Procedure for Dried Blood Spot Sampling and Storage

This method is to be utilized for Dried Blood Spot (DBS) Sample Collection and Sample Storage for the analysis of tenofovir (TFV), emtricitabine (FTC), tenofovir-diphosphate (TFV-DP), and emtricitabine-triphosphate (FTC-TP).

Personnel should use universal precautions for blood borne pathogens. This procedure can be performed outside a biosafety cabinet if consistent with local regulations.

Supplies:
- 4ml EDTA Vacutainer Evacuated Blood Collection Tubes
- Whatman Protein Saver Card #903 (Whatman 10534612 or Fisher Scientific #05-715-121)
- Whatman Plastic Sample Bags (Whatman 10548232 or Fisher Scientific #50-853-570)
- Desiccant pack (Whatman WB100003 or Fisher Scientific #09-923-360)
- Humidity indicator cards (Multisorb Des Manufacture # MS200032, DESCO Industries #13870 or Fisher Scientific # NC0281067)
- Whatman card drying rack (VWR catalogue # 89015-592)
- Latex or nitrile gloves preferably powder free
- Water proof marker, black
- 10-100 µL or 20-200 µL micropipette and appropriate tips with filters. Sites should check with local suppliers for appropriate tips for their micropipettes.

Processing Procedure:

1. **Blood Sample Collection:**
   1) Personnel should use universal precautions for blood borne pathogens.
   2) A 4mL EDTA Vacutainer (purple top) tube is used for blood collection and labeled with PID, Protocol name and sample ID, Date and collection time with permanent marker or affix appropriate label.
   3) Total of 4 mL of whole blood should be drawn by venipuncture from the consented subjects.
   4) Invert the blood tube several times and store at room temperature.
   5) Blood sample should be processed as soon as possible, but needs to be initiated within 24 hours of draw time due to stability of the anlaytes of interest.

2. **DBS Processing Procedure:** (See Figure 1 for properly spotted DBS card)
   1) The DBS should be processed as soon as possible from time of blood draw. No more than 24 hours should elapse. Store blood at room temperature until DBS can be processed.
      i) Note in laboratory documents if processing exceeds 24 hours from collection.
2. **DBS Processing Procedure: (con’t)**

2) Document in laboratory records (hard copy documents, LDMS or spreadsheet) the following information:
   i) protocol name/number
   ii) participant identification number (PID)
   iii) specimen draw date and time
   iv) specimen receipt date and time
   v) date and time of DBS processing (spot time)
   vi) date and time of DBS completion and storage

3) Ensure that both hands are gloved before handling the Protein Saver (DBS) card; Do not touch the areas where blood spots will be placed (the filter paper portion).

4) Label each DBS Card with the study protocol name, PID, specimen date and time. Use a waterproof pen. Alternatively, an LDMS-generated label may be used.

5) Assure the blood tube has been gently inverted several times before spotting.

6) Remove the stopper from the EDTA tube, place micropipette into specimen tube and draw up 25μL of blood into the pipette.

7) Spot the 25μL of blood onto designated circles of the DBS Cards.
   i) Pipet tip should be held approximately 3 mm above the spot location NOT touching the DBS card.
   ii) Dispense each 25μL of blood into the MIDDLE of the circle with one single dispensing motion.
   iii) DO NOT touch, press, or smear the spots.
   iv) Repeat steps until a total of 5 spots have been completed.

8) Dispose of any remaining blood as appropriate or save for further processing (plasma/PBMC/RBC) if desired.

9) Air-dry the cards in a card holder for at least 2 hours at ambient temperature without covering the spots. Overnight drying is acceptable.

10) After spots have dried, use the cover flap to cover the spots. Place DBS card in low gas-permeability plastic bags with humidity indicator and desiccant pack to reduce humidity.
   i) One card per bag only

**Storage:**

1) Store bag in a cardboard box at -20°C or -80°C.

2) The humidity indicator in the bag should be checked periodically as needed.

3) If indicator indicates too much humidity (color change from blue to pink - 40% to 50% level), replace the old desiccant pack and indicator card with a new one. Make sure to have gloves on so as to handle the DBS card properly and not disrupt the samples.
Shipping:

1) Ship samples to the Colorado Antiviral Pharmacology Laboratory at:
   a. Attn Lane Bushman,  
      UC Denver-Skaggs School of Pharmacy and Pharmaceutical Sciences  
      V20-4410  
      12850 E Montview Blvd  
      Aurora, CO 80045  
      303-724-6132

   b. DBS cards should be shipped on dry ice to the Pharmacology Laboratory.

   c. Check desiccant packs and humidity indicators before shipping and if applicable replace them.

   d. Place the boxes in water tight secondary containers (i.e. Tyvek bags) to protect from humidity while shipping.

   e. Provide a sample inventory spreadsheet (LDMS generated manifest acceptable) with each shipment containing the sample and participant information listed above.

Figure 1. Example of correctly spotted DBS card (25μL spot volume)

Note: 25μL spot volume does not completely fill target circle on DBS card.

Figure 2. Example of *incorrectly* spotted DBS card