CLINICAL AND LABORATORY STANDARDS INSTITUTE®

1st Edition

NBS08

Newborn Screening for Hemoglobinopathies

This guideline describes the recommended protocols for detecting hemoglobinopathies and thalassemias by population-based newborn screening using dried blood spot specimens. Early, presymptomatic detection to identify newborns with abnormal hemoglobins is critical because it improves treatment effectiveness.

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

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Newborn Screening for Hemoglobinopathies

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Abstract

Clinical and Laboratory Standards Institute guideline NBS08—*Newborn Screening for Hemoglobinopathies* describes the newborn screening (NBS) processes for testing dried blood spot specimens to detect hemoglobinopathies and thalassemias not usually evident at birth. Hemoglobinopathies and thalassemias are clinically significant congenital red blood cell disorders caused by structural or other hemoglobin abnormalities, resulting in various clinical manifestations. Early detection is critical, because without treatment, these conditions lead to increased morbidity and mortality. This guideline discusses various NBS approaches, including equipment considerations, laboratory screening methodologies, short-term and long-term follow-up processes, and future screening possibilities.

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Foreword

Since the implementation of universal newborn dried blood spot (DBS) screening, mortality in children with sickle cell disease (SCD) aged 1 to 4 years has decreased by 50%.[1-5] This dramatic decline in mortality is believed to result from multiple interventions, including early initiation of penicillin prophylaxis.[6] Timely interventions are made possible by early diagnosis, which results from newborn DBS screening coupled with comprehensive follow-up diagnostic testing.

Sickling disorders and thalassemias are among the most prevalent monogenetic diseases worldwide. The screening techniques currently used are primarily based on US experiences and have the longest history and most comprehensive use. The significant effects of hemoglobinopathies on US health care over time, particularly sickle cell anemia and other forms of SCD, can be measured by the availability of various SCD-related federal and state public health programs and funding streams.[7] Partially because of these programs, universal newborn DBS screening for hemoglobinopathies is now required in all 50 states, the District of Columbia, and many US territories. As of 2016, full-population hemoglobinopathy DBS screening also exists in eight Canadian provinces, seven European countries, and three Latin American countries. Additionally, pilot or targeted hemoglobinopathy screening exists in several other European, Latin American, Asian, Middle Eastern, and African countries.[8] Although newborn DBS screening has primarily focused on sickle hemoglobinopathies, there is increasing interest in newborn DBS screening for thalassemias, particularly in the Middle East and parts of Europe and Asia.

Various approaches to newborn DBS screening for hemoglobinopathies are used internationally. Variability exists not only in newborn screening (NBS) laboratory procedures but also in screening algorithms, results reporting, and patient follow-up. This guideline has been developed recognizing that although hemoglobinopathy NBS is expanding worldwide, no other informational, harmonizing resource is currently available. The comprehensive training and methodological guidance originally developed in the 1970s and 1980s are no longer readily available. Additionally, available hemoglobinopathy screening manuals are outdated.[9-11] Although some regulatory and quality improvement assistance is available to laboratories in the United States[12] and Europe,[13] it was not designed to harmonize the NBS program differences that currently exist. Guidance is needed to provide an accessible reference for basic newborn DBS screening and to assist NBS programs and medical professionals worldwide who may be initiating, expanding, or harmonizing newborn DBS screening for hemoglobinopathies.

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

Hemoglobinopathy, newborn screening, sickle cell anemia, sickle cell disease, thalassemia

Newborn Screening for Hemoglobinopathies

Chapter 1: Introduction

This chapter includes:

- Guideline's scope and applicable exclusions
- Background information pertinent to the guideline's content
- 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

Hemoglobinopathies are a group of inherited blood disorders characterized by the presence of structural hemoglobin variants or quantitative differences in globin chain production. Results reporting, particularly for carriers, may sometimes be complicated by regulatory and ethical issues. Although many newborn screening (NBS) programs primarily focus on detecting and reporting sickle cell disease (SCD), they may also detect and/or report the presumptive presence of other clinically significant hemoglobin disorders, including both α and β thalassemias, as well as heterozygotes (carriers).

