ITREMA

DRIED BLOOD SPOTS (DBS)

ITREMA STANDARD OPERATING PROCEDURE

FOR DBS SPOTTING

V1.0 (21 JUNE 2015)

NDLOVU CARE GROUP

UMC Utrecht

ZonMw

RHI 'S REPRODUCTIVE HEALTH & HIV INSTITUTE

Universiteit Utrecht

Radboudumc

RATIONALE FOR DBS

- Access to drug resistance testing in low- and middle-income countries is limited
  - High overall costs
  - Use of plasma, requiring cold-chain maintenance/transport
  - Limited availability of highly skilled laboratory workers

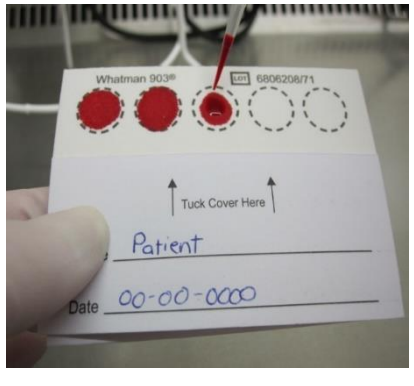
- Advantages of DBS
  - Easily collected through finger prick, or from sampled EDTA blood
  - Sufficiently stable at room temperature (up to 30 C°) for a maximum of two weeks*
  - No need for cold chain transport
  - Less costly to ship

* Aitken SC et al, Stability of HIV-1 Nucleic Acids in Dried Blood Spot Samples for HIV-1 Drug Resistance Genotyping. PLoS One. 2015 Jul 6;10(7):e0131541.

DBS HIV DRUG RESISTANCE TESTING: PROCESS

The basic process for DBS sampling and DBS-based drug resistance testing is as follows:

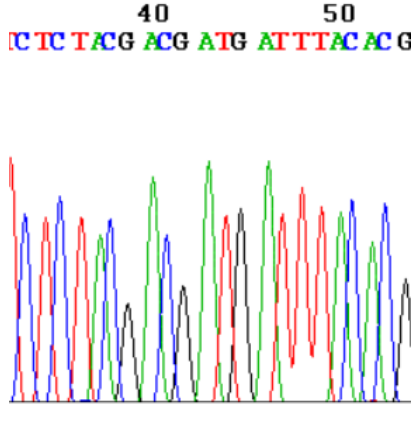
1. DBS preparation at local site
2. Posted to reference lab (by ordinary or parcel post)
3. DBS-based population-based sequencing of RT or PR-RT, and also for integrase
4. Interpretation by HIV drug resistance expert
5. feedback to clinician (by e-mail)



DBS spotting

Seal when adequately dried

Post the DBS



Sequencing and interpretation

Result e-mailed back to clinician

ITREMA

DRIED BLOOD SPOTS

PREPARATION, HANDLING, SAMPLING AND STORAGE

NDLOVU CARE GROUP

UMC Utrecht

ZonMw

RHI 'S REPRODUCTIVE HEALTH & HIV INSTITUTE

Universiteit Utrecht

Radboudumc

STEP 1

- Prepare the card for spotting by filling in the patient details prior to application of blood.

- Use multiple patient identifiers and the sampling date, in order to ensure accurate patient identification:
  - Example: "*Patient number/identifier*", "*Secondary identifier*", "*[date (DD/MMM/YYYY)]*"

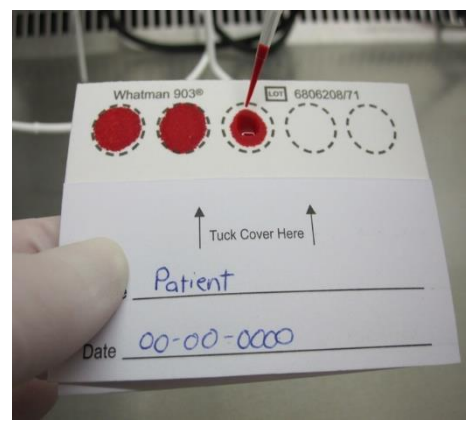
- Fold card so that the flap is used as a stand and the spots in the filter paper area do not touch the rest of the card*

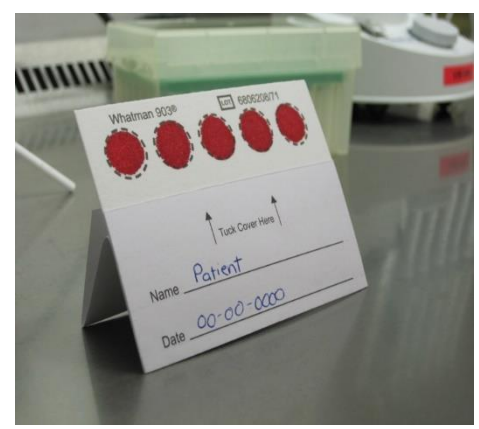
*Please don't touch the filter paper area and make sure that the filter paper doesn't touch any surface prior to application of blood.

