



<b>Title:</b>	Processing of Dried Blood Spots Standard Operating Procedure		
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## 1 Purpose

The purpose of this SOP is to document the procedures for collecting, packaging, storing and shipping samples collected as dried blood spots (DBS).

## 2 Scope

Users of the ACTG/IMPAACT Laboratory Manual

## 3 Background

Dried blood spots (DBS) have been used for newborn screening, therapeutic drug monitoring, antibody testing and nucleic acid testing for an extensive period of time. Dried blood spots provide an easy and inexpensive way to collect and store peripheral blood specimens from infants, children and adults and are especially important in low-resource settings where storing and shipping frozen plasma are difficult. Dried blood spots are prepared by applying a small amount of peripheral blood to filter paper cards from heel sticks (from infants), finger sticks (from adults), or anticoagulated whole blood. Finally, DBS can be shipped ambient as non-dangerous goods, which offers both flexibility and cost-savings.

## 4 Authority and Responsibility

- 4.1 The Network Laboratory Directors (or his/her designee) have the authority to establish, review and update this procedure.
- 4.2 The ACTG/IMPAACT Laboratory Technologist Committee (LTC) is responsible for the maintenance and control of SOP documentation.
- 4.3 The Laboratory Director is responsible for the implementation of this LTC SOP or laboratory-specific SOP and for ensuring that all appropriate personnel are trained. A laboratory SOP must:
  - 4.3.1 Include, without procedural modification, the portions of the current version of the LTC SOP that are used within the network site-affiliated laboratory
  - 4.3.2 Reference the current version of the LTC SOP
- 4.4 All laboratory technicians are responsible for reading and understanding this SOP prior to performing the procedures described.
- 4.5 The site PI and designees are responsible for understanding and adhering to the patient preparation and specimen collection components.

## 5 Reagents and Materials

- 5.1 Materials for all methods of specimen collection (see Figure 1)
  - 5.1.1 Alcohol swab

- 5.1.2 Whatman Protein Saver Card #903 (Whatman #10534612; Fisher Scientific #NC9307519)

Note: These guidelines refer specifically to DBS created from Whatman 903 paper. Other types of DBS paper are available (e.g. FTA-DMPK-A, FTA-DMPK-B, FTA-DMPK-C) and may be designated in a given protocol. Specific instructions with respect to blood volume, storage conditions and extraction methods should be provided in the protocol or by the manufacturer. Labeling and packaging requirements outlined in this document should be followed regardless of the paper used. Customized card configurations may be specified within a protocol. **Ensure that the appropriate cards are available before collecting any specimens.**

- 5.1.3 Gas-impermeable storage bag (Whatman #10548232; Fisher Scientific #50853570)
- 5.1.4 Desiccant pack (Whatman #10548234; Fisher Scientific #50853571)
- 5.1.5 Humidity indicator Cards (Multisorb Des Manufacture #MS200032; Fisher Scientific #NC9511648 or equivalent)
- 5.1.6 Whatman card drying rack (VWR catalogue #89015-592; Whatman #10537173; Whatman # 10539521 or equivalent)
- 5.1.7 Gloves, preferably powder-free
- 5.1.8 Waterproof marker
- 5.1.9 Glassine Envelopes, 3 1/4 inches x 4 7/8 inches, 100-pack (Whatman #10548236; Fisher Scientific # 50853572); optional

Figure 1: Materials needed for all methods of specimen collection.



- 5.2 Materials specifically needed for heel stick method
  - 5.2.1 Unistick 2 device (Fisher Scientific #22-0227)
  - 5.2.2 Dry sterile gauze pad
- 5.3 Materials specifically needed for finger stick method
  - 5.3.1 Lancet/needle
- 5.4 Materials specifically needed for venous blood method using Vacutainer® Tubes
  - 5.4.1 Vacutainer Evacuated Blood Collection Tubes
  - 5.4.2 Tourniquet
  - 5.4.3 Bandage/plaster

5.4.4 Vacutainer Needle

5.4.5 Vacutainer Needle holder

## 6 Biosafety

It is essential that universal precautions be taken while working with or collecting DBS specimens. Dried blood spots on filter paper are not considered to be a biohazard, but whole blood handled during specimen collection and reconstituted specimens obtained during processing may be hazardous. Appropriate personal protective equipment, including gloves and a lab coat/gown, should be worn at all times, to ensure safe handling of samples. If you should tear a glove, remove the torn one and replace it immediately. If a needle puncture should occur, follow your site/institution policy for handling work-related injuries.

