GEMS
GLOBAL EVALUATION OF MICROBICIDE SENSITIVITY

GEMS Standard Operating Procedure (SOP)

Procedure: DBS Preparation from Venipuncture Blood
Laboratory: HIV Drug Resistance Testing Laboratory
Effective Date: 17 September 2017

Initial Review and Approval		Date
GEMS Co-Director	Dr. Urvi Parikh, PhD	17 September 2017

Revision History	Date

Authorized to Approve:
Authorized to Review:

Personal Protective Equipment

Performance of this procedure will expose personnel to biohazardous material.
All specimens must be handled as infectious material as outlined in your Laboratory's Safety Manual. The technologist must take all precautions and adhere to all prescribed policies.

This procedure may expose you to:
[X] Bloodborne pathogens
[X] Hazardous reagents

To perform this procedure, you must use:
[X] Gloves
[X] Disinfectant: Diluted sodium hypochlorite (1:10 v/v solution)

Purpose

To outline the procedure to collect blood by venipuncture and spot onto Dried Blood Spot (DBS) cards for assessment of HIV Drug Resistance.

Scope

This SOP applies to all staff members involved in preparing DBS from venipuncture whole blood.

Background

Resistance to antiretroviral (ARV) drugs are a risk when an individual becomes HIV infected while using Pre-exposure prophylaxis (PrEP) for HIV prevention. The collection of a DBS after seroconversion will enable the determination of the presence of HIV drug resistance mutations known to cause ARV resistance. A drug resistance test will be performed using DBS collected from this procedure.

Equipment and Materials

Equipment:

- Blood tube rocker

Materials:

Kit Contents

- Rubber latex powder free gloves
- Alcohol or spirit swabs
- EDTA vacutainer blood collection tube
- Blood collection safety needle and cap
- Lancet
- Two unused DBS cards
- Transfer pipette (plastic dropper)
- One sealable plastic bag

- Desiccant packs
- Humidity indicator cards
- Pre-addressed shipping envelope with pre-printed G4S number

Other Contents of Box

- Barcode labelled stickers (5 unique identifiers)
- Lab Requisition forms
- Elastoplasts
- Tourniquet
- Sterile cotton wool
- Vacutainer holder
- DBS card drying rack
- DBS collection Job Aid
- Extra DBS cards

Materials Required (but not provided)

- Disinfectant: Diluted sodium hypochlorite (1:10 v/v solution)
- Sharps container for needle and blood collection tube disposal
- Protective clothing (lab coat) (optional)

Specimen Information

Specimen volume:

- Minimum volume required: one full EDTA containing tube of whole blood

Handling/storage Instructions:

- All whole blood specimens must be handled as infectious material as outlined in your laboratory's Safety Manual.
- Whole blood should be used for DBS preparation immediately or within 12 hours after collection. If not used immediately, blood tubes must be constantly gently mixed at ambient temperature until use through the use of a tube rocker or similar piece of equipment.
- Do not freeze whole blood.

Unacceptable Specimens:

- Blood that has coagulated or been stored at unacceptable temperatures.
- EDTA must be used as the anticoagulant. Other anticoagulants may give incorrect results.

CAUTION: Performance of this procedure will expose personnel to biohazardous material. All specimens must be handled as infectious material using Universal Precautions, including:

- Wear gloves at all times and change if contaminated
- Do not eat or drink in testing areas
- Ensure all spills and contaminated material are properly decontaminated using 10% sodium hypochlorite or chlorhexidine solution
- Ensure all consumables and unused blood specimens are disposed of in accordance with local regulations

Storage Requirements

- Store kit at ambient temperature.
- Dried DBS cards may be stored at ambient temperature in a sealable plastic bag with desiccant and a humidity indicator for up to 3 days.
- Ship dried DBS cards immediately or within 3 days of collection.

Quality Control

- Ensure blood collection tubes are within their stated expiration date.
- Note any blood collection or DBS card preparation issues or abnormalities on the data collection form.
- Use all materials and consumables only once.
- Avoid contamination of DBS cards by placing them on a clean surface, only handling them with gloves and never touching the defined circles where the blood will be spotted.

Procedure – Stepwise

Before beginning the procedure:

- HIV infection must be confirmed using local testing standards.
- The consent form must be reviewed and signed by the client or client's representative.

1. Remove the 5 identical barcoded stickers from the sample collection box. Affix one barcode labeled sticker to each of the following items:
    a. DBS card #1 (over the sample barcode icon shown on the front of the card)
    b. DBS card #2 (over the sample barcode icon shown on the front of the card)
    c. -Data collection form (in the box labelled 'Client ID/Barcode Sticker')
    d. Client's medical file (may vary between clinics; follow standard procedure)
    e. Blood collection tube (length wise near the middle of the tube; ensure the barcode will be able to be read by a barcode reader).
2. Record date of sample collection on all the 5 barcoded labeled stickers,
3. Complete the data collection form with all required client information.

