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

This document addresses the issues associated with specimen collection, the filter paper collection device, and the transfer of blood onto filter paper, and provides uniform techniques for collecting the best possible specimen for use in newborn screening programs.

A standard for global application developed through the NCCLS consensus process.

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Abstract

NCCLS document LA4-A4—*Blood Collection on Filter Paper for Newborn Screening Programs; Approved Standard—Fourth Edition* addresses the issues associated with specimen collection, the filter paper collection device, and the transfer of blood onto filter paper. The purpose of these considerations is to produce a functional 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 applying blood collected by heel-stick onto the preprinted circles of filter paper; (2) recommendations on the source of blood; (3) techniques for direct collection, and alternative collection procedures of specimens; (4) specifications for the filter paper, handling, and the mailing package; (5) specifications for the specimen collection device; and (6) the handling of blood spots collected on filter paper for DNA analysis.

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Foreword

This standard has been written for both manufacturers and newborn screening programs, with primary emphasis on the specimen collection centers and the specimen collection device (card). Specimens for newborn screening are usually collected by hospital personnel during the first few days of the neonate's life. This standard informs and instructs collection personnel on the essentials of collecting a high-quality specimen, for handling it after it has been collected, for transporting it to the testing facility, and for storing the residual specimen content 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 (e.g., fingerstick collections on filter paper to test for specific antibodies and for DNA diagnostic testing). See the most current edition of the NCCLS standard H4 —*Procedures for the Collection of Diagnostic Blood Specimens by Skin Puncture*, for an essential reference document for use with this standard.

The present standard replaces the third edition approved standard, LA4-A3, which was published in 1997. Several changes have been made in this edition; chief among them are the modifications (in Sections 3.1, 3.2, and 3.3) of the procedures for applying collected blood onto the preprinted circles of filter paper. Modifications were made that better describe the use of other sources of blood and their collection and application onto the filter paper. The current standard makes recommendations on the source of blood (Section 2), and techniques for the direct blood collection from the puncture site and other collection techniques (Section 3). Extensive enhancements were made to the specifications for specimen matrix and shipment, especially in the printing section (Section 4.1.3). Minimum data set requirements have been updated (Section 5.1). Modifications were made in the specimen collection device section to include quality control aspects for printing (Section 5.2). A new section has been included to cover storage of and access to specimens for biobanking (Section 7). Appendix A was updated and a figure was added to demonstrate drying techniques. This single-page appendix is detachable from the document and can be used as an instructional aid and displayed in the specimen collection center. In addition, a new appendix has been added that illustrates a blood spot drying device (Appendix D).

Standard Precautions

Because it is often impossible to know what might be infectious, all human blood specimens are to be 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 any pathogen and thus are more comprehensive than universal precautions which are intended to apply only to transmission of blood-borne pathogens. Standard precaution and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (*Guideline for Isolation Precautions in Hospitals*. Infection Control and Hospital Epidemiology. CDC. 1996;Vol 17;1:53-80), (MMWR 1987;36[suppl 2S]2S-18S), and (MMWR 1988;37:377-382, 387-388). For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials and for recommendations for the management of blood-borne exposure, refer to the most current edition of NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections.*

Key Words

Biobank, blood collection, DNA diagnostics, dried blood spots, filter paper, heel-stick puncture, neonatal screening, newborn screening

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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.[1] Unacceptable and poor quality specimens place an unnecessary burden on the screening facility, cause unnecessary trauma to the infant and anxiety to the infant's parents, potentially delay the detection and treatment of the affected infant, and could contribute to a missed or late diagnosed case. When the screening laboratory receives an unacceptable specimen, it should request another specimen according to criteria established by the testing laboratory. In all newborn screening programs, the turnaround time for analytic results is critical if treatment to prevent the adverse consequences of the condition (such as irreversible mental retardation or death) is to begin on time.

1.2 Specimen Acceptability

The only justification for refusing to analyze a specimen and declaring it unacceptable is that its analysis might yield unreliable, misleading, or clinically inaccurate values for a particular analyte. Since, by this definition, an unacceptable specimen gives no usable information, such specimens should not be analyzed, and those responsible for collecting the original specimen should be notified with all due haste so that an acceptable specimen can be obtained as soon as possible. If a specimen is analyzed, the laboratory is, in effect, acknowledging that the specimen is suitable for testing and is assuming responsibility for the reliability of the analytic values. Program-specific rules should be written and followed consistently with respect to handling specimens of insufficient quantity, especially for multianalyte test panels.

1.2.1 Other Considerations

The secondary goals of this standard are to delineate the minimum necessary information for the specimen collection form; to standardize the components of this form; to describe minimal requirements for the filter paper matrix on which the blood spots are collected; and to define the handling, shipping, and storage conditions for dried blood spot specimens.

1.3 Applications

This standard specifically addresses the collection of blood specimens for newborn screening programs[1] and applies to the collection of specimens used to detect such congenital disorders as primary hypothyroidism, phenylketonuria (PKU), galactosemia, congenital adrenal hyperplasia, biotinidase deficiency, maple syrup urine disease (MSUD), hemoglobinopathies and homocystinuria, among others. Many aspects of this standard are also appropriate and useful for the collection of dried blood spots used for DNA diagnostics, home collection devices, and 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 one year of age) and adults, the palmar surface of the finger's last phalanx is most frequently used. (See the most current edition of NCCLS document H4—*Procedures and Devices for Collection of Diagnostic Blood Specimens by Skin Puncture*.)

2 Source of Blood

2.1 Heel

Blood collected from the heel is preferred for newborn screening and should be collected from the most medial or lateral portion of the plantar surface of the heel. "Medial" is defined as closest to the midline of the body; "lateral" is defined as away from the midline of the body; and "plantar surface" as the walking surface of the foot (see Appendix A).[2,3,4] Previous puncture sites or the curvature of the heel must *not* be used.

2.2 Other

Cord blood, venous blood (dorsal hand vein or umbilical venous catheter specimens), and arterial blood (umbilical arterial catheter specimens) might be appropriate for special situations. (See Sections 3.1 through 3.4.) Consult local regulations and institutional policies for the collection of such specimens.

2.3 Unacceptable Sources

2.3.1 Sites From Which Blood Must Not Be Obtained:

(1) Central area of an infant's foot (arch), because this might result in injury to nerves, tendons, and cartilage and offers no advantage over puncturing the heel. (See the most current edition of NCCLS document H4— *Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture.*)

(2) Fingers of a newborn, since the distance from the skin's surface to the bone in the thickest portion of the last segment of each finger of newborns ranges from 1.2 to 2.2 mm, and the available lancets could easily damage the bone. In newborns, local infection and gangrene might be a complication of finger punctures.[4] (See the most current edition of NCCLS document H4— *Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture.*)

(3) Earlobe, because this might cause excessive bleeding.

(4) A swollen or previously punctured site, because accumulated tissue fluid will contaminate the blood specimen.

