

TAKING BLOOD FROM INFANTS FOR THE HIV DNA PCR TEST



STANDARD OPERATING PROCEDURE



ACRONYMS

ARV	Antiretrovirals
CCMT	Comprehensive care, management and treatment
DBS	Dried blood spots
DNA	Deoxyribonucleic acid
EDTA	Ethylene-diamine-tetra-acetic acid, a compound used to keep blood samples from clotting
HIV	Human immunodeficiency virus
µl	Microlitres
ml	Millilitres
PMTCT	Prevention of mother to child transmission
PCR	Polymerase chain reaction, a test method used to detect the genes of the virus
SOP	Standard operating procedure

This Standard Operating Procedure file

is designed to assist healthcare workers in taking blood from infants for the HIV DNA PCR test. This test is routine care for babies born to HIV-positive mothers. The PCR test ensures early diagnosis of HIV infection which improves the survival and quality of life of these babies.

We hope you find this SOP useful in your job of caring for mothers and babies.

HIV DNA PCR TEST

Two types of blood samples can be used for an HIV DNA PCR test:

1. Dried blood spots (DBS)

- Dried blood spots are technically easier to obtain and are suitable for blood sampling in the primary health care setting.
- The filter paper is framed, pre-printed with three circles and has space for labelling.
- Dried blood spots (DBS) can be collected from a heel-, toe- or finger-prick or by venous blood onto filter paper (DBS card).

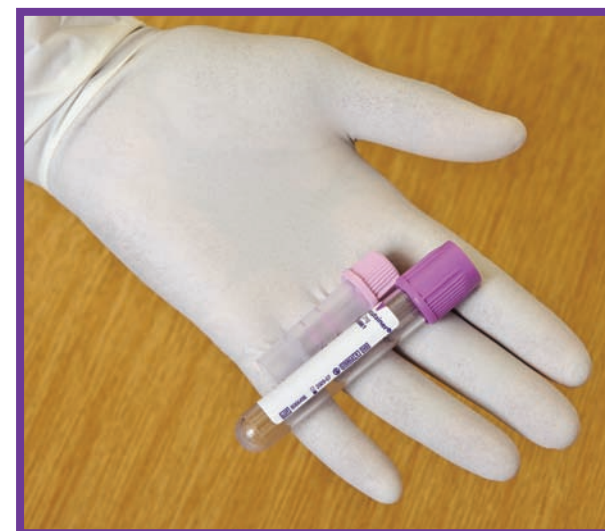
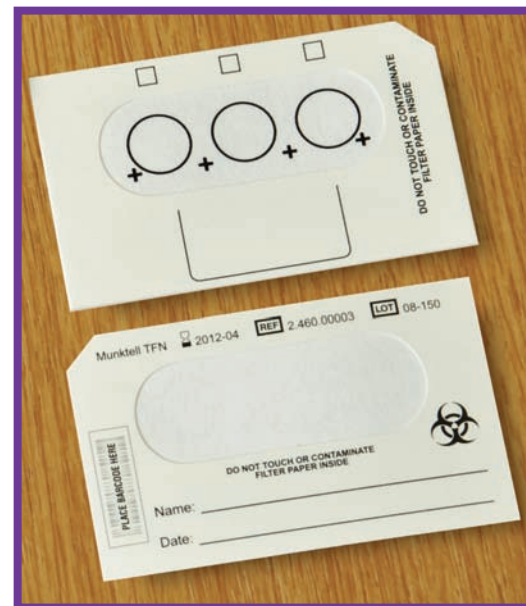
2. Whole blood in an EDTA/purple-top tube

(Either the 5 ml tube or the microtainer (BD microtainer with BD microgard closure; 8 mm diameter))

- Clotted whole blood samples interfere with HIV DNA PCR test results. Mix blood well to avoid clotting.

Remember:

- Universal precautions – handle all specimens as if they are capable of transmitting infectious agents.
- Explain the procedure to the mother and obtain informed consent.



MATERIALS REQUIRED

DBS collection kit

Instructions for performing the procedure are printed on the back of each kit.

