properly.
  - Provide orientation for all testing personnel.
  - Annually evaluate and document the competency of all testing personnel to perform authorized testing.

Testing Personnel Qualifications and Responsibilities

Qualifications

CLIA requires testing personnel who perform high-complexity testing to meet at least one of the following sets of qualifications (42 CFR §493.1489) (*13*):

- Be a doctor of medicine, doctor of osteopathy, or doctor of podiatric medicine
- Have an earned doctoral, master's, or bachelor's degree in a chemical, physical, biological, or clinical laboratory science or medical technology from an accredited institution
- Have an earned associate's degree in a laboratory science or medical laboratory technology from an accredited institution

Biochemical genetic testing. These CLIA qualification requirements apply to testing personnel who perform biochemical genetic testing. Laboratories should ensure that testing personnel have received adequate training, including on-the-job training, and demonstrate competency in high-complexity biochemical genetic testing before performing patient testing.

Newborn screening. Testing personnel who perform public health newborn screening must meet the CLIA qualification requirements for testing personnel for high-complexity testing and should also meet any additional state or local qualification requirement that might be more stringent than the CLIA requirements.

Responsibilities

CLIA requires persons who perform high-complexity testing to follow laboratory procedures for test performance, quality control, results reporting, documentation, and problem identification and correction (42 CFR §493.1495) (*13*). Personnel who perform biochemical genetic testing or newborn screening must meet these requirements.

Personnel Competency Assessment

CLIA requires laboratories to establish and follow written policies and procedures to assess employee competency, and if applicable, consultant competency (42 CFR §493.1235) (*13*). CLIA requirements for laboratory director responsibilities (42 CFR §493.1445[e][*13*]) specify that laboratory directors must ensure that policies and procedures are established for monitoring and ensuring the competency of testing personnel and for identifying needs for remedial training or continuing education to improve knowledge and skills. Technical supervisors are responsible for implementing the personnel competency assessment policies and procedures, including evaluating and ensuring competency of testing personnel (42 CFR §493.1451[b][8]) (*13*). Laboratories that perform testing for inherited metabolic diseases must meet these general personnel competency assessment requirements because regular competency assessment is an important element of ensuring all personnel are capable of performing their duties appropriately. Laboratories also should follow the applicable CMS guidelines to establish and implement policies and procedures specific for assessing and ensuring the competency of all types of laboratory personnel, including

technical supervisors, clinical consultants, general supervisors, and testing personnel, in performing duties and responsibilities (*74*). For example, the performance of testing personnel must be evaluated and documented at least semiannually during the first year a person tests patient specimens. Thereafter, evaluations must be performed at least annually; however, if test methods or instrumentation changes, performance must be reevaluated to include the use of the new test methods or instrumentation before testing personnel can report patient test results. Personnel competency assessments should identify training needs and ensure that persons responsible for test performance receive regular in-service training and education appropriate for the services performed (*74*).

Conclusion

The recommendations in this report are intended to provide laboratory professionals and others with information to ensure and improve the quality of laboratory testing performed for screening, detection, diagnosis, monitoring, and clinical management of persons with inherited metabolic diseases. Although the recommended practices are primarily intended for laboratories performing biochemical genetic testing and newborn screening, many recommendations reflect general good laboratory practices for ensuring the quality of laboratory services. Recommendations beyond the CLIA requirements are included as guidelines rather than requirements; therefore, general laboratories may also implement these recommendations, when appropriate, to improve testing quality.

Usefulness for Users of Laboratory Services

These recommendations serve as a resource for health-care professionals and other users of laboratory services to improve the delivery and usefulness of biochemical genetic and newborn screening test results in clinical and public health practices. In particular, these recommendations inform users of laboratory services about the responsibilities and practices to be expected of laboratories when providing biochemical genetic test results and newborn screening services. An understanding of these recommendations by users of laboratory services might prevent or reduce errors or problems relating to test selection, specimen submission, test performance, and reporting and interpretation of results. These practices should lead to improved use of biochemical genetic tests and better collaboration and follow-up to newborn screening, resulting in better health outcomes for patients and their families.

Usefulness for Evaluators of Laboratory Practices

These recommendations also are a resource for medical and public health professionals, including laboratory inspectors and surveyors, payers, and persons who evaluate laboratory practices and policies to improve quality assurance procedures and quality systems. The recommendations are intended to clarify CLIA requirements that are applicable to biochemical genetic testing and newborn screening for inherited metabolic diseases, provide recommendations for additional quality assurance practices that are not specifically addressed by CLIA requirements, and facilitate assessment of laboratory practices, especially the specific recommended quality management practices in addition to CLIA regulations.

Usefulness for Development of Future Professional Guidelines and Accreditation Standards

The recommendations in this report were developed with consideration of existing relevant regulatory requirements (both federal and state), accreditation standards, professional guidelines, and other standards. These CDC recommendations are expected to help address quality assurance concerns in laboratory practices and clarify or provide guidance for areas and issues that are inconsistent among existing standards. These recommendations also can serve as a resource for accrediting agencies that are evaluating whether laboratories are performing properly and adhering to established requirements, standards, and recommendations. In addition, these recommendations can be updated in future documents to reflect changes in laboratory testing for inherited metabolic diseases, such as new technologies or practices that might be adopted in the future.

Usefulness for Development and Use of Standards for Electronic Communication in Clinical and Public Health Practice

The recommendations in this report should be considered in the development of information technology systems to be used for biochemical genetic testing or newborn screening to ensure that they accommodate the nationally recommended laboratory practices. For example, the health information technology standards for newborn screening results reporting, created collaboratively by many federal agencies, advisory groups, and the newborn screening system vendors, are directly relevant to laboratory practices for newborn screening, particularly in the preanalytic and postanalytic phases of the testing process (*36–38,40*). Consistently following the CDC

recommendations can help expedite the delivery of newborn screening results and provide the data needed to manage and improve the newborn screening process.

Usefulness for In Vitro Diagnostic Manufacturers

The recommendations in this report focus on parameters important in the development and application of biochemical genetic and newborn screening tests, including introduction of new tests for patient testing, analytic and clinical validity, quality control, proficiency testing, and communication of test results to health-care providers and other users. Some of these parameters are already considered in the FDA review process for test systems, control materials, and other in vitro diagnostic products used in performing biochemical genetic or newborn screening tests. Therefore, an understanding of the recommendations in this report can assist in vitro diagnostic manufacturers in providing products in accordance with recommended good laboratory practices.

Usefulness for Patients and Families

Knowledge of these CDC recommendations might also help patients and their families to understand the test information that should be provided and the responsibilities of the laboratory. For example, understanding that laboratories must maintain the confidentiality of information regarding patients and their family members should facilitate the ability of patients and families to make informed decisions. In addition, knowledge of the recommended laboratory practices for retention of records, reports, and tested specimens should also be helpful for patients and families.