1.1 Scope

This guideline focuses on the NBS laboratory analytical processes for detecting SCD and other clinically significant hemoglobin disorders, including basic information about the biological and clinical features of clinically significant hemoglobinopathies detectable through NBS. It also provides information on preanalytical considerations affecting laboratory detection of hemoglobinopathies in NBS, including dried blood spot (DBS) specimen stability considerations. Various NBS procedures for hemoglobinopathy detection are discussed, with details of the methods included in the appendixes following a general template to allow easier comparisons between the different screening technologies. Terminology and reporting recommendations are included, along with other postanalytical NBS activities, including both short-term follow-up (STFU) activities (tracking and confirmatory testing) and long-term follow-up (LTFU) activities (outcome indicators, registries, care coordination, and access to services).

The guideline's overall purpose is to provide sufficient information for worldwide quality NBS process implementation, evaluation, and harmonization. This guideline may also inform policymaking for ensuring quality NBS results. Intended users of this guideline include:

- NBS laboratory and associated follow-up personnel
- Hospital personnel managing newborn DBS specimen collection activities, including:
  - Newborn DBS specimen collection supplies management
  - Newborn DBS specimen collection and transmittal process
  - NBS patient follow-up
  - NBS recordkeeping
- Medical personnel advising NBS programs and caring for affected newborns

- Manufacturers of laboratory kits and other products intended to be a part of the hemoglobinopathy NBS process

This guideline is not intended to provide:

- Details of confirmatory diagnostic laboratory testing processes
- Information about inappropriate NBS methods, such as solubility testing
- Models or recommendations for parent or provider education or for genetic counseling

1.2 Background

Although the first report of an individual with a sickling hemoglobin was published in 1910,[14] the first NBS programs for sickle cell anemia screening did not begin in the United States until 1975.[15] Expansion of NBS programs to require universal rather than targeted screening for hemoglobin disorders did not progress quickly. Although recommendations for universal NBS were published in 1987,[16] it took nearly 20 years for all US NBS programs to adopt universal hemoglobinopathy screening as part of their required screening panels.[4] As of January 2019, hemoglobinopathies associated with a hemoglobin S (Hb S) allele are included as three of the 35 primary conditions on the US Recommended Uniform Screening Panel (RUSP),[17] with all other hemoglobinopathies together counting as one of the 26 recommended secondary conditions.[18] Because there is insufficient evidence to meet primary condition selection criteria, Hb H disease, an α thalassemia more commonly seen in Southeast Asian, Middle Eastern, and Mediterranean populations, is included as a secondary condition, although it has been considered for inclusion as an RUSP primary condition.

With increasing population migration from high-prevalence regions, hemoglobinopathy NBS is becoming more common worldwide.[8] Disorders included on screening panels are generally based on the distribution and frequency of various hemoglobinopathies within the screened population. As examples, Asian and Mediterranean NBS programs focus on thalassemia screening, whereas NBS programs in sub-Saharan Africa focus on SCD screening. For hemoglobinopathies, universal NBS is recommended. Targeted NBS based on race, ethnicity, or country of origin is **not** recommended because of the possibility of missing cases due to genetic heterogeneity in mixed populations.

The original NBS methodology for hemoglobinopathies was electrophoresis on cellulose acetate membranes, using basic pH buffers.[19] Because this procedure did not sufficiently separate sickle hemoglobin (Hb S) from some nonsickling hemoglobins, a positive cellulose acetate screen was followed by a second electrophoresis using citrate agar and acidic buffers for any specimen exhibiting unusual or abnormal results. Using this two-tiered approach, results were interpreted together for a final screening result. However, electrophoretic test results from newborn DBS specimens collected in a filter paper matrix were difficult to interpret on specimens that were a few days old because of oxidative degradation. Over time, more sensitive and specific hemoglobinopathy laboratory procedures have been adapted for testing newborn DBS specimens.

