



Standard Operating Procedure for HIV-1 Quant Assay with CBNAAT



National AIDS Control Organisation
Ministry of Health & Family Welfare, Government of India



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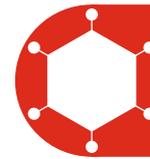
National AIDS Control Organisation
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Abbreviations



ART	Antiretroviral Therapy
ARTC	Antiretroviral Therapy Centre
CBNAAT	Cartridge Based Nucleic Acid Amplification Test
CD4	Cluster of differentiation 4
CDC	Centers for Disease Control and Prevention
CTD	Central TB Division
EDTA	Ethylene Diamine Tetra Acetic acid
EQA	External Quality Assessment
HIV	Human Immunodeficiency Virus
IMS	Inventory Management System
LT	Lab Technician
MO	Medical Officer
M&E	Monitoring and Evaluation
ml	Millilitre
NACO	National AIDS Control Organisation
NACP	National AIDS Control Programme
PCR	Polymerase Chain Reaction
PLHIV	People Living with HIV/AIDS
QA	Quality Assurance
QC	Quality Control
RNA	Ribonucleic Acid
RNTCP	Revised National TB Control Program
RPM	Revolutions per minute
RT-PCR	Real-Time Polymerase Chain Reaction
SACEP	State AIDS Clinical Expert Panel
SACS	State AIDS Prevention and Control Societies
TAT	Turnaround Time
TB	Tuberculosis
TRRF	Test Requisition cum Result Form
VL	Viral Load
WHO	World Health Organisation
°C	Degree Celsius





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Introduction

HIV treatment outcomes among people living with HIV (PLHIV) are dependent on monitoring the response to antiretroviral therapy (ART). Under NACO, PLHIV on ART were traditionally monitored by CD4 count and Viral Load (VL) was offered only in case of suspecting a clinical failure and/or drug resistance. In wake of the new WHO VL testing guidelines, National AIDS Control Programme (NACO) has developed the policy of routine Viral Load testing for monitoring of patients on ART⁽¹⁾. The main rationale behind recommending routine Viral Load monitoring as the preferred approach compared to immunological and clinical monitoring is to provide an early and more accurate indication of treatment failure and the need to switch to second-line ART drugs, reducing the accumulation of drug-resistance mutations and improving clinical outcomes.

Cartridge Based Nucleic Acid Amplification Test (CBNAAT) is a rapid, easy to use test with a short Turnaround Time (TAT) and is flexible (on-demand) to adapt to low throughput requirements in proximity to ART centres. It requires minimum infrastructure and training. It's recent validation carried out at the National AIDS Research Institute (NARI), Pune, ascertains the validity and performance of the CBNAAT and its integration into the national programme for management of PLHIV and HIV-TB patient care⁽²⁾. To support the scaling up of VL across the country, NACO and Central TB Division (CTD) have initiated the process to provide available spare capacity of CBNAAT for HIV-I VL testing. This document provides technical and operational guidance on HIV-I Viral Load assay using CBNAAT instrument.

1. Purpose and Scope:

The purpose of this document is to define the technical procedure and operational steps of Xpert HIV-I Quant Assay, performed on the GeneXpert Instrument Systems. In addition, the document outlines the steps and responsibilities of the staff in the sample collection, plasma separation, packaging, transportation, testing and report generation. The Standard Operating Procedure (SOP) will be implemented by the staff of ARTC and CBNAAT lab.

2. Staff Responsibilities:

2.1 Medical Officer at the ARTC

- 2.1.1 The Medical Officer (MO) at the ARTC will screen the patient for HIV-I VL test eligibility and prepare (initiates) the Test Requisition and Request Form (TRRF, Annexure-III) in triplicate, fill patient's details, reason for current VL test and the previous VL test details, if any, in the TRRF.
- 2.1.2 On receipt of the HIV-I Viral Load results from the CBNAAT laboratory, MO ARTC will take an appropriate action or refer to e-SACEP for further management (Refer to HIV-I VL testing algorithm). MO will suggest the next date of Viral Load test.