(5) Intravenous lines that are contaminated with substances (such as amino acid solutions) that might adversely affect the test results.

3 Techniques for Blood Collection on Filter Paper

3.1 Heelstick (Method of Choice)

3.1.1 Preliminary Steps

Ensure that the expiration date of the specimen collection device (card) has not passed. Complete the required patient information included on the collection device (card) either manually or electronically. In manual applications a ballpoint pen should be used; soft-tip pens will not copy through to the other sheets of paper. Address imprint devices (or adhesive labels) should never be used unless the handling process ensures that patient information is not obscured and the blood collection area is not compromised. Do not use a typewriter or printers that might compress the paper. Avoid touching the area within the circles on the filter paper section before, during, and after collection (blood spots) of the specimen. Do not allow

water, feeding formulas, antiseptic solutions, glove powder, hand lotion, or other materials to come into contact with the specimen card before or after use.

3.1.2 Precautions

Confirm the identity of the infant and ensure accuracy of the demographic data on the card. Wash hands vigorously before proceeding. All appropriate precautions, including wearing powder-free gloves (changing gloves between infants), should be taken for handling blood and disposing of used lancets in a biohazard container for sharp objects. Follow local recommendations regarding use of latex gloves in situations of latex allergy. (See the most current version of NCCLS document M29— *Protection of Laboratory Workers from Occupationally Acquired Infections.)*

3.1.3 Site Preparation

Warm the newborn's heel, since warming the skin-puncture site can help increase blood flow. A warm, moist towel or diaper at a temperature no higher than 42 °C may be used to cover the site for three minutes. This technique increases the blood flow sufficiently and will not burn the skin.[5] (See the most current edition of NCCLS document H4— *Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture.*) Acceptable heel warming devices are also commercially available. In addition, positioning the infant's leg lower than the heart will increase venous pressure. (**Caution:** Before topical anesthetic creams are used for a heel puncture, the testing laboratory should document that these creams do not produce analytic interferences.)

3.1.4 Cleaning the Site

The skin should be wiped with alcohol (isopropanol/water: 70/30 by volume, "70%"). Allow the skin to air dry.

3.1.5 Puncture

To obtain sufficient blood flow, puncture the infant's heel on the plantar surface of the heel with a sterile lancet or with a heel incision device.[1,6] The incision device provides excellent blood flow by making a standardized incision 1.0 mm deep by 2.5 mm long. (See the most recent edition of NCCLS document H4—*Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture*). Any puncture device used should be selected so that the puncture does not exceed 2.0 mm in depth (see reference 4 for more details). For infant safety, scalpel blades or needles must not be used to puncture the skin for blood collection. Disposable skin puncture lancets of different designs are commercially available for performing the heel stick on infants.[6] For worker safety, disposable skin puncture devices that protect the user from unintentional self-inflicted skin punctures should be used.[7]

In small, premature infants, the heel bone (calcaneus) might be no more than 2.0 mm beneath the plantar heel skin surface and half this depth at the posterior curvature of the heel. Studies indicate that for some infants (including full-term infants) a puncturing depth beyond 2.0 mm might be excessive and might cause bone damage.[6,8,9] In this situation other collection methods should be considered (see Section 2.2).

3.1.6 Direct Application

After the heel has been punctured, wipe away the first drop of blood with a sterile gauze pad or cotton ball and allow a large drop of blood to form. (Intermittently apply gentle pressure to the heel with the thumb, and ease this pressure as drops of blood form [see Section 3.1.6.1]). Touch the filter paper gently against the large blood drop and, in one step, allow a sufficient quantity of blood to soak through and completely fill a preprinted circle (Section 5.1 [14]) on the filter paper. *Do not press the filter paper against the puncture site on the heel*. Blood should be applied only to one side of the filter paper. Both sides of the filter paper should be examined to assure that the blood uniformly penetrated and saturated

the paper. After blood has been collected from the heel of the newborn, the foot should be elevated above the body, and a sterile gauze pad or cotton swab pressed against the puncture site until the bleeding stops. It is not advisable to apply adhesive bandages over skin puncture sites on newborns.[2] (For treatment of the puncture site after specimen collection, see the current edition of NCCLS document H4—*Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture*.)

3.1.6.1 Milking

Excessive milking or squeezing the puncture might cause hemolysis of the specimen or result in an admixture of tissue fluids with the specimen and might adversely affect the test result.

3.1.6.2 Layering

Do not apply layers of successive blood drops to the same printed circle. Applying successive drops of blood to already partially dried spots causes nonuniform analyte concentrations and invalidates the specimens.

3.1.7 Collection

Collect the required number of uniform blood spots. Failure to collect the appropriate number of blood spots might invalidate the specimen for all tests depending on screening program rules (see Section 1.2). If blood flow diminishes so that a circle is not completely filled, repeat the sampling technique using a new circle or, if necessary, a new blood collection card (see Sections 3.1.3 through 3.1.6) Consult local regulations and institutional policies concerning minimum numbers of blood spots required.

3.2 Capillary Tube

Although not the method of choice, specimens can be obtained by applying blood collected in sterile heparinized capillary tubes to the collection device (see Section 3.3). EDTA might cause interference with some laboratory tests (see Section 3.3). The capillary tube collection method may also apply to cord or venous blood transferred onto filter paper. (See the most current edition of NCCLS document H4—*Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture.*) Consult appropriate local regulations and institutional policies for specific applications.

3.2.1 Collection

Using a fresh capillary tube for each circle to be filled on the screening card, collect the appropriate volume of blood (75 µL or 100 µL in U.S. screening programs) (see Section 5.1 [14]) into a heparinized capillary tube. (**Note:** The appropriate volume of the patient specimen is defined by the screening program to match that of the test calibrators and controls.)

Touch the tip of the heparinized capillary tube to the blood drop formed at the heel puncture site (see Section 3.1.5). Allow blood to flow into the tube by capillary action. Fill rates might be improved by holding the tube in a near-horizontal position when touching to the blood drop. Collect the required number of uniform blood spots. Failure to collect the appropriate number of blood spots might invalidate the specimen for all tests depending upon screening program rules (see Section 1.2).

3.2.2 Application

After filling a capillary tube to the calibration mark, immediately apply the contents of that tube to the center of a single, preprinted circle on the filter paper, completely filling the circle. Waiting too long before application will allow cells and plasma to separate. To avoid damaging the filter paper fibers, do not allow the capillary tube to touch the filter paper. Actions such as “coloring in” the circle, repeated

dabbing around the circle, or any technique that might scratch, compress, or indent the paper should not be used. Do not reuse capillary tubes.

Apply blood to only one side of the filter paper. Do not apply multiple capillary specimens to the same circle, since caking or heterogeneous spreading will occur and might adversely affect test results. The directions in Section 3.4 should be followed to complete the procedure.

