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NBS05

Newborn Screening for Cystic Fibrosis

This guideline describes newborn screening laboratory
tests for detecting analytes and genetic markers associated
with cystic fibrosis (CF). It includes both the first-tier and
second-tier screening tests performed on newborn dried blood
spot specimens, as well as the screening strategies for
identifying newborns at increased risk for developing CF.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

Newborn Screening for Cystic Fibrosis

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Abstract

Clinical and Laboratory Standards Institute guideline NBS05—*Newborn Screening for Cystic Fibrosis* describes newborn screening (NBS) laboratory tests and screening strategies used worldwide to identify newborns at increased risk of developing cystic fibrosis (CF). CF is a common genetic disorder caused by variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Presymptomatic detection through NBS leads to early diagnosis and improves the outcomes of babies with CF. This guideline describes comprehensively the laboratory tests for detecting CF risk among newborns as well as recommendations for follow-up evaluation. It describes the use of immunoreactive trypsinogen assays and second-tier NBS testing, including DNA analysis for detecting specific *CFTR* variants and pancreatitis-associated protein assays. A core panel of *CFTR* variants for routine testing is discussed with guidance included on NBS program considerations for core panel expansion. This guideline is intended for use by NBS laboratory, follow-up, and program personnel; public health program administrators; medical laboratories; CF center personnel and organizations responsible for CF center networks; health care providers (eg, primary care providers, neonatologists, pediatricians, disease specialists); regulatory agencies; public health policy makers; and manufacturers of instruments, reagents, and related products for NBS testing.

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Foreword

Newborn screening (NBS) is a highly effective public health program that saves or improves the lives of thousands of babies every year.[1] NBS programs are organized, population-based public health services applying preventive medicine principles in defined regions to reduce morbidity and mortality from certain congenital disorders. NBS programs are part of NBS systems that include birthing facilities, public health programs, health care providers, and families. NBS's goal is presymptomatic detection of at-risk newborns through screening platforms for newborn dried blood spot (DBS) specimens that are analyzed in specialized NBS laboratories, newborn hearing screening, and cyanotic congenital heart disease screening. NBS programs are linked to medical follow-up programs for diagnosis and rapid initiation of specialized therapies. The organization of NBS programs features a system of care that includes preanalytical, analytical, and postanalytical activities. They include education, collection of DBS specimens, laboratory analysis, result reporting, linkage to clinical care (short-term follow-up), diagnosis, management, programmatic evaluation, evaluation of clinical outcomes (including long-term follow-up), QA, and quality improvement.

Cystic fibrosis (CF) is a common genetic disorder caused by genetic variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. These variants result in defective chloride transport. Affected children develop a life-threatening chronic disease with digestive defects leading to severe malnutrition and respiratory tract abnormalities that are associated with recurrent bronchopulmonary infections and persistent cough. Detecting newborns at risk for CF by identifying increased immunoreactive trypsinogen (IRT) concentrations in DBS specimens (often followed by DNA analysis of variants in the CF gene) provides an opportunity for presymptomatic detection before irreversible pathology develops.

This guideline describes newborn DBS screening algorithms for CF using IRT assays alone or, most commonly, in combination with second-tier DNA testing for detecting specific *CFTR* variants. CF NBS algorithms are among the first NBS models incorporating DNA technologies. Variations in the IRT/DNA method, including the use of pancreatitis-associated protein (PAP) testing after IRT testing, are also summarized with explanations of their advantages and disadvantages.

Despite its widespread use since rapid implementation began in 2005, CF NBS is complicated by several challenges and controversies regarding laboratory science and public health applications that have resulted in NBS programs adopting diverse approaches.[2] Implementing NBS for CF provides new opportunities for enhanced care, education, and research. However, the multitude of methodologies has highlighted the need for quality improvement and QA. For example, a variety of methods are used for reaching decisions regarding IRT concentrations cutoff values for in-range results and out-of-range results. In addition, given that over 2000 *CFTR* variants have been reported, some debate exists about which *CFTR* panels should be used for IRT/DNA NBS strategies. This consensus guideline on CF NBS provides a global resource for NBS programs to evaluate and refine their current procedures and practices for all aspects of the CF NBS system, including the challenging follow-up components of sweat chloride testing and genetic counseling.