2.2. ARTC Laboratory Technician

- 2.2.1 ARTC Laboratory Technician (LT) will complete the relevant sections of the TRRF and assign a Unique Viral Load test ID to each patient. Unique VL test ID (17 digit) is a

- combination of ART centre ID + patient's ART number + Viral load test number. (Refer Annexure-IV).
- 2.2.2 LT should ensure that all necessary materials / consumables required for sample collection is present at the ARTC. (Refer Annexure-I).
 - 2.2.3 LT is responsible for sample collection, separation of plasma (centrifuge) and preparation of aliquots.
 - 2.2.4 LT is responsible for packing and transportation of plasma aliquots along with two copies of TRRF.
 - 2.2.5 On receiving the information from CBNAAT LT about test completion and report preparation, ART LT should collect the reports (completed TRRF with results) from CBNAAT laboratory and submit to ARTC for further action.
 - 2.2.6 Maintain Viral Load Register at ARTC (Annexure-V)
 - 2.2.7 Enter data in Inventory Management System (IMS) (Refer: National Guidelines for HIV-1 Viral Load Laboratory Testing, Chapter-13)

2.3 CBNAAT Laboratory Technician

- 2.3.1 CBNAAT LT is responsible to receive and verify the plasma aliquots along with two copies of TRRF from the ARTC.
- 2.3.2 LT is responsible to follow sample acceptance and rejection criteria (refer viral load testing guidelines). In case of any discrepancy, LT informs ARTC immediately by phone and/or email.
- 2.3.3 On receiving the sample, LT will enter patient's details in VL laboratory register (Annexure V) at CBNAAT laboratory.
- 2.3.4 The laboratory technician is responsible to perform VL test as per the test protocol and SOP of the CBNAAT instrument.
- 2.3.5 LT should enter the result in the TRRF and get the result (TRRF) signed from the MO CBNAAT laboratory.
- 2.3.6 LT should inform the ARTC on completion of the test and report preparation.
- 2.3.7 One copy of TRRF is retained at CBNAAT laboratory and other copy of TRRF is handed over to ARTC LT.
- 2.3.8 LT is responsible for maintaining inventory of reagents and consumables required for Viral Load testing and storage of the CBNAAT HIV-1 VL cartridges at 2-8°C.

2.4 Medical Officer at CBNAAT Laboratory

- 2.4.1 The MO is responsible for supervising the entire process of VL testing.
- 2.4.2 On completion of the tests, MO will authorize the release of the test result.

3. Procedure

The procedure is divided under two broad headings: **Operational requirements and Technical requirements.**

3.1 Operational aspects of HIV-I Viral Load test using CBNAAT instrument

The RNTCP has installed 628 CBNAAT instruments across the country to diagnose TB and Rifampicin resistance and has plans to add approximately 500 additional machines in 2018. Using different cartridges, TB testing along with HIV-I VL estimation can be performed simultaneously on the same instrument. Hence, convergence of both the programmes is possible through integrated testing.

3.1.1 Work flow for HIV-I Viral Load testing

All HIV-I and HIV I & 2 co-infected patients who are registered under the program and have been on ART for more than six months are eligible for VL testing.

