KINGDOM OF CAMBODIA
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Ministry of Health

Standard Operating Procedure (SOP) for implementing Point-of-Care HIV Viral Load Testing for pregnant, breastfeeding women and exposed infants using GeneXpert Machines

November 2022

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Acknowledgements

The National Centre for HIV/AIDS, Dermatology and STD (NCHADS), National Maternity and Child Health Centre (NMCHC), and National Centre for Tuberculosis and Leprosy Control (CENAT) would like to acknowledge and appreciate all PMTCT-TWG members for their commitment and contribution to the development of this SOP for piloting Point-of-Care HIV viral load testing for pregnant and breastfeeding women and HIV exposed infants using GeneXpert machines.

The National Programs appreciate the efforts made by focal points of NCHADS (Dr. Kaoeun Chetra), NMCHC (Dr. Chorn Samang) and CENAT (Dr. Narith Ratha) in coordinating with all concerned partners during the development of this document. Thanks also go to experts from development partners, particularly US-CDC, WHO, FHI360, UNAIDS, and CHAI who provided suggestions in developing this SOP.

Phnom Penh, ..02/Dec/..2022

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Abbreviations

Abbreviation	Full terminology
ANC	Antenatal Care
ART	Antiretroviral Therapy/Treatment
CAC	Community Action Approach
CENAT	National Centre for Tuberculosis and Leprosy Control
CMC	Case Management Coordinator
CMA	Case Management Assistance
DBS	Dried Blood Spot
DMU	Data Management Unit
EID	Early Infant Diagnosis
EQA	External Quality Control
EMTCT	Elimination of Mother-to-Child Transmission (of HIV)
HC	Health Centre
HEI	HIV Exposed Infant
HP	Health Post
HTS	HIV Testing Service
IQS	Internal Quantitative Standard
LMU	Logistic Management Unit
LTFU	Loss to Follow-Up
NCHADS	National Centre for HIV/AIDS, Dermatology and STD
NMCHC	National Maternal and Child Health Centre
OD	Operational District
PAC	Paediatric AIDS Care
PASP	Provincial AIDS and STD program
PCR	Polymerase Chain Reaction
PHD	Provincial Health Department
PNC	Post-Natal Care
PMTCT	Prevention of Mother-to-Child Transmission (of HIV)
POC	Point-of-Care
PPE	Personal Protective Equipment
PW	Pregnant Women
RDT	Rapid Diagnostic Test
SOP	Standard Operating Procedure
SVA	Sample Volume Adequacy
TAT	Turn-around-Time
VCCT	Voluntary Confidential Counselling and Testing
VL	Viral Load

1. Introduction and Rationale

Some progress has been made towards the elimination of Mother-to-Child Transmission (eMTCT) of HIV. MTCT of HIV rate decreased approximately from 14.3% in 2018 to 13.6% in 2021[1]. Currently, HIV testing for pregnant women (PW) is conducted in all health centres (HC), maternity wards, and health posts (HP) where women received antenatal care (ANC). The viral load (VL) test is performed at only two sites, NCHADS laboratory in Phnom Penh and Siem Reap Provincial Hospital, whereas DNA-PCR testing for exposed infants is conducted only at NCHADS laboratory. Whole blood and dried blood spot (DBS) samples are transported from Maternity Wards and antiretroviral therapy (ART)/Paediatric AIDS Care (PAC) sites to the laboratories for testing. Because of the reliance on referrals, all polymerase chain reaction (PCR) tests for early infant diagnostics (EID) have a long turn-around time (TAT) and a high rate of loss to follow-up (LTFU), with 47% of DNA PCR result ≥ 2 weeks[2].

The Standard Operating Procedure (SOP) outlines the process to pilot point-of-care (POC) HIV viral load testing for pregnant and breastfeeding women and their HIV Exposed Infant (HEI). They focus on the processes that will need to be adapted or introduced to use available GeneXpert machines for PMTCT HIV viral load and PCR testing. This SOP should be used alongside the SOP and job-aides developed for eMTCT and HIV testing service (HTS) and clinical management guidelines such as existing testing algorithms and linkages to ART. The changes outlined below are about ***where*** the tests are conducted and ***how*** results are communicated across the system.

2. Objectives and expected outcomes

This innovation in POC HIV viral load testing seeks to improve patient experiences with HIV viral load testing and improve Prevention of Mother-to-Child Transmission (PMTCT) outcomes. It will achieve these objectives by:

- Reducing turn-around-time of VL test for PW and breastfeeding women and DNA PCR test for their HEIs
- Improving adherence to HIV testing algorithms and reducing LTFU for PW, breastfeeding women and HEIs
- Improving HIV viral load testing among pregnant women and breastfeeding women in accordance with national guidelines.

This is intended to have additional positive impact on PMTCT outcomes, including:

- Timely detection of HIV viral load among PW with increasing HIV viral load testing coverage
- Increasing percentage of HIV-positive women who achieved viral suppression and can safely breastfeed their HEIs
- Reducing morbidity and mortality of infants through early HIV case detection and treatment among HEIs
- Early and continued viral suppression among pregnant and breastfeeding women living with HIV

[1]Cambodia HIV Estimates 2022 based on AEM-spectrum modelling estimates
[2]eMTCT of HIV and Syphilis mock review findings, 2021

3. Role and responsibilities of national programs and involved services

POC VL Testing for PMTCT requires coordination across three national programs, and site-level hospital departments and health facilities provide involved services:

Table 1: Role and responsibilities of national programs and involved services

National Program	**Responsibilities:**
NCHADS	• Ensuring supply of test kits to site-level facilities • Joint training, monitoring and evaluation • Ensuring quality of test results • Ensuring smooth coordination across programs
CENAT	• Support site-level lab to perform testing • Joint training, monitoring and evaluation • Ensuring machine maintenance and quality of test results • Ensuring smooth coordination across programs
NMCHC	• Support site-levels (ANC and maternity services) for sample collection, referral to labs and linkage to ART sites • Joint training, monitoring and evaluation • Ensuring smooth coordination across programs • Ensuring regular progress report
Sub-national and Site Levels	
Provincial Health Department – Provincial AIDS/STD Program, Provincial TB Program, and Provincial MCH/PMTCT Program	Overseeing and coordinating across the programs and services in the province
ART/PAC Services	Providing clinical management (including ART), counselling and adherence support as well as follow-up tests for mothers and infants according to the relevant national guidelines.
Maternity wards	Sample collection of pregnant women and new-borns at delivery to refer to labs and link them to ART/PAC services
Antenatal care Services	Sample collection of pregnant women during ANC visits to refer to labs and link them to ART/PAC services.
Hospital Laboratory	Performing tests, delivering test results to relevant services and machine maintenance
Operational District - OD MCH coordinator	Tracing any HIV-positive mother and HEI for the caring and testing schedule and for those women who are lost to follow-up in their catchment areas
Operational District - OD active case management coordinator (CMC) and case management assistance (CMA)	Tracking and ensuring all PW are screened. Those identified with HIV reactive will receive confirmatory test, and if confirmed positive, women will be immediately enrolled in ART clinic and regularly follow-up

Successful implementation of POC testing requires strong commitment, collaboration, and frequent data sharing across these entities.

4. Site eligibility

POC testing for PMTCT will only be done in a sub-set of HIV testing sites in Cambodia. The site must:

- Have a voluntary confidential counselling and testing (VCCT) and ART clinic
- Have an existing GeneXpert machine, with spare capacity as defined by CENAT or other relevant body
- Have high patient volumes (≥ 15 tests per year) to ensure efficiency of testing
- Be located in a distant geography with limited access to EID testing

In 2022-2023, NCHADS, CENAT and NMCHC have agreed to initiate a POC viral load testing for PMTCT in 15 sites that satisfied the above criteria:

1. Siem Reap Provincial Hospital
2. Kampong Cham Provincial Hospital
3. Ang Rokar Referral Hospital
4. Preah Sihanouk Provincial Hospital
5. Stung Treng Provincial Hospital
6. Tboung Khmum Referral Hospital
7. Rattanakiri Provincial Hospital
8. Pailin Provincial Hospital
9. Kampong Speu Provincial Hospital
10. Thmor Kul Referral Hospital
11. Poipet referral hospital
12. Kampong Trach Referral Hospital
13. Cambodia-Japan Friendship Provincial Hospital-Mongkul Borei
14. Sampov Loun Referral Hospital
15. Serey Sophorn Referral Hospital

In all other sites, existing processes of testing will be followed.

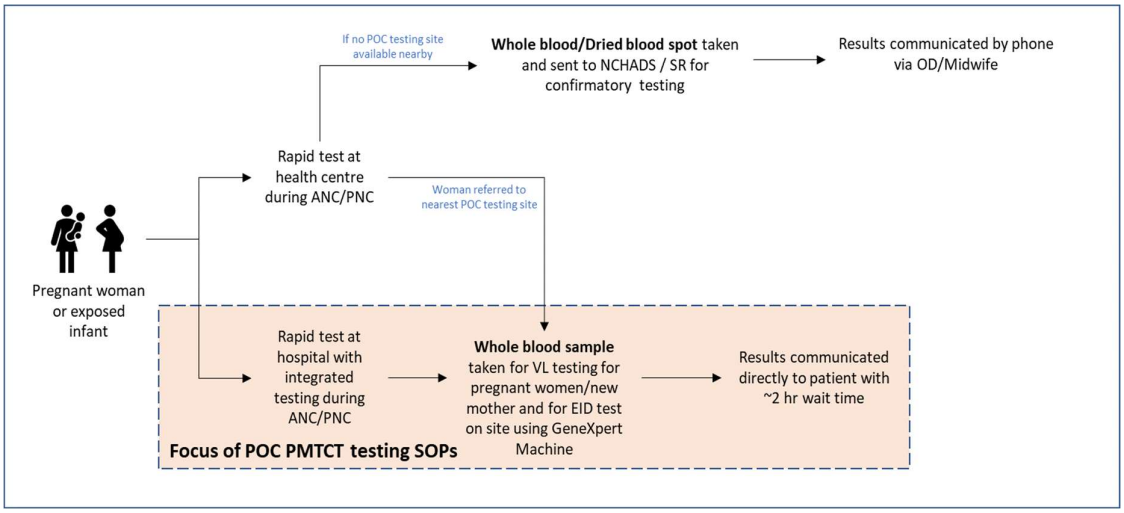


Figure 1: Focus of POC Testing SOP

The scale up of this approach will be considered after the evaluation and learning from the experience of the pilot implementation in these 15 sites.

5. Site-level Operating Procedures

5.1. Patient Flows

Certain changes are required in patient pathways at the referral/provincial hospitals conducting POC testing. Testing algorithms and linkages to care should continue to follow existing SOPs, but samples will be referred to POC labs instead of NCHADS lab. Below, we outline how women move between the maternity ward, laboratory, ART/PAC sites for testing during pregnancy and postpartum and for their HEIs.

5.1.1. Patient flows for HIV testing for pregnant and breastfeeding women

It is essential that pregnant women know their HIV status. When a pregnant woman presents herself at the maternity (for first ANC or any other later opportunities), the midwife should ask about their HIV status. In line with the existing PMTCT guideline - for pregnant women receiving ART before conception should conduct a viral load test immediately. For pregnant women receiving ART during pregnancy should conduct a viral load test after three months if initiate ART before 20 weeks of gestation, if initiate ART after 20 week of gestation, viral load test can be delayed until 34-36 weeks of gestation. All pregnant women should conduct a viral load testing at 34-36 weeks of gestation or at the latest at delivery. Regardless of when ART was initiated, for all breastfeeding women should conduct a viral load test three months after delivery and every six months thereafter.