STEP 2 (FOR EDTA-DERIVED WHOLE BLOOD)

- When using EDTA-derived whole blood:
  - Do not centrifuge the EDTA vacutainer tube
  - Gently invert the closed EDTA tube containing whole blood (5-10 times) to mix the blood thoroughly
  - Ensure adequately mixed whole blood
  - Carefully open the tube and pipette 50 µl whole blood*
  - Spot the whole blood on filter paper by applying directly to the center of the spot.
  - Filter tip can be held gently against the filter paper or slightly above it. Pipette the sample slowly to allow for efficient absorption
  - The card can either be on a surface at an angle or be held flat

*Please never touch or smear the blood spots and use examination gloves and conduct experiment in clean conditions





STEP 2 (FOR SKIN PRICK)

- When using skin prick:
  - Clean patient skin with an alcohol swab
  - Ensure alcohol is fully evaporated prior to sampling, as it may affect the quality of the sample
  - Pierce the skin using a dedicated skin prick lancet
  - Discard or wipe the first drop of blood using cotton wool
  - Spot blood on filter paper one drop at a time, by applying directly to the center of the spot, hovering slightly above the spot.
  - The card can either be held in the hand (without touching the filter paper), or placed on a surface at an angle

*Please never touch or smear the blood spots and use examination gloves and conduct experiment in clean conditions

STEP 3

- Allow the card to air dry for 4 hours, or preferably overnight. Cards can either stand on surface (figure 1A) or be placed in a drying rack (figure 1B).

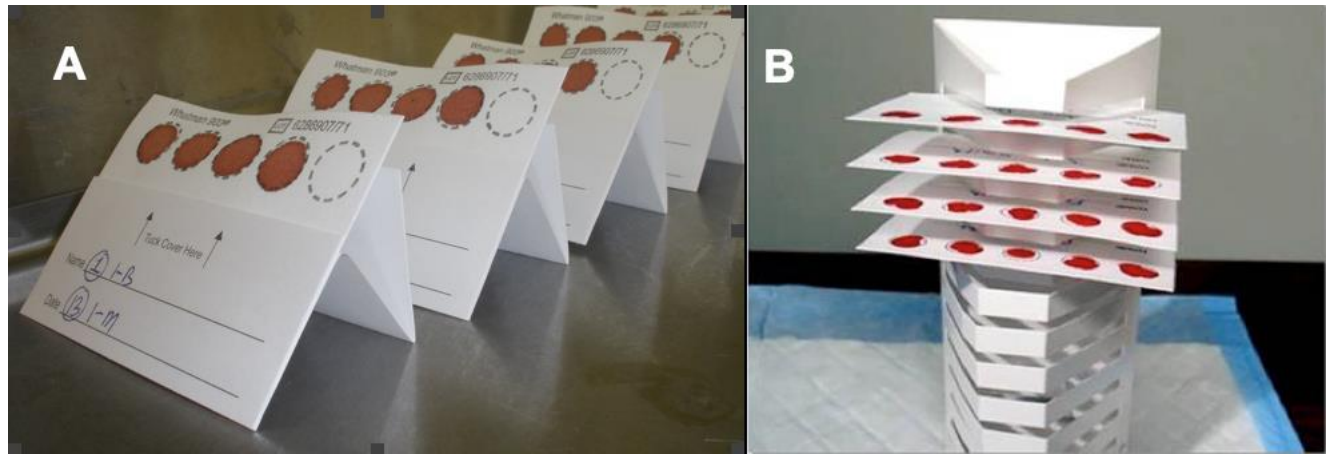


Figure 1: Drying options. On a flat surface at an angle *(A)*, or in a rack *(B)*

PACKAGING

- Ensure the filter paper is completely dry before packaging.
- When spots are completely dry, tuck the flap as illustrated on the card ("Tuck here") and insert in a plastic zip-lock bag with a silica desiccant for storage.
- To mail DBS specimens, use the basic triple-packaging system:
  - The filter paper matrix that contains the absorbed and dried blood.
  - Enclose the primary (filter paper) container with the use of a fold-over flap or an inner envelope
  - An outer envelope of sturdy, high quality paper.

STORAGE

- If DBS are intended for storage, they should be stored at -20° C or -80° C.
- For storage, DBS packed in plastic with silica dessicant can be put flat into empty cardboard freezer boxes. Next to their individual packaging all DBS per box must be packed into one zip-lock with an additional silica desiccant.
- Once a sample has been frozen, it cannot be sent at room temperature anymore and must be shipped on dry ice.

DELIVERY

- DBS specimens are considered as nonregulated, exempt materials by the International Air Transporter Association (IATA) and WHO.*
- DBS are not considered as biohazardous if packaged correctly
- DBS specimens can be shipped by mail or other carrier with no reasonable expectations of occupational exposure to blood or other potentially infectious material
- Individual samples should be shipped at room temperature.
- Indicate duration of storage and storage conditions prior to shipment, and date of shipment, on the card.

* Centers for Disease Control and Preventions. Shipping Guidelines for Dried-Blood Spot Specimens. 2017. Available at: https://www.cdc.gov/labstandards/pdf/nsqap/Bloodspot_Transportation_Guidelines.pdf [Accessed 2019 March 10].