The MO at the ARTC will screen the patient for HIV-I VL test eligibility and for those who require testing. MO will prepare/initiate the TRRF and direct them for the sample collection. LT at the ARTC will collect the whole blood (WB) sample, centrifuge WB, separate plasma and prepare plasma aliquots. These plasma aliquots along with the TRRF will be transported to CBNAAT laboratory within six hours of sample collection in transportation box with ice /gel packs. On receiving the plasma samples, LT at the CBNAAT laboratory will verify the samples with the TRRF. Once the verification is complete, the CBNAAT LT will run the test. On completion of the test, CBNAAT LT will enter the result in the TRRF and inform the ARTC. On receiving the information about test completion and report preparation, the ARTC LT will collect the signed TRRF report from the CBNAAT laboratory. The workflow of HIV-I VL testing is explained below:

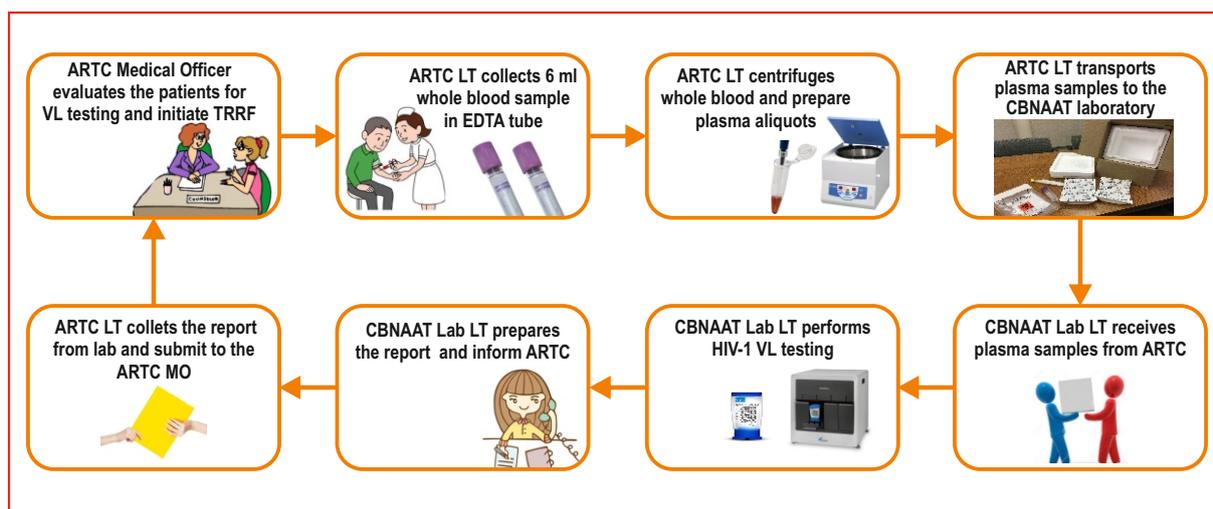


Figure-I: Workflow of HIV-I Viral Load testing

3.1.2 Sample collection, processing, storage and transport

The LT should ensure that the material required for sample collection, processing and temporary storage is available at the ARTC. Annexure-I provides a list of these consumables.

3.1.3 Sample Collection, Storage and Transport

- a. As soon as the patient arrives at the sample collection area, the LT verifies the patients identity by at least two identifiers (for example age, sex, ART number etc.) from the green book.
- b. LT will fill the relevant sections of the TRRF, label the sample collection (EDTA) tube and prepare the patient for sample collection.
- c. LT will collect 6 ml of whole blood sample in EDTA (Purple top) tube using sterile precautions from a peripheral vein. (*refer chapter 2 NACO HIV testing guidelines June 2015 for details on phlebotomy*)
- d. This whole blood sample will be processed at the ARTC to obtain the plasma. The vacutainer will be centrifuged at 2000-2500 RPM for 10 minutes. After centrifugation, plasma is separated in pre-labelled aliquot tube.
- e. Plasma sample should be stored and packed as per the instructions in the Annexure-II.
- f. The plasma aliquots should be transported to CBNAAT laboratory by ARTC LT in a sample transportation box.
- g. Two scenarios for sample transportation include:
 - (i) The ARTC and CBNAAT laboratory are either co-located or in the same premises. In that case, plasma should be separated within 2-3 hours of blood collection and must be transported in sample transport box using with cool gel packs on the same day.
 - (ii) If plasma is to be transported from an ARTC which is far away from CBNAAT laboratory: In that case, plasma is packed in a triple layer package and transported to the testing laboratory as per the sample transportation instructions in Annexure-II

