15.04.003 How to Collect an Acceptable Blood Spot Specimen

1. Identification of Newborn

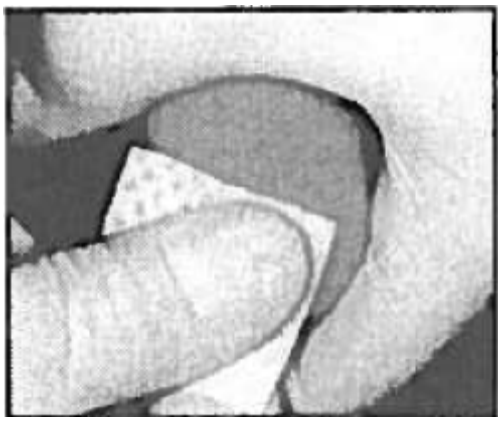
1. Most newborns will not have any form of identification or a health number, therefore the mother's identification is required to match the follow up request to the newborn patient.
2. Identify the newborn by matching to CPL request all of:
   - Manitoba Health six-digit number (health card)
   - Mother's surname (health card)
   - Baby's date of birth (copy of birth registration if available, or verbally otherwise).
   - Baby's surname (verbally)
   - For twins or multiples, their birth order (Twin A or Twin B, etc., verbally) first name is not useful.
3. Complete a blank CPL Newborn Screening card with all identifying information. For repeat collection requests, check the ☑Repeat box.

2. Preparation

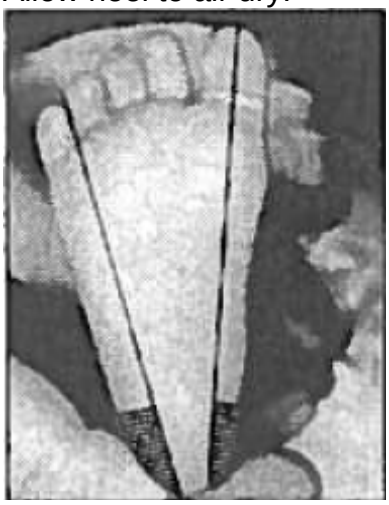
1. Wash hands vigorously
2. Wear powder-free gloves and change gloves between infants
3. Confirm identity of infant and ensure that all data elements on the form are complete, accurate and consistent.
4. Determine number of spots to collect. For first time collection or repeat request due to poor specimen collection, fill all circles. For repeat or subsequent collections in follow up to abnormal results, fill 2 or 3 circles.

3. Sampling Technique

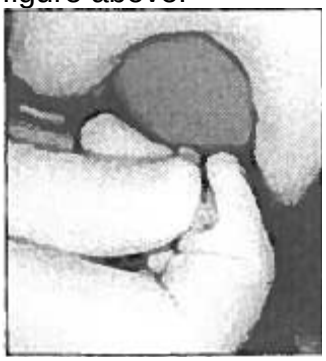
1. Warm heel for puncture (incision/stick) site. Heel warming devices containing an exothermic thermochemical composition are commercially available, or warm site with soft cloth, moistened with warm water (less than 42°C) for three to five minutes. In some situations, warming site may not be necessary to increase blood flow and volume.
2. Position the infant's leg lower than the heart to increase venous pressure.



3. Wearing gloves, wipe infants' heel with 70% isopropyl alcohol.
4. Allow heel to air dry.



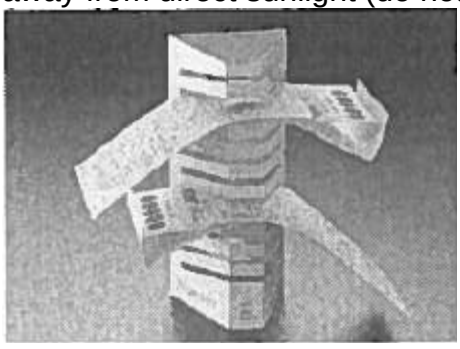
5. The puncture should be made within the shaded area as illustrated in the figure above.



6. Using a sterile lancet of recommended length, perform puncture (depth <2.0 mm) as illustrated or use an incision device. An incision device provides superior blood flow by making a standardized incision 1.0 mm deep by 2.55 mm long.