3.3 Dorsal Hand Vein

Although not the method of choice, blood collected from needle puncture of the dorsal hand vein (See the most current edition of NCCLS document H3—*Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture*) and its application directly onto the preprinted circles of the filter paper is possible.[a] Blood should not be drawn from an extremity into which IV fluids (including blood) are being or have been infused unless appropriate precautions are taken (see the most recent edition of NCCLS document H4—*Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture).* Consult appropriate local regulations and institutional policies for specific applications.

The routine practice of dorsal hand vein collection is discouraged. Problematic issues include:

(1) Test results might be affected by blood from different vessel sources.[10,11,12,13]
(2) Hand veins might be needed for IV fluids.
(3) Venous sampling is more invasive than a heel stick.

3.3.1 Collection and Application

Select appropriate size winged blood collection set (butterfly). Remove or shorten catheter length so blood can flow freely onto the circle on the filter paper. Use standard pediatric venous collection procedures. Collect the required number of blood spots. Failure to collect the appropriate number of blood spots might invalidate the specimen for all tests depending on screening program rules (see Section 1.2).

Syringe collection of blood for application onto a collection device (card) is not recommended because of lack of anticoagulant and time delays that could allow for clot formation and settling of cells producing heterogeneous specimens.

3.4 Umbilical Venous Catheter (UVC) or Umbilical Arterial Catheter (UAC)

Although not the method of choice, blood collected from umbilical catheters (venous or arterial) is acceptable in certain situations (e.g., sick babies or in very low birth weight babies). Although unknown, it is reasonable to expect that there might be some difference in analytic test results between blood taken from the heel and that collected by umbilical catheters. Consider repeat collection from the heel at a later time. (Consult appropriate local regulations and institutional policies.)

3.4.1 Collection and Application

Due to the fact that UAC or UVC are used to infuse antibiotics or other medicines, in order to clean the line, it is important that blood (e.g., 2 to 2.5 cc [mL]) be drawn from the line before the blood is collected for testing purposes. After cleaning the line, collect blood in a syringe and immediately apply appropriate volumes to the printed circles on the specimen collection card. It is important that the blood transfer be as quick as possible to avoid blood clotting that might invalidate the specimen for testing (see Section 3.3.1). The required number of blood spots should be collected. Failure to collect the appropriate number of blood spots might invalidate the specimen for all tests depending upon screening program rules.

[a] For extensive details of this technique and application methods, see M.E. Clagg in *Laboratory Medicine* [1989;20:248-250].

3.5 Specimen Handling and Transport

3.5.1 Drying

Avoid touching or smearing the blood spots. Allow the blood specimen to air dry on a horizontally level (see Appendix D), nonabsorbent, open surface for at least three hours at an ambient temperature of 15 °C to 22 °C. Keep the specimen away from direct sunlight (indirect room light is not usually detrimental unless accompanied by heat). Blood spots on the filter paper should not be heated, stacked, or allowed to touch other surfaces during the drying process.

3.5.2 Stacking

Since leaching (cross-contamination) between specimens might occur, specimen-to-specimen contact is not appropriate. Before placing the specimens in a container for transport (see Section 3.5.3), the dried blood spots on the collection card should be rotated 180° from the blood spots on the cards in the stack immediately above and below. If collection cards are separated by physical barriers, specimen rotation is not necessary. When stacking of exposed dried blood spots cannot be avoided, the following procedure should be used:

(1) A fold-over cover attachment can be added to the specimen collection device (see Section 5.2.3). This attachment, added when forms are manufactured, provides protection from contamination prior to blood collection, during specimen transportation (see Section 3.5.3), and during specimen storage after analysis (see Sections 3.5.4 and 7).

(2) Glassine paper can be placed between specimens.

3.5.3 Timing and Transport (Mailing)

Unless otherwise directed by the screening laboratory, the collection card should be transported or mailed to the laboratory within 24 hours after specimen collection, and the appropriate tracking documentation maintained. Daily courier transport is recommended whenever possible. Delays at collection sites should be avoided, and the shipping environment relative to possible delays should be structured to maximize transport efficiency. Use of sealed plastic bags or other air-impermeable shipping containers are not recommended and require humidity control (see Guidelines for Shipment: http://www.cdc.gov/od/ohs/biosfty/driblood.htm). Comply with local regulations and institutional policies.

Specimens should not be placed in hermetically sealed containers (e.g., plastic or foil bags). Federal postal and local transport regulations must be followed.[14] If local regulations require enclosure in water-tight plastic containers for transportation, then sufficient numbers of desiccant packages must be included to ensure minimal exposure of specimens to excessive moisture.[14] Indicator cards may be used to monitor humidity. Humidity and moisture are detrimental to stability of dried blood spot specimens and analyte recovery.[15,16] Specimens known to be biohazardous should be transported with special precautions.

3.5.4 Storage During and After Analysis

Following receipt in the newborn screening laboratory, the specimen should be stored in a manner allowing for easy access and analysis without analytic compromise. During the analytic process, storage in a low-humidity (less than 30%)[15] environment at ambient temperature is adequate. Low humidity and lower temperatures (4 °C) are suggested for program storage up to two years.[15] For storage periods beyond two years see Section 7.

In cases where residual specimens are to be disposed of, care should be taken to dissociate patient identifiers from the blood spots.[15] Blood spot disposal should comply with local regulations and institutional policies.

4 Specifications for Specimen Matrix

4.1 Collection Paper Specifications

4.1.1 Specifications

Physical characteristics of the paper should conform to specifications outlined in Appendix C (also see Section 5.2), and validation should be available from the paper manufacturer upon request.

4.1.2 Lot Number/Sources

The manufacturer's name and lot number for the filter paper must be indicated on the filter paper portion of all specimen collection devices with the exception of devices in which the filter paper is embedded in the device (so-called "collection cassettes"). In this instance the information should be printed on the device. In situations in which the sample collection area was designed for division into separate parts, traceability should be maintained for each part. The information regarding the filter paper lot number and the manufacturer are important to the quality assurance efforts of the testing laboratories.

Specimen collection devices (cards) in use by the collection centers at any one time should be limited, ideally to only one batch and manufacturer with a short transition phase to new batches or manufacturers. The potential effects of filter paper on analytic assays can be minimized by using the same lot number and manufacturer of collection devices (cards) for all specimens and calibrators.

4.1.3 Printing

The press must be thoroughly cleaned before printing. Any chemicals used to perform this task must not interfere in the analytic test procedures. The filter paper must not be calendered during the printing process. For this reason the use of a guillotine trimmer or any device that calenders (compresses) the filter paper must not be used to trim the cards. Lithographic printing is not an acceptable method for specimen filter paper. A dedicated ink delivery system should be used for filter paper printing. The dedicated glue system should be used for attachment of the filter paper to the demographic portion of the collection device.

4.1.4 Shelf Life

The printed collection device (card) for newborn screening has a shelf life of two years. Carbonless paper has a shelf life of approximately two years and is sensitive to temperature changes. When cards are removed from the shipping carton, they should be stored in their original wrapping and stacked in a manner that avoids compressing the filter paper. Compression of the filter paper will alter its performance characteristics.