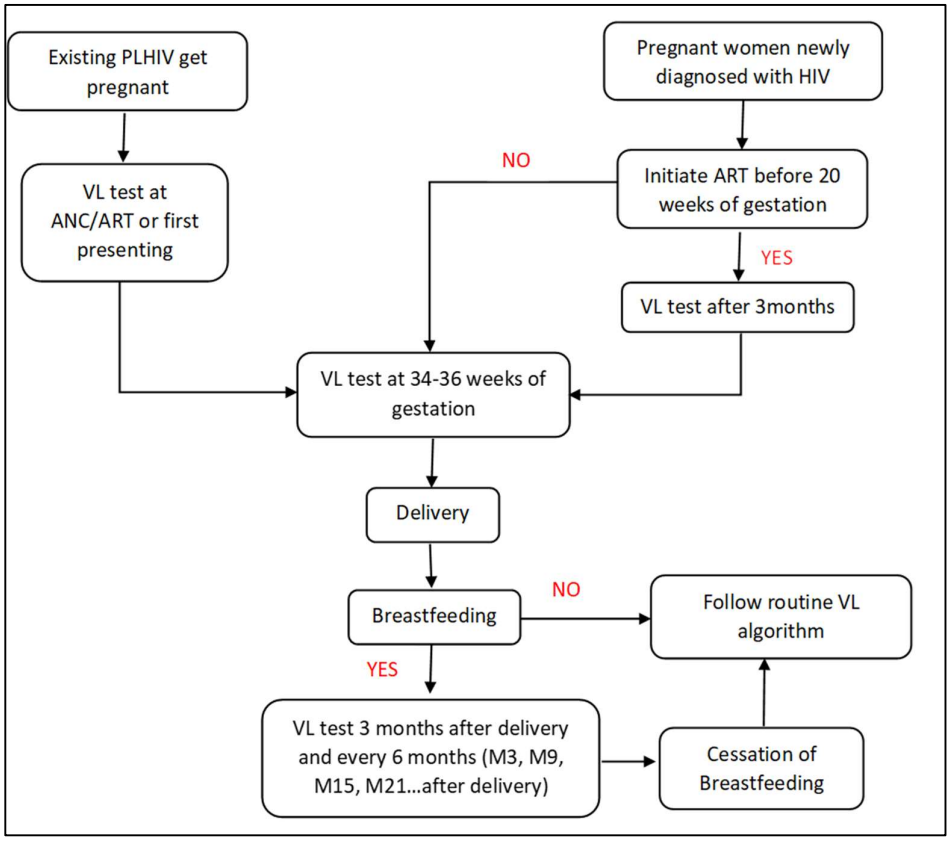


Figure 2: VL algorithm for pregnant and breastfeeding women[3]

[3] National HIV clinical management guidelines for adults and adolescents, Fifth edition 2020

Table 2: Patient flow for pregnant women with unknown HIV status and known HIV+ already on ART

Pregnant women with unknown HIV status	Pregnant women with known HIV+ and already on ART
1. HIV rapid diagnostic test at first ANC	1. At first ANC or fist presenting: VL test using POC GeneXpert machine
2. If positive: Referred to VCCT for confirmatory rapid test	2. At 34-36 weeks of gestation or latest at the delivery: VL test using POC GeneXpert machine. Advised to deliver at hospital and come for EID testing.
3. If confirmed positive at VCCT: Referred to ART site for HIV counselling and treatment. Advised to deliver at hospital and come for EID testing	3. If breastfeeding, conduct VL test three months after delivery and every six months thereafter using POC VL testing.
4. Three months after ART initiation: VL test using POC GeneXpert machine	
5. At 34-36 weeks of gestation or the latest at the delivery: VL test using POC GeneXpert machine	
6. If breastfeeding, conduct VL test three months after delivery and every six months thereafter using POC VL testing.	

Pregnant women with unknown HIV status

As outlined in the PMTCT guideline, all pregnant women who don't know their HIV status should be offered HIV testing and counselling at the first ANC visit or at delivery. If the HIV test was not done at the first ANC visit, it should be done at the later opportunity.

HIV Testing for pregnant women during pregnancy: for pregnant women who access for the first ANC, will be tested using HIV Rapid Diagnostic Test (RDT). Women who test reactive to HIV RDT are referred to VCCT to receive confirmatory test at the same day. If confirmed positive, the women are referred to the nearest ART services for immediate counselling and treatment. The ART site should schedule a VL test after 3 months on ART, f initiate before 20 weeks of gestation. Then, do VL at 34-36 weeks of gestation or at the latest at the delivery. If ART initiate after 20 weeks of gestation, the VL test scheduled at 3 months after ART can be delay to 34-36 weeks of gestation or at the latest at the delivery. This test will be conducted at the ART site using POC VL test as in the guidelines outlined in Section 2.3. Staff in the ART site will take a whole blood sample and complete the lab request form for VL test to send to POC lab for testing.

HIV Testing for pregnant women during labor: for pregnant women who do not know their HIV status at delivery, HIV RDT will be offered. If RDT is reactive, the women should be urgently given ARV as soon as possible while waiting for the result of confirmatory test. If the confirmatory test is negative, stop treatment and reassure the women that she is not infected and provide routine post-natal care (PNC). The women who found to be HIV+ at the delivery do not require viral load test at the delivery. The exposed infants will be provided prophylaxis treatment as high-risk HIV exposed infant.

Pregnant women known HIV+ and already on ART

At the maternity ward, any woman who already known their status should be provided routine ANC counselling. If the women have been on ART before conception, at the first ANC visit, staff in the maternity ward using the guidelines outlined in Section 5.3 will take a whole blood sample and complete the lab request form for VL test to send to POC lab for testing. If the women have the first ANC at an hospital without ART facilities, the woman will be referred to an ART site where the viral load test can be performed. At 34-36 weeks of gestation or at the latest at the delivery, the women should conduct VL. Staff in ART site will take a whole blood sample and complete the lab request form for VL test to send to POC lab for testing.

All HIV+ Pregnant women

All HIV positive pregnant women are enrolled in ART and pre-register at the maternity ward with expected delivery date. The women are advised to deliver at the RH maternity ward co-located with their ART service for safe delivery and to enable faster testing for exposed infants after delivery. After delivery, if the women decide to breastfeed her child, VL test should be conducted three months after delivery, and every six months thereafter to detect viraemic episodes during the post-natal period.

