



ACTG/IMPAACT LABORATORY STANDARD OPERATING PROCEDURE			
Title:	Dried Blood Spot Card Preparation Standard Operating Procedure		
SOP number:	LTC-SOP-55	Effective:	01Apr2023
Version:	v3.0	Last reviewed:	19Mar2012
Originator:	ACTG/IMPAACT Lab Technologist Committee	Pages:	12

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1. PURPOSE

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

2. BACKGROUND

Dried blood spots have been used for newborn screening, pharmacology and pharmacogenomic determination, antibody testing and nucleic acid testing for an extensive period of time. Dried blood spots are prepared by applying a small amount of blood to filter paper cards from anticoagulated blood collected from heel sticks (capillary blood, from infants) or anticoagulated venous whole blood.

3. SCOPE

Users of the [ACTG/IMPAACT Laboratory Manual](#) [8.1]

4. DEFINITIONS

Term	Definition
ACTG	AIDS Clinical Trials Group
DBS	Dried Blood Spot(s)
IMPAACT	International Maternal Pediatric Adolescent AIDS Clinical Trials Group
LDMS	Laboratory Data Management System
LPC	Lab Processing Chart
LTC	ACTG/IMPAACT Laboratory Technologist Committee
SOP	Standard Operating Procedure

5. RESPONSIBILITIES

- 5.1 The Network Laboratory Directors (or his/her designee) have the authority to establish, review and update this procedure.
- 5.2 The ACTG/IMPAACT Laboratory Technologist Committee (LTC) is responsible for the maintenance and control of SOP documentation.
- 5.3 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:
 - 5.3.1 Include, without procedural modification, the portions of the current version of the LTC SOP that are used within the network site-affiliated laboratory.

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5.3.2 Reference the current version of the LTC SOP.

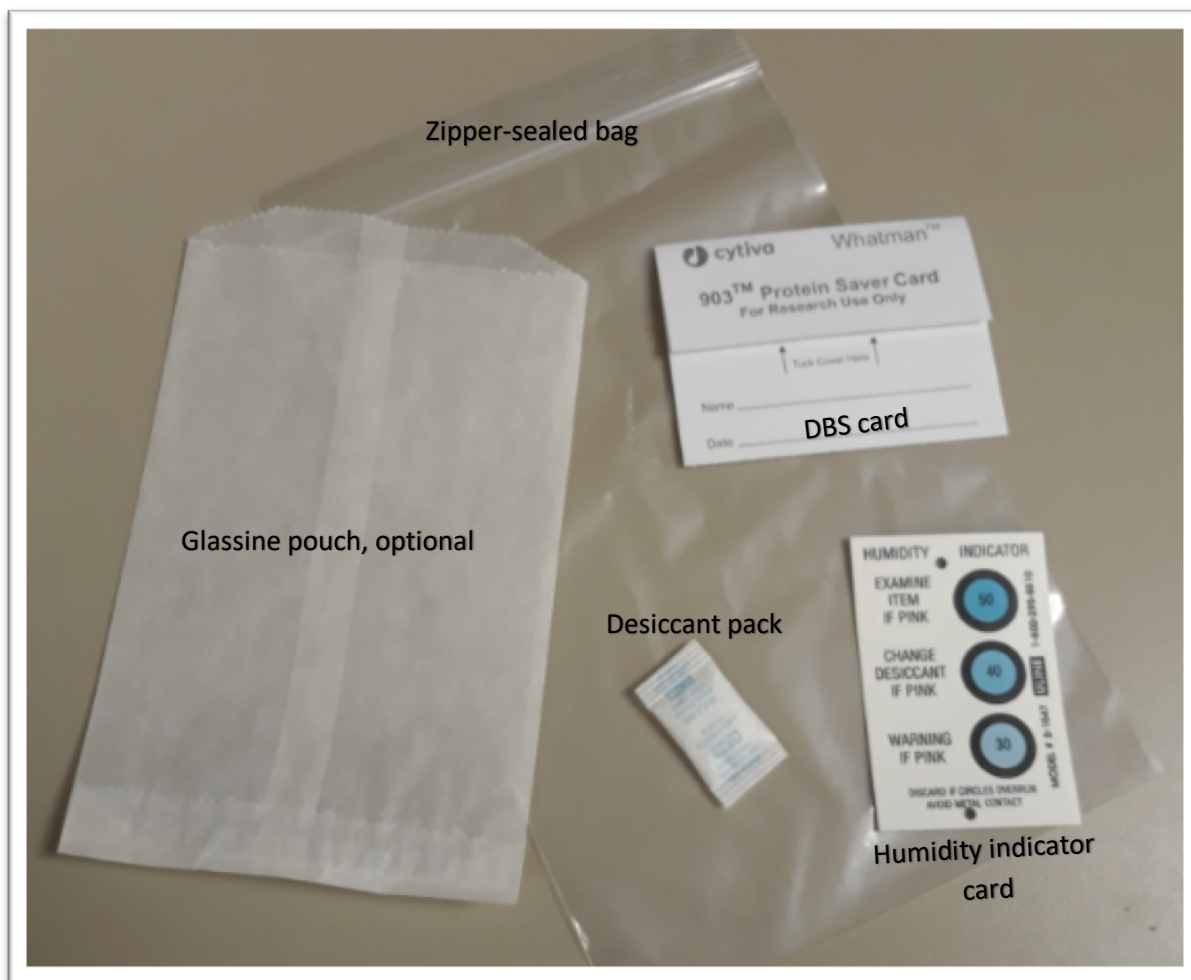
- 5.4 All laboratory technicians are responsible for reading and understanding this SOP prior to performing the procedures described.
- 5.5 The site principal investigator and designees are responsible for understanding and adhering to the patient preparation and specimen collection components.

