

STANDARD OPERATING PROCEDURE

Title: **Managing INDETERMINATE HIV PCR Test Results**

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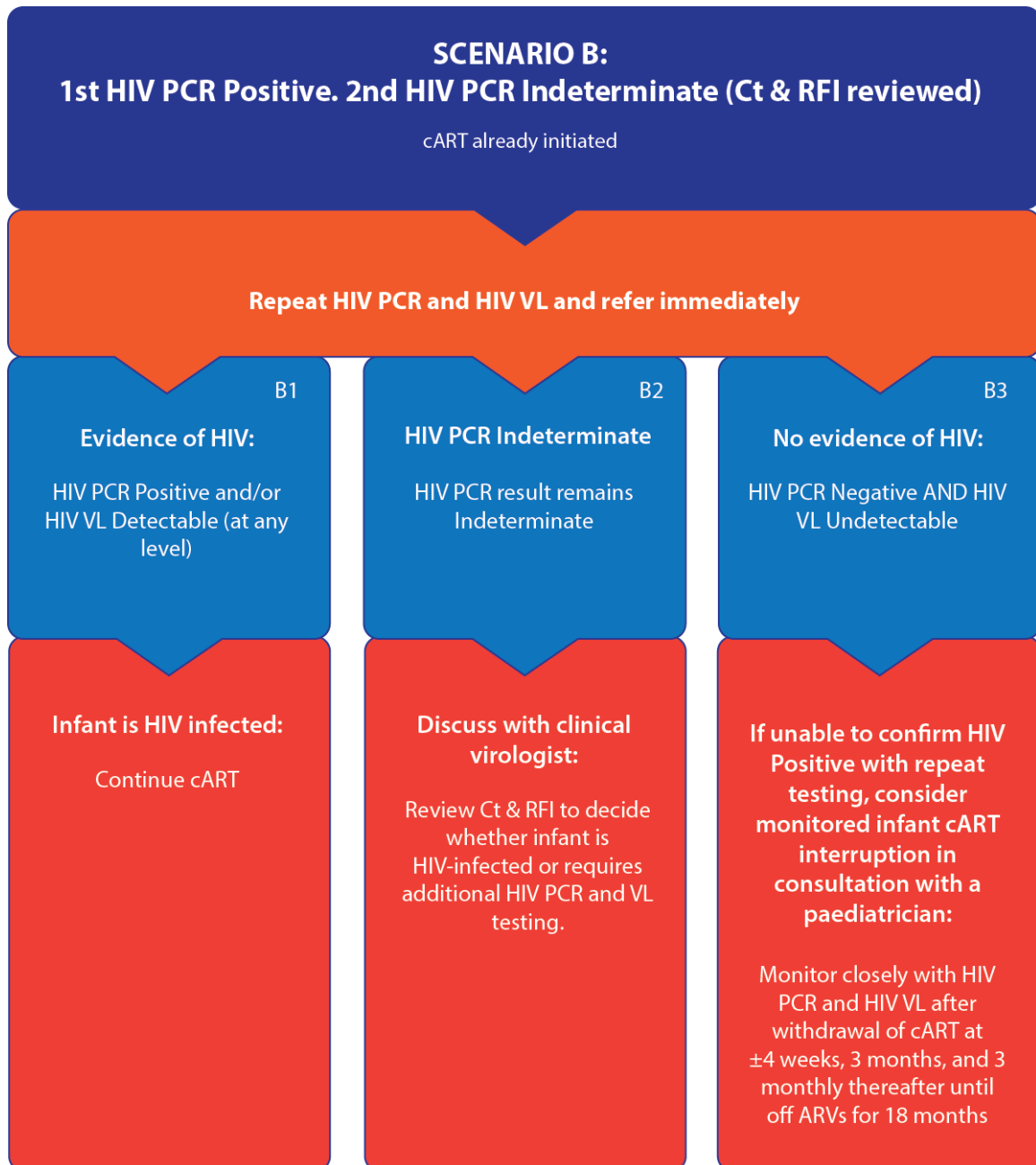
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Summary