The kit contains consumables for blood sampling and collection

Blood sampling

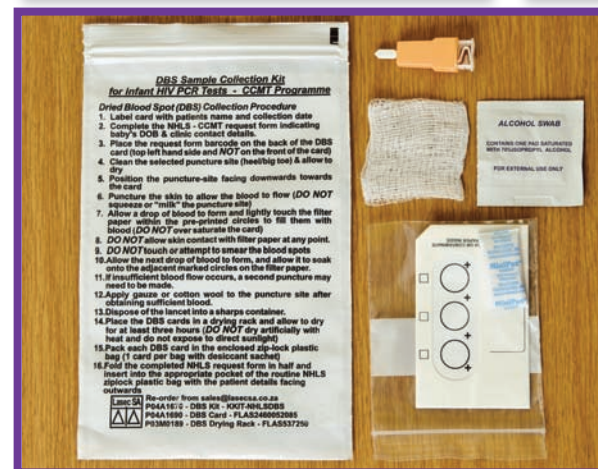
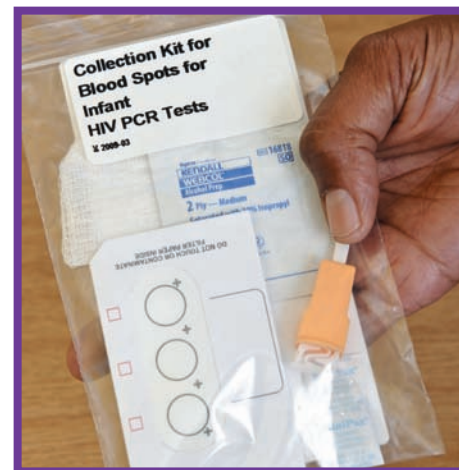
- Disinfectant for skin (eg alcohol wipe)
- Single use, loaded lancing device (eg Hemocue or similar device)
- Cotton wool or gauze

Collection

- DBS card
- Ziplock plastic bag
- Desiccant sachet

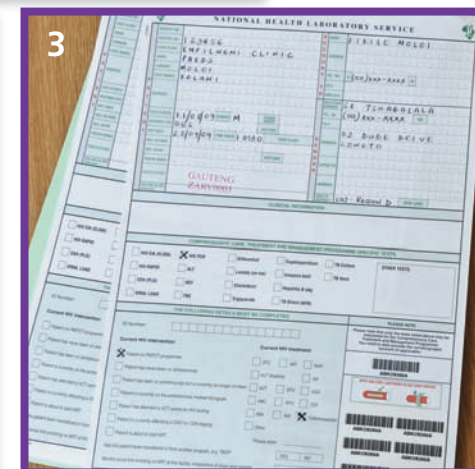
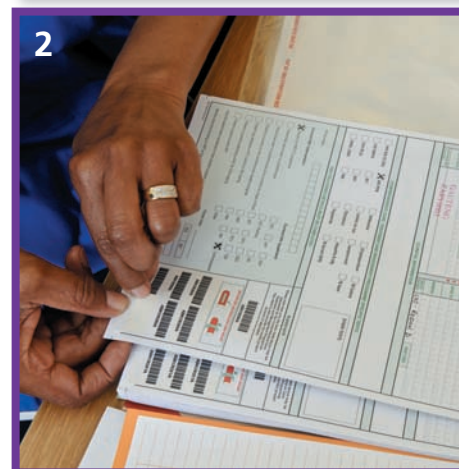
PLUS you'll need

- Powder-free gloves
- Drying rack
- Comprehensive Care, Management and Treatment (CCMT/ARV) NHLS laboratory request form with barcode
- A ziplock plastic bag for specimen packaging (biohazard bag)



PAPERWORK

1. Label the DBS card with the patient's name, date of birth, hospital or clinic number and the date that the sample was obtained. Use a ballpoint pen or other permanent marker directly on the paper.
2. Carefully stick the barcode from the NHLS form onto the back of the DBS card. Please take care to place the sticker carefully within the frame so that it does not extend beyond the edge of the card or onto the filter paper.
3. Complete the CCMT (ARV) NHLS laboratory form. Make sure you fill in the:
 - name of the hospital or clinic and health district
 - patient's name, date of birth and address
 - hospital or clinic number
 - date the specimen was taken
 - name and contact details of the healthcare worker
 - test requested (HIV PCR)
 - clinic details – specify whether on PMTCT programme or not
4. All HIV-exposed infants that require a DNA test will need cotrimoxazole prophylaxis



METHOD OF COLLECTION

1. Wash your hands
2. Put on gloves
3. Select the site:
 - young infants – heel or big toe
 - infants older than 9 months – finger
4. Clean the selected area of skin (heel, toe or finger) with a skin disinfectant swab and allow to dry for 30 seconds. Take care to keep away from bony prominences.

Remember:

Show the mother how to warm the baby's foot to increase circulation



Toe prick position

METHOD OF COLLECTION cont.

5. **Position the foot or hand with the puncture site downwards.** Read the instructions on the protective tab and check whether to, **twist** or **pull** off the tab. Press the loaded lancing device against the skin and push the white plunger.