## 7 Sample Chain of Custody

It is essential that each sample be logged into the LDMS. If DBS are prepared in the clinic and transported to the lab, immediately compare the inventory from the clinic to the specimens received. If there are ANY discrepancies, please notify the lab manager immediately as action needs to be taken to try and recover samples.

## 8 DBS Labeling

- 8.1 Log cards prepared using the venous blood methods should be entered into the LDMS with the primary type=BLD, additive=EDT (or appropriate additive if a different tube type is received) and derivative=DBS.
- 8.2 Log cards prepared using the heel or finger stick method should be entered into the LDMS with the primary type=FST (finger stick) or HST (heel stick), additive=NON, and derivative=DBS.
- 8.3 Ensure that each LDMS label contains the protocol (ACTG/IMPAACT) required identifiers, which include but are not limited to the patient ID, visit, sample dates, and draw times.
- 8.4 Number of aliquots per card
  - 8.4.1 For protocols that require each spot have a unique identifier: Log the DBS into the LDMS with the number of aliquots created based on the number of whole spots per card. For each complete spot, create one 50-80- $\mu$ L aliquot using the DBS derivative in the LDMS. If a spot is not complete, estimate the volume the spot contains (one drop is approximately 50 $\mu$ L of blood). Place each label near the DBS spot; each card will have up to 5 LDMS labels (see Figure 8).

- 8.4.2 For protocols which do not require that each spot have a unique identifier: Log the DBS into the LDMS; each aliquot may contain a volume of a spot (50-80uL) or the total volume of the card (250-400uL). Create one LDMS label if the total card volume is used; print multiple LDMS labels if each spot is to be labeled as a separate aliquot.

Note: Special-order DBS cards may be needed for labeling individual spots on cards.

- 8.4.3 Ensure that the label(s) are FIRMLY AFFIXED to the card/spots. For ease of handling it is also recommended to affix label(s) to the outside of the glassine envelopes or placed inside the bag so the label is viewable through gas impermeable bag after storing the card as detailed below.

Note: Labels affixed to the outside of the gas impermeable bags may fall off during storage in a freezer.

## 9 DBS Specimen Collection

### 9.1 Collection of dried blood spots from heel stick

The Unistick 2 device has a penetration depth of 1.8 mm and a convex tip for accurate positioning to eliminate the risk of accidental needle stick injury or cross-infection and reduce the risk of osteomyelitis of the heel.

- 9.1.1 Clearly label the Whatman Protein Saver 903 Card/spots with Patient ID and Date, Protocol, Visit ID using a marker or LDMS generated label(s).

Note: DBS cards collected for use in ACTG and IMPACT must include LDMS labels.

- 9.1.2 Prepare the Unistick 2 device by depressing the pink plunger until it clicks. Twist the plunger until it breaks off and remove it from the device. The Unistick 2 device is now ready to trigger.

- 9.1.3 Preferably, the baby should be in a supine position (lying down face up) with the knee at the edge of the table. This position allows for the foot to hang lower than the torso, improving blood flow. Alternatively, the baby may be held in the mother's arms.

- 9.1.4 Clean the incision area of the heel with an alcohol pad and allow the heel to air dry. Do not touch the incision site or allow the heel to come into contact with any non-sterile item or surface.

- 9.1.5 Wrap your index finger around the base of the heel and your thumb around the ankle.

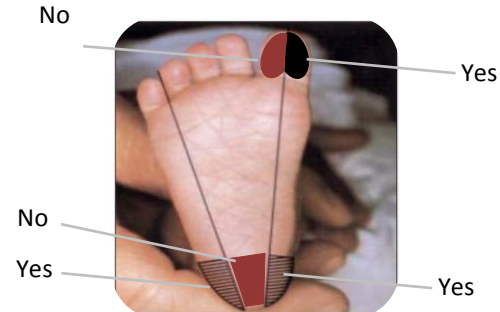
- 9.1.6 Position the device properly against the outside of the heel or big toe (see black shaded areas in Figure 2).