Overview of Changes

This guideline replaces the previous edition of the approved guideline, NBS05-A, published in 2011. Several changes were made in this edition, including:

- Reassessed IRT cutoff value guidelines and discussed the use of a floating rather than fixed cutoff value

- Revised recommendations regarding *CFTR* variant panels based on the most current information, including new biotechnologies such as next-generation sequencing

- Assessed using PAP for detecting newborns at risk for CF

- Discussed communication strategies related to detecting CF heterozygote newborns and providing genetic counseling

- Reviewed emerging issues related to using genetic and genomic sequencing in NBS

- Described the existing CF NBS algorithms

NOTE: The content of this guideline is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

Key Words

Cystic fibrosis, cystic fibrosis transmembrane conductance regulator gene, DNA analysis, genetic counseling, immunoreactive trypsinogen, newborn dried blood spot screening, pancreatitis-associated protein, quality assurance, sensitivity, variants

Newborn Screening for Cystic Fibrosis

Chapter 1:   Introduction

This chapter includes:

- Guideline's scope and applicable exclusions

- Standard precautions information

- "Note on Terminology" that highlights particular use and/or variation in use of terms and/or definitions

- Terms and definitions used in the guideline

- Abbreviations and acronyms used in the guideline

1.1   Scope

This guideline specifies recommendations for newborn screening (NBS) for cystic fibrosis (CF) and routine use of dried blood spot (DBS) specimens for identifying potentially affected newborns. This guideline also discusses the preanalytical, analytical, and postanalytical activities of CF NBS, including short-term follow-up (STFU) and long-term follow-up (LTFU) considerations.

This guideline describes:

- Screening methodologies for immunoreactive trypsinogen (IRT), pancreatitis-associated protein (PAP), and cystic fibrosis transmembrane conductance regulator (*CFTR*) gene variant analysis

- Screening algorithms currently used, including the use of IRT assays alone or in combination with DNA analysis for detecting specific *CFTR* variants through second-tier NBS with the IRT/DNA strategy

- Variations in the IRT/DNA strategy, including the use of PAP testing after IRT testing, with explanations of their advantages and disadvantages

- Selecting *CFTR* variant panels that enable equal detection of CF in all populations within the screening jurisdiction

- Reporting results

- Roles and responsibilities during STFU through diagnosis

This guideline recommends the use of *CFTR* variant panels in CF NBS algorithms when resources allow. The intended users of this guideline are NBS laboratory, follow-up, and program personnel; public health program administrators; medical laboratories; CF center personnel and organizations responsible for CF center networks; health care providers (HCPs) (eg, primary care providers, neonatologists, pediatricians); regulatory agencies; public health policy makers; and manufacturers of instruments, reagents, and related products used for NBS testing.

Although the need for confirmatory diagnostic testing is discussed, this guideline describes only some of the issues regarding satisfactory sweat chloride testing. In addition, this guideline:

- Is not intended to provide details of confirmatory diagnostic laboratory testing
- Does not include comparative cost information
- Does not cover CF prenatal carrier screening or prenatal diagnosis

1.2 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to "standard precautions." Standard precautions are guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of all known infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of bloodborne pathogens. Published guidelines are available that discuss the daily operations of diagnostic medicine in humans and animals while encouraging a culture of safety in the laboratory.[3] For specific precautions for preventing the laboratory transmission of all known infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all known infectious diseases, refer to CLSI document M29.[4]

1.3 Terminology

1.3.1 A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization whenever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in different countries and regions and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. CLSI recognizes its important role in these efforts, and its consensus process focuses on harmonization of terms to facilitate the global application of standards and guidelines.

NOTE: Mandates are generally reserved for CLSI standards but are occasionally allowed in CLSI guidelines. In CLSI guidelines, use of the term "must" is either 1) based on a requirement or 2) indicative of a necessary step to ensure patient safety or proper fulfillment of a procedure. The document development committee evaluated use of the term "must" and deemed it appropriate.

CLSI uses the globally applicable terms *preexamination, examination,* and *postexamination* in its documents. However, in the NBS laboratory, DBS specimens are "examined" to ensure they are satisfactory before they are "analyzed." Hence, for the purposes of CLSI NBS documents, the terms *preanalytical, analytical,* and *postanalytical* are used in place of *preexamination, examination,* and *postexamination.* Additionally, the term *analysis* is used in place of *examination.* Although contradictions among these terms may exist between new CLSI NBS documents and already published NBS documents, these contradictions will be reconciled as documents go through the routine revision process.