Acknowledgments

This report is based, in part, on contributions by Carol L. Greene, MD, Department of Pediatrics, University of Maryland School of Medicine; the Newborn Screening and Genetics in Public Health Practices Committee and Quality Assurance/Quality Control Subcommittee, Association of Public Health Laboratories; the Secretary's Advisory Committee on Heritable Disorders in Newborns and Children; the Secretary's Advisory Committee on Genetics, Health, and Society; Michele A. Lloyd-Puryear, MD, PhD, Sara Copeland, MD, Maternal and Child Health Bureau, Health Resources and Services Administration; Cathy Fomous, PhD, Office of Biotechnology Activities, National Institutes of Health; Judith Yost, MA, Penny Keller, Ronalda Leneau, MS, Division of Laboratory Services, Centers for Medicare & Medicaid Services; Alberto Gutierrez, PhD, Kellie Kelm, PhD, Office of In Vitro Diagnostic Device Evaluation and Safety, Food and Drug Administration; Rebecca Goodwin, JD, National Library of Medicine; Fred Lorey, PhD, Genetic Disease Screening Program, California Department of Public Health; Alan R. Fleischman, MD, March of Dimes; Jelili Ojodu, Elizabeth Jones, Association of Public Health Laboratories; Chunli Yu, MD, Department of Genetics and Genomic Sciences, Mt. Sinai School of Medicine; Erin Strovel, PhD, Division of Human Genetics, University of Maryland School of Medicine; Victor De Jesús, PhD, Marie Earley, PhD, Hui Zhou, MD, Division of Laboratory Sciences, National Center for Environmental Health—Agency for Toxic Substances and Disease Registry, CDC; William D. Dotson, PhD, Katherine Kolor, PhD, CGC, Office of Public Health Genomics, CDC; and Andrea Scott Murphy, CMP, CGMP, Amy Gargis, PhD, and Laurina O. Williams, PhD, Division of Laboratory Science and Standards, Laboratory Science, Policy and Practice Program Office, Office of Surveillance, Epidemiology, and Laboratory Services, CDC.

References

1. Raghuveer TS, Garg U, Graf WD. Inborn errors of metabolism in infancy and early childhood: an update. Am Fam Physician 2006;73: 1981–90.
2. Association of Public Health Laboratories. Newborn screening and genetics. Silver Spring, MD: Association of Public Health Laboratories; 2010. Available at http://www.aphl.org/aphlprograms/nsg/pages/default.aspx. Accessed February 2, 2012.
3. Watson MS, Mann MY, Lloyd-Puryear MA, Rinaldo P. Newborn screening: toward a uniform screening panel and system. Genet Med 2006;8(Suppl 1):1S–252S.
4. CDC. Good laboratory practices for molecular genetic testing for heritable diseases and conditions. MMWR 2009;58(No. RR-6).
5. McGovern MM, Benach M, Wallenstein S, Boone J, Lubin IM. Personnel standards and quality assurance practices of biochemical genetic testing laboratories in the United States. Arch Pathol Lab Med 2003;127:71–6.
6. National Center for Biotechnology Information. GeneTests. Seattle, WA: National Center for Biotechnology Information; 2012. Available at http://www.ncbi.nlm.nih.gov/sites/genetests/?db=genetests. Accessed February 2, 2012.
7. College of American Pathologists. Biochemical genetic testing proficiency survey summary reports. 2006.
8. CDC. Using tandem mass spectrometry for metabolic disease screening among newborns: a report of a work group. MMWR 2001;50(No. RR-3).
9. National Newborn Screening and Genetics Resource Center. National newborn screening status report. Austin, TX: National Newborn Screening and Genetics Resource Center; 2011. Available at http://genes-r-us.uthscsa.edu/nbsdisorders.pdf. Accessed February 2, 2012.
10. Response of the Secretary of Health and Human Services to the Secretary's Advisory Committee on Heritable Disorders in Newborns and Children, May 21, 2010. Available at http://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/recommendations/correspondence/uniformpanelsecre052110.pdf. Accessed February 2, 2012.
11. Dietzen DJ, Rinaldo P, Whitley RJ, et al. National Academy of Clinical Biochemistry laboratory medicine practice guidelines: follow-up testing for metabolic disease identified by expanded newborn screening using tandem mass spectrometry; executive summary. Clin Chem 2009;55:1615–26.
12. Public Health Service Act, 42 U.S.C. Sect. 263(a) (1999). Available at http://wwwn.cdc.gov/clia/pdf/phsa_353.pdf. February 2, 2012.
13. Centers for Medicare & Medicaid Services. 42 C.F.R. Part 493. Laboratory Requirements: Clinical Laboratory Improvement Amendments of 1988. Available at http://wwwn.cdc.gov/clia/regs/toc.aspx. Accessed February 2, 2012.
14. CDC. Clinical Laboratory Improvement Advisory Committee (CLIAC). Available at http://wwwn.cdc.gov/cliac/default.aspx. Accessed February 2, 2012.
15. Cowan TM, Strovel ET. Management and quality assurance in the biochemical genetics laboratory. Curr Protoc Hum Genet 2008;17.7.1–17.7.12
16. Hertzberg MS, Mammen J, McCraw A, Nair SC, Srivastava A. Achieving and maintaining quality in the laboratory. Haemophilia 2006;12 (Suppl 3):61–7.
17. Clinical and Laboratory Standards Institute. Application of a quality management system model for laboratory services; approved guideline, 4th ed. Wayne, PA: Clinical and Laboratory Standards Institute; Publication no. GP26-A4; 2011.
18. American College of Medical Genetics. Standards and guidelines for clinical genetics laboratories: clinical biochemical genetics. Bethesda, MD: American College of Medical Genetics; 2008. Available at http://www.acmg.net/pages/acmg_activities/stds-2002/f.htm. Accessed February 2, 2012.
19. Peters V, Garbade SF, Langhans CD, et al. Qualitative urinary organic acid analysis: methodological approaches and performance. J Inherit Metab Dis 2008;31:690–6.
20. Fowler B, Burlina A, Kozich V, Vianey-Saban C. Quality of analytical performance in inherited metabolic disorders: the role of ERNDIM. J Inherit Metab Dis 2008;31:680–9.
21. Jenny RW, Jackson KY. Proficiency test performance as a predictor of accuracy of routine patient testing for theophylline. Clin Chem 1993;39:76–81.
22. Parsons PJ, Reilly AA, Esernio-Jenssen D, et al. Evaluation of blood lead proficiency testing: comparison of open and blind paradigms. Clin Chem 2001;47:322–30.
23. Dequeker E, Cassiman JJ. Evaluation of CFTR gene mutation testing methods in 136 diagnostic laboratories: report of a large European external quality assessment. Eur J Hum Genet 1998;6:165–75.
24. European Research Network for Evaluation and Improvement of Screening, Diagnosis, and Treatment of Inherited Disorders of Metabolism (ERNDIM), Sheffield, UK. Available at http://www.erndim.unibas.ch. Accessed February 2, 2012.
25. College of American Pathologists. 2011 surveys & anatomic pathology education programs. Northfield, IL: College of American Pathologists. Available at http://www.cap.org/apps/docs/proficiency_testing/2011_surveys_catalog.pdf. Accessed February 2, 2012.
26. Clinical and Laboratory Standards Institute. Assessment of laboratory tests when proficiency testing is not available; approved guideline, 2nd ed. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. GP29-A2; 2008.
27. College of American Pathologists. Sample exchange registry for alternative assessment. Northfield, IL: College of American Pathologists. Available at http://www.cap.org/apps/docs/laboratory_resources/exchangeregistry/material_submission_form.pdf. Accessed February 2, 2012.
28. McGovern MM, Benach MO, Wallenstein S, Desnick RJ, Keenlyside R. Quality assurance in molecular genetic testing laboratories. JAMA 1999;281:835–40.
29. European Research Network for Evaluation and Improvement of Screening, Diagnosis, and Treatment of Inherited Disorders of Metabolism (ERNDIM). The Eurogentest project and ERNDIM. Interim report on biochemical genetic testing in Europe: deficits and needs and EQA. Sheffield, UK: ERNDIM. Available at http://www.erndim.unibas.ch/Meeting_Rep/06_dec_basel/Best%20practice%20EUGT%20UPDATED%20report%203Sept2007.pdf. Accessed February 2, 2012.
30. De Jesús VR, Mei JV, Bell CJ, Hannon WH. Improving and assuring newborn screening laboratory quality worldwide: 30-year experience at the Centers for disease Control and Prevention. Semin Perinatol 2010;34:125–33.
31. McCallister T, Himstedt L, Ross L, et al; Newborn Screening Workgroup of the Heartland Regional Genetics and Newborn Screening Collaborative. The heartland good spot/bad spot project. Presented at the 2010 National Newborn Screening and Genetic Testing Symposium, Orlando, FL; May 2010. Available at http://www.heartlandcollaborative.org/annualconference/documents/GoodSpotandBadSpot--McCallister.pdf. Accessed February 7, 2012.
32. National Newborn Screening and Genetics Resource Center. Austin, TX; 2011. Available at http://genes-r-us.uthscsa.edu. Accessed February 2, 2012.
33. Rinaldo P, Zafari S, Tortorelli S, Matern D. Making the case for objective performance metrics in newborn screening by tandem mass spectrometry. Ment Retard Dev Disabil Res Rev 2006;12:255–61.
34. Therrell BL, Hannon WH. National evaluation of U.S. newborn screening system components. Ment Retard Dev Disabil Res Rev 2006;12:236–45.