9.1.6.1 Draw an imaginary line from midpoint of the big toe to the heel and one from between the 4th and 5th toe to the heel.

9.1.6.2 Black areas indicates safe areas for puncture site (see Figure 2).

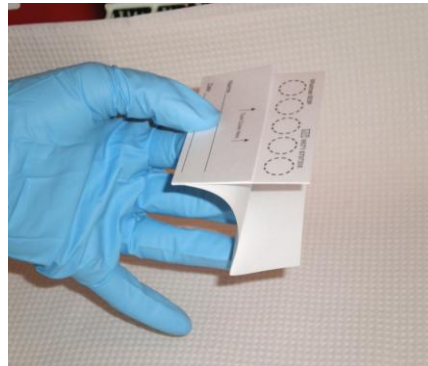
- 9.1.6.3 Do not puncture the back of the heel or Achilles tendon or the medial aspect of the big toe. Solid red areas indicate areas that are not safe for puncture (see Figure 2).

**Figure 2: Heel stick placement and device**



- 9.1.7 Trigger the Unistick 2 heel-stick device.
- 9.1.8 Wipe away the first drop of blood using a dry sterile gauze pad.
- 9.1.9 Hold the Whatman Protein Saver 903 Card without touching the filter paper (see Figure 3).

**Figure 3: Hold the Whatman Protein Saver 903 Card without touching the filter paper.**



- 9.1.10 Allow the blood from the heel to flow for collection onto the dried blood spot card by gently touching the filter paper card to the blood drop. Allow the card to absorb the blood until the circle is full. You should be able to obtain 5 spots of blood on a card. (You may need to squeeze the heel to obtain more blood. However, do not milk the heel or interstitial fluid will be mixed in with the blood.) Do not touch the DBS circle once blood is applied. See examples of good and bad spots in *Section 10: Examples of Invalid Spots and Invalid Collections of DBS*.
- 9.1.11 Gently press a sterile pad to the incision site until bleeding stops.
- 9.1.12 Monitor the baby's heel for late bleeding and inflammation. Place a sterile pad over the wound to prevent formation of a hematoma.

**Note:** Bandaging the baby's foot is controversial because of skin sensitivity. The incision should be monitored by the primary care nurse for bleeding and



inflammation. A bandage is not necessary as long as the bleeding has stopped before the child leaves the clinic.

9.1.13 Proceed to Section 7: Specimen Drying.

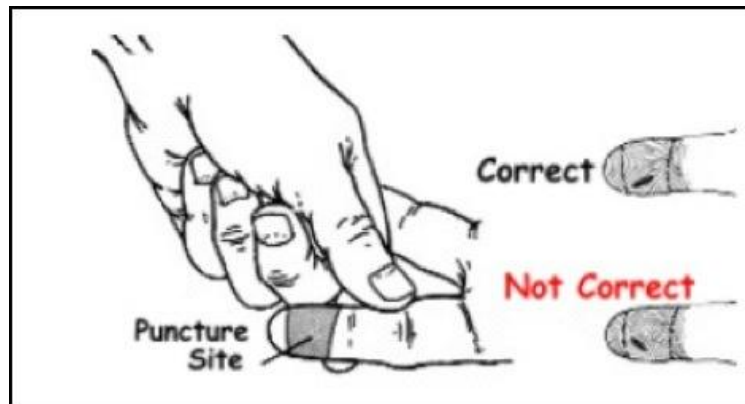
9.2 Collection of dried blood spots from finger stick

9.2.1 Clearly label the Whatman Protein Saver 903 Card with Patient ID and Date, Protocol, Visit ID using a marker or LDMS generated label(s).

Note: DBS cards collected for use in ACTG and IMPACT must include LDMS labels.