In addition, the maternity ward should inform the ART site of any HIV+ pregnant women/ breastfeeding women arriving for ANC/delivery or PNC. As outlined in Section 5.2, the result of VL test should be recorded in the ART database. If the women do not come for VL test, the case should be referred to the Active Case Management Assistant (CMA) or Case Management Coordinator (CMC) or Community Action Approach (CAC) for follow-up.

5.1.2. Patient flow for HIV testing for HIV-exposed infants

All HIV-exposed infants should receive HIV-DNA PCR test at birth and enrolled in PAC for additional testing in line with the infant diagnosis algorithm. Each HEI should receive a total of up to 3 PCR tests using POC GeneXpert machines:

- HIV DNA PCR at **birth (0-3 days or earliest opportunity prior to week 4 of age)**
- HIV DNA PCR test at **4-6 weeks of age**
- HIV DNA PCR test at **9 months of age**
- HIV antibody test at **18 months of age or 3 months after cessation of breastfeeding**, whichever occurs later.
- **If the infant tests positive on any of the above tests, a confirmatory PCR test should be conducted** right away using the GeneXpert machine. The infant should start ARV immediately while waiting for confirmatory test results.

Test at birth

Any HEI born to an HIV+ mother at an eligible POC testing health facility should receive their HIV DNA PCR at birth before the mother is discharged from the hospital. A midwife at the maternity ward will obtain a whole blood sample from the infant through a heel-prick and ARV prophylaxis should be started. The sample will be tested at the POC lab within the same day and results sent to both the maternity ward and ART clinic.

If the result of HIV DNA PCR at birth is positive, the mother and infant should immediately be referred to the ART clinic to initiate treatment, and confirmatory PCR test should be conducted.

If the result is negative, the maternity ward should provide necessary counselling to inform the HIV-positive mother of the importance of testing for their HEIs. They should be informed of the date of the test and their follow up PAC schedule.

Tests after birth

Whole blood samples for the HIV DNA PCR at 4- 6 weeks and 9 months of age will be collected at the ART/PAC clinic and sent to the POC lab for testing using GeneXpert. This will be done in line with the infant diagnosis algorithm. It will also be done for any HEI that did not receive a test at birth.

If the HIV DNA PCR is positive, ART should be started as quickly as possible and ARV prophylaxis should be stopped. A repeat HIV PCR test confirmatory is required, however do not delay ART initiation while waiting for the confirmatory test result.

If the result is negative, the PAC/ART clinic should provide necessary counselling to inform the HIV-positive mother of the importance of testing for their HEIs. The date of the next test should be scheduled.

The staff at ART/PAC should conduct active case management to trace known all HEIs who failed to show up for the scheduled visits and immediately inform OD/PHD coordinator or CMA/CMC/CAA team of any lost to follow up cases.

5.2. Site-level data collection and management

Health facility staff at ANC, maternity ward, VCCT, ART/PAC are responsible for registering referral card, lab form and mother-infant follow up forms, in line with the SOP above.

This project will be considered part of routine standard of care. All patient documents including HIV test result, must be kept in a confidential and secure place, and should only be accessed by health care providers directly involved in providing care to patient.

Labs will record the result in VL or EID logbook and dispatch/release results to the requester (i.e., either the maternity ward or ART/PAC site) following routine laboratory procedures, to enable quick delivery of results to the patient. In addition, if the test is requested at the maternity ward, the result will additionally be reported to ART/PAC site by lab and recorded in ART database by database officer. ART site will do monthly report and send to provincial AIDS and STD Program (PASP) manager. PASP send a backup file of data to NCHADS on monthly basis and share a consolidated report to NCHADS by quarterly. More details on data management above at provincial and national level can be found in section 6.5.

5.3. Sample Management

5.3.1. Whole Blood Sample collection

Every viral load test should be requested by Maternity/ART/PAC staff using a request form for HIV testing. The form should be fully completed and signed by the requester.

The patient brings the test request and the patient's file to the sample collection site.

The following steps will be taken by the sample collector:

- Label the patient ID on the collection tube
- Collect 4 mL whole blood in 5 mL EDTA tube using Vacutainer apparatus for VL
- Collect 200 µL whole blood in 500 µL EDTA microcontainer tube by heel-prick for EID

- Mix contents immediately by inverting the tube gently back and forth 8-10 times.

5.3.2. Sample handling, transport, and storage

Sample and test request form should be transported to the laboratory for processing as soon as possible by the person who collected the sample. If test cannot be done immediately, the laboratory staff should store the sample using the following procedures:

- Whole blood can be stored at 15–30 °C for up to 8 hours or at 2–8 °C for up to 72 hours
- If testing can't be done within 24h (less preferred situation), centrifuge at 3000 rpm for 15 minutes to obtain plasma
    - Plasma can be stored at 15–30 °C for up to 24 hours or at 2–8 °C for up to 6 days
    - Plasma samples are stable frozen (≤ -18 °C and ≤ -70 °C) for 6 weeks.

5.3.3. Sample processing

Up to four samples can be processed simultaneously: one for each GeneXpert cartridge. It is not necessary for all these to be the same test. For example, one HIV EID test can be performed alongside three TB tests.

Process for testing is outlined in section 5.4.1.