4.2 Paper Performance Characteristics

At least four physical properties of the filter paper should be measured by the paper manufacturer for each lot of paper and the specifications and results provided before the release of the lot.[17] (See Appendix B.) The following properties should be measured utilizing the 100-µL volumes of 55% hematocrit blood:

(1) Absorption capacity; serum retention volume of the 1/8-inch (3.2-mm) paper punch taken from the dried blood spot (humidity adversely affects the absorbance and should be controlled).[17]

(2) Homogeneity of the filter paper lot (spot-to-spot and sheet-to-sheet variability).

(3) Diameter of the circle for the dried blood aliquot.

(4) Absorption time for a 100-µL blood aliquot.

4.3 Ink Specifications

Printing ink must not interfere in the analytic test procedure. Data should be available that validate the test compatibility of the ink. Colored ink contrasting to black or blue ballpoint pens should be used for printing the demographic input part of the collection card to allow easy reading. Ink and the printing process must not produce an impermeable barrier in the filter paper for the blood spreading during collection (see Section 5.1 [14]).

4.4 Packaging Precautions

Packaging should not lead to compression of the filter paper. Chemicals or other types of specimens should not be packaged in the same container used for shipment of blood spot specimens.

5 Specimen Collection Device

5.1 Minimum Preprinted Information

The following is the minimum necessary information required to achieve the screening goals. Additional information may be included at the discretion of the screening programs in order to meet specific needs. Consult local regulations and institutional policies for deviations from minimum preprinted information.

(1) infant's name (last [and first if available]);

(2) mother's first and last name (optional: include mother's maiden name);

(3) sex;

(4) birth date (optional: include time of birth);

(5) date of specimen collection;

(6) infant's age (indicate if less than 24 hours; optional: include time of collection);

(7) patient identification number (e.g., medical record number; optional: include address and phone number);

(8) birth weight;

(9) submitter's identification and address (optional: include birth facility);

(10) physician's name (healthcare provider) and telephone number;

(11) name of newborn screening program and address;

(12) unique nonrepeating serial number (see Section 5.2.2);

(13) expiration date of specimen collection device;

(14) appropriate number of preprinted circles should be available with preprinted broken- or dotted-line circles on one side of the filter paper section (with optional printing of circles on both sides). (In the United States, the preprinted circle (1/2 inch [13 mm] internal diameter) is filled to the printed line by 75 µL of blood while 100 µL fills slightly beyond the print.); and

(15) manufacturer and lot number of filter paper indicated on the filter paper section, and manufacturer or printer listed on the patient information section of the form (optional: barcodes may be imprinted on the specimen collection device [see Section 5.2.2] and barcodes should contain a check sum digit).

5.2 Specimen Collection Device

The specimen collection device (card) for dried blood spots should *NOT* be purchased in the same manner as standard business forms used for the various routine documentation activities in laboratories. (In the United States, the manufacturer of specimen collection devices must comply with Food and Drug Administration's regulations; otherwise adhere to local regulations.) The supplier of finished devices must provide upon request the information listed below validating the homogeneity (spot-to-spot and card-to-card variability) and performance equivalency of the filter paper before and after printing:

(1) diameter of the circle for the dried blood aliquot (see Appendix B, Sections B3.2 and B3.6); and

(2) absorption time for a 100-µL blood aliquot (see Appendix B, Sections B3.2 and B3.6).

5.2.1 Patient Information Section

The patient information section of the specimen collection card must not cover (interfere with) the circles designated for blood spots unless it is removable prior to collection.

5.2.2 Specimen Device Numbers

Sequential numbers for each specimen collection device (card) should be preprinted on each page of the collection device and, if the blood-spot collection section is detachable from the card, on the filter paper component. The specimen identification number should be designed to maintain its uniqueness across time (suggested format: two-letter state abbreviation, two-digit year for year collection devices ordered, seven-digit sequence number, and an optional one-character check sum) (see Sections 3.5.4 and 5.1 [12]). This identification number may serve as a linkage identifier for other health programs or registries, including birth certificates, immunizations, newborn hearing screening, and birth defects. A barcode facsimile of the identification number printed on the collection device may be of use in automated systems, and multiple labels with this barcode may be attached to the collection device for removal and use with other records.

5.2.3 Contamination

A fold-over cover attachment can be included in the specimen collection card design (see Section 4.4) when manufactured to provide extra protection of the filter paper from contamination prior to blood collection and of the collected specimen during transportation to the laboratory. This attachment can be designed to be removed from the collection device at the newborn screening laboratory.

5.2.4 Color Coding

A portion of the demographic part of the collection device can be color tinted for easy identification of different lot numbers of filter paper.

5.2.5 Transition to a New Production Batch

Transition to a new batch of specimen collection devices should be phased in before the batch in use expires.

6 Blood Spot Handling for DNA Analysis

Amplification of DNA by polymerase chain reaction (PCR) for DNA analysis using dried blood spot specimens is possible. Specimens collected for newborn screening have been analyzed using these techniques by some programs to provide additional testing capabilities, thus specimen handling should encompass compatible procedures.[18,19,20]

6.1 Contamination

Collection devices and specimens should be handled with gloves at all times to prevent contamination from extraneous DNA by DNA-containing cell transfers from specimen handlers (see Section 5.2.3).

6.2 Specimen Contact

Dried blood spot specimens should not be allowed to come in direct specimen-to-specimen contact during handling, shipment, or storage (see Sections 3.5.2 and 4.4).

6.2.1 Specimen Carry-over

Blood spot punching and cutting tools used in DNA tests must be cleaned to ensure absence of carry-over contamination of DNA-containing cells between specimens. The tools can be cleaned between specimens by punching or cutting clean paper between specimens or treating the cutting surface of the tool with bleach or dilute hydrochloric acid.[18]

7 Extended Storage of Residual Specimens

Storage of residual specimens may constitute a biobank that is potentially an important resource for public health programs. Programs should have written policies and procedures for access to retained specimens. Issues of informed consent might limit the access to specimens.[21] Consult appropriate local regulations and institutional policies.

Residual dried blood spot specimens stored by the newborn screening laboratory for extended periods must be protected to ensure specimen integrity when analyzed following storage. One method is to store the specimens in low gas-permeable, zip-closure plastic bags with desiccant and humidity indicator cards. Humidity should be maintained below 30%.[15] For long-term storage (greater than two years) –20 °C is recommended.[15] Positive and negative blood spot controls, if available, should be stored with the residual specimens so that the specimen's integrity can be checked when it is removed from storage.[15] (See Section 6 for precautions if DNA analysis is anticipated.) Improper storage conditions might compromise specimens.

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Appendix A. How to Collect an Acceptable Blood Spot Specimen (Detachable)

A1 Preparation

A1.1 Wash hands vigorously.

A1.2 Wear powder-free gloves and change gloves between infants.

A1.3 Confirm identity of infant.

