सत्यमेव जयते

National Guidelines for
HIV-I Viral Load Laboratory Testing

NACO

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

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February 2018

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

Foreword

Government of India strives to attain the Sustainable Development Goal of elimination of AIDS by 2030. To achieve this fast track targets of 90-90-90 i.e. 90% of all people living with HIV know their status, 90% of all people with diagnosed HIV infection receive sustained antiretroviral therapy; and 90% of all people receiving antiretroviral therapy are virally suppressed; are to be attained by 2020. To measure viral suppression, third 90 it is essential that all patients on ART are monitored routinely by estimating their viral load once a year.

I am pleased to declare that NACO has taken necessary measures to initiate routine viral load testing by outsourcing and strengthening public health infrastructure by increasing facilities for testing. In this regard preparation for "National Guidelines for HIV-1 Viral Load Laboratory Testing" by Lab Services Division is at an appropriate time and shall provide necessary guidance to all concerned for routine Viral Load testing in the country.

I congratulate Lab Service Division for preparing these guidelines and reaffirm our commitment to eliminate AIDS epidemic by 2030.

(Sanjeeva Kumar)

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सत्यमेव जयते

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Ministry of Health & Family Welfare
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Message

Under National AIDS Control Organization (NACO), Ministry of Health and Family Welfare, Government of India there are more than 1 1 million PLHIV on ART at more than 530 ART Centers. On April 28, 2017 the Health Ministry launched "Test and Treat" policy for all PLHIV in the country. By adopting Test and Treat, NACO aims to significantly increasing the number of PLHIV on treatment in order to achieve epidemic control.

Currently, PLHIV on first line ART are monitored by six monthly CD4 count testing to monitor the treatment response. Those with immunological failure along with other supportive clinical criteria undergo targeted viral load testing for switching over to second line ART. Presently, NACO implements targeted HIV Viral Load testing through 10 Viral Load Testing Centers in the government run facilities across the country and around 18,000 tests are done annually.

In line with the 90-90-90 target for controlling HIV/ AIDS epidemic, the third 90 is about number of people whose infection is virally suppressed . In this regard, NACO plans to scale up viral load testing and introduce routine viral load monitoring of all patients on ART in a phased manner to achieve this 90 at the earliest. Under support from GFATM, 80 VL testing laboratories will be setup under NACO to meet the demand of Routine VL tests of more than a million per year. Hence, the need to develop a set of Viral Load Guidelines.

These National Guidelines for HIV-1 Viral Load Laboratory Testing will provide an update on Viral Load testing platforms under NACO, laboratory setup required for Viral Load testing, specimen collection and sample handling, testing and interpretation of Viral Load test results for clinical decision making.

I congratulate the Laboratory Services Division of NACO for bringing out these guidelines to provide complete information on technical aspects of Viral Load testing applicable across country.

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Preface

More than three decades have elapsed since the HIV/AIDS epidemic was first recognized in India. During this period, our country has responded in several ways, including formulating and implementing a series of strategic plans and interventions. Many interventions were geared towards preventing further spread of HIV/AIDS. In line with the 90-90-90 target for controlling HIV/AIDS epidemic, the third 90 is addressed in these "National Guidelines for HIV-1 Viral Load Laboratory Testing" i.e. 90% of those initiated on antiretroviral treatment should have suppressed viral load.

Towards the achievement of the third 90, NACO is scaling up the nationwide network of HIV-1 Viral Load testing facilities. These technical guidelines will provide all the required details to establish 70 more Viral Load testing laboratories in the countries. The guidelines cover all aspects required starting from specimen collection to testing at Viral Load Laboratories and interpretation of test results.

I thank all the national experts and contributors for their hard work in producing this technical document for Viral Load Laboratories.