9.2.2 Disinfect selected site on the finger and prick using a lancet/needle (See Figure 4).

**Figure 4: Disinfect selected site on the finger and prick using a lancet/needle.**



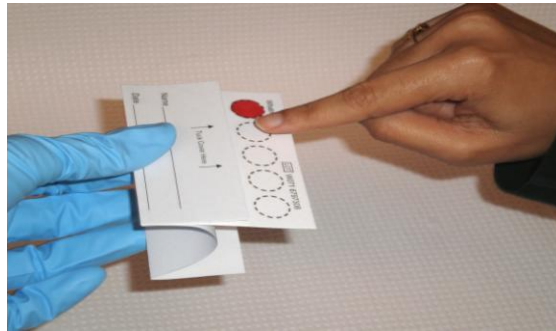
9.2.3 Hold the Whatman Protein Saver 903 Card without touching the filter paper (see Figure 3).

9.2.4 Uniformly saturate the entire circle by quickly and gently touching the drop of blood to the Whatman Protein Saver 903 Card (see Figure 5). **Do not press the puncture site to the filter paper or touch the Whatman Protein Saver 903 Card at any stage of collection.** Do not touch the DBS circle once blood is applied. See examples of good and bad spots *Section 10: Examples of Invalid Spots and Invalid Collections of DBS.*

9.2.5 After collecting 5 dried blood spots, clean the site and leave it un-bandaged.

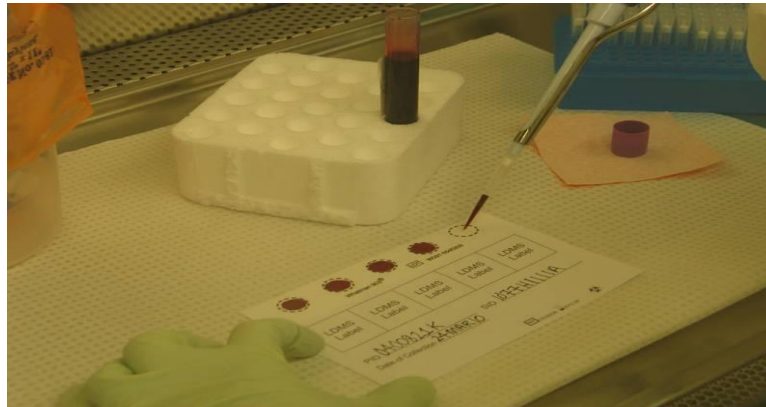
9.2.6 Proceed to Section 10: Specimen Drying.

Figure 5: Collection of DBS from Finger Stick



- 9.3 Collection of venous blood using EDTA tube (other tubes as determined by protocol team)
  - 9.3.1 Vacutainer Evacuated Blood Collection Tubes are designed to be filled with a predetermined volume of blood by vacuum. The rubber stoppers are color-coded according to the additive that the tube contains. Collect 1 EDTA (purple top) tube at each required time point. The total volume required per card will be 1 x 0.250 or 5 x .050 mL per spot (for 50  $\mu$ L spots) or 1 x 0.400 mL or 5 x 0.080 mL per spot (for 80  $\mu$ L spots). The total blood volume needed is dependent on the number of spots to be created for each sample.
  - 9.3.2 Label the tubes with the patient ID number, the date of collection, and the study and visit identifier using a marker or LDMS generated label(s).
  - 9.3.3 Fill the blood collection tube to the recommended volume so the anticoagulant is at the proper dilution.
  - 9.3.4 Gently invert the tube (8 to 10 times) to mix the blood thoroughly. After the blood is completely mixed, remove the cap, and apply 50 to 80  $\mu$ L of the whole blood to a single spot on the Whatman Protein Saver 903 Card (see Figure 6, do not touch the card with the pipette tip. Slowly expel blood from the tip and touch the drop to the paper, allowing the blood to absorb. Care must be taken when applying larger volumes of blood to ensure the spots do not run outside the circle). Repeat four times to fill all five spots on the card. This transfer of blood should be performed with a pipette and a disposable tip. A single tip may be used to load the card; cover and mix blood by inversion in between applications if creating more than one DBS card. Do not touch the DBS circle once blood is applied. See examples of good and bad spots Section 13: Examples of Invalid Spots and Invalid Collections of DBS.
  - 9.3.5 Proceed to Section 10: Specimen Drying.