6. **While holding the foot correctly, apply and release pressure to allow a drop of blood to form.** Do not squeeze or "milk" the puncture site as this may dilute the blood with tissue fluid.

Allow a large drop of blood to collect.

Once a drop of blood has formed, lightly touch the drop to the pre-printed circle on the filter paper (DBS card) allowing it to soak onto the circle. Allow the next drop of blood to form and soak it onto the next marked circle. Repeat until all three marked circles are adequately filled with blood. The pre-printed circles hold 50-75 μ l blood each when fully filled. The size of the blood spot and the penetration of the spot through to the reverse side of the card allow for some assessment of the blood volume. Samples with insufficient blood cannot be processed.

If insufficient blood flow occurs, a second puncture may need to be made. Do not excessively saturate the card with blood. Do not touch or attempt to smear the blood spots.

7. **Apply gauze (or cotton wool) to the puncture site after obtaining sufficient blood.**

8. **Dispose of the lancet into a sharps container.**

Heel prick:



Toe prick:



METHOD FOR DRYING

1. **Place the DBS cards in a drying rack to dry.** Place only one card per slot in the drying rack and do not allow the cards to touch each other.
2. **Allow to dry for at least three hours.** The blood spots should be a dark brown colour once properly dried. Do not dry artificially with heat and do not expose to direct sunlight.
3. **Dry completely before packing** – blood turns dark red/brown.



FEATURES OF ACCEPTABLE AND UNACCEPTABLE DBS SAMPLES

Acceptable:

1. Front of card

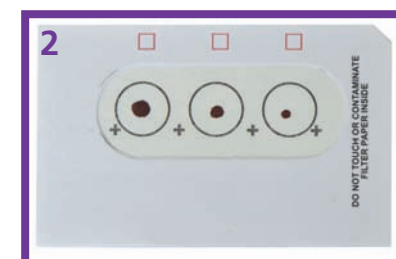
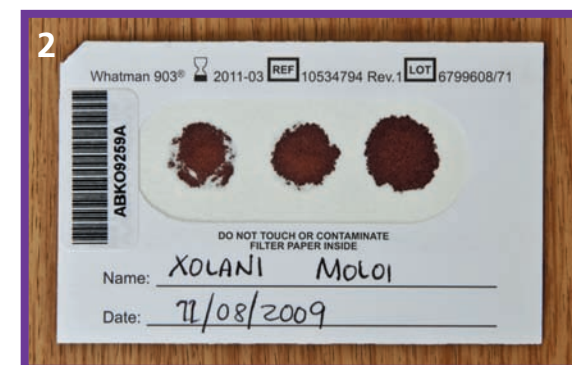
- All three pre-printed circles should be completely filled with blood.

2. Back of card

- The barcoded sticker from the CCMT (ARV) NHLS request form should be placed over the shaded barcode printed on the back of the card.
- The **size** of the blood spot and the **penetration** of the spot through to the reverse side of the card allow for some assessment of the blood volume.

Unacceptable:

1. NHLS requisition form correctly completed – but details on DBS card are not legible
2. Insufficient sample for processing – sample rejected
3. Blood spots should fill the circle and should not be 'smeared' or crusted.
4. Blood spotted outside the circle cannot be used.



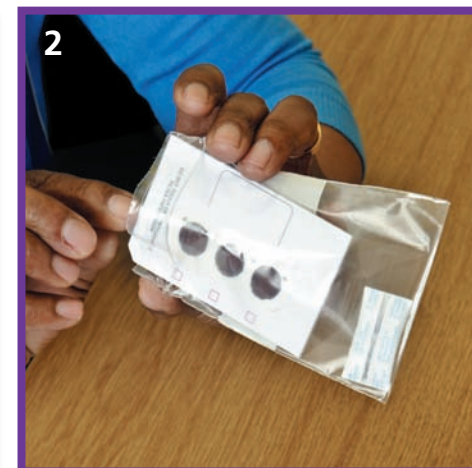
SUBMISSION TO LABORATORY

1. Fold the corresponding, completed CCMT (ARV) NHLS form in half and insert into the pocket of the larger plastic bag with the patient details facing outwards.

Ensure all information is provided on the CCMT (ARV) NHLS form.

2. After the blood spots have dried, place each card in a separate ziplock plastic bag. Insert one desiccant sachet and one DBS card per bag.
3. Place the packed DBS card into the larger ziplock bag containing the CCMT (ARV) NHLS form.
4. Place samples into the NHLS specimen collection box for transportation to the nearest NHLS PCR laboratory.