NOTES:

- *The DBS collection is expected to be completed at the time of HIV seroconversion confirmation. However, if for some reason the sample collection is not completed on the day of seroconversion confirmation, clients will be asked to return as soon as possible to complete the procedure.*
- *If DBS collection via venipuncture is not possible, fingerprick using the clinic's SOP may be used to collect the blood on one DBS card. A lancet is provided in the kit in case fingerprick needs to be done. The following procedure outlines procedures to prepare DBS using intravenous blood collection.*

Specimen Preparation (refer to DBS Collection Job Aid in HIV DR Box):
The health care worker should wash hands and put on gloves once hands are dry before starting procedure.

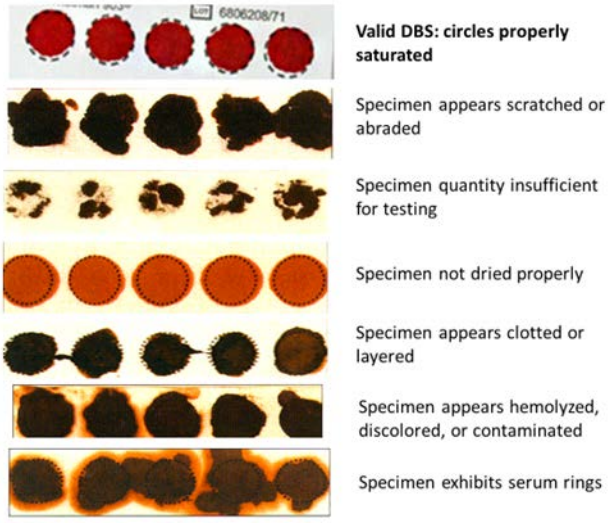
4. Prepare the client for venipuncture blood draw according to the clinic's SOP. Fill one EDTA containing blood tube with approximately 2 mL whole blood.
5. If the DBS card will not be prepared immediately following blood collection, place the tube containing blood on a moving rocker at ambient temperature in order to prevent separation and ensure constant mixing with the EDTA in the tube.

DBS Preparation:

The health care worker should wash hands and put on gloves once hands are dry before starting procedure.

6. Lay out both barcoded DBS cards onto a clean surface.
7. Gently invert the blood tube 2 to 3 times to ensure complete mixing then draw up at least 0.5 mL of blood using the transfer pipette.
8. Starting from left to right on the first DBS card, fill each circle on the first DBS card with two drops of blood by slowly depressing the bulb.
    a. Do not touch the card with the pipette tip.
    b. The drops should fall on the center of each circle.
    c. When absorbed, the blood drops should fill the entire outline of each circle.
9. Spot the second card in the same manner.
10. Once both cards have been successfully spotted with 5 spots per card, place them on a drying rack in a clean, dry space.
    d. The cards should be stored with blood spots facing up, and dried overnight or for at least 3 hours at ambient temperature.
    e. Protect the cards from rodents, insects and direct sunlight.
    f. Do not stack the cards on top of each other, or allow them to touch other surfaces during the drying process.
11. Dispose of all consumable materials and unused blood in accordance with local protocols and clean working areas with a 10% sodium hypochlorite or chlorhexidine solution when finished.

Examples of valid and invalid dried blood spots



Source: GE Healthcare Life Sciences. 2010. *Simple spot check*. GE Document 28984392

DBS Storage and Shipping:

12. Once the cards are dry fold the flap over each DBS card and place both cards in the gas-impermeable, sealable plastic bag.
13. Add 5 desiccant packs to the sealable plastic bag to remove excess moisture and one fresh humidity indicator card.
    a. 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^{0}C for 3-4 hours in a drying oven; then cool 10 minutes. IMMEDIATELY RETURN card and desiccant pack to sealable plastic bag.
14. Place the sealable bag containing the DBS cards and the lab requisition form into the pre-addressed shipment envelope provided in the kit.
15. Immediately notify the courier company (G4S) for pick-up of the package for transportation to Kisumu KEMRI laboratory. The account number will be indicated on the package envelope and will cover the cost of the transportation.
16. The sample must be shipped to the laboratory as soon as possible and no later than 3 days of sample collection. Temporarily store DBS cards at room temperature in a dark place (drawer) in a secured location before shipment.
17. If for any reason, direct shipment is not feasible within 3 days, contact the GEMS Coordinator (contact listed below) and alternate instructions will be provided
    b. Note: Ship DBS cards as non-dangerous goods (they are exempt biological specimens according to ICAO and IATA).