35. CDC. Laboratory standardization and quality assurance programs: newborn screening quality assurance program. Atlanta, GA: CDC; 2012. Available at http://www.cdc.gov/labstandards/nsqap.html. Accessed February 2, 2012.
36. Abhyankar S, Lloyd-Puryear MA, Goodwin R, et al. Standardizing newborn screening results for health information exchange. AMIA Annu Symp Proc 2010:1–5.
37. National Library of Medicine. Newborn screening coding and terminology guide: data standards for electronic reporting. Bethesda, MD: National Library of Medicine; 2011. Available at http://newbornscreeningcodes.nlm.nih.gov/hl7. Accessed February 2, 2012.
38. Downs SM, van Dyck PC, Rinaldo P, et al. Improving newborn screening laboratory test ordering and results reporting using health information exchange. J Am Med Inform Assoc 2010;17:13–8.
39. Public Health Informatics Institute. Newborn dried blood spot (NDBS) screening implementation guide for laboratory orders, U.S. realm. Decatur, GA: Public Health Informatics Institute; 2011. Available at http://www.phii.org/sites/default/files/resource/pdfs/PHII-NDBS-Lab-Orders-Impl-Guide-OML%5EO21-HL7-251_v1%200%201_2011-10-12_final.pdf. Accessed March 15, 2012.
40. Public Health Informatics Institute. Newborn dried blood spot (NDBS) screening implementation guide for laboratory results, U.S. realm. Decatur, GA: Public Health Informatics Institute; 2011. Available at http://www.phii.org/sites/default/files/resource/pdfs/PHII-NDBS-Lab-Results-Impl-Guide-ORU%5ER01-HL7-251_v1%200%201_2011-11-01_final.pdf. Accessed March 15, 2012.
41. Hannon WH, Lim T, Adam B, Therrell B. Outcomes from tandem mass spectrometry (MS/MS) workshops in the United States and the performance evaluation of MS/MS laboratories. Southeast Asian J Trop Med Public Health 2003; 34(Suppl 3):121–6.
42. Hoeltge GA, Phillips MG, Styer PE, Mockridge P. Detection and correction of systematic laboratory problems by analysis of clustered proficiency testing failures. Arch Pathol Lab Med 2005;129:186–9.
43. Matern D, Tortorelli S, Oglesbee D, Gavrilov D, Rinaldo P. Reduction of the false-positive rate in newborn screening by implementation of MS/MS-based second-tier tests: the Mayo Clinic experience (2004–2007). J Inherit Metab Dis 2007;30:585–92.
44. Rinaldo P, Hahn S, Matern D. Clinical biochemical genetics in the twenty-first century. Acta Paediatr Suppl 2004;93:22–6.
45. Rinaldo P, Lim JS, Tortorelli S, Gavrilov D, Matern D. Newborn screening of metabolic disorders: recent progress and future developments. Nestle Nutr Workshop Ser Pediatr Program 2008;62:81–93.
46. Rinaldo P, Tortorelli S, Matern D. Recent developments and new applications of tandem mass spectrometry in newborn screening. Curr Opin Pediatr 2004;16:427–33.
47. Tan IK, Gajra B, Lim MS. External proficiency testing programmes in laboratory diagnoses of inherited metabolic disorders. Ann Acad Med Singapore 2006;35:688–93.
48. Therrell BL, Hannon WH, Pass KA, et al. Guidelines for the retention, storage, and use of residual dried blood spot samples after newborn screening analysis: statement of the Council of Regional Networks for Genetic Services. Biochem Mol Med 1996;57:116–24.
49. Barshop BA, Greene CL. Laboratory referral practices in biochemical genetics in the United States. Mol Genet Metab 2009;98:149–51.
50. New York State Department of Health. Approved laboratories. Albany, NY: New York State Department of Health, 2012. Available at http://www.wadsworth.org/labcert/clep/CategoryPermitLinks/CategoryListing.htm. Accessed February 2, 2012.
51. Washington State Department of Health. Office of Laboratory Quality Assurance. Olympia, WA: Washington State Department of Health; 2011. Available at http://doh.wa.gov/hsqa/fsl/lqa_home.htm. Accessed February 2, 2012.
52. CDC. Newborn Screening Quality Assurance Program: 2010 annual summary report. Atlanta, GA: CDC; 2011. Available at http://www.cdc.gov/labstandards/pdf/nsqap/nsqap_summaryreport_2011.pdf. Accessed February 2, 2012.
53. CDC. Impact of expanded newborn screening—United States, 2006. MMWR 2008;57:1012–15.
54. European Research Network for Evaluation and Improvement of Screening, Diagnosis and Treatment of Inherited Disorders of Metabolism (ERNDIM). ERNDIM schemes. Sheffield, UK: ERNDIM, 2012. Available at http://cms.erndimqa.nl/home.aspx. Accessed February 7, 2011.
55. Society for Inherited Metabolic Disorders. Listing of laboratories and tests in biochemical genetics (UCSDW3BG). West Linn, OR; 2010.
56. American College of Medical Genetics. Standards and guidelines for clinical genetics laboratories. Bethesda, MD: American College of Medical Genetics; 2008. Available at http://www.acmg.net/am/template.cfm?Section=laboratory_standards_and_guidelines&template=/cm/htmldisplay.cfm&contentid=3136. Accessed March 15, 2012.
57. Clinical and Laboratory Standards Institute. Evaluation of precision performance of quantitative measurement methods; approved guideline, 2nd ed. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. EP5-A2; 2004.
58. Clinical and Laboratory Standards Institute. Interference testing in clinical chemistry; approved guideline, 2nd ed. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. EP07-A2; 2005.
59. Clinical and Laboratory Standards Institute. Method comparison and bias estimation using patient samples; approved guideline, 2nd ed. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. EP9-A2; 2002.
60. Clinical and Laboratory Standards Institute. Evaluation of matrix effects; approved guideline, 2nd ed. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. EP14-A2; 2005.
61. Clinical and Laboratory Standards Institute. Protocols for determination of limits of detection and limits of quantitation; approved guideline. Wayne, PA: Clinical and Laboratory Standards Institute; Publication no. EP17-A; 2004.
62. Clinical and Laboratory Standards Institute. Estimation of total analytical error for clinical laboratory methods; approved guideline. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. EP21-A; 2003.
63. Clinical and Laboratory Standards Institute. Defining, establishing and verifying reference intervals in the clinical laboratory; approved guideline, 3rd ed. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. C28-A3; 2008.
64. Clinical and Laboratory Standards Institute. Gas chromatography/mass spectrometry (GC/MS) confirmation of drugs; approved guideline, 2nd ed. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. C43-A2; 2010.
65. Clinical and Laboratory Standards Institute. Mass spectrometry in the clinical laboratory: general principles and guidance; approved guideline, 2nd ed. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. C50-A2; 2007.
66. Clinical and Laboratory Standards Institute. Continuous quality improvement: integrating five key quality systems; approved guideline, 2nd ed. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. GP22-A2; 2004.
67. Clinical and Laboratory Standards Institute. Using proficiency testing to improve the clinical laboratory; approved guideline, 2nd ed. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. GP27-A2; 2007.
68. Clinical and Laboratory Standards Institute. Development and use of quality indicators; approved guideline. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. GP35-A; 2010.

