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Taking blood from infants for the HIV PCR test

Standard Operating Procedure









Department: Health REPUBLIC OF SOUTH AFRICA.



Acronyms



ARV	Antiretrovirals
ССМТ	Comprehensive Care, Management and Treatment
DBS	Dried Blood Spots
EDTA	Ethylenediaminetetraacetic acid, used to prevent blood clotting
НСТ	HIV Counselling and Testing
HIV	Human Immunodeficiency Virus
HPCSA	Health Professions Council of South Africa
μΙ	Microlitres
ml	Millilitres
PCR	Polymerase Chain Reaction, a test method used to detect the genes of the virus
РМТСТ	Prevention of Mother To Child Transmission
PICT	Provider-Initiated Counselling and Testing
SANC	South African Nursing Council
SOP	Standard Operating Procedure

This Standard Operating Procedure is

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

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

HIV PCR test

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

- 1. Dried blood spots (DBS)
- Dried blood spots are technically easier to obtain and are suitable for blood sampling in the primary healthcare setting.
- The DBS card has 5 pre-printed circles with perforated edges and space for labelling.
- Dried blood spots can be collected from a heel-, toe- or fingerprick or prepared from venous blood.
- 2. Whole blood in an EDTA/purple-top tube

(Either a microtainer or a 4ml tube)

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

Remember:

- In accordance with the HCT policy, a history of maternal HIV status should be obtained at every visit to identify all HIV-exposed infants that require a PCR test. If indicated, offer PICT to mothers.
- Explain the procedure to the mother, including when to return for results, and obtain informed consent.
- Universal precautions handle all specimens as if they are capable of transmitting infectious agents.





Materials required

1. DBS collection kit

Instructions for performing the procedure are printed on the back of each kit. The kit contains consumables for:

2. Blood sampling

- Disinfectant for skin (e.g. alcohol swab)
- Single use, loaded lancing device (e.g. Hemocue)
- Cotton wool or gauze

3. Collection

- DBS card
- Desiccant sachet
- Ziplock plastic bag

4. PLUS you'll need

- CCMT (ARV) NHLS laboratory request form with barcoded stickers
- Powder-free gloves
- Drying rack
- Biohazard bag: a ziplock plastic bag for specimen packaging

Remember:

Never touch the pre-printed circles on the DBS card either before or after blood collection.









Paperwork

1. DBS card:

Check the expiry date. Label 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. Complete the CCMT (ARV) NHLS laboratory request form with:

- Name of the hospital or clinic
- · Hospital or clinic number
- · Patient's name, date of birth, address and telephonic contacts
- Name, contact details and SANC / HPCSA number of healthcare worker
- · Date the specimen was taken and specimen type
- Clinical details specify whether on PMTCT programme or not
- Test requested (HIV PCR)
- **3. Place a barcoded sticker from the CCMT (ARV) NHLS** laboratory request form onto:
 - The DBS card over the shaded outline on the left side of the card taking care not to overlap the pre-printed circles or the patient details recorded on the card.
 - The Road to Health booklet and the clinic's PCR register to facilitate tracking of the PCR test result.

4. Complete the PMTCT page of the Road to Health booklet.

5. Prescribe cotrimoxazole and antiretroviral (if required) prophylaxis for HIV-exposed infants.





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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 the skin disinfectant swab and allow to dry for 30 seconds. Take care to keep away from bony prominences.











- **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 firmly 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. Lightly touch the drop to the pre-printed circle on the 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 at least three marked circles are filled with blood.
 - The pre-printed circles hold $\pm 75 \mu l$ blood each when completely filled.
 - Samples with insufficient blood cannot be processed since the PCR result may be unreliable.
 - If insufficient blood flow occurs, a second puncture may be required.
 - Do not excessively saturate the card with blood or attempt to smear the blood spots.
- **7.** Apply pressure to the puncture site using gauze (or cotton wool) to stop further bleeding.
- 8. Dispose of the lancet into a sharps container.

Heel prick method













Toe prick method













Method for drying

- 1. DBS card drying rack.
 - Place one DBS card into each slot of the drying rack without allowing the cards to touch each other.