Whole blood may be held at;

- a. 15°–30 °C (Room Temperature) for up to six hours,

After centrifugation, plasma may be held at;

- b. 2°–8° C for up to five days
- c. Frozen (≤ -70 °C) for up to five years

Plasma must be removed from the primary collection tube after centrifugation for storage

Refer: Viral Load Testing guideline-2018

3.1.4 Receipt of plasma samples and Acceptance/ Rejection criteria

a. Receipt of plasma samples at CBNAAT Laboratory:

1. CBNAAT LT receives the plasma sample along with two copies of TRRF from the ARTC.
2. On receiving the plasma samples, the patient details are recorded in Viral Load Register at Testing Laboratory (Annexure-VI) and are verified based on acceptance and rejection criteria.
3. In case of sample rejection, CBNAAT LT informs respective ARTC immediately by phone and/or email. CBNAAT LT also returns the respective TRRF back to the ARTC requesting for a repeat sample.
4. The issue and resolution must be recorded in the VL register (“Remarks” section) present at the laboratory .

5. Once the sample is accepted, then LT performs the test.

b. Acceptance criteria

1. Sample properly labelled
2. Sample tube integrity maintained, no leakage
3. Sample label matched with TRRF
4. Adequate volume
5. Clear plasma
6. Transport box temperature maintained within 2- 8°C on receipt

c. Rejection criteria

1. Haemolysed sample
2. Grossly lipemic samples
3. Contaminated samples
4. Inadequate volume (less than 1.2 ml of plasma)
5. Leaking tubes
6. Improperly labelled sample
7. Label not matching with TRRF
8. Samples from HIV-2 infected individuals
9. Improper storage and transportation
10. All rejected samples should be retained till fresh sample is either received or as per the protocol.

3.2 Technical aspects of HIV-I Viral Load test by CBNAAT

3.2.1 Instrument

GeneXpert instrument (Cepheid, USA) is a CBNAAT that uses reverse transcriptase polymerase chain reaction (RT-PCR) technology for rapid quantitation of HIV-I in human plasma from HIV-I infected individuals.



Figure-2: GeneXpert CBNAAT Instrument with HIV-I VL Assay Cartridges

3.2.2 Principle

GeneXpert Systems automate and integrate sample preparation, nucleic acid extraction and

amplification, and detection of the target sequence in simple or complex samples using real-time reverse transcriptase PCR (RT-PCR). The systems consist of an instrument, personal computer, and preloaded software for running tests and viewing the results. The systems require single-use disposable cartridges that contain the RT-PCR reagents and carry out the sample extraction and the RT-PCR processes. Because the cartridges are self-contained, cross-contamination between samples is minimized. Each cartridge also includes reagents for two internal controls for quantitation of HIV-1 RNA. The internal controls are used to monitor the presence of inhibitor(s) in the RT and PCR reactions. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity and dye stability.

3.2.3 Analytical Measurable Range (AMR)

The Analytical Measurable Range (AMR) for Xpert HIV1 quant is in the range of 40 to 10,000,000 copies/ml.

3.2.4 Sample requirement

- a. Sufficient volume is critical to obtain valid test results. Therefore, minimum of 1.2 ml plasma should be pipetted directly into the test cartridge by precision pipette however, if using the transfer pipette included in the kit, a minimum of 1.2 ml plasma is required.
- b. Plasma samples stored at 2°– 8°C should be removed from the refrigerator and equilibrated to room temperature (20°– 35°C) prior to testing. Plasma samples should be vortexed for 15 seconds before use. Cloudy samples should be clarified by a quick (10 second) centrifugation at 2200 rpm.

