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.

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Blood Collection on Filter Paper for Newborn Screening Programs; Approved Standard—Sixth Edition

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Abstract

Clinical and Laboratory Standards Institute document NBS01-A6—Blood Collection on Filter Paper for Newborn Screening Programs; Approved Standard—Sixth Edition addresses the issues associated with specimen collection, the filter paper segment of the specimen collection device (card), and the transfer of blood onto filter paper. The purpose of these considerations is to produce a practical standard that will result in uniform techniques for collecting the best possible specimen for use in newborn screening programs. Issues addressed in the standard include: (1) procedures for skin puncture and pain management; (2) procedures for applying blood collected by heelstick directly onto the preprinted circles of the filter paper; (3) recommendations on the source of blood; (4) other techniques for collection of blood spot specimens; (5) specifications for the filter paper, handling, and the mailing package; (6) specifications for the specimen collection device (card); (7) the handling of blood spots collected on filter paper for DNA/RNA analysis; and (8) the storage and retention of residual 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 specimens for newborn screening (NBS), the manufacturers who develop testing methodologies, and the NBS programs that perform testing. This standard is written with primary emphasis on specimen collection and the specimen collection device (card). 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. Furthermore, the 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 for DNA/RNA testing). See CLSI documents GP42 and MM13, which are essential reference documents to use alongside this standard.

This edition of NBS01 replaces the fifth edition of the approved standard, LA04-A5, which was published in 2007. Substantial efforts were made by the document development committee and its contributors to ensure global applicability of this standard by including a wide array of international experts representing many countries around the world (ie, Argentina, Canada, Denmark, Finland, Korea, Norway, Philippines, Spain, Trinidad and Tobago, the United Kingdom, and the United States). The committee deliberations produced a revised standard that will better serve the global community and harmonize the collection and application of high-quality blood spot specimens for NBS worldwide.

Issues addressed in this revised edition of the standard include:

- Procedures for applying blood that was collected by different techniques in the preprinted circles (targets) on the specimen collection device (card) (see Section 5)
- Collection of blood with transfer devices (see Section 5.2)
- The analysis of poor-quality and less-than-ideal specimens (see Section 1.2)
- Pain management strategies during skin puncture (see Section 5.1.3.1)
- Interfering substances (see Section 5.2 and Appendix D)
- Recommendations on the sources of blood (see Section 4)
- Minimal and optional information captured with the collected specimen (see Section 6)
- Specifications for the filter paper portion of the specimen collection device (card) (see Section 7)
- Procedures for handling, shipping/mailing of specimens (see Section 5.5)
- Handling of blood spot specimens for DNA analysis (see Section 8)
- Short- and long-term storage of specimens (and residuals) (see Section 9)

Additionally, Appendix C (which focuses on filter paper evaluation protocol) was rewritten and updated with a table and figure. A new Appendix D was added on patient conditions and treatments affecting NBS results. Laminated sheets are available for Appendixes A and B. Appendix B illustrates representative examples of poor and good specimen quality that can be used as an instructional aid and displayed in the
specimen collection center. An updated instructional video consistent with the content of NBS01 will be available in 2013.

**Key Words**

Biobank, blood collection, DNA diagnostics, dried blood spots, filter paper, heelstick puncture, neonatal screening, newborn screening, sharps safety device, skin puncture device
Blood Collection on Filter Paper for Newborn Screening Programs; Approved Standard—Sixth Edition

1 Scope

1.1 Specimen Quality

The primary goal of this standard is to improve and ensure the quality of blood spots collected from newborns. Poor-quality and unsatisfactory specimens place an unnecessary burden on the screening system. Retesting requires additional follow-up, which, if not completed in a timely manner, could result in missed or late diagnosed cases, and can cause unnecessary trauma to the newborn and anxiety to the parents. Because of the complexity and diversity of the specimens that might be encountered and the influence of specimen quality on test results and their interpretation, the specimen criteria and handling procedures should address the common variances. Laboratory staff should immediately request another specimen (or the missing information) when the screening laboratory receives a poor-quality specimen (or one missing critical patient information). In all newborn screening (NBS) programs (NSPs), the turnaround time for results is critical if treatments to alter the adverse consequences of a condition (such as irreversible brain damage or death) are to commence in a timely manner.

1.2 Less-Than-Ideal and Unsatisfactory Specimens

NBS specimens may be collected in less-than-ideal circumstances and their quality may not be optimal for some, or all, of the NBS test categories. Some typical circumstances considered less than ideal may include specimens that are collected too early, too soon after a transfusion, before adequate feeding has occurred, or from a sick newborn. Other collection problems, eg, transit delays and missing information, may also affect the reliability of NBS results. Individuals responsible for collecting and submitting specimens should be made aware of these and other potential problems that can affect screening results (see Appendix D).

Nomenclature such as less-than-ideal, poor-quality, unsatisfactory, unacceptable, or invalid may be used in describing dried blood spot (DBS) specimens that are not properly collected. Such specimens are those with insufficient quantity of blood; clotting; smearing or contamination; inadequately filled circles; oversaturation with blood; scratching or abrading by capillary tube spotting; incomplete drying before mailing; or those that are usable to test for some, but not all conditions (see Appendix B). The standard operating procedures of the laboratory should address whether DBS specimens that are considered less than ideal (“unsatisfactory”) meet the established quality criteria. For all less-than-ideal specimens, a second specimen should be requested immediately and the request documented and tracked. NBS policies and protocols relating to submission of less-than-ideal specimens should seek to eliminate any confusion regarding results that might arise from such specimens. Similar policies should also exist for the screening laboratory. Protocols involving less-than-ideal specimens should be carefully followed.