6. EQUIPMENT, REAGENTS AND CONSUMABLES

- 6.1 Whatman 903™ Protein Saver Card (Cytiva Whatman™ #10534612 (US) / #10531018 (EU); Fisher Scientific #05-715-121)
Note: These guidelines refer specifically to DBS created from Whatman 903™ paper.
- 6.2 Moisture-proof zip-sealing storage bags (Cytiva Whatman™ #10548232; Fisher Scientific #09-800-16)
- 6.3 Desiccant pack (Cytiva Whatman™ #10548239; Fisher Scientific #09-928-142)
- 6.4 Humidity indicator cards (Multisorb Des Manufacture #MS200032; Fisher Scientific #NC9511648 or equivalent)
- 6.5 Glassine pouch, 8cm x 12cm, 100-pack (Whatman #10548236 or equivalent) or weighing paper, 152 x 152mm (Fisher Scientific #09-898-12C or equivalent); optional
- 6.6 Gloves, powder-free
- 6.7 Waterproof marker
- 6.8 Class II biosafety cabinet, splash shield, or dead air box.
- 6.9 Micropipettes and filter barrier tip
- 6.10 Whatman card drying rack (Cytiva Whatman™ #10539521 or equivalent)
- 6.11 ≤ -20°C freezer (without automatic defrost preferred)
- 6.12 Fiberboard storage / freezer box

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Figure 1: Materials needed to package a DBS specimen



7. PROCEDURES

7.1 Laboratory Data Management System [8.2]

7.1.1 Entering the DBS as a single card into the LDMS:

- 7.1.1.1 Create one LDMS label with the primary volume equal to the total amount of blood received and the derivative volume is equal to the total volume of blood on the card (uL).

Note: Each spot may contain a volume of 25-80uL with the total volume of 125-400uL for the card.

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7.1.1.2 LDMS code: BLD or HST/DPE/DBS.

7.1.2 Entering the DBS as individual spots into the LDMS:

7.1.2.1 Create five LDMS labels with the primary volume equal to the blood volume received and the derivative volume equal to each spot (25-80uL).