Current methodologies for both first-tier and second-tier screening tests include isoelectric focusing (IEF),[20] high-performance liquid chromatography (HPLC),[21] and capillary electrophoresis (CE), in various combinations. Less common screening methodologies, which continue to be refined for NBS, include tandem mass spectrometry (MS/MS) and various molecular methods. Brief background discussions of each of these techniques are included in this guideline, with more details provided in the appendixes. In addition, more-extensive procedural details not commonly available for cellulose acetate and citrate agar electrophoresis are provided to assist resource-limited programs seeking a less expensive screening alternative. In the current screening environment, particularly in developed economies, commercial kit manufacturers provide the majority of newborn DBS screening quality control materials. External quality assessment through proficiency testing is available through organizations in both the United States[12] and Europe.[13]

specimen – discrete portion of a body fluid, breath, hair, or tissue taken for examination, study, or analysis of one or more quantities or properties assumed to apply for the whole[24]; **NOTE:** For newborn screening, the dried blood spot specimen is collected.

thalassemia – a condition resulting from reduced or absent synthesis of one or more of the globin chains or occurring when a structurally abnormal hemoglobin is synthesized at a reduced rate (eg, hemoglobin E and hemoglobin Constant Spring).

1.4.3 Abbreviations and Acronyms

ACS	acute chest syndrome
CE	capillary electrophoresis
DBS	dried blood spot
ddNTP	dideoxynucleoside triphosphate
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
ESI	electrospray ionization
FAS	hemoglobin FAS
FSA	hemoglobin FSA
Hb	hemoglobin[a]
Hb F	fetal hemoglobin
HPFH	hereditary persistence of fetal hemoglobin
HPLC	high-performance liquid chromatography
IEF	isoelectric focusing
LTFU	long-term follow-up
MCH	mean corpuscular hemoglobin
MCV	mean corpuscular volume
mRNA	messenger ribonucleic acid
MS/MS	tandem mass spectrometry
NBS	newborn screening
NGS	next-generation sequencing
PCR	polymerase chain reaction
PCR-RFLP	polymerase chain reaction–restriction fragment length polymorphism
pH	negative logarithm of hydrogen ion concentration
pI	isoelectric point
POC	point-of-care
QA	quality assurance
QI	quality improvement
RBC	red blood cell
RNA	ribonucleic acid
RUSP	US Recommended Uniform Screening Panel
SCD	sickle cell disease
STFU	short-term follow-up
VOC	vaso-occlusive crisis
WBC	white blood cell

[a] The term "hemoglobin" is abbreviated in this guideline only when it precedes a specific hemoglobin variant. Otherwise, it is written out in the text.

Chapter 2: Hemoglobinopathy Nomenclature, Pathophysiology, and Clinical Manifestations

This chapter includes:

- Nomenclature and hemoglobin development
- Basic pathophysiology and clinical manifestations of SCD
- Potential complications associated with sickle cell trait
- Basic pathophysiology and clinical manifestations of thalassemias

Hemoglobinopathies are clinically heterogeneous and result from mutations or deletions in the α and β globin genes. The clinically significant hemoglobin disorders that result from these mutations are broadly classified into two genetically distinct groups: SCD and thalassemias. SCD is caused by a structurally abnormal hemoglobin, Hb S, inherited in the homozygous state or in combination with either another variant (eg, Hb C, Hb D, Hb E, Hb O-Arab) or a β thalassemia mutation. Thalassemias are caused by mutations or deletions that result in reduced or absent synthesis of α or β globin chains. A thalassemia phenotype may also occur when structurally abnormal hemoglobin is synthesized at a reduced rate (eg, Hb E) or as a result of an unstable hemoglobin (eg, Hb Constant Spring).