- The laboratory diagnosis of HIV in infants <18 months of age requires two HIV PCR positive results, each on a separate specimen, as per National HIV Paediatric testing guidelines of 1 June 2015 (alternatively, one HIV PCR positive result in association with an HIV viral load that is detectable on a separate specimen is also diagnostic of HIV)
- An HIV PCR result can be positive, negative or indeterminate
- An indeterminate HIV PCR result means that the test is inconclusive (i.e. it is not clearly positive or negative) and requires immediate FURTHER TESTING to determine whether the infant is HIV infected or not and REFERRAL.
- Repeat HIV PCR and HIV viral load testing needs to be performed as a matter of urgency and the patient managed accordingly (see Flow Diagram Scenario A for managing initial HIV PCR indeterminate cases and Flow Diagram Scenario B for managing confirmatory HIV PCR indeterminate cases)
- Infants in which the diagnosis of HIV remains inconclusive or where discordant results have been obtained (i.e. a positive HIV PCR followed by a negative HIV PCR and undetectable HIV viral load) need to be managed by a multidisciplinary team and should be discussed as a matter of urgency with a specialist clinician and pathologist (see contact details below). Repeat HIV testing and clinical monitoring is required until an HIV status is established
- It is important to remember that infants cannot be considered HIV-uninfected unless repeat testing occurs at least 4 weeks after infant prophylaxis (or cART has been discontinued), and six weeks after cessation of breastfeeding
- Counseling the mother/primary caregiver regarding the indeterminate result is of paramount importance to ensure successful follow-up and arriving at a definitive diagnosis (see COUNSELING box below)

Flow Diagram A:

Flow Diagram B:



Introduction

According to the National HIV Paediatric testing guidelines of 1 June 2015, all HIV-exposed infants should be tested for HIV infection, using a molecular based assay such as PCR (polymerase chain reaction), at birth, 10 weeks of age and 6 weeks after stopping breastfeeding if still under 18 months of age at that time.¹ In children receiving prolonged nevirapine prophylaxis to 12 weeks of age, an additional HIV PCR test is required at 18 weeks (or 14 weeks of age in some provinces).

The most common specimen collected from an infant is capillary whole blood from a heel prick spotted onto a cotton based paper card, which is dried at the site of collection. This is known as a dried blood spot (DBS) and requires three full spots per card. EDTA (purple top tube) anti-coagulated whole blood is also suitable and can replace a DBS; the minimum volume is 250µl (0.25mL).

The HIV PCR results should generally be available within THREE working days of reaching the nearest NHLS PCR laboratory.

Reporting of an HIV PCR result has 4 options:

- 1) **POSITIVE**, meaning that HIV is detected in the sample,
- 2) **NEGATIVE**, meaning that HIV is not detected in the sample,
- 3) **INDETERMINATE** result or
- 4) **OTHER** results *e.g.* 'insufficient sample'; 'clerical error'; 'invalid result', etc. These infants require submission of a repeat sample for HIV PCR as soon as possible.

Purpose

This SOP provides guidance for NHLS laboratory staff and the relevant clinical care providers on managing INDETERMINATE HIV PCR results.

Definitions:

ART:	Antiretroviral therapy
cART:	Combination antiretroviral therapy
Ct:	Cycle threshold
NDoH:	National Department of Health
DBS:	Dried Blood Spot
DCST:	District Clinical Specialist Team paediatrician or paediatric nurse
EDTA tube:	Blood collection tube with a purple top to prevent coagulation
EID:	Early Infant Diagnosis
HAST:	HIV AIDS STD

HIV:	Human Immunodeficiency Virus
NHLS:	National Health Laboratory Service
Lab:	EID NHLS laboratory
LIS:	Laboratory Information System
MCDS:	Minimal Clinical Data Set
NVP:	Nevirapine
PCR:	Polymerase chain reaction
PMTCT:	Prevention of mother-to-child transmission
RFI:	Relative fluorescence intensity
RTHB:	Road to Health Booklet
SOP:	Standard Operating Procedure
TAT:	Turnaround time
VL:	Viral load