7.1.2.2 LDMS code: BLD or HST/DPE/DBS.

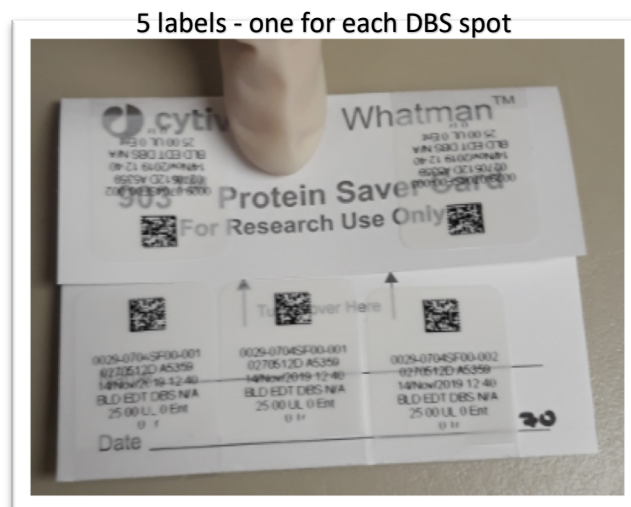
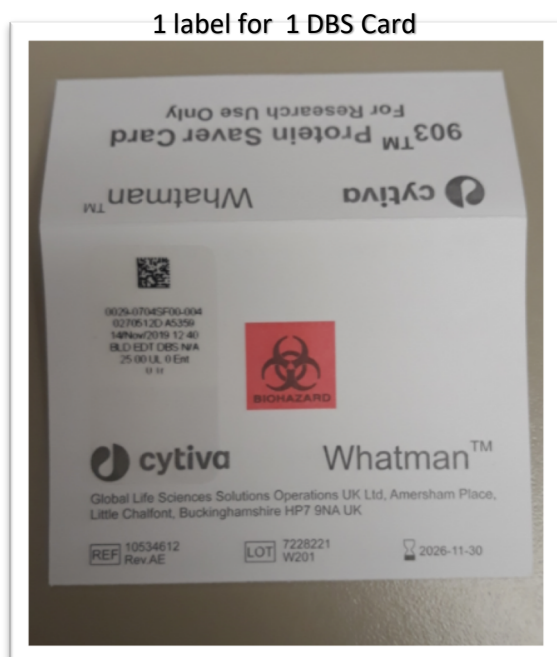
7.2 Labeling

7.2.1 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.

7.2.2 Ensure that the label(s) are FIRMLY AFFIXED to the card.

Note: Labels should be scannable through the bag. An extra set of labels may be placed inside the bag for easy scanning.

Figure 2: DBS labeling examples – one card vs. 5 DBS spots



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7.3 Preparation

7.3.1 Gently invert the tube (8 to 10 times) to mix the blood thoroughly.

Note: Capillary blood from a heel stick should be collected in a microtainer containing an anticoagulant to prevent clotting prior to or after DBS preparation.

7.3.2 Remove the cap, and apply 25 to 80 μ L of the whole blood to each spot.

Note: Do **not** touch the card with the pipette tip. Slowly expel blood from the tip to the center of the spot, allowing the blood to drop to the paper and fully absorb within the circle.