69. Clinical and Laboratory Standards Institute. Blood collection on filter paper for newborn screening programs; approved guideline, 5th ed. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. LA4-A5; 2007.
70. Clinical and Laboratory Standards Institute. Newborn screening follow-up; approved guideline. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. ILA27-A; 2006.
71. Clinical and Laboratory Standards Institute. Newborn screening for preterm, low birth weight, and sick newborns; approved guideline. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. ILA31-A; 2009.
72. Clinical and Laboratory Standards Institute. Newborn screening by tandem mass spectrometry; proposed guideline. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. ILA32-P; 2009.
73. Clinical and Laboratory Standards Institute. Collection, transport, preparation, and storage of specimens for molecular methods; approved guideline. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. MM13-A; 2005.
74. Centers for Medicare & Medicaid Services. Clinical Laboratory Improvement Amendments: Appendix C: survey procedures and interpretive guidelines for laboratories and laboratory services. Baltimore, MD: Centers for Medicare & Medicaid Services; 2011. Available at http://www.cms.gov/clia/03_interpretive_guidelines_for_laboratories.asp. Accessed February 2, 2012.
75. Food and Drug Administration. Guidance for industry and FDA staff. Commercially distributed analyte specific reagents (ASRs): frequently asked questions. Silver Spring, MD: Food and Drug Administration; 2007. Available at http://www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm071269.pdf. Accessed February 2, 2012.
76. Food and Drug Administration. Guidance for industry and FDA staff. Class II special controls guidance document: newborn screening test systems for amino acids, free carnitine, and acylcarnitines using tandem mass spectrometry. Silver Spring, MD: Food and Drug Administration; 2004. Available at http://www.fda.gov/downloads//medicaldevices/deviceregulationandguidance/guidancedocuments/ucm071031.pdf. Accessed February 2, 2012.
77. New York State Department of Health. Clinical laboratory evaluation program: survey standards. Albany, NY: New York State Department of Health; 2012. Available at http://www.wadsworth.org/labcert/clep/standards.htm. Accessed February 2, 2012.
78. State of Washington Department of Health. Chapter 246-338 WAC: medical test site rules. Olympia, WA: Washington State Department of Health; 2006. Available at http://www.doh.wa.gov/hsqa/fsl/documents/lqa_docs/wac-changes-4-03-07.pdf. Accessed February 2, 2012.
79. College of American Pathologists. Commission on Laboratory Accreditation. Chemistry and toxicology checklist. Washington, DC: College of American Pathologists; 2006. Available at http://www.pipettecal.com/cap%20checklist.pdf. Accessed February 2, 2012.
80. College of American Pathologists. Commission on Laboratory Accreditation. Molecular pathology checklist. Washington, DC: College of American Pathologists; 2007. Available at http://www.cap.org/apps/docs/laboratory_accreditation/checklists/molecular_pathology_sep07.pdf. Accessed February 2, 2012.
81. College of American Pathologists. Laboratory accreditation standards relating to ensuring clinical validity of genetic tests. Washington, DC: College of American Pathologists; 2010.
82. College of American Pathologists. Commission on Laboratory Accreditation. Laboratory general checklist. Washington, DC: College of American Pathologists; 2007. Available at http://www.cap.org/apps/docs/laboratory_accreditation/checklists/laboratory_general_sep07.pdf. Accessed February 2, 2012.
83. National Newborn Screening & Genetics Resource Center. Performance evaluation and assessment scheme (PEAS) for newborn screening systems. Austin, TX: National Newborn Screening and Genetics Resource Center; 2006. Available at http://genes-r-us.uthscsa.edu/nbs_peas.htm. Accessed February 2, 2012.
84. International Organization for Standardization. ISO 15189: medical laboratories—particular requirements for quality and competence. Geneva, Switzerland: International Organization for Standardization; 2007.
85. Organisation for Economic Co-operation and Development. OECD guidelines for quality assurance in molecular genetic testing. Paris, France: Organisation for Economic Co-operation and Development; 2007. Available at http://www.oecd.org/dataoecd/43/6/38839788.pdf. Accessed February 2, 2012.
86. European Research Network for Evaluation and Improvement of Screening, Diagnosis and Treatment of Inherited Disorders of Metabolism (ERNDIM). Quality control in inherited disorders of metabolism (IDM) laboratories: ERNDIM schemes and theoretical aspects. Sheffield, UK: ERNDIM; 2002. Available at http://www.erndim.unibas.ch/pdf/aspect.pdf. Accessed February 2, 2012.
87. CDC. Clinical Laboratory Improvement Advisory Committee (CLIAC). September 10–11, 2008: meeting summary. Atlanta, GA: CDC; 2008. Available at http://wwwn.cdc.gov/cliac/cliac0908.aspx. Accessed February 2, 2012.
88. Federal Advisory Committee Act of 1972. Pub. L. No. 92-463, 5 U.S.C. app (1972). Available at http://www.gsa.gov/graphics/ogp/without_annotations_R2G-b4T_0Z5RDZ-i34K-pR.pdf. Accessed February 2, 2012.
89. CDC. Clinical Laboratory Improvement Advisory Committee (CLIAC). February 9–10, 2010: meeting summary. Atlanta, GA: CDC; 2010. Available at http://wwwn.cdc.gov/cliac/cliac0210.aspx. Accessed February 2, 2012.
90. International Organization for Standardization. ISO 9001: Quality management systems—requirements. Geneva, Switzerland: International Organization for Standardization; 2008.
91. International Organization for Standardization. ISO 17025: General requirements for the competence of testing and calibration laboratories. Geneva, Switzerland: International Organization for Standardization; 2005.
92. Clinical and Laboratory Standards Institute. Laboratory documents: development and control; approved guideline. 5th ed. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. GP2-A5; 2006.
93. Clinical and Laboratory Standards Institute. Training and competence assessment; approved guideline. 3rd ed. Wayne, PA: Clinical and Laboratory Standards Institute. Publication no. GP21-A3; 2009.
94. Whitely RJ, Hannon WH, Dietzen DJ, Rinaldo P. Pre-analytical, analytical, and post-analytical issues related to follow-up testing of positive newborn screens [Chapter 2]. In: Bennett MJ, editor. Follow-up testing for metabolic diseases identified by expanded newborn screening using tandem mass spectrometry. Washington, DC: National Academy of Clinical Biochemistry; 2009. Available at http://www.aacc.org/members/nacb/lmpg/onlineguide/publishedguidelines/newborn/documents/expandednewbornchapter2.pdf. Accessed February 2, 2012.
95. Khoury MJ, Gwinn M, Yoon PW, Dowling N, Moore CA, Bradley L. The continuum of translation research in genomic medicine: how can we accelerate the appropriate integration of human genome discoveries into health care and disease prevention? Genet Med 2007;9:665–74.
96. Burke W. Clinical validity and clinical utility of genetic tests. Curr Protoc Hum Genet 2009; Chapter 9: Unit 9.15.
97. Grosse SD, Kalman L, Khoury MJ. Evaluation of the validity and utility of genetic testing for rare diseases. Adv Exp Med Biol 2010;686:115–31.
98. National Newborn Screening and Genetics Resource Center. National newborn screening information system database. Austin, TX: National Newborn Screening and Genetics Resource Center; 2012. Available at http://nnsis.uthscsa.edu/xreports.aspx?xreportid=5. Accessed February 2, 2012.