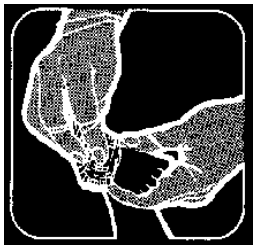
A2 Sampling Technique

A2.1 Wearing gloves, wipe infant's heel with 70% isopropyl alcohol.

A2.2 Allow heel to air dry.

A2.3 The puncture should be made within the shaded area as illustrated in the drawing above.

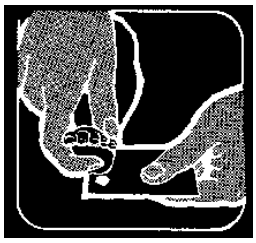
A2.4 Using a lancet of recommended length, perform puncture (depth <2.0 mm) as illustrated.



A2.5 Gently wipe off first drop of blood with sterile gauze or cotton ball. (Initial drop contains tissue fluids which might dilute sample.)

A2.6 Wait for formation of large blood droplet.

A2.7 Apply gentle pressure with thumb and ease intermittently as drops of blood form.



A2.8 Gently touch the filter paper card to the blood drop and fill each printed circle with a SINGLE application of blood. Apply blood to one side only. Observe the saturation of each printed circle as the blood flows through the filter paper.

A2.9 All used items should be disposed of in an appropriate biohazard container.

A2.10 After the specimen is collected, elevate the infant's foot and, using sterile gauze, briefly apply gentle pressure to the puncture site until the bleeding stops. Do not apply adhesive bandages.

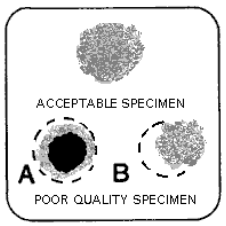
A2.11 Allow blood specimen to AIR DRY THOROUGHLY, on a horizontally level, nonabsorbent, open surface, such as a drying rack or plastic-coated test tube rack, *for a minimum of 3 hours* at ambient temperature. (Do not stack or heat.)

Drying rack figure reprinted with kind permission of Schleicher & Schuell BioScience, Inc.

A2.12 After the specimen has dried, place in an approved container for transport. (See local regulations.)

A3 Pitfalls

A3.1 Failure to allow residual alcohol to dry might dilute the specimen and adversely affect test results.



A3.2 Puncturing the heel on posterior curvature will permit blood to flow away from puncture, making proper spotting difficult. DO NOT USE PREVIOUS PUNCTURE SITES.

A3.3 *Milking* or squeezing the puncture might cause hemolysis and admixture of tissue fluids with specimen.

A3.4 Do not layer successive drops of blood on the target spot (Example A). If blood flow diminishes to incompletely fill circles, REPEAT sampling technique A2.1 through A2.10. Note Example B for poor quality specimen with inadequate blood.

A3.5 Avoid touching the area within the circle before and after blood collection. Do not allow water, feeding formulas, antiseptic solutions, powder from gloves or other materials to come into contact with the specimen card before or after use.

A3.6 Do not place the specimens in the transport container until thoroughly dry. Insufficient drying adversely affects test results. Use of sealed plastic bags requires desiccation. Ideally, transport specimens within 24 hours of collection.

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Appendix B. Protocol for Testing the Absorption Characteristics of Filter Paper

B1 Scope

This protocol is designed for comparing production lots of filter paper by measuring absorption characteristics and is provided only as a working example. No recommended or suggested status is either intended or implied. Since the paper punch is a volumetric measurement for an analytic method, a high degree of uniformity is essential to minimize variance from the lot-to-lot filter paper transitions for specimens, calibrators, and quality control materials.

B2 Reagents and Supplies

(1) Whole human blood. Human blood that has passed the expiration date for human use and that exhibits no hemolysis or high lipid content may be obtained from the Red Cross Blood Bank and used as a matrix. Freshly drawn, human whole blood preserved in ACD (acid citrate dextrose solution) to blood bank ratios may also be used. The hematocrit should be adjusted to 55% before use. Severely outdated blood might adversely influence the measured absorption characteristics of the paper.

(2) High specific activity ^{125}I-L-thyroxine.

(3) A single-hole, paper hole puncher, 1/8-inch (3.2 mm) diameter disks (performance calibrated).

(4) Filter paper cut in sizes of intended use or collection cards.

(5) Saline (155 mmol sodium chloride/L; 0.9% w/v) injection, U.S.P.

B3 Preparation of Blood Enriched With Radiolabeled Thyroxine

B3.1 Hematocrit

Red blood cells (RBCs) are separated from plasma by centrifugation (at least 1820 x g for 5 minutes). The RBCs are washed with saline (3x). For intact cell preparations, nonwashed RBCs may also be used. The hematocrit of the RBCs should be determined (usually about 90% for washed RBCs). The volume of serum or plasma needed to adjust the hematocrit to 55 ± 1% should be calculated and this volume of serum or plasma added to the RBCs. The hematocrit of this plasma-RBC mix should be determined before use. Lysed RBC preparations can be an acceptable test matrix after confirmatory studies have validated the proportional equivalence of performance. Lysed RBC preparations should be prepared by a freeze-thaw cycle after adjusting and confirming the hematocrit.

Taking extreme care that the blood (55% hematocrit) is constantly and gently mixed, an aliquot of approximately 15 mL of blood should be transferred to a 25-mL volumetric flask and approximately 30 µCi (11.1 x 10^8 ßq) of ^{125}I-L-thyroxine added and brought to volume with blood (55% hematocrit).

B3.2 Filter Paper Production Lots

Filter paper lot numbers to be tested should be stored for at least 24 hours under identical conditions of humidity and temperature. A statistically valid sampling procedure should be used for testing the filter paper lots.

B3.3 Total Count Tubes

The filter paper should be suspended in a flat, horizontal position. Maintaining continuous gentle mixing, aliquots of 100 µL of the ^{125}I-L-thyroxine-enriched blood should be applied to the filter paper (see Section B3.1). Wet blood spots should not be allowed to come in contact with any surface. At least two spots of blood should be applied to each sheet. Total count tubes (at least four) are prepared by taking an aliquot from the ^{125}I-L-thyroxine-enriched blood previously prepared as described in Section B3.1. Using extreme care that the blood is constantly and gently mixed, at least four independent 1:100 dilutions in saline should be prepared. Aliquots of 100 µL taken with a positive displacement pipette from these 1:100 dilutions will yield satisfactory count rates. The conditions of treatment for the total count tubes should simulate that of the blood spots.

B3.4 Drying

The spotted sheets of filter paper are allowed to air dry at ambient temperature overnight.

B3.5 Blood Spot Punches

Punches of 1/8-inch (3.2-mm) diameter are taken from center and four peripheral locations (N, S, E, and W) of each blood spot on each sheet of paper. For homogeneity testing (see Section B5.2), only punches from the peripheral locations are used.

