CLINICAL AND LABORATORY STANDARDS INSTITUTE®

7th Edition

NBS01

Dried Blood Spot Specimen Collection for Newborn Screening

This standard highlights specimen collection methods, discusses acceptable techniques for applying blood drops or aliquots to the filter paper section of the specimen collection device, and provides instructions on proper specimen drying, handling, and transport to ensure quality specimens are consistently obtained for newborn screening analysis.

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

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Dried Blood Spot Specimen Collection for Newborn Screening

Víctor R. De Jesús, PhD
Gustavo JC Borrajo, PhD
Joanne Mei, PhD
Will Adams
Kerry Chunko, BS
Saswati Das, MD, MBBS
M. Christine Dorley, MS, MT(ASCP)
Donald Dunbar, PBT(ASCP)
Houria El Mouden, MS
Roanna George, BSc, MSc, FRCPath
Patrice Karyn Held, PhD
Kellie B. Kelm, PhD
Jennifer O. Macdonald, MPH, BSN, RN
Christine McRoberts, MLT
Kimberly Noble Piper, BS, CPH, CPHG, RN
Joseph M. Quashnock, PhD, HCLD/CC(ABB), FAACC
Jeanette Schier-Pugsley, BS, MBA, RAC
Bradford L. Therrell, Jr., MS, PhD
Kim Turner, MSQA, MT(ASCP), CQA(ASQ)
Joesph Wiencek, PhD
Raquel Yahyaoui, MD, PhD

Abstract

Clinical and Laboratory Standards Institute standard NBS01—*Dried Blood Spot Specimen Collection for Newborn Screening* provides descriptive, stepwise processes and procedures for collecting blood spot specimens for newborn screening. This standard was developed and continues to be updated to promote uniform practices for demographic newborn data and specimen collection. This standard informs and instructs health care professionals who collect and submit dried blood spot specimens on procedures for skin puncture and pain management; application of blood collected by heelstick directly onto the preprinted circles of the filter paper; blood source recommendations and other blood spot specimen collection techniques; filter paper, handling, and mailing package specifications; specimen collection device specifications; handling blood spots collected on filter paper for DNA and/or RNA analysis; and storage and retention of residual specimens. An expanded appendix section provides additional images and descriptions of unacceptable blood spot specimens.

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Foreword

Since 1982, CLSI has acknowledged the need to provide and update instructions to health care professionals who collect and submit dried blood spot (DBS) specimens for newborn screening (NBS), manufacturers who develop testing methodologies and equipment, and NBS programs that perform testing. This standard is written with primary emphasis on the specimen collection device and specimen collection. Specimens for NBS may be collected by hospital personnel, midwives, or other health care workers during the first few days of the newborn's life. This standard informs and instructs personnel on the essentials of correctly collecting a high-quality specimen, handling it after it has been collected, transporting it to the testing facility, and storing the residual specimen that remains after laboratory testing. Additionally, this standard is applicable to other testing procedures for which blood collected on filter paper is used as a specimen source (eg, fingerstick collections on filter paper to test for specific antibodies and DNA and/or RNA testing). CLSI documents GP42[1] and MM13[2] are essential reference documents to use alongside this standard.

In this edition of NBS01, several chapters and appendixes were revised, as outlined in the Overview of Changes. One of the main updates is the reformatting of the standard to follow a sequential process. An overview of this process is provided in the newly added flow chart (see Figure 1 in Chapter 2). Substantial efforts were made by the document development committee and its contributors, international experts representing many countries (ie, Argentina, Canada, India, Spain, United Kingdom, and United States), to better serve the global community and harmonize the collection and application of high-quality DBS specimens for NBS worldwide.

Overview of Changes

This standard replaces the previous edition of the approved standard, NBS01-A6, published in 2013. Several changes were made in this edition, including:

- Revising title
- Introducing easy-to-follow step-action tables that include comments for each action (as applicable), consistent with CLSI's goal to make standards and guidelines more user friendly
- Adding definitions for check sum character, collection device//specimen collection device (for newborn screening), dried blood spot, expected range, false-negative screening result, false-positive screening result, incision, in-range result, medical device, newborn screening program, newborn screening system, out-of-range result, puncture, re-collection, and specimen acceptability
- Adding information clarifying use of the term "filter paper" as the blood collection (specified filter paper) section of the specimen collection device, which also has a section for recording demographic and other requested information covered in Subchapters 1.1, 3.1, and 3.1.1 and in the Chapter 3 introduction
- Updating filter paper considerations (see Subchapters 3.2 and 3.2.1) and shelf life (see Subchapter 3.4.4)
- Adding information on identifying the patient (see Subchapter 4.3) and verifying patient and specimen identification at the time of collection (see Subchapter 4.5)
- Adding information and references for acceptable blood specimen sources and collection sites (see Subchapter 4.6), including new Figures 2 and 3 and optimal capillary puncture depth (see Subchapter 4.6.1)
- Adding discussion on preventing specimen contamination and preanalytical DNA analysis considerations (see Subchapter 5.1)