3.2.5 Reagents and Instruments

- a. CBNAAT HIV-1 VL Assay Cartridges with Integrated Reaction Tubes
- b. Bead 1, Bead 2 and Bead 3 (freeze-dried) 1 of each per cartridge
- c. Lysis Reagent (Guanidinium Thiocyanate) 2.0 ml per cartridge
- d. Rinse Reagent 0.5 ml per cartridge
- e. Elution Reagent 1.5 ml per cartridge
- f. Binding Reagent 2.4 ml per cartridge
- g. Proteinase K Reagent 0.48 ml per cartridge
- h. Disposable 1 ml Transfer Pipettes



Figure-3: CBNAAT HIV-1 VL Assay Cartridges

3.2.6 Storage and handling of CBNAAT HIV-I VL Assay Cartridges

- a. The HIV-I VL cartridge should be stored between 2-8°C
- b. Expired cartridges should not be used
- c. As these are single use cartridges, cartridges used once should not be re-used
- d. Cartridge should not be opened until ready to use
- e. Cartridge that has been dropped or shaken after the sample has been transferred to the cartridge should not be used as this may yield invalid results.

3.2.7 Testing process

The cartridge is a single-use, disposable, closed system that contains all the reagents for HIV-I RNA extraction, amplification and detection.

- a. HIV-I VL cartridges and plasma samples should be brought to room temperature prior to pipetting plasma into the cartridge.
- b. After opening the lid of the test cartridge, 1 ml of plasma is transferred using the precision pipette or 1.2 ml of plasma while using the transfer pipette.
- c. The lid of the cartridge is closed.
- d. The HIV-I VL cartridge is scanned and loaded on the GeneXpert system.
- e. The test is completed in 90 min based on the pre-fed programme for HIV-I Viral Load testing. For details, refer to the manufacturer's instructions

4. Quality Control (QC)

Includes activities used to monitor the testing process and ensure that the test run is valid. It refers to processes used to minimize errors during the analytical phase of testing. QC is implemented by using control materials which can be internal from the kit and a set of controls arranged from external sources other than the kit i.e. not belonging to the kit.

4.1 Internal Quality Control (IQC)

Internal to test kit: In CBNAAT cartridge, Quality control is inbuilt. Each test includes a Sample Volume Adequacy (SVA), a Sample Processing Control (SPC) and Probe Check Control (PCC). Result validation is based on inbuilt high and low controls

4.2 Turn Around Time (TAT)

HIV-I VL monitoring provides an early and more accurate indication of treatment failure and the need to switch the treatment regimen. Thus, it is very critical that the TAT, defined as the time between sample collection at ARTC and delivery of report is kept to a minimum. The TAT for any CBNAAT laboratory should not exceed 72 hours. In case of any possible delays; ARTC should be informed on a priority basis.

4.3 Annual Maintenance Contract (AMC), Maintenance and Calibration

All CBNAAT instruments should be under AMC wherein the manufacturer is responsible for scheduled maintenance and calibration. The ancillary equipment like centrifuge, refrigerator deep freezer, micropipette etc. should undergo periodic preventive maintenance and calibration as per NABL I I 2 requirements.

5. External Quality Assessment (EQA) Programme

It involves the evaluation of laboratory performance using an external agency / facility. Important elements includes proficiency testing.

5.1 Proficiency Testing

The CBNAAT laboratory should participate in the National EQA Programme.

6 Interpretation of Results

The results are interpreted automatically by the GeneXpert System from measured fluorescent signals and embedded calculation algorithms displayed in the View Results window. The system generated output include the following results:

S No.	Results	Interpretation
1	HIV-1 DETECTED #copies per ml	HIV-1 target nucleic acids are detected
2	HIV-1 DETECTED <40 copies per ml	HIV-1 target nucleic acids is detected below the analytical measurement range
3	HIV-1 NOT DETECTED	The HIV-1 target nucleic acids are not detected
4	NO RESULT	Indicates that insufficient data were collected or the operator stopped a test that was in progress.
5	INVALID/ ERROR	Repeat test according to the instructions

7 Safety

- 7.1 All biological samples, including used cartridges should be treated with standard biosafety precautions.
- 7.2 Protective disposable gloves, laboratory coats and eye protection should be used when handling samples and reagents.
- 7.3 When processing more than one sample at a time, open only one cartridge; add sample and close the cartridge before processing the next sample.
- 7.4 Follow BMW rules (2016) or local guidelines for disposal of HIV-1 VL cartridge and biological samples.