The blood spot punches and total count tubes are counted in a gamma counter with a preset count error rate of at least 1%.

B3.6 Absorption Time and Aliquot Diameter

The absorption time and the diameter should be measured for blood spots produced by 100-µL aliquots of blood (hematocrit 55 ± 1%) [see Sections B2 and B3.1]. Absorption time target: 12 seconds; range 5 seconds to 30 seconds. Diameter target: 16 mm; with reference range of 15 mm to 17 mm. If measured mean values are outside target, manufacturer and reference evaluator must decide on the acceptability of the lot for distribution. Ragged edges or mottling of dried blood spot should be recorded if observed. At least one previously measured production lot of paper should be used as a control lot. The control lots along with the test lot should be blind-coded. A coded randomized (using a random number table) sample set should be prepared for the absorbance rate measurements. Measurement of the control lot is to assure comparability of testing among lots of paper.

B4 Absorption Volume

The mean value(s) for the counts per minute within a 1/8-inch (3.2-mm) punch can be equated to the blood volume of the punch by using the total counts per unit volume (total count tubes, Section B3.3). With this blood volume and the hematocrit of the blood preparation used, a serum volume per 1/8-inch (3.2-mm) punch can be determined.

B5 Statistical Analysis[1, 2]

B5.1 Quality Control

A routine quality control procedure must be established using one selected lot of filter paper and control limits determined for each test method (use a minimum of ten analytic runs to set temporary working control limits) prior to applying the test for evaluation of absorption volume, aliquot diameter, and absorption time characteristics of filter paper lots. Standard quality control charts should be maintained for each of the parameters.

B5.2 Variance

The collected data should be analyzed for among-punch location (within-spot) variance, among-spot (within-sheet) variance, and among-sheet variance of the filter paper. The total variability should be apportioned into these three components, and the fraction of each to the total, and its corresponding coefficient of variation should be evaluated. A hierarchical, nested analysis-of-variance is used to assess the homogeneity of the paper lot. An F-test is used to test equivalence of the mean values (counts/minute/punch) of the lots of paper. If lot means are significantly different (statistically) from one another, then the manufacturer and the reference evaluator must decide whether or not to accept the filter paper lot for distribution.

B5.3 Serum Absorption Volume and Confidence Intervals

The mean serum volume for the 1/8-inch (3.2-mm) punch should be 1.54 ± 0.17 µL for a punch from a 100-µL blood aliquot with intact RBCs-55% hematocrit, or 1.30 ± 0.19 µL for a punch from a 100-µL lysed RBC-blood aliquot (these data for mean values and 95% confidence intervals were based on the previous analysis of ten acceptable lots of filter paper).[3] Lot numbers with measured mean values falling outside the 95% confidence interval should be rejected after confirmation by a repeated analysis.

References to Appendix B

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Appendix C. Filter Paper Specifications

These specifications are by no means complete, but they provide a sense of the type and degree of the requirements. For additional information, the user should contact ASTM, 100 Barr Harbor Drive, West Conshohocken, PA 19428-2959.

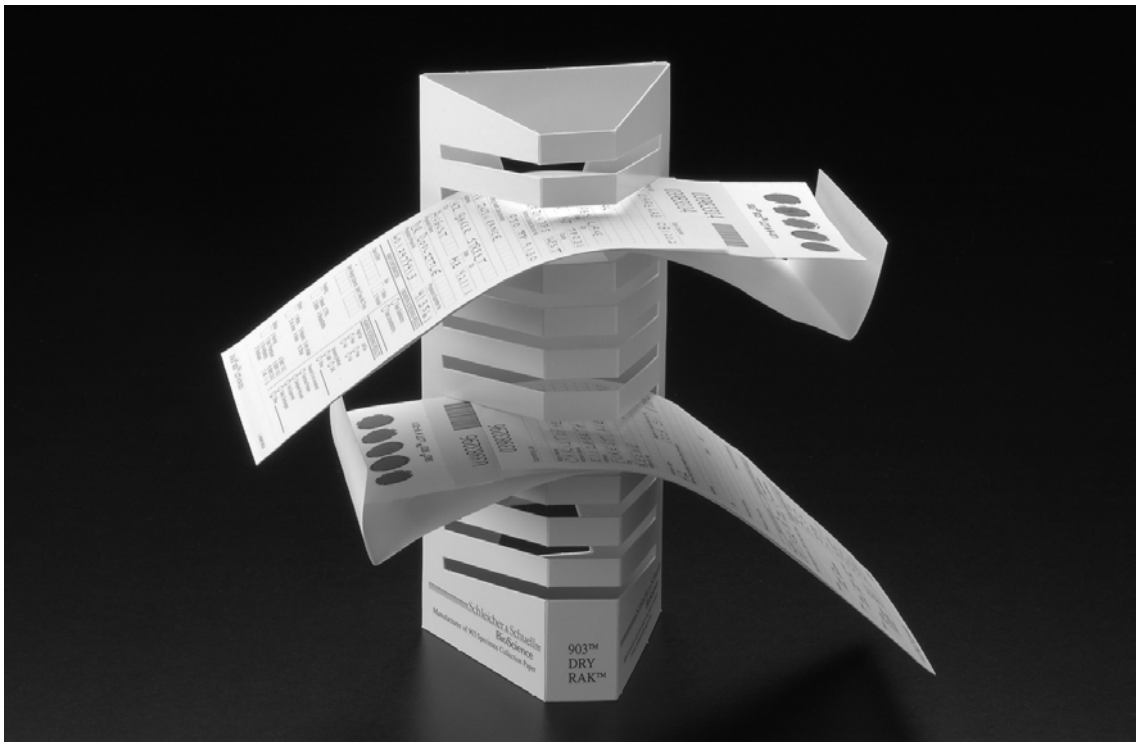
(1) Filter paper should be made of 100% pure cotton fiber, with no wet-strength additives.

(2) Basis weight should be 110 lb ± 5% per ream (179 g/m^2 ± 5%). A ream is defined as 500 sheets 24" x 36" (ASTM D646-96).

(3) The pH should be 5.7 to 7.5 (Test method ISO 6588:1981).

(4) Ash %: 0.1% maximum (Test method A of ASTM D586-97a).

Appendix D. Blood Spot Drying Device



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NCCLS consensus procedures include an appeals process that is described in detail in Section 9 of the Administrative Procedures. For further information, contact the Executive Offices or visit our website at www.nccls.org.

Summary of Comments and Committee Responses

LA4-A3: *Blood Collection on Filter Paper for Neonatal Screening Programs; Approved Standard—Third Edition*

Section 1.3, Applications (formerly Section 2.3)

1. Defining the age that fingersticks can be done might be helpful. I know it is not allowed, but it would reinforce the point if the standard were defined.