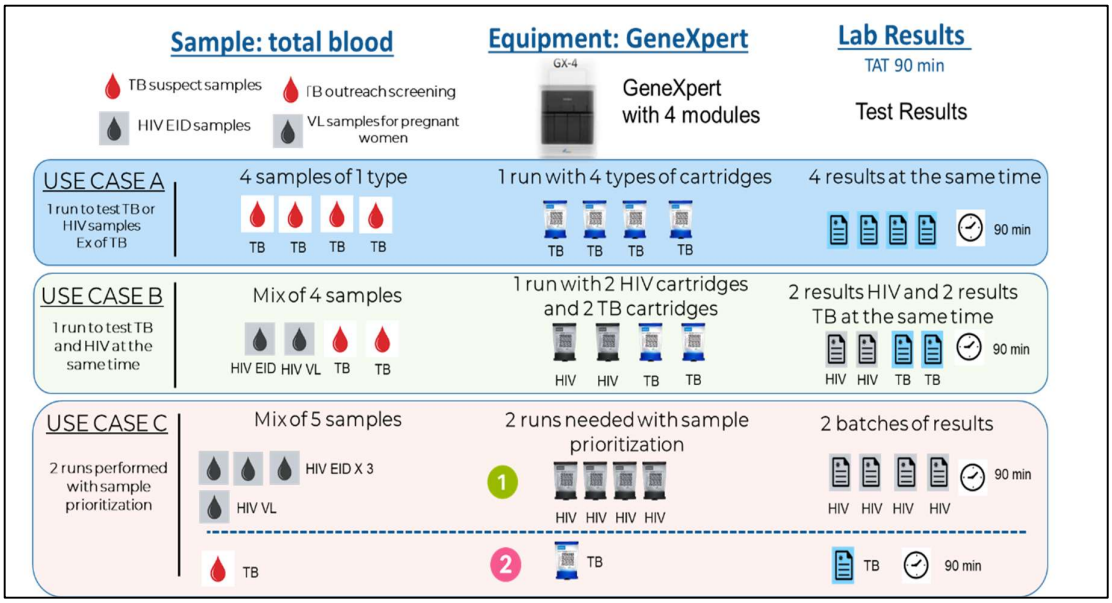


Figure 3: Example of simultaneous sample processing

If more than four samples that require PCR testing are received by the laboratory staff, tests should be performed in order of greatest need. Guidance will be provided during trainings and in job-aides to help determine the appropriate testing order.

After 90 minutes, results from the GeneXpert machine should be recorded in line with the data management SOP outlined in section 5.2.

5.4. Testing procedure

5.4.1. Testing

Table 3: Testing procedures for VL and EID using GeneXpert

	Testing procedure for VL using Xpert HIV-1 Viral Load	Testing procedure for EID using Xpert HIV-1 Qual
1	Prepare at least 1.2 ml plasma sample. If using frozen or refrigerated samples, place at room temperature (RT) until completely thawed and equilibrated to RT	Label the sample reagent (SR) bottle with the sample ID
2	Vortex the equilibrated sample for 15 seconds. If the specimen is cloudy, centrifuge it for a few seconds.	Label the side of the cartridge with the sample ID
3	Label the side of the cartridge with the sample ID	Open the cartridge lid
4	Open the cartridge lid	Pipette 750ul of SR the sample using the provided pipette into the sample chamber of the cartridge
5	Pipette a minimum of 1 mL of the sample using the provided pipette	Mix the whole blood by inverting the tube 7-10 times
6	Slowly empty the pipette into the sample chamber of the cartridge	Immediately transfer 100ul of whole blood using the provided pipette into the sample chamber of the cartridge
7	Close the lid firmly	Close the lid firmly
8	Start the test on your GeneXpert System within 4 hours after preparing the cartridges	Start the test on your GeneXpert System within 30 minutes after preparing the cartridges

Running the test

1. Turn on GeneXpert instrument
2. Turn on computer
3. Login using user ID and password
4. Click on “Create Test”
5. Scan cartridge barcode
6. Fill in the information of patient and sample ID
7. Click on “Start Test”
8. A green light will flash on the module. Load the cartridge into module and close the door.

5.4.2. Results interpretation

Upon completion of the test, the result can be viewed in 90 minutes. Click the “View Results” to view and generate a PDF report file. The results are interpreted automatically by the GeneXpert Instrument System from the measured florescent signals and embedded calculation algorithms. Results will clearly be shown in the “View Results” window.

Table 4: GeneXpert results and interpretation

Cartridge type	Result	Interpretation
Xpert HIV-1 Viral Load	HIV-1 DETECTED XX copies/mL	HIV-1 RNA is detected at XX copies/mL • HIV-1 RNA has quantitative value within the analytical measurement range.
	HIV-1 DETECTED >1 X 10^7 copies/mL	HIV-1 RNA is detected above the analytical measurement range.
	HIV-1 DETECTED <40 copies/mL	HIV-1 RNA is detected below the analytical measurement range.
	HIV-1 NOT DETECTED	HIV-1 RNA is not detected.
	INVALID	Presence or absence of HIV-1 RNA cannot be determined.
	ERROR	Presence or absence of HIV-1 RNA cannot be determined.
	No RESULT	Presence or absence of HIV-1 RNA cannot be determined.
Xpert HIV-1 Qual (EID)	HIV-1 DETECTED	HIV-1 target nucleic acids are detected.
	HIV-1 NOT DETECTED	HIV-1 target nucleic acids are detected.
	INVALID	Presence or absence of HIV-1 target nucleic acids cannot be determined.
	ERROR	Presence or absence of HIV-1 target nucleic acids cannot be determined.
	No RESULT	Presence or absence of HIV-1 target nucleic acids cannot be determined.

Repeat test using a new cartridge for **INVALID**, **ERROR** or **No Result** according to procedures in section 5.4.1.

- **INVALID**: This happens when the sample was not processed properly which resulted in PCR problem, e.g. Inhibition.
- **ERROR**: This happens when an assay was aborted due to machine and Environmental error, e.g. probe check control failure, maximum pressure limit exceeded, GeneXpert module failure and others.
- **No Result**: This happens when insufficient data was collected, e.g. when a run was stopped.

5.4.3. Internal Quality control

Each GeneXpert test cartridge is a self-contained test device with an in-built control for each sample. The internal controls enable the system to detect specific failure modes within each sample. The below controls will be checked on the GeneXpert machine before the test start running:

- Instrument System Control (Check Status): the software checks the optics, temperature of the module and the mechanical integrity of each cartridge.