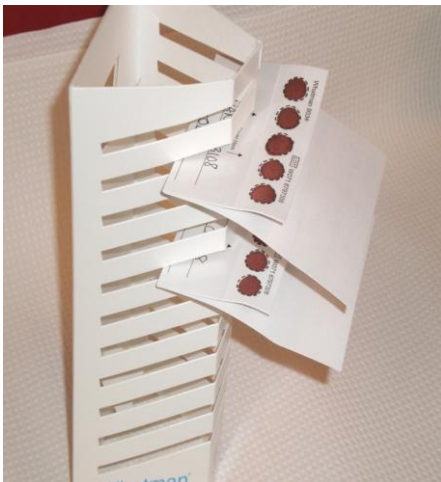
**Figure 6: Collection of DBS from a vacutainer tube (This is a customized Whatman DBS card)**



## 10 Specimen Drying

- 10.1 Allow the blood spot to air dry without the card flap covering the spots in a clean, dry place (i.e. biosafety cabinet, see drying racks in Figure 7) that is protected from rodents, insects and direct sunlight for at least 4 hours (drying overnight may be necessary in areas with higher humidity; less drying time may be warranted for some analytes, e.g. malaria parasites). Do not heat, stack or allow DBS to touch other surfaces during the drying process

**Figure 7: Whatman Protein Saver 903 Card drying rack and common drying rack**



- 10.2 Tuck in the flap of the Whatman Protein Saver 903 Card as indicated on the card. If the DBS card does not have a protector flap, it is recommended to put the DBS card in a glassine bag to protect spots from chemicals in the desiccant and humidity indicator.

Note: Protocols must define if the DBS card should be stored whole or cut into partitions for storage.

- 10.3 Seal the card (or card fractions) in a gas-impermeable, zip-lock bag containing a desiccant pack and humidity indicator. Store no more than one card per bag (See Figure 8).

Note: Do not use electric dryer or oven to dry cards. Store no more than one DBS card per bag. Do not heat, stack or allow DBS to touch other surfaces during the drying process. Use a minimum of 3 desiccant packs per zip lock bag. Humidity cards may be recharged before use. If the humidity card is pink at the 30% level, you may recharge the indicator card and desiccant pack by heating at 50-60°C for 3-4 hours in a drying oven; then cool 10 minutes. IMMEDIATELY RETURN card and desiccant pack to sealable plastic bag.

**Figure 8: Labeling and Aliquoting of DBS cards (Not all protocols will require the cutting of DBS cards for storage)**



10.4 Go on to Section 11: DBS Specimen Storage or Section 12: DBS Specimen Shipping

## 11 DBS Specimen Storage

Variable storage conditions have been reported for DBS generated from Whatman 903 paper. In general, extreme temperatures (>37°C) in the presence of high humidity are not acceptable storage conditions because they promote microbial growth and alter elution of analytes off the paper. Stability of proteins and nucleic acids (HIV DNA, HIV RNA, and HBV DNA) have been demonstrated after storage in a refrigerator (2 to 8°C) or at room temperature (15 to 30°C) for up to 1 year. Stability of genotypic HIV drug resistance testing has been demonstrated after storage in a refrigerator (2 to 8°C) or at room temperature (15 to 30°C) for up to 12 weeks. However, for optimal preservation, long term storage (> 90days) at ≤ -20°C is recommended. Protocols must define the storage conditions for DBS based on the analyte being tested, the assay being used, and the paper used for collection.

11.1 Store the DBS bag according to protocol MOPS (if available) and/or LPC until shipped to the processing or receiving laboratory. The protocol team will notify sites when to ship samples to the receiving laboratory.

11.1.1 Make sure the patient identifier and date (or LDMS generated label) are on the DBS Whatman Protein Saver 903 Card and on the outside of the bag, and that the bag is sealed (see Figure 8).

Note: Labels affixed to the outside of the gas impermeable bags may fall off during storage in a freezer.

11.1.2 Store DBS cards in sealed bags in a 2" fiberboard freezer box (regardless of the storage temperature) and inventory using the LDMS storage module.