7.3.3 Repeat four times to fill all five spots on the card (unless fewer spots are required).

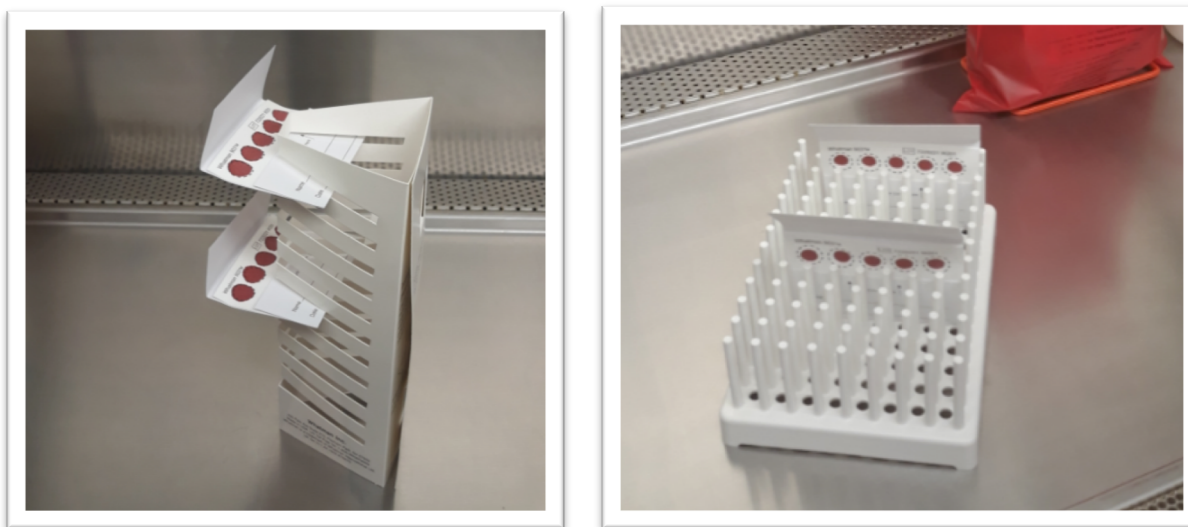
Note: Refer to Appendix 1 and 2 for examples of spotting techniques.

7.4 Drying

7.4.1 Allow the blood spot to air dry without the card flap covering the spots in a clean, dry place that is protected from direct sunlight for 2-4 hours (drying overnight may be necessary in areas with higher humidity).

7.4.2 Do not heat, stack or allow DBS to touch other surfaces during the drying process.

Figure 3: DBS drying rack examples



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7.5 Packaging

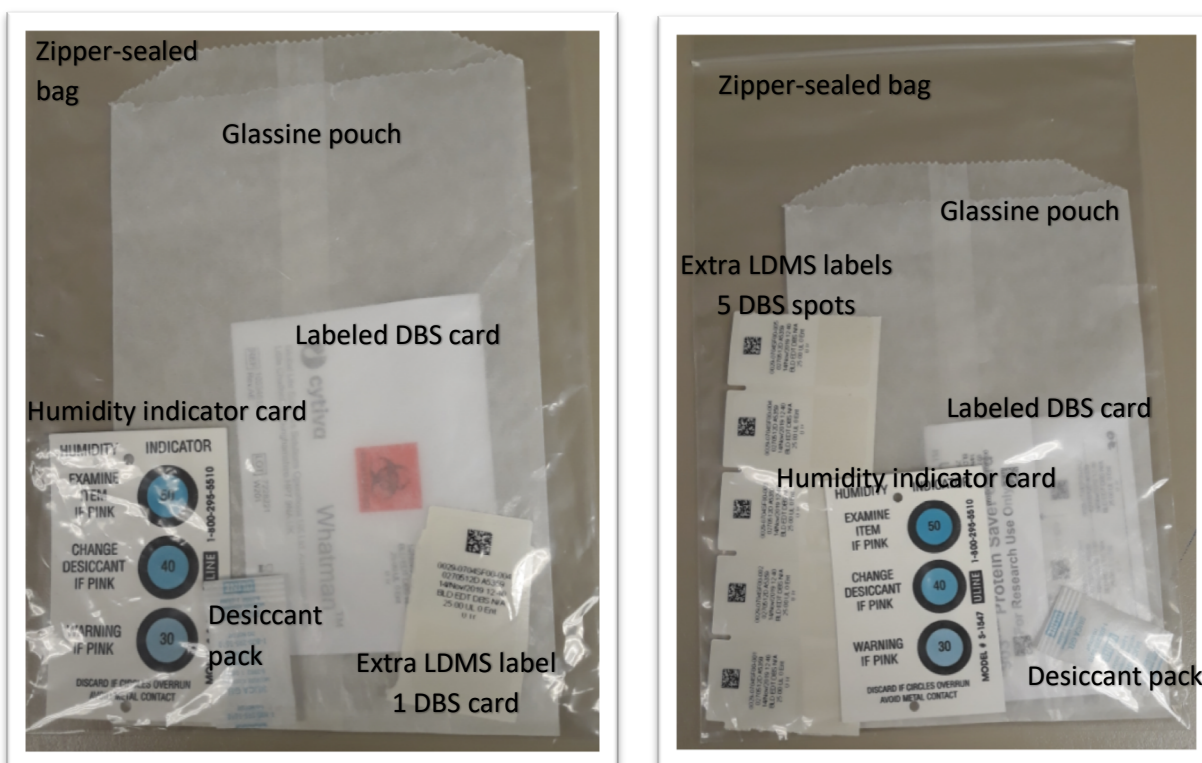
7.5.1 Tuck in the flap of the Whatman Protein Saver 903 Card as indicated on the card.