- Reagent Control (Probe Check): fluorescence readings are measured in the reaction tube for each probe and compared to default settings established by Cepheid.
- Sample Volume Adequacy (SVA): ensures that the sample was correctly added to the cartridge. The SVA verifies that the correct in-volume of sample has been added in the sample chamber.
- Internal Quantitative Standard (IQS): there are two Armored RNA® (IQS High and IQS Low) constructs in the form of a dry bead that goes through the whole assay process. They are used to verify correct sample processing. Additionally, they detect specimen associated inhibition of the RT-PCR reaction.
- Sample Processing Control (SPC) for Xpert HIV-1 Qual: ensure correct sample processing and verifies analysis and presence of the organism and detects PCR inhibition.

5.4.4. Sample rejection

The sample rejection will be documented in the Sample rejection logbook. Sample and test request form will be returned to the clinician. Samples should be rejected if:

- Sample is unlabelled or mislabelled
- Sample without request form
- Sample name and request form do not match
- Sample breakage or leakage
- Sample not collected in an appropriate container
- Insufficient volume of sample

5.4.5. Safety and waste management

Safety during testing

Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Personal Protective Equipment (PPE) should always be used when performing tests such as gown and gloves.

Waste disposal

GeneXpert HIV cartridges contain Guanidinium Thiocyanate, toxic chemical (to human and environment) and must be incinerated at a high temperature (≥ 850°C) within the second combustion chamber with a retention time of 2 seconds. All wastes should be followed proper waste disposal procure in the lab or National guidelines or Infection prevention control SOP.

5.5. Equipment maintenance

GeneXpert maintenance will be conducted: daily, weekly, monthly, and annually based on the below procedures. This is done to ensure quality of HIV and TB testing and to prevent equipment break-down. Lab managers are responsible for ensuring maintenance is carried out by the trained technician.

Material needs

The whole maintenance process involves cleaning with a sterile wipe (no cotton), with a 10% bleach solution prepared within one day. Once dry, after 5 minutes, wipe with a 70% ethanol solution. This requires the following materials:

- 10% Bleach solution
- 70% Ethanol solution

- Lint-free wipes
- Disposable gloves
- Water
- Brush

5.5.1. Daily maintenance

Every day, the lab technician will:

- Discard used cartridges, in line with waste management SOP
- Close all module doors
- Clean workbench around the instrument
- Whenever the GeneXpert system is not in use, switch it off and cover the GeneXpert instrument with a dust cover.

5.5.2. Weekly maintenance

Once per week, the lab technician will:

- Reboot the GeneXpert, computer and software by switching off and wait for 10 second then switch on.

5.5.3. Monthly Maintenance

Table 5: GeneXpert maintenance procedure

Maintenance area	Steps
Clean the instrument surface	1. Moisten a lint-free wipe with 70% ethanol solution 2. Wipe all outside surfaces of the instrument 3. Wipe table surfaces around the instrument
Clean the cartridge bay	1. Moisten a lint-free wipe with a 1:10 solution of household chlorine bleach 2. Wipe the inside of the cartridge bay, the inside of the door and the top lip of the door 3. Wait 2 minutes 4. Repeat step 1, 2, 3 for three times 5. Moisten a lint-free wipe with 70% ethanol solution 6. Wipe the parts described above with the ethanol solution
Clean plunger rods	1. Moisten a lint-free wipe with a 1:10 solution of household chlorine bleach 2. After the plunger rods are lowered, gently wipe the plunger rods 3. Wait 2 minutes (never longer than 5 minutes) 4. Repeat step 1, 2, 3 for three times 5. Moisten a lint-free wipe with 70% ethanol solution 6. Wipe the plunger rod with the ethanol solution

Clean module PCR tube slots	1. Wear laboratory gloves 2. Remove cartridges from the modules 3. Make sure that all the bristles are fully inserted (up to the shoulder of the plastic shank of the brush) 4. Brush the inside of the slot with up and down movements 5. Rotate the brush for approx. 1800 and back, then repeat the previous step 2 times 6. Clean each module for at least 30 sec.
Clean the fan filter	1. Turn off the system 2. Unclip 4 clips one by one 3. Remove the filter fan 4. clean with water and soap 5. Dry fan filter between two paper towels until completely dry before putting it back
Archive tests	1. Click on data management, then archive test 2. Select tests you want to archive, then click ok 3. Name will be given automatically, click on save 4. When archive is ready, click ok 5. Save data on a CD or CD-RW, go to GX folder in computer 6. In the export folder, write click on the data file and send to CD or CD-RW 7. On the DVD RW Drive in my computer, click on burn to disc

5.5.4. Yearly maintenance

Yearly calibration of GeneXpert modules will be done once a year, or every 2,000 modules run using Xpert® Check. This will be conducted by the CENAT calibration team yearly based on a defined schedule. If the machine reaches 2,000 runs before the scheduled maintenance date, laboratory managers should inform CENAT to initiate earlier calibration. If calibration fails, CENAT will send the calibration summary to the supplier (Cepheid) for evaluation.

5.5.5. Curative maintenance

If the module needs to be replaced, the site should inform CENAT as soon as possible. CENAT will collect the broken modules and send them for replacement. The waiting time for replacements is 1-2 months. When the modules of GeneXpert is broken, the site can request back-up modules from CENAT to avoid disruption to testing.

6. Operating Procedures for National and Provincial Levels

6.1. Procurement and Supply Chain Management

The HIV-1 Qual (EID) and HIV-1 Quant (VL) cartridges and other lab consumables are quantified and submitted to the logistic management unit (LMU) of NCHADS for review and approve by NCHADS management team and Global Fund (refer to procurement guidelines of NCHADS). Once the stock arrives, it is stored at NCHADS for management and distribution. The referral hospital sites are required to report the stock data/request form to OD. OD is responsible to compile all request forms and send to PHD for approval. Provincial hospitals send the stock data/request form directly to PHD. PHD submit all the stock data/request form to LMU/NCHADS quarterly to refill stock. Shortage of stock within a quarter can be reported directly to NCHADS (refer to logistic and supply management of NCHADS).

