

BLOOD COLLECTION ON FILTER PAPER FOR NEWBORN SCREENING PROGRAM

1.0 PURPOSE

- 1.1** The purpose of this procedure manual is to detail the proper collection of blood on filter paper for testing in the newborn screening laboratory.
- 1.2** The collection facility is to follow these instructions to ensure reliable results from the screening.

2.0 SPECIMEN

2.1 Conditions for patient preparation

2.1.1 Source of blood

2.1.1.1 Collect the blood from the infant's heel using the most medial or lateral portion of the plantar surface of the heel, where "medial" is defined as the 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 instructions page NCCLS Appendix A. Section A2.3). Do not use previous puncture sites or the curvature of the heel.

2.1.1.2 Do not perform skin punctures for obtaining blood specimens on the central area of a newborn infant's foot (area of the arch). This may result in injury to nerves, tendons, and cartilage and offers no advantage over puncturing the heel.

2.1.1.3 Do not perform skin punctures on the fingers of newborns or infants. 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 with available lancets the bone could easily be damaged. In newborns, local infection and gangrene may be a complication of finger punctures.

2.1.2 Time of specimen collection and data reporting procedure

2.1.2.1 A specimen should be collected at greater than 24 hours of age or before the infant is discharged from the hospital regardless of age. Collect a specimen no later than 7 days of age.

2.1.2.2 Specimens collected when infant is less than 24 hours old are tested but results reported as inconclusive, and a repeat is requested. The repeat should be collected as soon as possible.

2.1.2.3 Infants and children with known metabolic disorders who are being followed by the Office of Laboratory Services must use the same collection procedures as those specified for newborn infants, except finger-sticks may be used for children six months of age or older – see Section I.A.3.

2.2 Technique for specimen collection (See instruction sheets in addition to the following):

2.2.1 Complete the required information on the blood collection form. Use a ball-point pen. Do not use soft-tip pens because the information will not copy through to the other sheets of paper. Do not use a typewriter on the blood collection forms. Before and during collection of the specimen, avoid touching the area within the circles on the filter paper, and after the specimen has been collected, do not touch the blood spots. Do not allow water, feeding formulas, antiseptic solutions, or other materials to come into contact with the specimen. The blood collector should wear gloves, take all appropriate precautions for handling blood, and dispose of used lancets in a biohazard container for sharp objects.

2.2.2 Warming the skin-puncture site can increase blood flow through the site. A warm, moist towel or infant heel warmer at a temperature no higher than 42°C may be used to cover the site for 3 minutes. This technique increases the blood flow sufficiently and will not burn the skin. Also, holding the infant's leg in a position lower than the heart will increase venous pressure.

2.2.3 Clean the skin with an alcohol swab [isopropanol-water (70/30 by volume, "70%")]. Wipe off the excess alcohol with dry sterile gauze and allow the skin to air-dry. Alcohol residue remaining on the skin may dilute the specimen and adversely affect test results.

2.2.4 To obtain sufficient flow of blood, puncture the infant's heel with a sterile lancet with tips 2.0 mm long. Wipe away the first drop of blood with sterile gauze.

2.2.5 Gently touch the filter paper against a large drop of blood and, in one step, allow a sufficient quantity of blood to soak through to fill a preprinted circle on the filter paper. Apply blood to only one side of the filter paper.

Examine both sides of the filter paper to make sure that the blood 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. (For treatment of the puncture site after specimen collection, see NCCLS Appendix A, Section A2.10.)

2.2.5.1 Milking or squeezing the puncture site may cause hemolysis of the specimen and mix tissue fluids with the specimen.

2.2.5.2 Do not layer successive drops of blood to the printed circle. If blood flow diminishes so that circles are not completely filled, repeat the sampling technique using a new circle. Do not apply blood more than once to the same collection circle on the filter paper.

2.2.5.3 USE OF A CAPLLARY TUBE FOR SPECIMEN COLLECTION IS NOT ACCEPTABLE as it does not present a homogenous sample and becomes problematic with tests that require DNA amplification.

2.2.5.4 Apply the blood to only one side of the filter paper. Do not use multiple applications – caking or heterogeneous spreading will occur that may adversely affect test results.

2.2.6 Collect the required blood spots, one drop of blood in each preprinted circle of the filter paper.

2.2.7 Avoid touching or smearing the blood spots. Allow the blood specimen to air-dry in a suspended horizontal position for at least 3 hours at ambient temperature (15 to 22°C), but not in direct sunlight (indirect room light is not usually detrimental). Do not heat, stack, or allow blood spots on the filter paper to touch other surfaces during the drying process.

2.2.8 When the specimen has dried, place the biohazard flap over the filter paper and place the specimen in the pre-addressed mailing envelope.