Note: 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.

7.5.2 Seal the card in a gas-impermeable, zip-sealing bag containing a desiccant pack and humidity indicator.

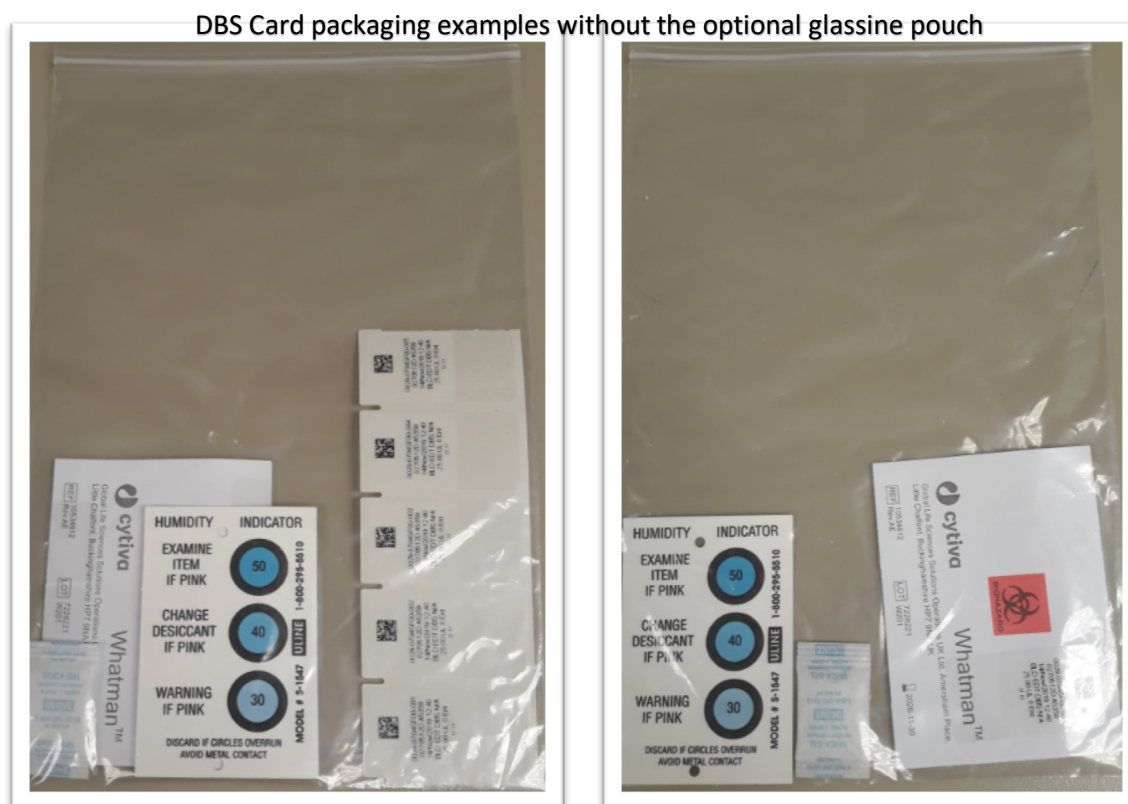
Note: Use at least 1 desiccant pack and store no more than one card per bag.