11.1.3 Store DBS cards in a clean, dry area of lab that has no exposure to direct sunlight, is free of insects and rodents, and where ambient temperatures will not exceed 30°C.

11.1.4 Check humidity indicators in bags weekly while DBS are stored at room temperature or in a refrigerator. Change desiccant packs if indicator color changes from blue to pink (see Figure 9). Document the date of any observed humidity changes on the humidity card and in the LDMS; add a new humidity indicator and leave the old and new humidity indicators in the bag. Make sure bags are sealed tightly.

Figure 9: Example of Humidity Indicator



## 12 DBS Specimen Shipping

- 12.1 Ship DBS Whatman Protein Saver 903 Cards to a testing laboratory, or transfer the stored DBS cards to a freezer ( $\leq -20^{\circ}\text{C}$ ) within 3 months of collection (or as defined in the protocol).
- 12.2 Shipping conditions:
- 12.2.1 Check protocol for required shipping conditions to be used for shipping DBS samples to testing laboratories and/or to the specimen repository.
- 12.2.2 DBS cards that are stored at room temperature (15 to  $30^{\circ}\text{C}$ ) may be shipped under ambient conditions or on dry ice (see instructions below). Inspect DBS specimens for contamination or humidity change, and replace desiccants prior to shipping.
- 12.2.3 DBS cards stored in a refrigerator may be shipped under ambient conditions or on dry ice (see instructions below), but the DBS cards must be equilibrated to room temperature (minimum of 30 minutes) prior to shipping under ambient conditions; inspect DBS specimens for contamination or humidity change, and replace or add additional desiccants prior to shipping; document any changes in the humidity indicator in the LDMS.
- 12.2.4 DBS cards stored at  $\leq -20^{\circ}\text{C}$  may be shipped on dry ice or under ambient conditions. If the frozen cards are to be shipped under ambient conditions, the DBS cards must be equilibrated to room temperature (minimum of 30 minutes) prior to shipping under ambient conditions; inspect DBS specimens for contamination or humidity change, and replace or add desiccants prior to shipping; may record this information in the LDMS.

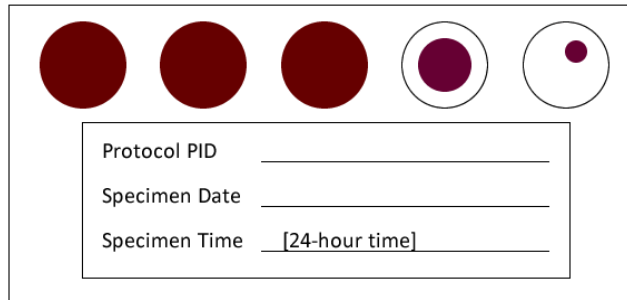
- 12.3 Ship DBS cards as non-dangerous goods (they are exempt biological specimens according to ICAO and IATA).
- 12.4 If shipping specimen boxes:
- 12.4.1 Secure boxes with a rubber band so they do not open during transit.
- 12.4.2 Place boxes in a water tight secondary container (i.e. Tyvek® bags) to protect the specimens from humidity during transit.
- Note: The box may be placed in a second ziplock bag with additional dessicants before placing into the Tyvek bag for shipping to provide additional protection against humidity.
- 12.4.3 Tyvek® bags containing boxes may be placed in larger shipping envelope or box.
- 12.5 If shipping individual specimen cards:
- 12.5.1 Place individual specimen cards in water tight secondary containers (i.e. Tyvek® bags) to protect the specimens from humidity in transit.
- Note: The individual specimen card may be placed in a second zip lock bag with additional dessicants before placing into the Tyvek bag for shipping to provide additional protection against humidity.
- 12.5.2 Place the Tyvek® bags containing individual DBS cards in a shipping envelope (ambient) or into an approved container filled with dry ice (frozen). Follow shipping guidelines for dry ice shipments.
- 12.6 Ship DBS cards by overnight or second-day courier services. Be sure to follow the ACTG/IMPAACT shipping instructions for ambient and/or dry ice shipments <http://www.hanc.info/labs/labresources/procedures/Pages/actnShippingDemo.aspx>
- 12.6.1 ACTG/IMPAACT Shipping Documents
- 12.6.1.1 Provide an LDMS Diskette or CD (place in disk/CD mailer).
- 12.6.1.2 Provide an LDMS shipping manifest for all boxes. Shipping manifest(s) must exactly match the label information and order in the associated shipment, including the global specimen IDs.
- 12.6.1.3 Provide an LDMS Box map for all boxes. Box maps must exactly match the label information and order in the associated shipment, including the global specimen IDs.
- 12.6.1.4 Provide any relevant CRFs. Confirm with receiving lab if CRFs are required. Do NOT send CRFs to the Repository.
- 12.6.2 Courier Information and Notification
- 12.6.2.1 Record courier service on ACTG/IMPAACT Specimen Shipment Notice.
- 12.6.2.2 Record courier air bill number on ACTG/IMPAACT Specimen Shipment Notice.