6.2. Training and Capacity Building

6.2.1. Training

Health providers at Maternity, VCCT/ART, PAC services and Laboratory, OD/PHD PMTCT coordinators, CMA/CMC/CCA will be trained by the NCHADS, CENAT, NMCHC with technical assistance from partners. The initial training will be conducted for 2 days, including on-site training for laboratory staff. Trainees will be taught how to conduct whole blood sample collection for pregnant women and infant, how to perform GeneXpert HIV for VL and EID test and how to record data and report results.

6.2.2. Job aides

Job aides on specimen collection, HIV testing for VL and EID using GeneXpert, data management of POC program will be developed and distributed to sites. It should be posted at the wall near where the testing is done. Job aides are essential to support health providers and clients in understanding better and following the procedures required.

6.2.3. Staff proficiency testing

Staff proficiency test should be conducted annually to evaluate the lab staff capacity and ensure high-quality testing and accuracy of results.

- Proficiency test result on GeneXpert HIV-1 VL testing (Xpert VL) ≥ 80%
- Proficiency test result on GeneXpert HIV-1 Qual testing (Xpert EID) ≥ 80%

6.3. Site-level Support

6.3.1. Motivation

The POC laboratory will receive a payment for results to encourage prioritisation of GeneXpert testing. For each VL or EID test conducted, laboratory staff will receive $1 per test. The testing number are reported to OD/PHD per month and send to NCHADS for review and approval. The payment will return to the finance at OD/PHD and paid to the lab staff.

6.3.2. Supportive supervision

Joint supportive supervision visits between the NCHADS, CENAT and NMCHC is critical to monitor POCprogram performance and provide objective feedback for program improvement. In order to ensure the quality of POC service delivery, joint-supervision will be conducted every six months by NCHADS, CENAT and NMCHC and partners. Supervision visits should not only aim to mitigate problems but also identify strengths and successes using a standard supervision checklist.

6.3.3. Loss to follow-up support by ODs

OD MCH Coordinators, in consultation with the PASP are responsible for conducting active case management to trace any HIV+ mother and exposed infants who are lost to follow up until cessation of breastfeeding or infection is ruled out.

6.4. External quality assessment (EQA)

To ensure the quality of testing, external quality assessment should be conducted periodically. EQA measures the performance of the tests and of the operator performing testing. Inter-laboratory comparison will be used for EQA. 10 samples (HIV VL and EID) that run by GX machines from each site will be collected and sent to NCHADS lab for testing and compare the result. The TAT for the retesting must be accomplished in a timely manner allowing for immediate corrective actions.

6.5. Data management and Monitoring

6.5.1. Data collection and analysis

The POC Sites submit monthly report to OD, and OD send to PHD. PASP will compile the data and send to NCHADS on quarterly basis. Data Management Unit of NCHADS will review the data sent by PHD to ensure consistency, accuracy and completeness of reported data and consolidate report to the national programs.

DMU will analyse the data for program evaluation and review quarterly using programmatic indicators, including volume of EID, TB and VL tests. Comparisons of programmatic outcome indicators within the integration sites will be made. Data will be analysed to review data of core indicators to identify improvements in implementation and to identify sites that require additional support in implementing POC testing.

6.5.2. Information sharing

The selected data and data analysis will be shared by DMU/NCHADS to CENAT and NMCHC for feedback. Also, share the feedback from the national centres to provincial and OD level on the progress and possible solutions to overcome challenges.

6.5.3. Monitoring indicators

Measuring the progress and compiling the results of the POC implementation is a crucial part of program monitoring. It is critical to hold quarterly meetings with POC sites under the direction of NCHADS, CENAT and NMCHC to evaluate data of core indicators to see how far the implementation has progressed, identify obstacles, and outline feasible solutions to overcome those challenges.

Table 6: Programmatic and Operational Indicators

Testing Quality and Testing volume	Number of EID tests done (disaggregated - positive, negative, invalid/error)
	Number of confirmatory tests for HIV DNA PCR Positive (disaggregated – positive, negative, invalid/error)
	Number of VL tests done for pregnant women (copies/ml to determine viral suppression)
	Number of VL tests done for breastfeeding women (copies/ml to determine viral suppression)
	Percentage of error/invalid results, per test type
Performance Impact	EID turnaround time (from sample collection to result available)
	Percentage of infants born to women living with HIV received a virological test for HIV within 2 months of birth (GAM indicator)
	Percentage of HEI with PCR 1 negative receive PCR 2 result
	Percentage of HEI with both PCR 1 and PCR 2 negative, receive PCR 3 result
	Percentage of HEI confirmed HIV positive and initiate treatment
	Percentage of pregnant/breastfeeding women tested for VL with previously high VL received EAC/follow up care

	Number of mother-baby pairs recorded in the line list with known HIV outcome of HEI at 18 months or 3 months after cessation of breastfeeding (whichever comes later)

