STANDARD OPERATING PROCEDURE FOR HVL DBS VENOUS SAMPLE COLLECTION

**Purpose**
Describe the procedures for collection and preparation of Dried Blood Spots (DBS) Specimens on filter paper cards for use in HIV Viral Load testing by using Venous blood.

**Scope**
This procedure applies to all blood samples collected from sites selected for Viral load sample collection.

**RESPONSIBILITIES**
HCWs at the selected sites are responsible for following this procedure.

Laboratory Personnel are responsible for the Quality assurance of this procedure.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Reagents</th>
<th>Supplies</th>
<th>Equipment</th>
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</thead>
<tbody>
<tr>
<td>• 4ml EDTA tube</td>
<td>• P100 single-channel pipettor,</td>
<td>• Universal pipette tips or yellow (100 μl size)</td>
<td>• NA</td>
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<tr>
<td>• Whatman 903 collection cards</td>
<td>• Plastic containers with</td>
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### STANDARD OPERATING PROCEDURE FOR HVL DBS VENOUS SAMPLE COLLECTION

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- 10% Bleach (Jik)
- Waste containers
- Skin disinfectant alcohol wipe
- Safety blood collection set with adapter and holder
- Absorbent gauze
- Bandages
- Disposable powder-free, latex or non-latex gloves
- Desiccant packs
- Glassine envelopes
- Humidity indicators
- 903 dry rack
- Lab markers

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**Sample**  
Whole blood collected in K3 EDTA or K2 EDTA vacuum tubes

**Specimen management**  
DBS cards *must* be labeled with the Patient Identification (PID) number and date of blood spot collection.
Completely dried DBS cards must be stored in a proper plastic sample bag together with desiccant packets and a humidity indicator card. If the indicator card changes the color (e.g., blue to pink. See Appendix A), it must be replaced and new desiccant packets will be added.

DBS cards have to be completely dry before packaging and storage.

DBS cards can be stored safely at RT up to 30°C for 9 weeks and in the event of high humidity for a maximum of 3 weeks.

**Special Safety Precautions**

- All specimens must be regarded as potentially infectious.

- Refer to Health Laboratory Safety and Waste Management Manual for safety considerations.
Quality control

- Patient identification number and date will be used on patient register book, Laboratory request form, on vacuum tubes, on DBS cards and on the packing manifest.

- **Powder free gloves** must be worn while preparing spots on the DBS cards.

- Use only the supplies provided.

- Fill each DBS circle with a 100 µl of blood. The under-filled DBS cards will not provide enough materials for HIV viral load testing.

- If a DBS circle is overfilled, it is fine. DO NOT “erase” the overfilled portion (e.g., liquid eraser)

- Do not expose DBS cards to sunlight as the direct light and heat will deteriorate the specimens

- Completely dried DBS cards must be packaged with a humidity card indicator and desiccants.

- Put one card per drying slot to avoid sample contamination (see appendix B)

Procedure:

**A: Preparation**

- Wash hand vigorously

- Wear powder free gloves and change gloves between patients

- Confirm Patient ID to ensure all data elements on the form are complete, accurate and consistent

**B: Phlebotomy**

- Write name and patient identification number on EDTA tube using undeletable/permanent marker pen

- Use toniquet to locate the vein
- Clean the puncture site with alcohol swab or disinfectant. Do not touch the site again after cleaning.

- Insert the tube into a holder then insert into the vein of the patient with bevel upward and insert EDTA tube into the holder and push gently to allow the needle to pierce the top of the vacuum tube. Wait till blood stops flowing to the tube.

- Remove the tube and mix gently by inverting several times to mix the blood with anticoagulant.

- In people with low blood pressure vacuum tube system may not be applicable and therefore syringe or butterfly needle may be used.

**C; DBS Preparation**

- Blood specimens will be collected using universal precautions and universal blood collection procedures in one EDTA 4mls vacuum tube.

- Label Whatman 903 DBS cards with Patient Identification number and collection date (*the blood spots have to be made within 2 hours of blood collection*) per patient.

- Invert the blood collection tube 3-4 times to mix the whole blood. Carefully open the blood collection tube.

- Add 100 μl of whole blood to each of the 5 pre-printed circles on the filter paper card, using a P100 or pipette. Mix the blood up and down 3-5 times with the pipette before a new circle on the card is filled. Add the sample slowly into the center of the circle. *Do not* touch the surface of the cards with the pipette tip. Duplicate this procedure with the second card.

- Discard pipette tips in a container with 10% Bleach/Jik.

- Clean the pipette between samples to avoid cross-contamination.
Repeat steps the above steps for the rest of the patient specimens.

- Place the completed DBS cards horizontally on the dry rack without touching the spots at Room Temperature overnight in a safe location and away from direct sunlight.
  - After DBS cards are completely dry, place each DBS card in an individual glassine envelope.
  - Place 10 glassine envelopes containing DBS cards (5 patients) or less in a plastic card bag glassine envelopes.
  - Insert 1 desiccant packet per card (e.g., 10 desiccants for 10 DBS cards).
  - Place *only* 1 humidity indicator card into the bag. Press to expel any air within the bag and seal tightly.
  - If the humidity indicator card changes the color (e.g., blue to pink. See appendix A.) Before DBS cards are transported to the storage facility, the humidity indicator will be replaced and five new desiccant packets will be added. Record this action.
  - The packaged DBS cards should be kept in a dry and safe location at Room Temperature (18 – 30°C) with no direct exposure to sunlight until bi-weekly pick up to the central storage facility.
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### Reference
11.0 APPENDIX A

11.1 Humidity Indicator Card Example:

11.2 Related Procedures

1. SOP for DBS sample transportation
2. SOP for HIV Viral Load DBS specimen Processing

Appendix A: Humidity Indicator
Appendices

Appendix B: How to dry DBS in a drying rack
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