In CLSI NBS documents, the terms *newborn* and *infant* have distinct meanings. *Newborn* indicates a person from birth to 28 days old, and *infant* indicates a person from 29 days to 1 year old. In situations that could apply to both (or either) age groups, the term *baby* is used.

In this guideline, the terms *variant, genetic variant,* and *pathogenic variant* are used instead of the previously used term, *mutation,* to describe a DNA sequence that varies from a reference DNA sequence in the *CFTR* region. A *genetic variant* may be CF-causing (pathogenic), likely CF-causing (likely pathogen), likely benign or benign (non–CF-causing), or a variant of unknown significance (VUS).[5,6] The

Chapter 3:   Path of Workflow

This chapter includes:

- Introduction to the NBS for CF process
- Process flow chart for CF NBS

All NBS programs follow a process for detecting newborns at increased risk for screened diseases, including CF. The NBS laboratory may be involved in some or all of these activities. An NBS laboratory may develop and control these processes or may participate in organizationally defined processes. The NBS laboratory needs to be knowledgeable of its role in each process so it can meet regulatory, accreditation, and reporting requirements in the most efficient manner possible. Although NBS laboratorians may not collect the DBS specimens, they need to know the standard for acceptable specimens (see CLSI document NBS01[85]). Although NBS laboratorians may not be directly involved in STFU, they should still be responsible for initiating communication to those who have responsibility for STFU procedures.

This guideline follows NBS processes as they logically progress through preanalytical, analytical, and postanalytical processes. Figure 1 shows the sequence of necessary activities and considerations for CF NBS. The process elements are accompanied by the corresponding NBS05 chapters and subchapters that cover these topics.

Chapter 6:   Analytical Activities – Strategy for Screening Methods and Models

This chapter includes:

- CF NBS models
- Selecting an NBS strategy
- Determining the IRT cutoff concentrations
- IRT/DNA model and variations, including extremely high IRT with no variants
- IRT/IRT models
- IRT/PAP/DNA model
- Comparing CF NBS models

6.1   Cystic Fibrosis Newborn Screening Models

CF NBS models are multitier algorithms, all of which begin with IRT measurement and most of which incorporate *CFTR* variant analysis. The most common and recommended strategy involves an initial IRT measurement followed by DNA analysis (IRT/DNA model) for one or more variant *CFTR* alleles on those specimens with high IRT concentrations. Variations of the IRT/DNA strategy have been developed recently that are based on either demonstrating persistent hypertrypsinogenemia before DNA analysis (IRT/IRT/DNA model) or incorporating a sequential interrogation of DNA through various methods of *CFTR* EGA to identify two variant alleles before sweat chloride testing is recommended (IRT/DNA/EGA model). The IRT/IRT and IRT/PAP/DNA models are also discussed, though these strategies are used less frequently, and are not recommended for widespread use. Other approaches have been described in the literature.[60,130,131]

In all CF NBS models, newborns with CF screen-positive results are referred for diagnostic evaluation including sweat chloride testing. Ideally, newborns should be seen by medical experts as soon as possible after receiving the CF screen-positive results, and the CF diagnosis should be made before 4 weeks of age.[44] In some cases, the diagnosis may follow the initiation of intervention in babies with a presumptive positive diagnosis so necessary interventions are not delayed.

NBS programs may receive information from families or clinicians identifying newborns with older siblings known to have CF. Programs may perform *CFTR* testing on the DBS of the newborn sibling regardless of the IRT results.

6.2   Selecting a Newborn Screening Strategy

Initial selection of an NBS model, the IRT cutoff concentration, and the *CFTR* variant panel depend on predetermined thresholds for sensitivity and an evaluation of local population variation in both IRT concentration and *CFTR* gene variants in the NBS program's jurisdiction. Cutoff values chosen for IRT concentrations should fall between the 95th and 99th percentiles to achieve appropriate sensitivity, with a multivariant panel that offers sufficient coverage of the population screened. The various models used by NBS programs are discussed in Subchapters 6.4 to 6.6, and Table 1 compares their sensitivity, PPV, and other performance statistics.

6.3   Determining the Immunoreactive Trypsinogen Cutoff Concentration

6.3.1   Setting an Initial Immunoreactive Trypsinogen Cutoff Concentration

Most programs choose an IRT cutoff concentration that represents the 95th to 99th percentile of all specimens tested. NBS programs should periodically evaluate the numbers of false-positive and false-

Related CLSI Reference Materials[*]

C34   **Sweat Testing: Specimen Collection and Quantitative Chloride Analysis. 4th ed., 2019.** This guideline describes methods for all aspects of sweat testing, including collection and analysis, results evaluation and reporting, and quality control.