Figure 4: DBS packaging Examples



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Figure 4 (continued): DBS packaging Examples



7.6 Storage

- 7.6.1 The Lab Processing Chart (LPC) must define the storage conditions for DBS based on the analyte being tested.
- 7.6.2 Store the prepared DBS cards in a fiberboard freezer box and inventory using the LDMS storage module.
- 7.6.3 Freeze storage boxes should be stored at $\leq -20^{\circ}\text{C}$ unless otherwise instructed in the LPC.
- 7.6.4 Quality Control

Note: Quality control is not routinely required and should only be performed when pulling specimens for shipment or preparing to ship a full box.

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7.6.5 If the humidity card is pink at the 30% level, allow the specimen to acclimate to ambient temperature, then add a new indicator card (include date and initial on the new indicator card), and add 1-2 additional desiccant packs.

7.6.6 Return specimen to freezer storage container.

7.6.7 Change the condition code for the DBS to HUM. Add a comment in LDMS that a new humidity indicator and desiccant packs were added.

7.7 Shipping

7.7.1 DBS cards stored at $\leq -20^{\circ}\text{C}$ should be shipped on dry ice unless otherwise instructed in the LPC.

7.7.2 Ship boxes per [ACTG/IMPAACT Network Guidelines for Shipping and Receiving Biological Substance, Category B Specimens](#) [8.1].

7.7.3 Pre-notify the recipient per International Air Transport Association (IATA) regulations (refer to the LPC for special shipping instructions).


8. REFERENCES

- 8.1 *ACTG/IMPAACT Laboratory Manual*. (n.d.). Office of HIV/AIDS Network Coordination. <https://www.hanc.info/resources/sops-guidelines-resources/laboratory/actg-impaaclaboratory-resources.html>
- 8.2 *Lab Data Management System*. (n.d.). Frontier Science Foundation. <https://www.ldms.org/>
- 8.3 *Blood Spot Check*. (n.d.). Nemours Children's Health. <https://www.nemours.org/content/dam/nemours/wwwv2/filebox/service/support/Acceptable%20Blood%20Spot.pdf>

9. INQUIRIES

Contact the ACTG/IMPAACT LTC Leadership at actg.ltcleadership@fstrf.org for questions and comments related to these procedures.

10. NETWORK LAB CENTER SOP APPROVAL

NAME AND TITLE	SIGNATURE	DATE OF APPROVAL
Grace Aldrovandi, MD ACTG/IMPAACT Network Laboratory Principal Investigator		24 May 2023