Note: DBS samples are very stable and, if necessary, can be kept overnight or over the weekend before being submitted to the laboratory. Store in a cool place, but not in the fridge.



WHOLE BLOOD COLLECTION

Whole blood samples can be collected by:

- A. Heel/Finger prick method
- B. Formal venipuncture

Remember:

Take care to mix whole blood samples well as clotted samples cannot be processed by the laboratory.
Check the expiry date on the EDTA/purple-top tube.

Materials required:

Blood sampling

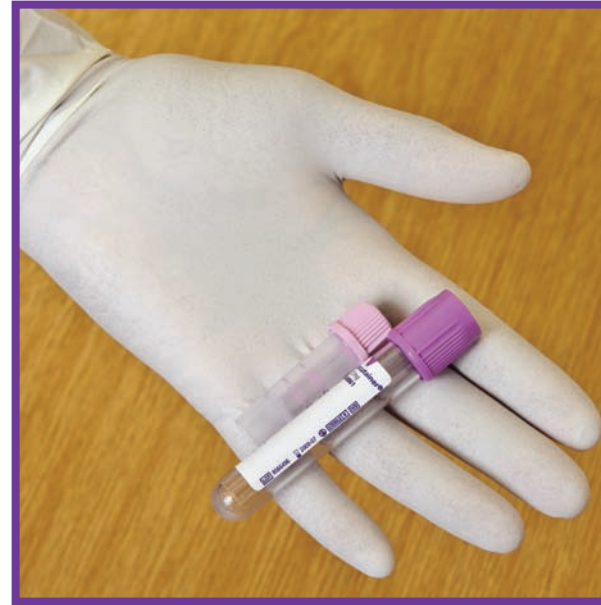
- Disinfectant for skin (eg alcohol wipe)
- Single use, loaded lancing device (eg Hemocue) or needle and syringe
- Cotton wool or gauze

Collection

- EDTA purple-top tube (5 ml or BD microtainer with BD Microgard closure; 8mm diameter)

PLUS *you'll need*

- Powder-free gloves
- CCMT (ARV) NHLS laboratory request form with barcode
- A ziplock plastic bag for specimen packaging (biohazard bag)



STEPS FOR WHOLE BLOOD COLLECTION

- Complete the CCMT (ARV) NHLS laboratory request form with all the patient's details, as per DBS collection.
- Label the EDTA/purple-top tube with the corresponding barcode from the completed CCMT (ARV) NHLS laboratory request form.
- Collect blood in EDTA/purple-top tube by using one of the following methods:

A. Heel/Toe or Finger prick method

1. Clean the proposed puncture site and position as described on page 10.
2. Puncture heel/toe or finger using the loaded lancing device.
3. Allow drops of blood to collect and fall into the purple top microtainer, gently shaking the tube after each drop to prevent clotting. Do not squeeze at the puncture site as this will dilute the blood with tissue fluid.
4. Ideally there should be 500 µl of blood (minimum volume of 250 µl). Place the lid on the microtainer and invert several times to prevent the formation of clots.



STEPS FOR WHOLE BLOOD COLLECTION

B. Formal venipuncture

Blood can also be sampled into 5 ml EDTA purple-top tubes. Collect at least 1 ml of whole blood. Package the whole blood sample in the same way as explained on page 16. Store in a cool place, but not in the fridge. The specimen must reach the laboratory within four days.

RECORD KEEPING

For PCR testing the following record keeping is required:

1. A correctly completed **NHLS request form**. This is necessary so that the laboratory can inform the clinic if there is a problem with the PCR test and deliver patient reports to the correct facility. It also allows for monitoring of infant testing rates.
2. **The clinic PCR testing register** to document infants who have been tested and to ensure that PCR test results are obtained and communicated to parents or caregivers. Remember to document the CCMT site to which HIV-infected infants have been referred for care.
3. **The specimen transport check list** must be completed as a record of the PCR sample being transported to the laboratory for analysis.
4. The infant's **Road to Health Card** to maintain complete medical records indicating that a PCR test has been done, the date it was done and the test result.

CONSUMABLES FOR DBS TESTING NEED TO BE ORDERED FROM THE NEAREST NHLS LABORATORY

Full DBS kit

Item	Supplier	Oracle Item#
DBS kit	Merck	P04A2644
DBS kit	Lasec	P04A2806

If DBS kits are unavailable, the contents of the kit can be ordered separately.

Components

Item	Supplier	Oracle Item#
Safety lancets	Hemocue	P02S0377
Framed DBS cards	Merck	P02B0291
Desiccant sachets	Lasec	P04D0323
Drying rack	Lasec	P03M0189

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