2. Dry for at least three hours at room temperature.

• Do not dry artificially with heat or expose to direct sunlight.

3. Dry completely before packing.

• Properly dried blood spots are dark red or brown in colour.







Features of acceptable and unacceptable DBS samples

Acceptable:

- 1. At least three pre-printed circles should be completely filled with blood.
- 2. The CCMT (ARV) NHLS laboratory barcoded sticker should be affixed and the DBS card completely and accurately labelled.

Unacceptable: 🗶

The laboratory cannot process these DBS cards and repeat samples will be required to obtain a PCR result:

- 3. Patient details on DBS card are not legible.
- 4. Patient details on DBS card and CCMT (ARV) NHLS laboratory request form do not match and no barcode sticker is affixed.
- 5. Insufficient sample for processing.
- 6. Blood spotted outside the pre-printed circle and DBS cards containing clotted/crusted blood.

















Submission to laboratory

- 1. Fold the corresponding, completed CCMT (ARV) NHLS laboratory request form in half and insert into the pocket of the larger biohazard plastic bag with the patient details facing outwards. Check that all information is provided on the CCMT (ARV) NHLS laboratory request form.
- 2. Each properly dried DBS card should be packed into the ziplock plastic bag provided in the DBS collection kit, with one desiccant sachet.
- 3. Place the packed DBS card into the larger biohazard ziplock bag containing the CCMT (ARV) NHLS laboratory request form. Check that the patient details on the DBS card and request form match.
- 4. Record that the sample has been submitted to NHLS on the specimen transport checklist.
- **5.** Place samples into the NHLS specimen collection box for transportation to the nearest NHLS PCR laboratory.

Remember:

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 or in the fridge.

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Whole blood collection

Whole blood samples can be collected by:

- 1. Heel/Finger prick method
- 2. Formal venipuncture

Remember:

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

Materials required (refer page 4)

Blood sampling

- Disinfectant for skin (e.g. alcohol swab)
- Single use, loaded lancing device (e.g. Hemocue) or needle and syringe
- Cotton wool or gauze

Collection

• EDTA/purple-top tube (microtainer or 4ml)

PLUS you'll need

- CCMT (ARV) NHLS laboratory request form with barcode
- Powder-free gloves
- · Biohazard bag: a ziplock plastic bag for specimen packaging



Steps for whole blood collection

Paperwork (refer page 6)

- Place a barcoded sticker from the CCMT (ARV) NHLS laboratory request form onto the EDTA/purple-top tube.
- Indicate on the CCMT (ARV) NHLS laboratory request form that the sample is whole blood.

Method of collection (refer page 8)

Collect blood in EDTA/purple-top tube by:

1. Heel-, toe- or finger-prick method

- 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.
- Ideally there should be 500µl of blood (minimum volume of 250µl).
- Place the lid on the microtainer and invert several times to prevent the formation of clots.

2. Formal venipuncture

Blood can also be sampled into 4ml EDTA/purple-top tubes. Collect at least 1ml of whole blood to avoid dilution by EDTA.

Submission to laboratory (refer page 16)

Store in a cool place or fridge to reach the laboratory as soon as possible but within 4 days.









Record keeping

For PCR testing the following record keeping is required:

- **1.** A correctly completed **CCMT (ARV) NHLS laboratory request form** is necessary so that the laboratory can inform the clinic if there is a problem with the PCR sample and deliver patient reports to the correct facility. It also allows for monitoring of infant testing rates from the NHLS database.
- 2. The clinic PCR testing register to document infants who have been tested and to ensure that PCR test results are obtained from NHLS and communicated to parents or caregivers. Remember to document the CCMT site to which HIV-infected infants have been referred for ARVs.
- **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 Booklet** 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

ltem	Supplier	Oracle Item
DBS kit	Roche	P01B2527
Drying rack	Lasec	P03M0189

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> 9th edition: October 2011 For more information please contact: Prof Gayle Sherman: gayle.sherman@nhls.ac.za Sister Tsakani Mhlongo: tsakani.mhlongo@nhls.ac.za Mrs Megan Murray: infant.dx@nhls.ac.za or 011 489 8543

Disclaimer: the patient name used in this publication is fictitious.