Responsibilities

Clinical care givers

1. Identify HIV-exposed neonates or infants
2. Counsel the care provider and obtain consent for testing
3. Collect the blood specimen as required
4. Complete the NHLS request form with complete minimal clinical data set (MCDS) to ensure results can be returned accurately to the correct clinician in time and that there is a record of the parent's/caregiver's physical address and telephone contact numbers to link infants to care. Ensure the NHLS requisition barcode sticker is placed into the infant's RTHB for ease of tracing the PCR result.
5. For a follow-up specimen, please specify all previous HIV PCR and HIV VL results on the request form with either the laboratory barcode or episode number.

Pathologist and lab staff

1. Provide HIV PCR results within the agreed TAT
2. Review results according to the corresponding SOP and authorize results
3. Prioritize indeterminate HIV PCR results since these infants may be HIV infected and run the risk of increased morbidity and mortality due to delayed cART initiation or unnecessary cART in an uninfected infant
4. Ensure there is a record-keeping system for all indeterminate HIV PCR results
5. Ensure there is a notification system for the relevant clinical staff for all indeterminate HIV PCR results

Procedures:

What does an indeterminate HIV PCR test result mean?

An indeterminate result means that

- The HIV PCR test is inconclusive *i.e.* it is not clearly positive or negative
- Either HIV is present at very low levels that can only just be detected or HIV is absent.

Either way, **urgent repeat** HIV testing is essential to establish the child's HIV status. Such children may either be HIV-infected or uninfected on additional testing. Repeat blood samples should preferably be EDTA (purple top) anti-coagulated whole blood for HIV PCR **AND** HIV VL. If this is not possible, submit DBS for repeat HIV PCR testing and refer immediately for EDTA whole blood sampling for HIV VL testing.

Indeterminate results occur infrequently (in less than 1% of all HIV PCR tests) but are problematic to manage. Uncertainty of the infant's HIV infection status can result in poor outcomes *e.g.* lifelong treatment in HIV-uninfected infants, or morbidity and mortality due to delayed treatment in HIV-infected infants.

An **HIV-infected child** has at least TWO positive HIV virological assays (either an HIV PCR or HIV VL) on TWO separate samples.

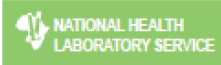

An **HIV-uninfected child** has a negative HIV virological assay on a sample taken ± 4 weeks after all infant ARV prophylaxis has been discontinued provided that no breastfeeding has occurred in the last 6 weeks. These children require follow up testing as per the national guidelines.

Responding to indeterminate results requires a multidisciplinary approach from clinicians and pathologists. Depending on the referral structures in each district, the primary clinician should urgently seek advice for each case from more specialized clinicians (*e.g.* DCST paediatricians, paediatric infectious disease specialists) and pathologists based at the NHLS HIV PCR laboratories as well as PMTCT/HAST program managers.

Every primary clinician should have contact details of specialist clinicians, program managers and their NHLS virology laboratory from the outset. Accurate completion of the NHLS requisition form with patient and clinician contact details facilitates this multidisciplinary approach and should include at least the following: clinic/hospital name, name and surname of patient, date of birth, sex, file number, patient address and contact details, specimen type and collection date, and the health care workers name, registration number and contact details [refer to the Minimal Clinical Data Set – MCDS SOP]. Please take special care to ensure that the details on the request form reflect those on the specimen (*i.e.* ensure that the name, surname and barcode on the form and on the specimen are the same).

Figure1: Example of an NHLS request form

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Actions

The actions required by Clinical Staff:

The actions required following an indeterminate result are described in two broad scenarios **A** and **B** (see flow diagram above)

Scenario A: The first HIV PCR test has an **indeterminate** result:

Action: Repeat an HIV PCR **AND** HIV VL test immediately and refer (as per national guidelines page 27). Do not await the repeat HIV PCR and VL results before referring (see 'Referrals' section below).