99. McHugh DM, Cameron CA, Abdenur JE, et al. Clinical validation of cutoff target ranges in newborn screening of metabolic disorders by tandem mass spectrometry: a worldwide collaborative project. Genet Med 2011;13:230–54.
100. National Conferences of State Legislatures. Genetic privacy laws. Washington, DC: National Conferences of State Legislatures; 2008. Available at http://www.ncsl.org/issuesresearch/health/geneticprivacylaws/tabid/14287/default.aspx. Accessed February 2, 2012.
101. The Commonwealth of Massachusetts. Title XVI: public health. Available at http://www.malegislature.gov/laws/generallaws/parti/titlexvi/chapter111/section70g. Accessed February 2, 2012.
102. Legislative Council, State of Michigan. Public Health Code (Excerpt) Act 368 of 1978. Sect. 333.17520 (2000). Available at http://www.legislature.mi.gov/(S(0em5or45cvz1tjqiyyqte1vv))/documents/mcl/pdf/mcl-333-17520.pdf. Accessed February 2, 2012.
103. Nebraska Legislature. Sect. 71.551 as amended (2012). Available at http://www.legislature.ne.gov/laws/statutes.php?statute=s7105051000. Accessed February 2, 2012.
104. New York State. Civil Rights Law, Sect. 79-I (2002). Available at http://www.wadsworth.org/labcert/regaffairs/clinical/79-l_1_2002.pdf. Accessed February 2, 2012.
105. South Dakota Legislature. Chapter 34-14-22. S.L. 2001. Chapter 184, Sect. 2. Available at http://legis.state.sd.us/statutes/displaystatute.aspx?statute=34-14-22&type=statute. Accessed February 2, 2012.
106. Holtzman NA. Promoting safe and effective genetic tests in the United States: work of the task force on genetic testing. Clin Chem 1999;45:732–8.
107. Texas Department of State Health Services. Newborn screening—use and storage of dried blood spots after NBS. Austin, TX: Texas Department of State Health Services; 2011. Available at http://www.dshs.state.tx.us/lab/nbsbloodspots.shtm. Accessed February 2, 2012.
108. Minnesota Department of Health. Minnesota newborn screening program: parental options. St. Paul, MN: Minnesota Department of Health; 2010. Available at http://www.health.state.mn.us/newbornscreening/optout.html. Accessed February 2, 2012.
109. Centers for Medicare & Medicaid Services. Laboratory demographics lookup. Baltimore, MD: Centers for Medicare & Medicaid Services; 2012. Available at http://www.cms.gov/clia/20_clia_laboratory_demographic_information.asp#topofpage. Accessed February 2, 2012.
110. Food and Drug Administration, 21 C.F.R. Subchapter H—Medical devices. Part 820: Quality System Regulation. Silver Spring, MD: Food and Drug Administration, 2011. Available at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=820&showfr=1. Accessed February 2, 2012.
111. Centers for Medicare & Medicaid Services. CLIA approved proficiency testing programs. Baltimore, MD: Centers for Medicare & Medicaid Services; 2011. Available at http://www.cms.gov/clia/downloads/ptlist.pdf. Accessed February 2, 2012.
112. Centers for Medicare & Medicaid Services. Clinical Laboratory Improvement Amendment (CLIA) proficiency testing: dos and don'ts. Baltimore, MD: Centers for Medicare & Medicaid Services; 2008. Available at http://www.cms.gov/clia/downloads/cliabrochure8.pdf. Accessed February 2, 2012.
113. De Jesús VR, Lim TH, Meredith NK, et al. Proficiency testing results for low free carnitine and hydroxyisovalerylcarnitine by tandem mass spectrometry: impact of laboratory cutoff practices. Available at http://www.aphl.org/conferences/proceedings/documents/2010/2010_aphl_nbs_genetic_testing_symposium/029dejesus.pdf. Accessed February 2, 2012.
114. CDC. HIPAA privacy rule and public health: guidance from CDC and the U.S. Department of Health and Human Services. MMWR 2003;52(Suppl).
115. US Department of Health and Human Services. Does the HIPAA privacy rule permit doctors, nurses, and other health care providers to share patient health information for treatment purposes without the patient's authorization? Washington, DC: US Department of Health and Human Services; 2005. Available at http://www.hhs.gov/hipaafaq/providers/treatment/481.html. Accessed February 2, 2012.
116. Centers for Medicare & Medicaid Services. Certification boards for laboratory directors of high complexity testing. Baltimore, MD: Centers for Medicare & Medicaid Services; 2012. Available at http://www.cms.gov/clia/16_certification_boards_laboratory_directors.asp#topofpage. Accessed February 2, 2012.