- **This has been addressed in Section 1.4 with the additional sentence: "With older children (greater than one year of age) and adults, the palmar surface of the finger's last phalanx is most frequently used. (See the most current edition of NCCLS document H4—*Procedures and Devices for Collection of Diagnostic Blood Specimens by Skin Puncture.*)"**

Section 2.2, Other (formerly Section 2.4)

2. Is cord blood acceptable?

- **Yes, consult local regulations and institutional policies for recommended uses of cord blood.**

Section 3.1.1, Preliminary Steps

3. "Address imprint devices should be carefully used so that…" I would encourage this section be stated more strongly to discourage the use of the imprint devices.

- **Section 3.1.1 has been modified with the addition of the following statement: "Address imprint devices (or adhesive labels) should never be used unless the handling process ensures that patient information is not obscured and the blood collection area is not compromised."**

Section 3.1.5, Puncture

4. Nurses cannot control the depth of the heel puncture on an infant when they are using a sterile lancet that has a blade longer than the recommended puncture depth of approximately 2.0 mm. Since the intent is to prevent injury to the foot, it would appear that the standard needs to be reworded to be more specific regarding depth of puncture and/or the length of the blade of a sterile lancet.

- **The comment was reviewed and the selection of the sterile lancet for a depth of a puncture no greater than 2.0 mm was addressed in Section 3.1.5.**

Section 3.5.1, Drying (formerly Section 3.1.8)

5. A picture of a filter paper drying horizontally might be helpful. I recommend a comment not to dry under or close to fluorescent lights.

- **A picture of a filter paper drying horizontally has been included in Appendix D. The text in Section 3.1.8 (Section 3.5.1 in revised document) has been modified to include: "…(indirect room light is not usually detrimental unless accompanied by heat)."**

Section 3.5.3, Timing and Transport (Mailing) (formerly Section 3.1.10)

6. Is the "h" an acceptable abbreviation?

- **"Hours" has been spelled out.**

Section 3.5.4, Storage During and After Analysis (formerly Section 3.1.11)

7. How is "Storage" related to "Techniques for Blood Collection on Filter Paper?"

- **Sections 3.1.8, Drying; 3.1.9, Stacking; 3.1.10, Transport (Mailing); and 3.1.11, Storage have been changed to Sections 3.5.1 through 3.5.4 and moved to Section 3.5, Specimen Handling.**

Section 3.2, Capillary Tube

8. Regarding the reference to NCCLS document 4, would this reference be easily accessible to a health care provider? References to previous documents are cumbersome.

- **Reference to NCCLS document H4 has been removed from that statement, because it was not essential.**

9. There are so many elements to newborn screening that benefit from standardization, that anytime "exceptions" (such as capillary tube and dorsal hand vein) enter into the process, there is increased risk of missing identifying a newborn with one of the diseases, not to mention increased cost to the system (for provider education and quality assurance) and less cost-effectiveness overall.

- **The working group has reviewed the comments and believes that none of the collection methods can be completely excluded based on scientific evidence. Appropriate changes have been made in Sections 3.2 and 3.3 to identify the risks and limitations associated with each collection method.**

10. Regarding blood draws from a central line, can it never be used or is there an acceptable timeframe after the IV has been discontinued?

- **The following statement has been added to Section 3.3: "Blood should not be drawn from an extremity into which IV fluids (including blood) are being or have been infused unless appropriate precautions are taken…Consult appropriate local regulations and institutional policies for special applications."**

Section 3.2.1, Collection

11. I recommend a statement to check the expiration date on the filter paper. You might explain why they expire.

- **"Expiration date of specimen collection device" has been added to Section 5.1 (13) and "Ensure that the expiration date of the specimen collection device (card) has not passed" has been added to Section 3.1.1.**

Section 3.2.2, Application

12. I recommend a statement not to touch the filter paper.

- **The following statement has been added to Section 3.2.2: "To avoid damaging the filter paper fibers, do not allow the capillary tube to touch the filter paper."**

Section 4.1.1, Specifications

13. A manufacturer changed its filter paper manufacturing process, claiming that the old and new filter papers were similar and met NCCLS specifications even though the absorption capacities were significantly different. Consequently, it appears that the actual NCCLS recommendation define too wide "desirable" specifications. We suggest that in the next NCCLS revision of this document that some of the specifications be reconsidered in the narrowest way.

- **Section 4.1.1 has been modified to include the statement "validation should be available from the paper manufacturer upon request." While tightening the acceptable limits could be achieved, there is no evidence in the available data that this would improve the overall performance of the system. All filter papers used for newborn screening should be validated to meet criteria provided in this standard (Appendix B, ref. 3).**

Sections 4.1.2, Lot Number/Sources and 5.1, Minimum Preprinted Information

14. Section 4.1.2 states " The manufacture's name and lot number for filter paper must be indicated on all specimen collection devices (cards; filter paper portion)" and in Section 5.1(12) "manufacturer and lot number of filter paper indicated on the filter paper section." Our interpretation of these sections allows for the printing of the above required information directly on the filter paper or for this information to be printed on paper which is secured to the filter paper in what is intended to be a permanent attachment. It is our belief that this method meets the intent in which the above standards were written.

- **The intent of the quoted statements in Sections 4.1.2 and 5.1(12) is to assure that the manufacturer (source) and the production lot number of the filter paper are always attached to the filter paper with the collected blood and available to the user laboratory, even if separated from the information section of the collection device. The information regarding the filter paper lot number and the manufacturer is important to the quality assurance efforts**

of the testing laboratories. For clarity Section 4.1.2 has been modified to read: "The manufacturer's name and lot number for the filter paper must be indicated on the filter paper portion of all specimen collection devices with the exception of devices in which the filter paper is embedded in the device (so-called "collection cassettes"). In this instance the information should be printed on the device. In situations in which the sample collection area was designed for division into separate parts, traceability should be maintained for each part. The information regarding the filter paper lot number and the manufacturer are important to the quality assurance efforts of the testing laboratories."

15. Feeding status is really only relevant to reliability of results for PKU when it is tested by the Guthrie method. Since a number of states have moved on to fluorometric methods or HPLC, there is not a universal need for this.

- **Feeding status has been removed as a minimum information requirement.**

16. It would be clearer to state, "date and time of specimen collection" in Section 5.1 (6), and important to add "time" to "birth date" in Section 5.1 (2). Most laboratory and follow-up database systems automatically determine based on birth date & time, and collection date and time, whether or not the specimen is considered drawn early and therefore the baby is in need of a repeat specimen. Birth time is also extremely useful to follow-up in the case of multiple births (twins/trips etc.) when no first name is listed, and as boy A or B get their actual first names.

- **Text was changed in Section 5.1(2) [now 5.1(4) in the revised document] with the addition of "(optional: time of birth)" and in 5.1 (6) with the addition of "(indicate if less than 24 hours)."**

17. Birthplace should be added, because it is valuable in matching the correct baby for tracking and follow-up. Often times the submitter is NOT the same as the birthplace (e.g., home births, transferred babies, repeat specimens).