A1: The repeat HIV PCR is positive and/or HIV VL is detectable (*i.e.* any value above the detection limit of the assay): the child is likely HIV infected. Infant cART initiation should not be delayed by further testing. Although these cases require a confirmatory HIV PCR and/or VL to definitively establish a positive HIV infection status, the clinical team must consider each case individually. In some cases an indeterminate HIV PCR result (depending on Ct/RFI values) followed by a positive HIV PCR and/or detectable HIV VL result may be enough to establish a diagnosis of HIV infection. If not, confirmatory HIV PCR and HIV VL tests are required.

Action: cART initiation following submission of specimen for confirmatory HIV PCR and HIV VL if necessary.

A2: The repeat HIV PCR is negative or indeterminate again **AND** the HIV VL is undetectable: HIV infection cannot be excluded in the presence of antiretroviral prophylaxis (*e.g.* daily NVP) or within 4 weeks of discontinuing prophylaxis.

Action: Complete the infant ART prophylaxis course (*i.e.* infant NVP syrup, usually for 6 weeks) and repeat HIV PCR **AND** HIV VL 4 weeks later. Monitor the child clinically every 2 weeks. If the child becomes symptomatic for HIV infection, repeat testing immediately. Healthcare workers should have a low threshold for repeat HIV PCR testing at any opportunity before 10-18 weeks.

Scenario B: The first HIV PCR is positive but the second, confirmatory HIV PCR is indeterminate:

Action: Repeat the HIV PCR **AND** HIV VL test immediately and refer. Do not await the repeat HIV PCR and VL results before referring (see 'Referrals' section below).

B1: The repeat HIV PCR is positive and/or HIV VL is detectable (*i.e.* any value above the detection limit of the assay): the child is confirmed HIV-infected because HIV would have been detected twice on separate samples.

Action: Continue cART.

B2: The repeat HIV PCR is indeterminate and HIV VL is undetectable.

Action: Review the Ct and RFI in consultation with a clinical virologist to decide whether the infant can be considered HIV-infected or whether HIV PCR and HIV VL require repeating.

B3: The repeat HIV PCR is negative **AND** the HIV VL undetectable: HIV infection cannot be excluded in the presence of antiretroviral prophylaxis (daily NVP) or cART if already initiated.

Action: The best approach for these infants should be determined within the multidisciplinary team. It is vital to keep the patient's caregiver informed and supported (see 'Counselling Suggestions' below) and the patient kept in close clinical follow up. The same approach should be followed for infants with repeatedly indeterminate HIV PCR results.

In all cases, a clear plan should be documented, communicated and adhered to. If the diagnosis remains unclear despite all attempts to resolve, the last resort is a monitored treatment interruption, if treatment has been started, with follow-up testing at one month, three months, and three monthly thereafter for a minimum of 18 months off ART.

The actions required by Pathologist and Laboratory staff

HIV VIROLOGY LABORATORIES

Indeterminate results, currently defined as Ct >33 and/or RFI <5, should be treated as urgent and only authorized after careful review by a virology registrar or consultant. The Ct & RFI values should be documented on the LIS. Consider:

- TRAK search to identify previous HIV PCR and HIV VL test result(s)
- Contact clinician who sent the HIV PCR test and/or designated centralized responsible person for district or province (*e.g.* paediatric infectious disease specialist/PMTCT coordinator/DCST paediatrician) to discuss case and request repeat samples as soon as possible
- Repeat HIV PCR test **AND** HIV VL (if possible on DBS) on the 'indeterminate' sample after alerting the field i.e. don't delay result in laboratory
- Where clinicians cannot be reached, kindly refer these cases and all available information to Drs Gayle Sherman (gayles@nicd.ac.za) or Ahmad Haeri Mazanderani (ahmadh@nicd.ac.za).

References:

1. South African National Department of Health. National Consolidated Guidelines for the Prevention of Mother-to-Child Transmission of HIV (PMTCT) and the Management of HIV in Children, Adolescents and Adults. Pretoria: Department of Health, 2015.