Appendix A

Abbreviations

ACMG	American College of Medical Genetics
APHL	Association of Public Health Laboratories
CAP	College of American Pathologists
CLIA	Clinical Laboratory Improvement Amendments of 1988
CLIAC	Clinical Laboratory Improvement Advisory Committee
CLSI	Clinical and Laboratory Standards Institute (formerly National Committee on Clinical Laboratory Standards [NCCLS])
CMS	Centers for Medicare & Medicaid Services
CPT	current procedural terminology
ERNDIM	European Research Network for Evaluation and Improvement of Screening, Diagnosis, and Treatment of Inherited Disorders of Metabolism
FDA	U.S. Food and Drug Administration
HHS	U.S. Department of Health and Human Services
HIPAA	Health Insurance Portability and Accountability Act of 1996
HL7	Health Level Seven International
HRSA	Health Resources and Services Administration
NIH	National Institutes of Health
NLM	National Library of Medicine
NSQAP	CDC Newborn Screening Quality Assurance Program
QMS	quality management system
SACGHS	Secretary's Advisory Committee on Genetics, Health, and Society
SACHDNC	Secretary's Advisory Committee on Heritable Disorders in Newborns and Children

Appendix B

Glossary

accuracy	Closeness of agreement between a measurement result and the true value or the accepted reference value
alternative performance assessment	Quality assessment activities for a test for which proficiency testing is not available or not used for the purpose of verifying the accuracy of the test results
amino acid	Basic structural units of proteins, the measurement of which (in protein and peptide hydrolysates, physiological fluids, and numerous other samples) provide important information for fundamental studies and the diagnosis of many pathological conditions, especially those resulting from inborn errors of metabolism
analyte	Component represented in the name of a measurable quantity
analyte-specific reagent	Antibodies (both polyclonal and monoclonal), specific receptor proteins, ligands, nucleic acid sequences, and similar reagents which, through specific binding or chemical reactions with substances in a specimen, are intended for use in a diagnostic application for identification and quantification of an individual chemical substance or ligand in biological specimens (21 CFR §864.4020[a])
analytical sensitivity	In quantitative testing: the change in response of a measuring system or instrument divided by the corresponding change in the stimulus; in qualitative testing: the test method's ability to obtain positive results in concordance with positive results obtained by the reference method
analytical specificity	Ability of a measurement procedure to measure solely the measurand
biochemical genetic testing	A diverse spectrum of laboratory analyses of metabolites, enzyme activities, and functional assays for evaluation, diagnosis, treatment monitoring, disease management, and assessing a person's risk for carrying a specific disease trait (i.e., carrier status assessment), such as inborn errors of metabolism
clinical sensitivity	The proportion of patients with a well-defined clinical disorder whose test values are positive or exceed a defined decision limit
clinical specificity	The proportion of patients who do not have a specified clinical disorder whose test results are negative or within the defined decision limit
clinical validity	The accuracy with which a test predicts designated intermediate or final clinical outcomes
competency assessment	Evaluation of a person's ability to perform a test, including all aspects of testing, from specimen collection to reporting of results
confirmatory test	A test to prove or disprove the presence of a specific condition identified by screening tests (for newborn screening using dried blood spot specimens, this testing is from a specimen other than the screening specimen)
control material	A device, solution, or lyophilized preparation intended for use in the quality control process to monitor the reliability of a test system and to maintain its performance within established limits
current procedural terminology (CPT) codes	Current procedural terminology code set maintained by the American Medical Association
critical values	Test results that require immediate notification of the clinician for patient evaluation or treatment

cut-off value	Quantitative value of the analyte that is used as the decision point between a positive and a negative result
drift	A slow or systematic change of a metrological characteristic of a measuring instrument or system, such as accuracy, trueness, and precision
genetics	The study of inheritance patterns of specific traits
genome	The complete genetic content of an organism
genotype	The genetic makeup of an organism or group of organisms with reference to a single trait, set of traits, or an entire complex of traits
Health Level Seven International (HL7)	A standards-development organization that has produced international standards for electronic reporting of laboratory results and orders
in-range result	Newborn screening result that is within the expected range of normal or negative test results established for a particular condition
informed consent	A process by which persons voluntarily confirm their willingness to participate in a particular testing act after having been informed of all aspects of the act that are relevant to the decision to participate in the act
internal control material	A control material that is placed in the same reaction tube as the specimen being analyzed and therefore is subjected to exactly the same internal conditions and external parameters as any analyte present in the tube
International Classification of Diseases (ICD)	The international standard diagnostic classification for the coding of diseases, signs and symptoms, abnormal findings, complaints, social circumstances, and external causes of injury or diseases, as maintained by the World Health Organization
limit of detection (LOD)	The lowest amount of analyte in a sample that can be detected (with stated probability), although the amount might not be quantified as an exact value
limit of quantitation (LOQ)	The lowest concentration at which an analyte can be quantitatively determined with stated acceptable precision and trueness under stated experimental conditions; might be equal to the limit of detection or could be at a higher concentration
measurand	Quantity to be measured
newborn screening	A system that identifies, shortly after birth, infants who are at increased risk for genetic and other congenital conditions so that treatment can begin as soon as possible; need to confirm positive newborn screening results with additional diagnostic testing
nonwaived testing	Test systems, assays, or examinations that have not been determined to be waived testing. Nonwaived testing encompasses moderate- and high-complexity testing for which CLIA regulations provide requirements for laboratory certification, quality systems, performance assessment, and laboratory personnel
out-of-range result	Newborn screening result that is outside the expected range of normal or negative test results established for a particular condition, including carrier results and any need for additional testing
phenotype	The observed biochemical, physiological, and morphological characteristics of an individual as determined by the genotype and the environment in which the genotype is expressed; also, in a more limited sense, the expression of a particular gene or genes