The potential exists for specimens to be deemed “unsatisfactory” for analysis and/or for result reporting for one, many, or all analytes based on information accompanying the specimen or on the quality and/or amount of specimen received. It is important for the program to establish policies governing when and why a specimen should be considered less than ideal, of poor quality, or unsatisfactory. Data and specimens should be considered both independently and in combination when developing such policies.

In cases in which a specimen has been determined to be less than ideal or of poor quality, its analysis could yield unreliable, misleading, or clinically inaccurate results, and appropriate caution must be exercised. If such a specimen is analyzed, then the laboratory is acknowledging that the specimen is valuable for testing and will assume responsibility for the reliability of the analytical values and whether...
or not to report such results, and will track the receipt of a second specimen. Program-specific rules should be followed consistently with respect to handling, and analysis of, these specimens of less-than-ideal quality.

Because timely detection of a condition is critical for achieving maximum intervention benefits, testing and reporting of results from poor-quality (less-than-ideal) specimens may be considered. When testing and reporting results is permitted from specimens that deviate from typical quality specimens, the laboratory should follow established written procedures and document the properties of the poor-quality specimen on the data report. Specimens that are of poor quality (less than ideal) but still meet the minimum laboratory criteria for analysis should have results confirmed by a valid second specimen. Poor-quality (less-than-ideal) specimens may be analyzed and/or have their results reported only if considered appropriate by the authorities overseeing the NSP. (Adhere to local rules, regulations, and institutional policies.) **NOTE:** Analysis of poor-quality specimens should not distract from efforts to educate those who collect specimens and the constant pursuit of the collection of high-quality specimens (see Appendix B).

In addition to specimen quality, several patient conditions and treatments exist that are known to interfere with the reliability of NBS results. The tables in Appendix D provide lists of various conditions and treatments known to interfere with the reliability of screening results. For more specific information about these conditions and treatments, refer to CLSI document NBS03.

### 1.2.1 Other Considerations

The secondary goals of this standard are to delineate the minimum necessary patient information to capture on the specimen collection device (card); standardize the components of this device; describe minimum requirements for the filter paper matrix on which the blood spots are collected; and define the handling, shipping, retention, and storage conditions for DBS specimens.

### 1.3 Applications

This standard specifically addresses the collection of blood specimens for NSPs and applies to the collection of specimens used to detect congenital conditions (e.g., aminoacidopathies, fatty acid oxidation and organic acid disorders, endocrinopathies, hemoglobinopathies, cystic fibrosis, infectious diseases, immunodeficiency disorders). Many aspects of this standard are also appropriate and useful for the collection of DBS used for DNA/RNA molecular methods, for at-home specimen collection, and for a variety of new tests. In addition, most elements of this standard are applicable to blood collection on filter paper from fingerstick punctures of adolescents and adults. With older children (greater than 1 year of age) and adults, the palmar surface of the finger’s last phalanx is most frequently used (see CLSI document GP42).

### 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 blood-borne pathogens. The Centers for Disease Control and Prevention address this topic in published guidelines that address the daily operations of diagnostic medicine in human and animal medicine while encouraging a culture of safety in the laboratory. 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.
The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The quality management system approach applies a core set of “quality system essentials” (QSEs), basic to any organization, to all operations in any health care service’s path of workflow (i.e., operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager’s guide. The QSEs are as follows:

- Organization
- Personnel
- Process Management
- Nonconforming Event Management
- Customer Focus
- Purchasing and Inventory
- Documents and Records
- Assessments
- Facilities and Safety
- Equipment
- Information Management
- Continual Improvement

NBS01-A6 addresses the QSE indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Path of Workflow

A path of workflow is the description of the necessary processes to deliver the particular product or service that the organization or entity provides. A laboratory path of workflow consists of the sequential processes: preexamination, examination, and postexamination and their respective sequential subprocesses. All laboratories follow these processes to deliver the laboratory’s services, namely quality laboratory information.

NBS01-A6 addresses the clinical laboratory path of workflow steps indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.
Related CLSI Reference Materials*

GP42-A6  Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard—Sixth Edition (2008). This document provides a technique for the collection of diagnostic capillary blood specimens, including recommendations for collection sites and specimen handling and identification. Specifications for disposable devices used to collect, process, and transfer diagnostic capillary blood specimens are also included.

M29-A3  Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005). 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.

MM13-A  Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline (2005). This document provides guidance related to proper and safe biological specimen collection and nucleic acid isolation and purification. These topics include methods of collection, recommended storage and transport conditions, and available nucleic acid purification technologies for each specimen/nucleic acid type. A CLSI-IFCC joint project.

NBS02-A2  Newborn Screening Follow-up; Approved Guideline—Second Edition (2013). This guideline describes the basic principles, scope, and range of follow-up activities within the newborn screening system.

NBS03-A  Newborn Screening for Preterm, Low Birth Weight, and Sick Newborns; Approved Guideline (2009). This guideline outlines the recommended protocols for screening preterm, sick, or low birth weight infants for hearing loss and disorders detectable through dried blood spot testing.

NBS06-A  Newborn Blood Spot Screening for Severe Combined Immunodeficiency by Measurement of T-cell Receptor Excision Circles; Approved Guideline (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.

* 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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