M29   **Protection of Laboratory Workers From Occupationally Acquired Infections. 4th ed., 2014.** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

MM01   **Molecular Methods for Clinical Genetics and Oncology Testing. 3rd ed., 2012.** This document provides guidance for the use of molecular biological techniques for detection of mutations associated with inherited medical disorders, somatic or acquired diseases with genetic associations, and pharmacogenetic response.

MM17   **Verification and Validation of Multiplex Nucleic Acid Assays. 2nd ed., 2018.** This guideline includes recommendations for analytical validation and verification of multiplex assays, as well as a review of different types of biological and synthetic reference materials.

MM19   **Establishing Molecular Testing in Clinical Laboratory Environments. 1st ed., 2011.** This guideline provides comprehensive guidance for planning and implementation of molecular diagnostic testing, including strategic planning, regulatory requirements, implementation, quality management, and special considerations for the subspecialties of molecular genetics, infectious diseases, oncology, and pharmacogenetics.

NBS01   **Blood Collection on Filter Paper for Newborn Screening Programs. 6th ed., 2013.** This document highlights specimen collection methods, discusses acceptable techniques for applying blood drops or aliquots to the filter paper segment of the specimen collection device, and provides instructions on proper specimen handling and transport to ensure quality specimens are consistently obtained for newborn screening analysis.

NBS02   **Newborn Screening Follow-up. 2nd ed., 2013.** This guideline describes the basic principles, scope, and range of follow-up activities within the newborn screening system.
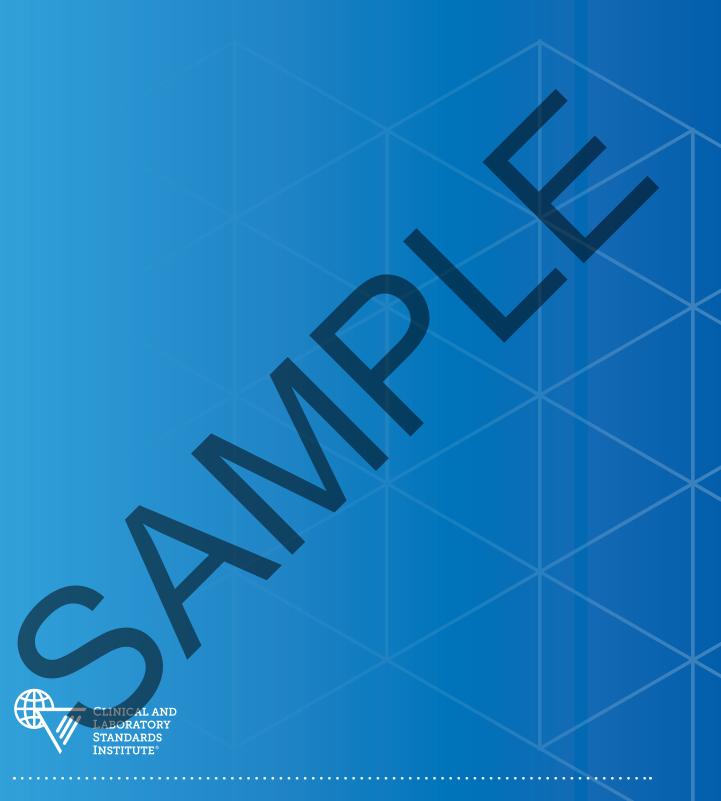
NBS03   **Newborn Screening for Preterm, Low Birth Weight, and Sick Newborns. 2nd ed., 2019.** This guideline describes the recommended protocols for screening preterm, low birth weight, and sick newborns for hearing loss, critical congenital heart defects, and diseases detectable through newborn dried blood spot screening.

QMS01   **A Quality Management System Model for Laboratory Services. 5th ed., 2019.** This guideline provides a model for medical laboratories to organize the implementation and maintenance of an effective quality management system.

QMS06   **Quality Management System: Continual Improvement. 3rd ed., 2011.** This guideline considers continual improvement as an ongoing, systematic effort that is an essential component of a quality management system. A continual improvement program may consist of fundamental processes and common supporting elements described in this guideline.

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[*] CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.

SAMPLE

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