precision	Closeness of agreement between independent test results from the same sample obtained under stipulated conditions, often determined by assessing repeatability and reproducibility
proficiency testing	A program in which multiple samples are periodically sent to members of a group of laboratories for analysis or identification (or both) in which each laboratory's results are compared with those of other laboratories in the group or with an assigned value (or both) and are reported to the participating laboratory and others
repeatability	Closeness of agreement between independent test results for the same measurand under the same conditions
reproducibility	Closeness of agreement between independent test results for the same measurand under changed conditions
qualitative test	A characterization applied to laboratory tests that detect or identify a particular analyte, constituent, or condition
quality assessment	A group of activities to monitor and evaluate the total testing process to help ensure that test results are reliable, improve the testing process, and promote good quality testing practices
quality control	Operational techniques and activities that are used to fulfill requirements for quality; the procedures used to detect and correct errors that occur due to test system failure, adverse environmental conditions, and variance in operator performance, as well as the monitoring of the accuracy and precision of the test performance over time
quality management system (QMS)	Coordinated activities to direct and control an organization with regard to quality
quantitative test	A characterization applied to laboratory tests that provide results expressed in a numerical amount or level (concentration) of an analyte in a sample or specimen
reagent	A substance that produces a chemical or biological reaction with a patient specimen that allows detection or measurement of the analyte for which the test is designed
reference interval	The range of test values expected for a designated population of persons (e.g., 95% of persons that are presumed to be healthy [or normal])
reference material	Material sufficiently homogeneous and stable with respect to one or more specified properties (quantitative or qualitative) that has been established to be fit for its intended use in a measurement process; might be used to calibrate a measurement system, to assess a measurement procedure, to assign values to other materials, and for quality control; can only be used for a single purpose in a given measurement
reportable range of test results	The span of test result values over which the laboratory can establish or verify the accuracy of the instrument or test system measurement response
total testing process	Series of activities or path of workflow for performing testing that can be divided into three major phases: preanalytic, analytic, and postanalytic
trueness	Closeness of agreement between the average of an infinite number of replicate-measured quantity values and a reference quantity value
waived test	A test system, an assay, or an examination that has been found to meet the statutory criteria specified in the Public Health Service Act (§353[d][3]) (*12*)

Appendix C

Sources of Reference Materials and External Quality Assessment Programs for Biochemical Genetic Testing and Newborn Screening

Organization	External quality assessment and proficiency testing			Quality control
	Sample type	Analytes	Frequency	
CDC Newborn Screening Quality Assurance Program	Dried blood spots	Analytes detected for >30 biochemical markers covering all disorders listed in the recommended uniform screening panel	**For U.S. newborn screening laboratories:** four testing events per year, including three events each consisting of five challenge samples and one event consisting of 25 challenge samples **For international newborn screening laboratories:** three testing events per year, each consisting of five challenge samples	This program provides dried blood spot quality control materials for 28 biochemical markers encompassing all disorders listed in the recommended uniform screening panel.
College of American Pathologists	Plasma	Acylcarnitines: qualitative and quantitative	Two testing events per year, each consisting of five challenge samples	This program does not provide reference or quality control materials for biochemical genetic testing or newborn screening.
	Serum	Carnitine, qualitative and quantitative		
	Plasma or urine	Amino acids: qualitative and quantitative		
	Urine	Glycosaminoglycans (mucopolysaccharides): qualitative and quantitative Organic acids: qualitative and quantitative		
European Research Network for Evaluation and Improvement of Screening, Diagnosis, and Treatment of Inherited Disorders of Metabolism (ERNDIM)	Lyophilized, spiked human serum	30 relevant amino acids	One shipment of eight samples per year	This program also provides previously analyzed proficiency test samples as quality control materials.
	Lyophilized, spiked human urine	Special assays in urine: 5-hydroxyindoleacetic acid (5-HIAA), free carnitine, creatine, creatinine, galactitol, guanidinoacetate, homovanillic acid (HVA), lactic acid, mucopolysaccharides, orotic acid, pipecolic acid, sialic acid, and succinylacetone	One shipment of eight samples per year	
	Lyophilized, spiked human serum	Special assays in serum: 3-hydroxybutyrate, 7-dehydrocholesterol, very long-chain fatty acids (C22/24 and 26:0), free carnitine, creatine, galactose, guanidine acetic acid, homocysteine, lactic acid, methylmalonic acid, phytanic acid, pipecolic acid, pristanic acid, and pyruvic acid	One shipment of eight samples per year	
	Lyophilized, spiked human urine	Quantitative organic acids in urine: 15 analytes incorporated, but each year different choice made	One shipment of eight samples per year	
	Lyophilized, spiked human urine	Purines and pyrimidines in urine: 5-hydroxymethyluracil, adenine, adenosine, 5-aminoimidazole-4-carboxamide ribonucleotide, creatinine, deoxyadenosine, deoxyguanosine, deoxyinosine, deoxyuridine, dihydrothymine, dihydrouracil, guanosine, hypoxanthine, inosine, orotic acid, orotidine, pseudouridine, thymidine, thymine, uracil, uric acid, and xanthine	One shipment of eight samples per year	

(*Continued*)

Organization	External quality assessment and proficiency testing			Quality control
	Sample type	Analytes	Frequency	
European Research Network for Evaluation and Improvement of Screening, Diagnosis, and Treatment of Inherited Disorders of Metabolism (ERNDIM)	Lyophilized protein, liquid white blood cell pellets	Cystine in white blood cells, related to protein	Eight pairs of protein and white blood cell pellets per year	This program also provides previously analyzed proficiency test samples as quality control materials.
	Lyophilized homogenates of leukocytes and Epstein-Barr virus–transformed lymphoblastoid cells	Lysosomal enzymes: galactose-6-sulfate sulfatase, β-galactosidase, β-glucuronidase, β-hexosaminidase A, β-hexosaminidase A+B, α-iduronidase, galactosylceramidase, sphingomyelinase, β-mannosidase, and α-*N*-acetylglucosaminidase	One shipment of eight samples per year	
	Heat-treated human urine	Qualitative organic acids in urine: analytes dependent on specific disorders	Three shipments of three samples per year	
	Dried blood spots on filter paper	Qualitative blood spot acylcarnitine, analytes dependent upon disorder	Two shipments of three samples per year	
	Lyophilized human plasma/serum	Congenital disorders of glycosylation, sialotransferrin isoforms	Six samples per year	
	Human urine	Urine mucopolysaccharides: quantitative (related to creatinine) and qualitative analysis	One shipment of six samples per year	
Reference Institute for Bioanalytics (Germany)	Dried blood spots	Neonatal thyroid stimulating hormone and 17-hydroxyprogesterone screening	Four times per year, each consisting of eight samples	This program does not provide reference or quality control materials for biochemical genetic testing or newborn screening.