2.1 Hemoglobin Expression During Development

The oxygen-carrying capacity of the RBCs relies on hemoglobin, a tetramer with four heme molecules and four globin chains. There are four major types of globins: α, β, γ, and δ. Normal adult hemoglobin is composed of two α globin and two β globin chains, each of which has an iron-containing heme ring. The composition of the non-α chains determines the hemoglobin type: Hb F has two γ globin chains ($\alpha_2\ \gamma_2$), Hb A has two β chains ($\alpha_2\ \beta_2$), and Hb A2 has two δ chains ($\alpha_2\ \delta_2$). Before birth, the β globin gene is silent, and the α and γ globin genes are expressed to form Hb F. The switch from Hb F to Hb A is tightly regulated during fetal development, such that Hb F accounts for approximately 80% and Hb A accounts for 20% of total hemoglobin in the newborn. Hb F declines sharply as γ globin is replaced by β globin (see Figure 1). By approximately 6 months of age, healthy infants produce mostly Hb A, a small amount of Hb A2, and a negligible amount of Hb F.

Newborns with β globin gene mutations causing SCD or β thalassemia produce Hb F and have normal hemoglobin levels until 3 to 4 months of age, when Hb F concentration declines in response to the developmental switch from γ gene expression to β gene expression. Instead of normal Hb A, Hb S is produced in SCD. There is absent or reduced production of Hb A in β thalassemia. As Hb F is replaced by dysfunctional hemoglobin (in SCD) or by insufficient amounts of Hb A (β thalassemia), affected infants gradually develop clinically significant anemia. Infants with SCD exhibit sickle cells in the peripheral blood and are at increased risk for developing life-threatening complications. In infants with β^0 thalassemia, ineffective erythropoiesis and severe anemia necessitate the initiation of regular transfusions as early as 6 months of age. The Hb F to Hb S switch in infants with SCD occurs more gradually than the Hb F to Hb A switch in unaffected infants.[25] Hb F concentrations in toddlers with SCD stabilize by age 3 or 4 years and are generally constant throughout childhood. Ranging from 3% to 20%, the Hb F concentration averages approximately 10% of total hemoglobin in children with SCD,[26] compared with an average of 0.5% to 2% in unaffected individuals.

Chapter 3: Overview of Newborn Screening Programs for Hemoglobinopathies

This chapter includes:

- NBS program components
- Path of workflow for hemoglobinopathy screening

NBS programs are organized, population-based public health services that apply preventive medicine principles to reduce morbidity and mortality from certain congenital disorders. They are part of an NBS system composed of six parts: education, screening, STFU, diagnosis, management, and evaluation (LTFU).[105] NBS promotes presymptomatic risk detection by testing newborn DBS specimens in centralized NBS laboratories linked to specialty clinical follow-up programs for diagnosis and rapid initiation of specialized therapies.

Hemoglobin disorder screening usually occurs in conjunction with screening for other inherited disorders using a portion of the DBS specimen collected for NBS. A description of the preferred specimen collection, packaging, and transportation process, technical aspects of the specimen collection device, and appropriate patient demographic data to be submitted are detailed in CLSI document NBS01.[106] Specimens collected following transfusion are generally unacceptable for hemoglobinopathy NBS. More detailed guidance regarding specimens obtained from preterm and transfused newborns can be found in CLSI document NBS03.[107]

This guideline describes NBS preanalytical, analytical, and postanalytical processes, with thorough consideration of factors relevant to hemoglobinopathy NBS. The path of workflow for hemoglobinopathy NBS is shown in Figure 2.

Related CLSI Reference Materials*

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.

C62 **Liquid Chromatography-Mass Spectrometry Methods. 1st ed., 2014.** This document provides guidance to the clinical laboratorian for the reduction of interlaboratory variance and the evaluation of interferences, assay performance, and other pertinent characteristics of clinical assays. This guideline emphasizes particular areas related to assay development and presents a standardized approach for method verification that is specific to mass spectrometry technology.

MM09 **Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine. 2nd ed., 2014.** This document addresses diagnostic sequencing using both automated capillary-based sequencers and massively parallel sequencing instruments. Topics include specimen collection and handling; isolation and extraction of nucleic acid; template preparation; sequence generation, alignment, and assembly; validation and verification; ongoing quality assurance; and reporting results.

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.

NBS04 **Newborn Screening by Tandem Mass Spectrometry. 2nd ed., 2017.** This guideline serves as a reference for the multiple activities related to operating a tandem mass spectrometry laboratory as part of public and private newborn screening programs.

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

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