- **Birth facility has been included as an optional element in Section 5.1.**

18. Why is it important to have the manufacturer and lot number of FP also imprinted on the patient information section of the form? The unique identifier number or bar code would seem more useful, to have on both the blood spot section and the identifying information section, in case (and it does happen) the blood spots are separated from the identifying information.

- **Section 4.1.2 was modified to clarify this requirement.**

19. The parent's address and phone should be optional. This is essential information for follow-up.

- **The statement, "Consult local regulations and institutional policies for deviations from minimum preprinted information," was added to Section 5.1.**

Section 5.2.1, Patient Information Section (formerly Section 5.2.2)

20. The following sentence is unclear: "Any covering piece (attached to the information section) for protection of the filter paper must be removable for blood collection and drying."

- **Section 5.2.1 was modified to read: "The patient information section of the specimen collection card must not cover (interfere with) the circles designated for blood spots unless it is removable prior to collection."**

Section 5.2.2, Specimen Device Numbers (formerly Section 5.2.3)

21. Delete: "…if the blood spot section is detachable from the card." Even if it is not made to be detachable, there are circumstances in which laboratory personnel intentionally cut away some of the blood spots, (e.g., 1 or 2 from a 5 circle card), and send them on to other laboratories for testing. Therefore, the numbers should always be preprinted on both parts of the collection device.

- **This has been adequately addressed as written. To require the addition of a preprinted number to the filter paper portion for all blood collection devices where the filter paper is not detachable would increase the cost unnecessarily for the majority of the users. If one intends to routinely remove one or more spots, filter paper designed with multiple numbers can be ordered for this purpose by the user. For random use of a selected spot from a collected specimen, removal of the spot by cutting and transfer of the number by hand is probably the most cost effective procedure.**

Appendix A

A2.8

22. Recommend statement to apply blood to one side only.

- **Since there is a possibility that the collection device could be flipped from side to side during collection, the working group has added the statement, "Apply blood to one side only." This statement already exists in Sections 3.1.6 and 3.2.2, however, Appendix A is a stand-alone page detachable from the document.**

A2.10

23. People might misunderstand the elevate statement. I am assuming you mean briefly.

- **Section A2.10 was revised to read: "After the specimen is collected, elevate the infant's foot and, using sterile gauze, briefly apply gentle pressure to the puncture site until the bleeding stops. Do not apply adhesive bandages."**

A2.11

24. A picture might be useful.

- **A picture has been added.**

A2.12

25. What is an approved container?

- **The statement, "See local regulations," has been added to Section A2.12. Changes were made also in Section 3.5.3 that address the transport of blood spot specimens.**

A3.5

26. Change "Avoid touching area within circle before collection and blood spots after collection" to avoid touching the area within the circle before and after blood collection.

- **The suggested change was made.**

A3.6

27. Add statement "do not use plastic bags."

- **Section A3.6 was revised to read: "Do not place the specimens in the transport container until thoroughly dry. Insufficient drying adversely affects test results. Use of sealed plastic bags requires desiccation. Ideally, transport specimens within 24 hours of collection."**

Summary of Delegate Comments and Working Group Responses

LA4-A4: *Blood Collection on Filter Paper for Newborn Screening Programs; Approved Standard—Fourth Edition*

General

1. I would find it easier to follow if H4 was referenced only, rather than including so much within this standard. It would keep this concise so specifics are more easily found.

- **The working group's intent was that the standard include all essential information for use of the standard without requiring the user to refer to other standards. In most cases the citation is only a reference to H4 for additional information, and the text is specific for blood spot collection and to assure consistency among the standards.**

2. How many days after birth should PKUs be drawn?

- **Ideally, the specimen for PKU should be detected after some feeding has occurred to challenge the metabolic system and so the general recommendation is for PKU sampling after 24 hours of age; however, samples drawn earlier can also give valid results. Studies have suggested that samples drawn earlier than 24 hours should have a different "expected range" against which the test results should be compared. This standard concentrates on specimen collection and submission and is not intended to provide laboratory testing advice. Time for collection may depend upon local regulations and therefore varies among programs.**

Section 3.5.3, Timing and Transport (Mailing)

3. It suggests the sample should be in the lab within 24 hours, but ours are mailed to a state lab and if a weekend/holiday is involved, this process could take 3 to 4 days. Are our results valid?

- **As stated in Section 3.5.3, "Unless otherwise directed by the screening laboratory, the collection card should be transported or mailed to the laboratory within 24 hours after specimen collection, and the appropriate tracking documentation maintained." This does not imply that the specimen must arrive at the laboratory within 24 hours; rather, that the collecting entity should get the specimen moving towards the laboratory within that period of time. While some laboratories may not be open on weekends, there is often mail pick-up so that specimens may be refrigerated over the weekend, thus providing a more controlled environment for the specimen.**

NOTES

The Quality System Approach

NCCLS subscribes to a quality 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 through a gap analysis. The approach is based on the model presented in the most current edition of NCCLS HS1—*A Quality System Model for Health Care.* The quality system approach applies a core set of "quality system essentials (QSEs)," basic to any organization, to all operations in any healthcare service's path of workflow. The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The quality system essentials (QSEs) are:

Documents & Records	Equipment	Information Management	Process Improvement
Organization	Purchasing & Inventory	Occurrence Management	Service & Satisfaction
Personnel	Process Control	Assessment	Facilities & Safety

LA4-A4 addresses the quality system essentials (QSEs) indicated by an "X." For a description of the other NCCLS documents listed in the grid, please refer to the Related NCCLS Publications section on the following page.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessment	Process Improvement	Service & Satisfaction	Facilities & Safety
GP2-A4					X						M29-A2

Adapted from NCCLS document HS1— *A Quality System Model for Health Care.*

Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, GP26-A2 defines a clinical laboratory path of workflow which consists of three sequential processes: preanalytical, analytical, and postanalytical. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

LA4-A4 addresses the clinical laboratory path of workflow steps indicated by an "X." For a description of the other NCCLS documents listed in the grid, please refer to the Related NCCLS Publications section on the following page.

Preanalytic					**Analytic**		**Postanalytic**	
Patient Assessment	Test Request	Specimen Collection	Specimen Transport	Specimen Receipt	Testing Review	Laboratory Interpretation	Results Report	Post-test Specimen Management
X H4-A4	X H4-A4	X H4-A4	X	X				X

Adapted from NCCLS document HS1— *A Quality System Model for Health Care.*

Related NCCLS Publications*

GP2-A4 **Clinical Laboratory Technical Procedure Manuals; Approved Guideline—Fourth Edition (2002)**. This document provides guidance on development, review, approval, management, and use of policy, process, and procedure documents in the laboratory testing community.

H4-A4 **Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture; Approved Standard—Fourth Edition (1999)**. A consolidation of H4-A3 and H14-A2, this standard provides detailed descriptions and explanations of proper collection techniques, as well as hazards to patients from inappropriate specimen collection by skin puncture procedures.

M29-A2 **Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Second Edition (2001).** This document provides guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting; specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure.

* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.

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