- Adding information regarding proper documentation, which includes using a procedures manual, confirming patient identification, documenting consent or refusal, and confirming the demographic data and the expiration date on the collection device (see Subchapter 5.2.1.1)
- Adding an alternative for warming the newborn's heel (see Subchapter 5.2.2.1)
- Clarifying the benefits of single-use permanently retractable puncture devices for both the newborn and worker safety (see Subchapter 5.2.3)
- Adding comment on making a single puncture that the specimen collector must not perform immediate repeat puncture at the same site (see Subchapter 5.2.3, step-action table step 8)
- Adding instructions on postcollection care (see Subchapter 5.2.4, step-action table step 10, and Subchapter 5.3.3, step-action table step 9)
- Adding information about anticoagulants to heelstick with capillary tube collection and application (see Subchapter 5.3)
- Updating information on DBS specimen acceptability and added an image of a good-quality DBS specimen in the new Figure 4 (see Subchapter 5.7.3.1)
- Indicating that records should include documentation showing delivery date and time, as well as the individual who received the specimen (see Chapter 6 introduction)
- Revising Appendix A to maintain consistency with the updated collection method instructions, which are covered in Chapter 5, and:
  - Adding preliminary precaution steps (see Appendix A, section A1) before site preparation
  - Dividing former sampling techniques section into three sections:
    - Site preparation (see Appendix A, section A2)
    - Puncture (see Appendix A, section A3)
    - Direct application, collection, and preparation for transport (see Appendix A, section A4)
  - Revising former section on pitfalls to precautions (see Appendix A, section A5)
    - Revised bullets to provide more concise instructions
- Revising title of Appendix B and replacing previously used figures with new images of individual DBS, each with an explanation of the specimen quality issue shown
- Updating Appendix C per its source document and clarifying the scope and purpose of this protocol for testing the absorption characteristics of filter paper
- Adding interferences affecting NBS results to Table D1 in Appendix D:
  - Removing hypothyroidism treated with carbamazepine from the list of maternal conditions
  - Adding the following maternal conditions:
    - Intravenous iodinated contrast administration
    - Glutaric aciduria type I
    - Medium-chain acyl-CoA dehydrogenase deficiency

  - Adding the following newborn conditions:
    - Biliary atresia, septicemia, and trisomies
    - Hemolytic anemia
    - Jaundice
    - Meconimum ileus
    - Renal insufficiency
    - Very low birth weight
  - Adding the following treatments used in special care baby units/neonatal intensive care units:
    - Cefotaxime
    - Selected antiseizure medications
- Moving filter paper specifications formerly in Appendix E to Chapter 3, Specimen Collection Device Considerations (see Subchapter 3.1)
- Updating Appendix F (formerly Appendix G):
  - Added "Repeat analysis to validate previous NBS results" to list of possible uses for residual DBS specimens that need prioritization

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

KEY WORDS

Biobank
Blood collection
DNA analysis
Dried blood spots
Filter paper
Heelstick puncture
Newborn screening
Puncture device
Specimen collection device

Chapter 1

Introduction

This chapter includes:

- Standard's scope and applicable exclusions
- Background information pertinent to the standard's content
- Standard precautions information
- Terminology information, including:
  - Terms and definitions used in the standard
  - Abbreviations and acronyms used in the standard

Dried Blood Spot Specimen Collection for Newborn Screening

1 Introduction

1.1 Scope

This standard describes the collection of blood specimens for newborn screening (NBS) programs, including equipment, sources of blood, collection sites, and techniques for blood spot specimen collection.[3] This standard is intended to improve and ensure the quality of dried blood spot (DBS) specimens collected from newborns to rapidly identify newborns at risk for some congenital disorders (eg, inborn errors of metabolism, endocrinopathies, immunodeficiency disorders).[3,4]

NBS01 includes detailed instructions for the preferred method of blood collection (heelstick with direct application) and also provides easy-to-follow illustrated instructions (see Appendix A) for the steps to be taken by personnel who work at facilities other than the NBS laboratory to ensure acceptable-quality blood spot specimens are collected and to prevent the need for specimen re-collection. Poor-quality specimens (shown in Appendix B) may result in false-positive or false-negative NBS test results and/or the inability to screen the baby. The need for re-collection results in additional follow-up, which if not completed in a timely manner, could result in a missed or delayed diagnosis, unnecessary trauma to the newborn, and anxiety to the parents or guardians.

In all NBS programs, results turnaround time is critical if treatments to alter the adverse consequences of a screened disease (eg, irreversible brain damage or death) are to commence in a timely manner. The specimen criteria and handling procedures describe common variances among different sources of NBS specimens that might be tested and the influence of specimen quality on NBS test results and their interpretation.[5]

In addition, this standard specifies:

- Minimum necessary information to provide on or with the specimen collection device
- Requirements for the two collection device components:
  - A section for recording demographic and other requested information
  - A blood collection (specified filter paper) section for applying and drying the blood drops
- Minimum requirements for the filter paper matrix, including the protocol for testing the absorption characteristics of filter paper (see Appendix C) on which the blood spots are collected
- Acceptable alternative methods and instructions for collecting blood specimens for NBS testing from the following sources:
  - Venous blood
  - Umbilical catheter blood
  - Femoral catheter blood
- Requirements for handling, shipping, retention, and storage conditions for DBS specimens