Sources: College of American Pathologists. 2011 surveys & anatomic pathology education programs. Northfield, IL: College of American Pathologists. Available at http://www.cap.org/apps/docs/proficiency_testing/2011_surveys_catalog.pdf. Accessed February 2, 2012.

CDC. Laboratory standardization and quality assurance programs: newborn screening quality assurance program. Atlanta, GA: CDC; 2012. Available at http://www.cdc.gov/labstandards/nsqap.html. Accessed February 2, 2012.

European Research Network for Evaluation and Improvement of Screening, Diagnosis and Treatment of Inherited Disorders of Metabolism (ERNDIM). ERNDIM schemes. Sheffield, UK: ERNDIM, 2012. Available at http://cms.erndimqa.nl/Home.aspx. Accessed February 7, 2011.

European Research Network for Evaluation and Improvement of Screening, Diagnosis and Treatment of Inherited Disorders of Metabolism (ERNDIM). Control materials. Sheffield, UK: ERNDIM, 2012. Available at http://cms.erndimqa.nl/control-materials.aspx. Accessed February 2, 2012.

Reference Institute for Bioanalytics (Referenzinstitut für Bioanalytik). Survey programs 2012. Bonn, Germany: Stiftung für Pathobiochemie und Molekulare Diagnostik; 2012. Available at http://www.dgkl-rfb.de/index_e.shtml. Accessed February 2, 2012.

Clinical Laboratory Improvement Advisory Committee
Biochemical Genetic Testing Good Laboratory Practices Workgroup (2009–2010)

Chairperson: Carol L. Greene, MD, University of Maryland School of Medicine, Baltimore, Maryland.

Members: Bruce Barshop, MD, PhD, University of California — San Diego, Rady Children's Hospital, San Diego, California; Michele Caggana, ScD, New York State Department of Health, Albany, New York; Joel Charrow, MD, Children's Memorial Hospital, Chicago, Illinois; Tina Cowan, PhD, Stanford University Medical Center, Stanford, California; Harry Hannon, PhD, Atlanta, Georgia; Julie Ann Neidich, MD, Quest Diagnostics—Nichols Institute, San Juan Capistrano, California; Stephen Raab, MD, University of Colorado Denver, Aurora, Colorado; David L. Smalley, PhD, Tennessee Department of Health, Nashville, Tennessee; Erin Strovel, PhD, University of Maryland School of Medicine, Baltimore, Maryland; V. Reid Sutton, MD, Baylor College of Medicine, Houston, Texas; Georgirene D. Vladutiu, PhD, The Buffalo General Hospital, Buffalo, New York; Emily S. Winn-Deen, PhD, RxDx Advisors, Livermore, California.

Clinical Laboratory Improvement Advisory Committee (2010–2011)

Chairperson: Elissa Passiment, EdM, American Society for Clinical Laboratory Science, Bethesda, Maryland.

Members: Ellen Baron, PhD, Stanford University Medical Center, Palo Alto, California; Christine Bean, PhD, New Hampshire Department of Health and Human Services, Concord, New Hampshire; Susan Cohen, Bethesda, Maryland; Judy Daly, PhD, Primary Children's Medical Center, Salt Lake City, Utah; Nancy Elder, MD, University of Cincinnati, Cincinnati, Ohio; Merilyn D. Francis, The MITRE Corporation, McLean, Virginia; John Fontanesi, PhD, University of California — San Diego, School of Medicine, San Diego, California; Julie Gayken, HealthPartners and Regions Hospital, Bloomington, Minnesota; Geraldine Hall, PhD, Cleveland Clinic Foundation, Cleveland, Ohio; Carol L. Greene, MD, University of Maryland School of Medicine, Baltimore, Maryland; Norman Harbaugh, Jr., MD, Kids Health First Pediatric Alliance, Atlanta, Georgia; Lee H. Hilborne, MD, David Geffen School of Medicine at University of California — Los Angeles, Los Angeles, California; Paul Kimsey, PhD, California Department of Public Health, Richmond, California; James Nichols, PhD, Baystate Medical Center, Springfield, Massachusetts; Gary Overturf, MD, University of New Mexico School of Medicine, Albuquerque, New Mexico; Stephen Raab, MD, University of Colorado at Denver, Aurora, Colorado; Linda Sandhaus, MD, Case Western Reserve University Hospitals and Medical Center, Cleveland, Ohio; Paula Santrach, MD, Mayo Clinic, Rochester, Minnesota; Jared N. Schwartz, MD, Presbyterian Healthcare, Charlotte, North Carolina; David L. Smalley, PhD, Tennessee Department of Health, Nashville, Tennessee; Gail Vance, MD, Indiana University School of Medicine, Indianapolis, Indiana; Emily Winn-Deen, PhD, Rx Dx Advisors, Livermore, California; Rosemary Zuna, MD, The University of Oklahoma Health Science Center, Oklahoma City, Oklahoma.

Designated Federal Officials: Thomas L. Hearn, PhD, National Center for Emerging and Zoonotic Infectious Diseases, May Chu, PhD, Office of Surveillance, Epidemiology, and Laboratory Services, CDC, Atlanta, Georgia.

Ex-Officio Members: Alberto Gutierrez, PhD, Center for Devices and Radiological Health, Food and Drug Administration, Rockville, Maryland; Judith Yost, MA, Center for Medicaid and State Operations, Centers for Medicare & Medicaid Services, Baltimore, Maryland; Devery Howerton, PhD, Office of Surveillance, Epidemiology, and Laboratory Services, CDC, Atlanta, Georgia.

Liaison Representative: Luann Ochs, MS, Advanced Medical Technology Association, Washington, DC.

The *Morbidity and Mortality Weekly Report (MMWR)* Series is prepared by the Centers for Disease Control and Prevention (CDC) and is available free of charge in electronic format. To receive an electronic copy each week, visit MMWR's free subscription page at *http://www.cdc.gov/mmwr/mmwrsubscribe.html*. Paper copy subscriptions are available through the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402; telephone 202-512-1800.

Address all inquiries about the *MMWR* Series, including material to be considered for publication, to Editor, *MMWR* Series, Mailstop E-90, CDC, 1600 Clifton Rd., N.E., Atlanta, GA 30333 or to *mmwrq@cdc.gov.*

U.S. Government Printing Office: 2012-523-218/73226 Region IV ISSN: 1057-5987