8 Monitoring and Evaluation

- 8.1 Supervisory visits will be conducted periodically to access the compliance of the testing process. NACO and SACS will conduct on-site visits to the CBNAAT laboratory to systematically review the laboratory's practices and implementation of national guidelines. These visits will also aid the laboratories in improving internal processes and root cause analysis.
- 8.2 Monthly data from ARTC and CBNAAT Laboratory will be compiled and sent to SACS and NACO.

Monthly indicators for monitoring CBNAAT HIV-1 VL test include the following:

a. ARTC



1. Number of patients eligible for VL testing
2. Number of samples referred from ARTC to CBNAAT laboratory for testing
3. Number of PLHIV with Viral Load > 1000 copies/ml
4. Number of reports received within 72 hours of referral to CBNAAT Lab
5. Clinical management of patient, referral to e-SACEPS

b. CBNAAT laboratory

1. Number of samples received for HIV-1 VL testing
2. Number of samples rejected (according to sample acceptance & rejection criteria)
3. Number of samples tested
4. Number of reports generated and informed to ARTC within TAT (within 72 hours of referral from ARTC)
5. Number of HIV-1 VL cartridge utilized/stock register (including wastage) in the reporting week

The formats of TRRF, Viral Load Registers at ARTC and CBNAAT laboratory are attached as Annexure III, V & VI.



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5. World Health Organization Prequalification of In Vitro Diagnostics Public Report, Product: Xpert® HIV-I Viral Load with GeneXpert® Dx, GeneXpert® Infinity-48, GeneXpert® Infinity-48s and GeneXpert® Infinity-80 (July/2017, version 2.0).
6. Cepheid Xpert® HIV-I Viral Load Technical report (2015)
7. Bio-Medical Waste Management Rules, Ministry of Forest, Environment and Climate change, Government of India, <http://www.iwma.in/BMW%20Rules,%202016.pdf> (2016)



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List of equipment and consumables required at the ARTC and CBNAAT laboratory

S No.	Sample Collection (ARTC)	CBNAAT Laboratory
1	Gloves	Gloves
2	Laboratory coat	Laboratory coat
3	TRRF	Micro-pipette
4	VL Laboratory Register (Sample Collection)	VL Laboratory Register (Testing)
5	EDTA tubes (6 ml)	GeneXpert System (4.6a or higher version)
6	Centrifuge, Refrigerator and Deep Freezer (2- 8°C and -20°C)	Air conditioner, Desktop/Laptop
7	SOPs for sample collection, handling and storage	Labels & Marker pens
8	SOPs for centrifugation, triple layer packaging and transport	HIV-1 VL cartridges and transfer pipette
9	Tube Rack, Pasteur Pipettes, Aliquots	Tube Rack
10	Labels & Marker pens	Thermometer
11	Syringe, Needle, etc.	Scanner & Printer
12	Needle destroyer and Puncture proof container	Ethanol (For dis-infection)
13	Transportation box/ Cold pack, Packing material/ Ziploc Bags with Biohazard sign	Internet connection
14	4% & 1% Sodium hypochlorite	4% & 1% Sodium hypochlorite; Ethanol
15	First aid & Spill management kit	First aid & Spill management kit
16	BMW buckets with colour coded bags	BMW buckets with colour coded bags



Sample Storage and Packing for Transportation

A. Specimen Storage conditions for VL testing using CBNAAT

Whole blood may be held at;