7. ANNEXES

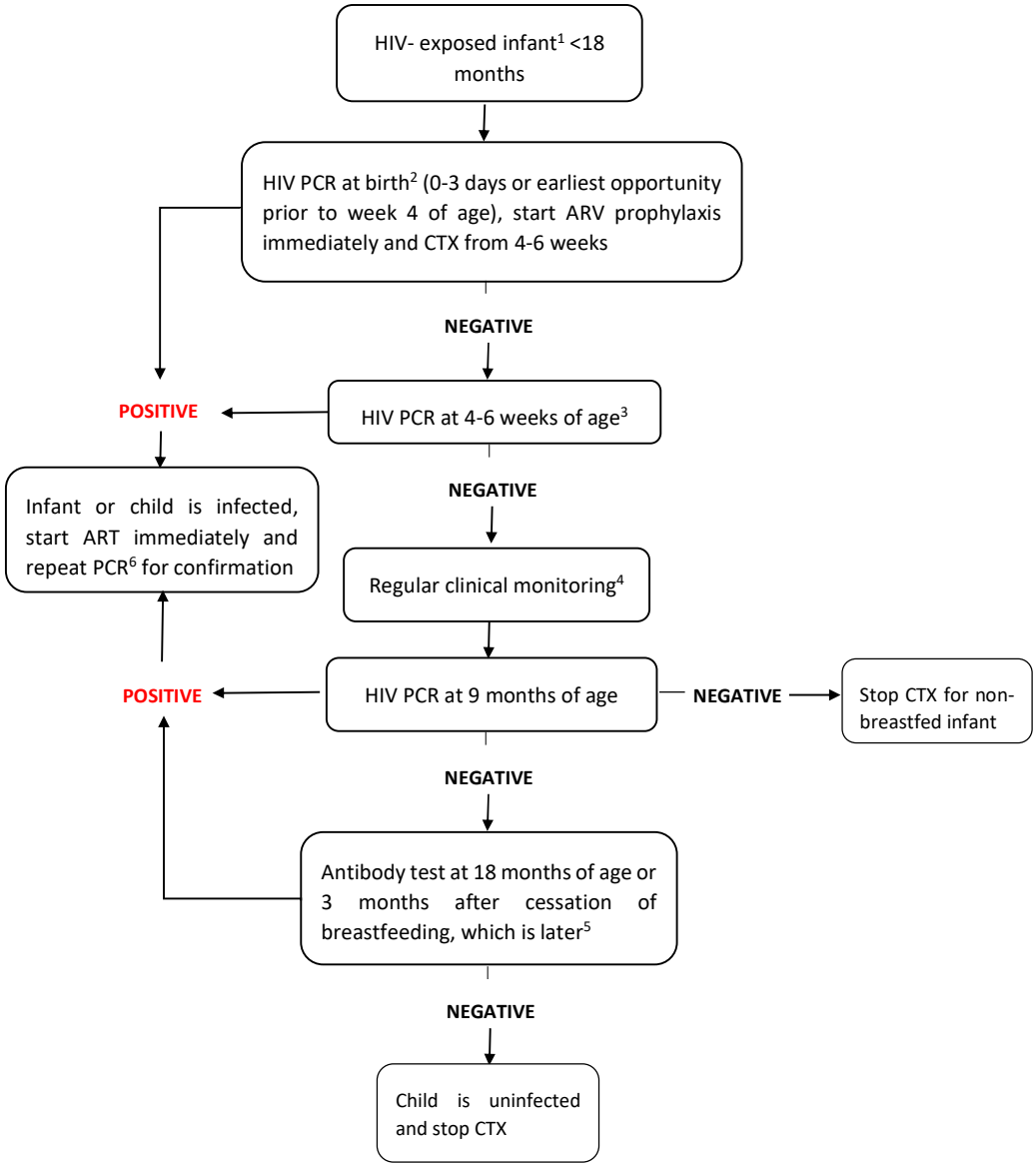
ANNEX 1: Table of site selection for POC

	GeneXpert Sites	City/Province	No. of GeneXpert machine	Unused GeneXpert Capacity (%)	ART site	No. EID test per year (3 tests per infant)[a]	No. of VL test per year (2 tests per mother)[b]
1.	Siem Reap Provincial Hospital	Siem Reap	1	54%	Yes	60	40
2.	Kampong Cham Provincial Hospital	Kampong Cham	2	66%	Yes	99	66
3.	Ang Rokar Referral Hospital	Takeo	1	75%	Yes	15	10
4.	Preah Sihanouk Provincial Hospital	Preah Sihanouk	1	73%	Yes	60	40
5.	Stung Treng Provincial Hospital	Stung Treng	1	75%	Yes	15	10
6.	Tboung Khmum Referral Hospital	Tbong Khmum	1	83%	Yes	15	10
7.	Rattanakiri Provincial Hospital	Rattanakiri	1	85%	Yes	30	20
8.	Pailin Provincial Hospital	Pailin	1	90%	Yes	30	20
9.	Kampong Speu Provincial Hospital	Kampong Speu	1	56%	Yes	60	40
10.	Thmor Kul Referral Hospital	Battambang	1	61%	Yes	27	16
11.	Poipet Referral Hospital	Banteay Meanchey	1	73%	Yes	45	30
12.	Kampong Trach Referral Hospital	Kampot	1	60%	Yes	21	14
13.	Cambodia-Japan Friendship Provincial Hospital-Mongkul Borei	Banteay Meanchey	1	76%	Yes	15	10
14.	Sampov Loun Referral Hospital	Battambang	1	77%	Yes	30	20
15.	Serey Sophorn Referral Hospital	Banteay Meanchey	1	82%	Yes	12	8

a. Number of infants (2020 data) multiply by DNA PCR test per infants following the testing algorithm.

b. Number of pregnant women (2020 data) multiply by VL test per pregnant women following the testing algorithm.

ANNEX 2: Diagnosis of a known HIV-exposed infant < 18 months of age



1. *All women should be offered HIV testing during pregnancy or delivery. Infants of HIV seropositive mothers are considered HIV-exposed. Some women may not be identified before the baby is born. Healthcare workers should ask mothers whether they have been tested when they bring their babies for EPI visits, and what the test results showed. Active case management should be conducted to trace known HIV-exposed infants who fail to show up for scheduled visits.*
2. *Birth testing is recommended and may identify high-risk intrauterine infections.*
3. *If birth HIV PCR is negative, the child may still be infected and further tests must be performed at around 4-6 weeks and 9 months, to increase detection of HIV-infected infants. Caregivers should not be told that the birth PCR test indicates the child is not infected, as this test has low sensitivity.*

4. *At any time during follow-up, if an HIV-exposed infant develops signs and symptoms of HIV infection (e.g. recurrent infections, thrush >6 weeks of age, hepatomegaly, failure to gain weight, neurological development problem), HIV testing should be conducted at that time.*
5. *If breastfeeding extends beyond 18 months, the final diagnosis of HIV status can only be assessed at the end of breastfeeding. If breastfeeding ends before 18 months, the final diagnosis of HIV status with antibody testing can only be assessed at 18 months. Antibody testing should be undertaken at least 3 months after cessation of breastfeeding (to allow for development of HIV antibodies).*
6. *Start ART without delay. At the same time, retest to confirm infection. If the second PCR test is negative, a third PCR test should be performed before interrupting ART. Once a child is confirmed to have HIV-infection, there is no need for subsequent repeat testing.*