REFERRALS:

Referrals can mean seeking advice from clinicians and/or virologists or sending the patient to a specialist referral center urgently.

1. NHLS laboratories and virologist contact details

Groote Schuur EID lab, Western Cape (0214045254/ 0214045202)

Dr Marvin Hsiao (0214045200/ 0834451592 after hours)

Tygerberg EID lab, Western Cape (0219389355/ 0219389557)

Dr Jean Maritz (0219389057/ 0833633736 after hours)

Dora Nginza EID Lab, Eastern Cape (0414644635)

Dr Howard Newman (0413956152/ 0832646070 after hours)

Umtata EID lab, Eastern Cape (0413956152)

Dr Howard Newman (0413956152/ 0832646070 after hours)

Universitas EID lab, Free State and Northern Cape (0514053162)

Dr Daniel Morobadi (0514053162/ 0823134770 after hours)

Inkosi Albert Luthuli Central Hospital EID Lab, KwaZulu Natal (0312402800)

Dr Kerusha Govender (0312402822/ 0837799199 after hours)

Tshwane Academic Division EID lab, Gauteng (0123192257)

Dr Ahmad Haeri Mazanderani (0123192670/ 0826428609 after hours)

Charlotte Maxeke Johannesburg Academic EID Lab, Gauteng (0114898809)

Dr Lucia Hans (0114898408/ 0842068074 after hours)

Chris Hani Baragwanath Academic EID Lab, Gauteng (0114898708)

Dr Jeannette Wadula (0114898726/ 0828035699 after hours)

2. Contact details for treating paediatric clinicians; DCST paediatricians or paediatric nurses; PMTCT or HAST managers & co-ordinators

Every facility should have the contact details of clinicians or mentors who can assist with management of complex paediatric HIV cases. Alternatively, facilities should consult their NHLS HIV PCR laboratory virologists.

3. Telephonic helplines

Right to Care Paediatric HIV Helpline 082 352 6642

KZN Paediatric Infectious Disease Helpline 0800 006 603

National HIV & TB Health Care Workers Hotline 0800 212 506

COUNSELING SUGGESTIONS for HIV PCR indeterminate results

The mother/primary caregiver should be consulted regarding decisions about cART initiation. Any decision must consider the practical implications of where and how treatment will be continued. Infant feeding should be carefully discussed considering that breastfeeding improves outcome in HIV-infected infants. Maternal adherence to ART during breastfeeding should be stressed. All cases should urgently be brought to the attention of the relevant HIV clinic. Engagement of the family should be encouraged but the mother should guide the level of family involvement. Monitor the mother's well-being including adequate ART care and monitoring, TB screening and adequate psychosocial support. It is important to document discussions with the mother in the infant's bed letter and RTHB. The mother should have the clinic contact numbers and clinical course and decisions should be documented in the infant's road to health booklet to facilitate communication between different health care providers.

Where possible, to improve compliance, aim for continuity of care at a single facility preferably with a single healthcare worker.

The guiding principles of counseling in these cases should include:

1. The mother/primary caregiver must be involved with honest and frank information at every stage.
2. The message must be communicated that there is a team involved with the infant's care, that guidelines and resources exist to determine the final outcome. However, the length of this process is uncertain. Follow-up care and clear communication, both verbal and written, is critical especially for mobile mothers.
3. The team may not know the answer to the diagnostic dilemma at present but is aware how stressful this is and will undertake to find the solution in consultation with the mother and the necessary experts. At this stage it is critical that the follow-up care is monitored and tracked to reassure the mother/family that somebody is pursuing the problem. In the absence of a clear answer this should provide some level of relief.
4. A clear plan should be documented, communicated and adhered to. In the event of an unclear diagnosis despite all attempts to come to a clear solution, the last resort will be a monitored treatment interruption, if the infant is on cART, with follow-up testing at one month, three months, and three monthly thereafter for a minimum of 18 months off ART.

Note that these families need increased adherence support as they may be confused by the indeterminate results and the lack of a final confirmed diagnosis may contribute to poor adherence to ART.