- ▶ 15°– 30 °C (room temperatures) for up to six hours

After centrifugation, plasma may be held at;

- ▶ 2°– 8° C for up to five days
- ▶ Frozen (≤ -70 °C) for up to five years

Plasma must be removed from the primary collection tube after centrifugation for storage
Refer: *Viral Load Testing guideline-2018*

B. Packaging Viral Load Samples for Transportation

Two scenarios for sample transportation include:

- (a) The ARTC and CBNAAT laboratory are either co-located or are in the same premises. In that case, plasma should be separated within 2-3 hours of blood collection and must be transported in sample transport box within six hours at room temperature on the same day.
- (b) If plasma is to be transported from an ARTC which is far away from CBNAAT laboratory, plasma is packed in a triple layer package and transported to the testing laboratory. Triple packaging includes three layers.
 - c) Primary Receptacles
 - ▶ Tube containing sample for Viral Load testing
 - ▶ The tube must be watertight and leak proof
 - ▶ Must be appropriately labelled
 - ▶ Wrapped in enough absorbent material to absorb all liquid in case of breakage or leakage
 - d) Secondary Packaging
 - ▶ The aim of this layer is enclosure and protection of the primary receptacle
 - ▶ This again must be watertight and leak proof
 - ▶ Several wrapped primary receptacles may be placed in a single secondary packaging. This can be a specially designed screw cap container or a zip lock bag. The second layer of packaging has a rack or similar item to keep samples from moving around too much.
 - e) Outer Packaging
 - ▶ This layer protects secondary packaging from physical damage while in transit
 - ▶ All the documents like TRRF and any other documentation required should be placed in this layer.
 - ▶ Must be a sturdy container with a latch or able to be taped shut. The outer container can be an insulated box like a thermocol or a cold container. The outside of the 3rd container should remain clean so as to be easily handled without any need for PPE.

- e. HIV positive blood sample are “BIOLOGICAL SUBSTANCE, CATEGORY B” (UN 3373) and each package should display following information. The shipper’s (sender’s, consignor’s) name, address and telephone number, the receiver’s (consignee’s) name, address and telephone number.
- f. The proper shipping name “BIOLOGICAL SUBSTANCE, CATEGORY B” should be mentioned adjacent to the diamond shape.

JobAID for Sample packaging

<p>Step 1: Place cooler in box for transport</p>	<p>Step 2: Add frozen ice packs to cooler. Temp for whole blood should be 2-8 °C.</p>	<p>Step 3: Cover frozen ice packs with absorbent material (paper towels, kimwipes, etc.)</p>
		
<p>Step 4: Add specimen racks, place in zip-top bag, close and add to cooler.</p>	<p>Step 5: Add racks and more frozen ice packs to minimize movement.</p>	<p>Step 6: Close cooler with lid and keep closed unless more specimens are added.</p>
		

Unique Viral Load Test IDs

Each patient will be assigned a unique viral load test ID (17 digit) which is a combination of his/her ART centre ID + patient's ART number + Viral load test number.

The ART number is unique for all the patients at an ART centre and is present for all patients on ART. The viral load test ID can be created using the methodology given below.

Viral load test ID is a number which represents the number of times a particular test type has been conducted. The first test will take the number 1, second test 2 and so on.

For instance –

If a patient with ART number –00876 from BJMC ART centre (ART centre ID: ART-MH-PNA-01) is undergoing his/her second viral load test, then the unique viral load test ID will be -

Unique Viral load Test ID = ART centre ID (10 digit) + Patient's ART number (5 digit) + Viral load test number (2 digit)

Unique Viral load Test ID: ART-MH – PNA-01 – 00876 – 02

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National AIDS Control Organisation
Ministry of Health & Family Welfare, Government of India
www.naco.gov.in

6th & 9th Floor, Chanderlok Building, 36 Janpath, New Delhi - 110 001
Tel: 011-43509999, 23731778, Fax: 011-23731746