Intended users of this standard include:

- Hospital personnel, midwives, or other health care workers who collect DBS specimens during the first few days of a newborn's life
- NBS laboratory personnel who perform testing
- NBS follow-up personnel

3.2.1.2 Submitter and Newborn Screening Program Information

The minimum necessary submitter and NBS program information provided with each newborn DBS specimen should include:

- Submitter's identification and address or submitter code, if linked to address
  - The submitter is intended to be the facility, clinic, birthing center, or individual who is sending the specimen for analysis. The submitter is also a contact person when an additional specimen is required (eg, due to insufficient specimen quantity).
  - Optional: birth facility name and address
- Health care provider's name (or primary clinic name) and telephone number
  - The health care provider is intended to be the individual who will provide medical care for the newborn. The health care provider also serves as a contact when an additional specimen is required (eg, due to retesting or follow-up) and the newborn is no longer accessible to the submitter.
- NBS program name and laboratory address

3.2.1.3 Specimen Number and Filter Paper Information

The minimum necessary specimen collection device information provided with each newborn DBS specimen should include:

- Unique nonrepeating serial number on each page of the specimen collection device (see Subchapter 3.2.3)
- Specimen collection device expiration date
- Appropriate number of preprinted circles, with preprinted broken- or dotted-line circles on one side of the filter paper section (with optional printing of circles on both sides) if printed circles are required by the NBS program
  - Local regulations and institutional policies should be consulted for circle diameter size.
  - For example, a preprinted 12- to 13-mm internal diameter circle is filled to the printed line by 75 µL of blood, whereas 100 µL fills slightly beyond the print.
- The manufacturer, filter paper grade (eg, 903 or 226), and lot number, all of which must be indicated on the filter paper section and the information section of the specimen collection device
  - Only filter paper grades that meet specifications described in Table 2 should be used for newborn DBS specimen collection.
  - Optional: Bar codes may be imprinted on the specimen collection device (see Subchapter 3.2.3).
    - Bar codes may contain a check sum character to validate proper entry of its unique nonrepeating serial number into the computer system.
    - The preferred symbology Code 128 should be used because of its accuracy and brevity; other codes (eg, Code 39, Codabar, NW7, Interleaved 2 of 5) should not be used (see CLSI document AUTO02[28]).

3.2.2 Optional Preprinted Information Fields

Additional optional information may be collected per NBS program preference and based on local regulations and institutional policies. This information may also be captured and transmitted electronically if appropriately

References

1 CLSI. *Collection of Capillary Blood Specimens.* 7th ed. CLSI standard GP42. Clinical and Laboratory Standards Institute; 2020.

2 CLSI. *Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods.* 2nd ed. CLSI guideline MM13. Clinical and Laboratory Standards Institute; 2020.

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Related CLSI Reference Materials[a]

AUTO02 **Laboratory Automation: Bar Codes for Specimen Container Identification. 2nd ed., 2005.** This document provides specifications for use of linear bar codes on specimen container tubes in the clinical laboratory and for use on laboratory automation systems.

GP33 **Accuracy in Patient and Specimen Identification. 2nd ed., 2019.** This standard specifies the processes required to ensure accurate patient and specimen identification in manual and electronic systems across the health care organization. Processes include system design considerations, differences in requirements for patients with or without identification bands, and provisions for patients with communication barriers.

GP41 **Collection of Diagnostic Venous Blood Specimens. 7th ed., 2017.** This standard provides procedures for the collection of diagnostic venous blood specimens, including line draws, blood culture collection, and venipuncture in children.

GP42 **Collection of Capillary Blood Specimens. 7th ed., 2020.** This standard provides procedures for collection of capillary blood specimens. Specifications for collection sites, puncture depth, and disposable devices used to collect, process, and transfer diagnostic capillary blood specimens are also included.

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.

MM13 **Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods. 2nd ed., 2020.** This guideline provides recommendations on proper and safe biological specimen collection and nucleic acid isolation and purification. Topics include collection methods, recommended transport and storage conditions, and available nucleic acid isolation and purification technologies for each specimen and nucleic acid type.

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.

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

Related CLSI Reference Materials (Continued)

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 dried blood spot screening.

NBS06 **Newborn Blood Spot Screening for Severe Combined Immunodeficiency by Measurement of T-cell Receptor Excision Circles. 1st ed., 2013.** This document addresses the detection of severe combined immunodeficiency (SCID) by population-based newborn screening using dried blood spot specimens to measure T-cell receptor excision circles. SCID is a lethal disorder of infancy that is not evident at birth, and effective treatment requires presymptomatic detection.

NBS07 **Newborn Blood Spot Screening for Pompe Disease by Lysosomal Acid α-Glucosidase Activity Assays. 1st ed., 2017.** This report discusses the detection of Pompe disease (PD) by population-based newborn screening using dried blood spot specimens to measure acid α-glucosidase enzyme activity. Classic infantile-onset PD is a lethal disorder that is not evident at birth, and therapy effectiveness is improved by presymptomatic detection.

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