12.6.2.3 Advance notification of shipment must be made to the recipient. The preferred method of notification is to FAX or email completed ACTG/IMPAACT Specimen Shipment Notice.

### 13 Examples of Invalid Spots and Invalid Collections of DBS

13.1 Samples with variable blood volumes.

Sample Volume: 3 Large OK; **1 Medium, 1 Small not usable**



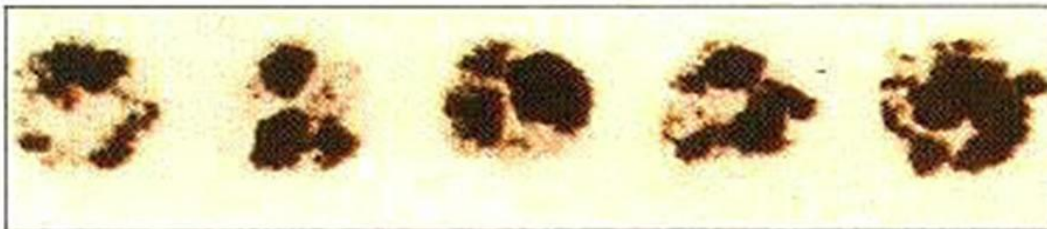
13.2 Spot with incomplete absorption resulting in insufficient blood volumes.

This specimen is invalid because quantity of blood is insufficient for testing. This may have been caused by the following:

13.2.1 Removing filter paper before blood has completely filled circle or before blood has soaked through to the other side

13.2.2 Applying blood to the DBS card with a capillary tube

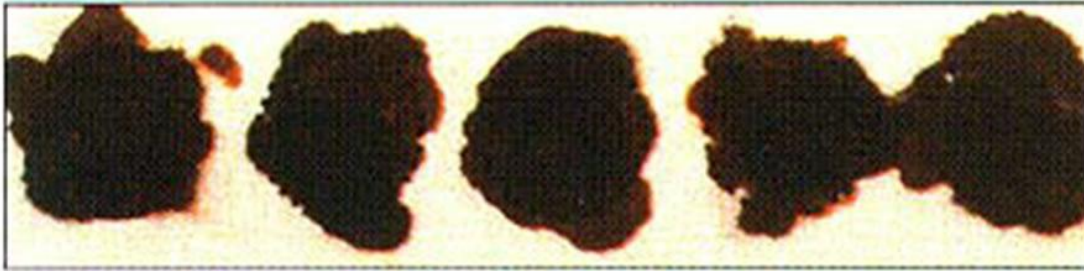
13.2.3 DBS paper coming in contact with ungloved hands or substances such as hand lotion or powder, either before or after blood specimen collection.





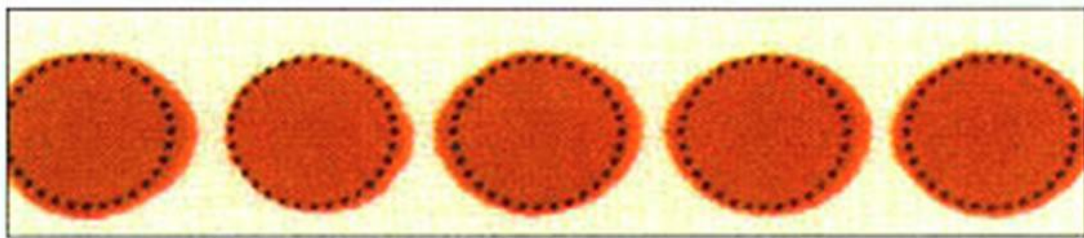
13.3 Sample that was potentially rubbed against the child's heel

This specimen is invalid because it appears scratched or abraded.