2.2.9 Mail the collection card to the laboratory within 24 hours after collecting the specimen. **AVOID MAILING DELAYS AT THE COLLECTION SITES.** (See Section 4.0 Handling Conditions: Transport and Storage)

3.0 TYPE OF SPECIMEN

3.1 Blood collected on filter paper specifically manufactured for collection of neonatal blood specimen. It is produced under FDA approved protocol to ensure homogeneity and uniform volumetric absorption of blood. Quality control checks of each lot number are made by Centers for Disease Control (CDC).

3.2 Amount of specimen required

3.2.1 Optimum – 5 preprinted circles filled with one drop of blood and saturated to opposite side of filter paper card (Preferred in case it is necessary to repeat a test.)

3.2.2 Minimum – 4 preprinted circles filled with one drop of blood and saturated to opposite side of filter paper card.

3.3 Acceptable collection materials

3.3.1 Collection facilities (i.e. hospitals, doctor's offices, county health departments, clinics, etc.) MUST USE Newborn Screening Kits provided by the Office of Laboratory Services (OLS).

3.3.2 Newborn Screening Kits are ordered by collection facilities from the OLS Container section using forms provided with Newborn Screening Kits.

3.3.3 Collection facilities should:

3.3.3.1 Designate one person to whom Newborn Screening Kits should be sent. This prevents kits from being misplaced within the facility.

3.3.3.2 Order a three month supply of the kits.

3.3.3.3 Discard all cards on hand when a new shipment is received. Storing cards for more than three months may create absorption problems which could result in unsatisfactory specimens.

3.4 Stability of specimens

3.4.1 Specimens are stable for seven days from the date of collection until the date of receipt in the laboratory provided they have not been exposed to adverse climatic conditions during handling and/or transit.

3.5 Criteria for an unacceptable specimen

3.5.1 Specimen quantity not sufficient

3.5.2 Specimen contaminated

- 3.5.3** Specimen too old – more than seven days has elapsed between date of collection and date received in laboratory.
- 3.5.4** Information requested on report form not provided
- 3.5.5** Specimen layered – blood applied on top of blood or applied on both sides (front and back) of specimen card
- 3.5.6** Blood collection form with no specimen
- 3.5.7** Infant’s name not on blood collection form
- 3.5.8** Blood did not soak completely through the filter paper
- 3.5.9** Gestational age not provided

3.6 Action taken by laboratory when a specimen is unacceptable

- 3.6.1** Specimen will be reported as unacceptable with a description of why specimen was unacceptable.
- 3.6.2** A repeat specimen is requested.
- 3.6.3** Specimen is tested, but results are not reported.
- 3.6.4** Abnormal results are reported to Division of Infant and Child Health, Office of Maternal, Child and Family Health (OMCFH) who notifies the infant’s physician and requests a repeat specimen.
 - 3.6.4.1** If an abnormal result for an unacceptable specimen is obtained, the decision concerning whether or not to notify OMCFH will depend upon:
 - 3.6.4.1.1** The condition of the specimen
 - 3.6.4.1.2** Test performed ex: For a QNS specimen a low Gal-1-PO₄ UT test result would be expected; therefore, when the actual result is low, OMCFH would not be notified.

3.7 Physical characteristics of the specimen that may compromise test results:

- 3.7.1** Specimen too old

- 3.7.2 Specimen contaminated – that is, by touching with fingers, liquids, etc.
- 3.7.3 Specimen quantity not sufficient
- 3.7.4 Specimen layered i.e., blood applied on top of blood or applied on both sides of specimen card
- 3.7.5 Specimen too dilute due to “milking” of infant’s heel.
- 3.7.6 Swirling pattern due to application with a capillary

4.0 HANDLING CONDITIONS: TRANSPORT AND STORAGE

- 4.1 Specimen should be mailed to the laboratory within 24 hours after collection.
- 4.2 Dried blood specimens must not be packaged in airtight, leak-proof plastic bags because heat build-up and moisture accumulation in the inner environment of a sealed plastic bag can adversely affect the dried specimen. The use of desiccant packs can reduce moisture accumulation, but shipping conditions remain uncontrolled and any desiccant has limited effectiveness.
 - 4.2.1 However, if a specimen dried onto filter paper is reasonably believed to contain an etiologic agent (that is, if there is clear underlying evidence that one or more etiologic agents are present), the packaging envelope should also bear a biohazard label to meet the requirements of the regulation.
- 4.3 Specimen mail is picked up twice each morning from the post office and these specimens are tested on the same day. Any specimens received in the afternoon are placed in the refrigerator in a desiccator for testing the next morning.

5.0 SPECIMEN COLLECTION KIT

- 5.1 A newborn specimen kit for specimen collection facilities includes:
 - 5.1.1 Blood collection form
 - 5.1.2 Pre-addressed envelope
 - 5.1.3 Newborn Screening Specimen Collection Instructions
 - 5.1.4 Re-order form for Newborn Screening specimen kits

6.0 NOTES

6.1 Questions regarding specimen collection should be directed to the office of laboratory services at (304) 558-3530 ext. 2510.