7. Gently wipe off first drop of blood with sterile gauze or cotton ball (initial drop contains tissue fluids, which might dilute sample).
8. Wait for formation of large blood droplet.
9. Apply gentle pressure with thumb around the heel but not near the puncture site, and ease intermittently as drops of blood form.

10. 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.
11. All used items should be disposed of in an appropriate biohazard container.
12. After the specimen is collected, elevate the infant's foot and, using sterile gauze or cotton ball, briefly apply gentle pressure to the puncture site until the bleeding stops. Do not apply adhesive bandages.
13. Allow blood specimen to AIR DRY THOROUGHLY, on a horizontally level, non-absorbent, open surface, such as a drying rack or plastic-coated test tube rack, *for a minimum of three hours* at ambient temperature. Keep specimen away from direct sunlight (do not stack or heat).



14. After the specimen has dried, place in an approved container for transport (see local regulations).

4. Pitfalls

- Failure to allow residual alcohol to dry might dilute the specimen and adversely affect test results.
- Puncturing the heel on posterior curvature will permit blood to flow away from puncture, making proper spotting difficult.

IMPORTANT: Do not use previous punctures sites.

- "Milking" or squeezing the puncture might cause hemolysis and admixture of tissue fluids with specimen.
- Do not layer successive drops of blood on the target spot. If blood flow diminishes to incompletely fill circles, REPEAT Sampling Technique in section 2.
- Avoid touching the area within the circle before and after blood collection. Do not allow water, feeding formulas, antiseptic solutions, or powder from gloves or other materials to come into contact with the specimen card before or after use.
- 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.

5. Specimen Handling and Transport

5.1 Drying

- Avoid touching or smearing the blood spots.
- Allow the blood specimen to air dry on a horizontally level, non-absorbent, open surface for at least three hours at an ambient temperature of 18°C to 25°C.
- If a protective flap is present, keep it away from the specimen during drying.
- Keep the specimen away from direct sunlight (indirect room light is not usually detrimental unless accompanied by heat or surfaces cleaned with bleach).
- Blood spots on the filter paper should not be heated, stacked, or allowed to touch other surfaces during the drying process.
- Specimens should not be placed in (hermetically) sealed containers and transported through pneumatic tubes prior to adequate drying.

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, the dried blood spots on the collection card should be rotated 180° from the blood spot 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 folder-over cover attachment can be added to the specimen collection device. This attachment, added when forms are

manufactured, provides protection from contamination prior to blood collection, during specimen transportation, and during specimen storage after analysis. Blood spots must be thoroughly dry before the flap is closed over spots.

2. Glassine paper can be placed between specimens.

5.3 Timing and Transport

- The dried blood specimen should be transported or mailed to the laboratory within 24 hours after specimen collection, and the appropriate tracking documentation maintained with periodic review for timely delivery assurance. Daily courier transport is recommended whenever possible. Delays at collections ties should be avoided, and the shipping environment relative to possible delays should be structured to maximize transport efficiency.
- "Standard Precautions" are to be followed in collecting and preparing these specimens for shipment.
- Dried spot specimens must not be packaged in airtight, leak-proof sealed containers (e.g. plastic or foil bags) because the lack of air exchange in the inner environment of a sealed container causes heat buildup and moisture accumulation. Heat, direct sunlight, humidity, and moisture are detrimental to stability of dried blood spot specimens and analyte recovery. The inclusion of desiccant packs will aid in preventing moisture accumulation, but shipping conditions are uncontrolled, and desiccant has limited effectiveness

6. References

- Clinical and Laboratory Standards Institute (CLSI). *Blood Collection on Filter Paper for Newborn Screening Programs: Approved Standard—Fifth Edition*. CLSI document LA4-A5 (ISBN 1-56238-644-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007.

7. Approval and Revisions

This document was approved by: R Van Caeseele 15.04.003 2014.01.27

Paul Van Caeseele, MD FRCPC Date
Medical Director, CPL

Version	Changes	Date
1	New	2014.01.27
1.1	Section 3, 6., incision depth should be 1.0mm, not 10mm.	2014.02.03