13.4 Sample not allowed to air dry before placing in storage bags

This specimen is invalid because the specimen was not dry before mailing. DBS must dry a minimum of 4 hours (preferably overnight, especially in areas with high humidity) before packaging and shipping.



13.5 Sample where blood was clotted and did not soak into paper

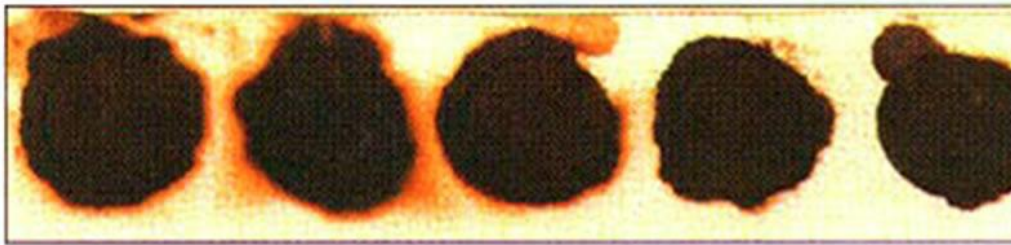
This specimen is invalid because the specimen appears clotted or layered. The volume of specimen will not be uniform between spots resulting in errors during the testing process. This may have been caused by:

13.5.1 Touching the same circle on the filter paper to blood drop several times.

13.5.2 Filling circle on both sides of filter paper



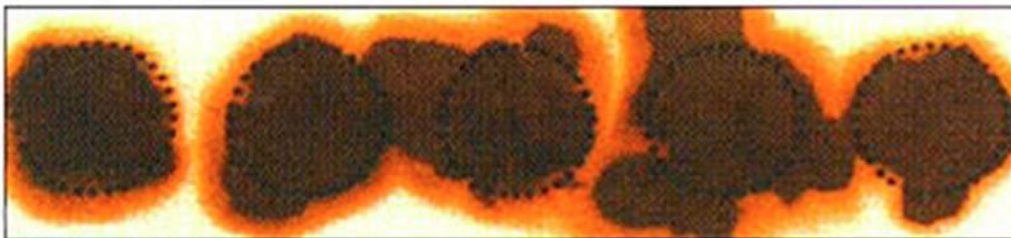
13.6 DBS with contamination or hemolyzed blood



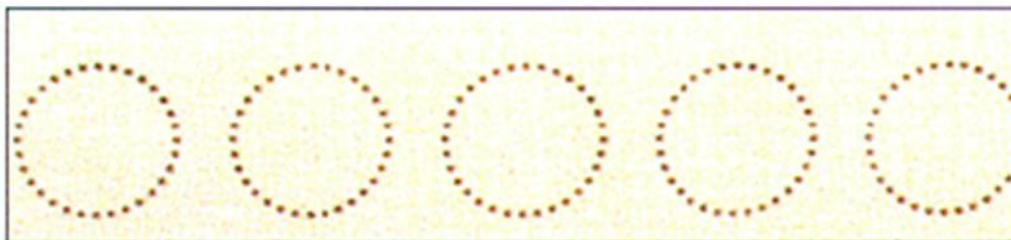
13.7 DBS where serum separated from cells

This specimen is invalid because the specimen exhibits serum rings i.e. serum becomes separate from cells. This may have been caused by the following:

- 13.7.1 Not allowing alcohol to dry at puncture site before making skin puncture
- 13.7.2 Allowing filter paper to come in contact with alcohol, hand lotion, etc
- 13.7.3 Squeezing area surrounding puncture site excessively
- 13.7.4 Drying specimen improperly
- 13.7.5 Applying blood to filter paper with a capillary tube



13.8 DBS where no blood was added to spot



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