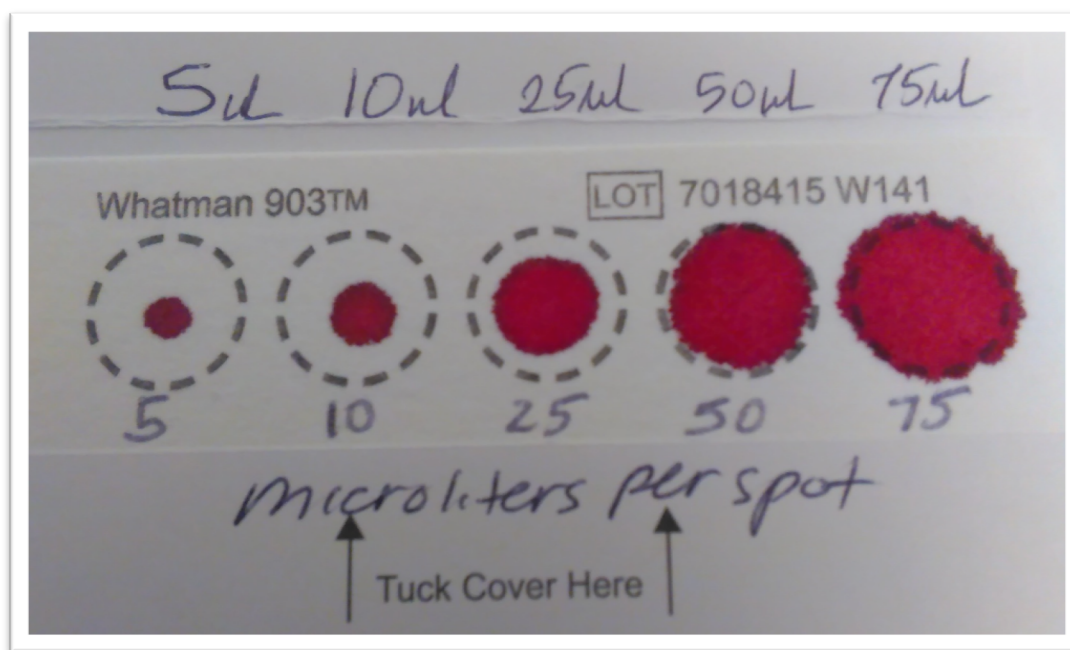
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11. REVISION HISTORY OR RECORD RETIREMENT

VERSION #	EFFECTIVE DATE	REPLACES	DATE OF REVISION	RATIONALE FOR [REVISION/RETIREMENT]
1.0	11Mar2009	NA	19Mar2012	NA
2.0	19Mar2012	Version 1.0	03Mar2023	Organized according to CLSI standards; updated procedures, materials, references.
3.0	01Apr2023	NA	NA	Removed blood collection references and streamlined processing to reflect current practices.










12. APPENDICES

12.1 Appendix 1: Example of Spots with Variable Blood Volumes



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12.2 Appendix 2: Examples of Acceptable and Unacceptable DBS Specimens [8.3]

ACCEPTABLE SPECIMEN	
	<ul style="list-style-type: none"> No foreign substances have contaminated the filter paper. The blood fills all printed circles and is applied evenly on one side of the filter paper only, free of layering and clots. The specimen dried in a horizontal position for at least three hours, away from direct contact with surfaces, heat, and sunlight. The specimen is sent to the appropriate location no more than 24 hours after collection.
UNACCEPTABLE SPECIMENS	POSSIBLE CAUSES
No Blood 	<ul style="list-style-type: none"> Failure to obtain any blood
Quantity of blood insufficient 	<ul style="list-style-type: none"> Filter paper circle incompletely filled or not saturated Blood applied to filter paper with needle or capillary tube Contamination of surface of filter paper circle
Scratched or abraded blood spots 	<ul style="list-style-type: none"> Blood applied improperly with capillary tube or by other means
Wet or discolored blood spots 	<ul style="list-style-type: none"> Specimen not properly dried before mailing
Supersaturated blood spots 	<ul style="list-style-type: none"> Excess blood applied to the filter paper, usually with capillary tube or needle Blood applied to both sides of the filter paper
Diluted blood spots 	<ul style="list-style-type: none"> Puncture site squeezed or "milked" to expel blood Exposure of blood spots to direct heat Contamination of filter paper before or after blood collection by gloved or ungloved hands or by substances such as alcohol, feeding or antiseptic solutions, hand lotion or powder
Clotted or layered blood spots 	<ul style="list-style-type: none"> Touching the same filter paper circle to a blood drop several times Filling the circle from both sides of the filter paper
Serum rings evident in blood spots 	<ul style="list-style-type: none"> Alcohol not allowed to dry completely before skin puncture is made Allowing filter paper to come in contact with alcohol, water, hand lotion, etc. Squeezing the area around the puncture site excessively Drying the specimen improperly Applying blood to filter paper with a capillary tube

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13. LABORATORY SOP REVIEW

LABORATORY STAFF NAME	DATE OF CONFIRMATION OF UNDERSTANDING