(Dr. Naresh Goel)

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Table of Contents

Abbreviations

ANC	Anti-Natal Care
ART	Antiretroviral therapy
bDNA	Branched DNA
BSC	Biosafety cabinet
CAPA	Corrective and preventive action
CD4	Cluster of differentiation 4
CO2	Carbon dioxide
CoE	Centre of Excellence
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide Triphosphate
EDTA	Ethylene Diamine Tetra Acetic acid
EQA	External Quality Assessment
FEFO	First Expired First Out
HEPA	High Efficiency Particulate Air
HIV	Human Immunodeficiency Virus
IA	Internal Auditor
IATA	International Air Transport Association
IC	Internal Control
ICTC	Integrated Counselling & Testing Centre
ID	Identity Document
ILC	Inter Laboratory Comparison
IMS	Inventory Management System
ISO	International Organization for Standardization
LIMS	Laboratory Information Management System
LJ CHART	Levey-Jennings Chart
LLOQ	Lower Limit of Quantification
LT	Lab Technician
M&E	Monitoring and Evaluation
ml	Millilitre
MMLV	Moloney Murine Leukemia Virus
MRM	Management Review Meeting
MSDS	Material Safety Data Sheet
NASBA	Nucleic Acid Sequence Based Amplification Assays
OI	Opportunistic Infections
PCR	Polymerase Chain Reaction

PEP	Post-Exposure Prophylaxis
PLHIV	People Living with HIV/AIDS
PPE	Personal Protective Equipment
PT	Proficiency Testing
QA	Quality Assurance
QC	Quality Control
QMS	Quality Management System
RCA	Root Cause Analysis
RNA	Ribonucleic Acid
RT	Reverse Transcriptase
RT-PCR	Real-Time Polymerase Chain Reaction
SACEP	State AIDS Control Expert Panel
SCM	Supply chain management
SD	Standard Deviation
SDS	Safety Data Sheet
TB	Tuberculosis
TMA	Transcription-Mediated Amplification
TO	Technical Officer
VL	Viral Load
CV	Coefficient of Variation
^{o}C	Degree Celsius

1 Introduction

1.1 Current status of HIV epidemic

The prevalence of HIV for the year 2015 among adult (15-49 years) population as estimated by the National AIDS Control Organization (NACO), Ministry of Health and Family Welfare, Government of India is 0.26% (0.30% among males and 0.22% among females).The adult HIV prevalence at national level has continued its steady decline from an estimated peak of 0.38% in 2001-03 through 0.34% in 2007 and 0.28% in 2012 to 0.26% in 2015.India is estimated to have around 86 thousand new HIV infections in 2015 and that shows a 66% decline in new infections from 2000 and 32% decline since 2007. Between 2000 and 2015, new HIV infections dropped from 2.51 lakhs to 86 thousand, a reduction of 66% against a global average of 35%.In 2015, an estimated 67.6 thousand people died of AIDS-related causes nationally. AIDS related death declined by 54% between 2007 and 2015 against a global average of 41% decline during 2005-2015. It is estimated that the scale-up of free ART since 2004 has saved cumulatively around 4.5 lakhs lives in India until 2014. This decline in prevalence, new infections and the mortality is consistent with the rapid expansion of access to ART in the country.This has been achieved by the efforts of all the stakeholders with a well-coordinated effort from NACO (India HIV Estimation 2015:Technical Report, NACO).

In October 2014, UNAIDS has set an ambitious target of 90-90-90 to help end the AIDS epidemic by the year 2030. By the year 2020 the policy aims to have 90% of all people living with HIV (PLHIV) to know their HIV status, 90% of all diagnosed HIV infected individuals to receive sustained antiretroviral therapy [ART] and amongst those individuals receiving ART 90% to be virally suppressed. As per the 2015 estimate the total number of PLHIV in India is 21.1 lakhs and of these 6.47% are children less than 15 years of age. Among these 10.7 lakhs including 57,230 children are receiving ART as on December 2016 (MPR, May 2017, NACO). India, the country with third largest PLHIV population also took up this challenge so that it can substantially reduce the epidemiological burden of HIV infection. Reaching these targets on time will require comprehensive strengthening of the programme, especially in early diagnosis and treatment, expanded access to second-line and third line ART and long-term retention in care. In order to achieve these targets, Government of India has adopted WHO guidelines of Test and Treat Policy in March 2017 where in patients diagnosed with HIV will be started on ART irrespective of the CD4 count. Towards the achievement of the third 90 in the 90-90-90, it is important to scale up the nationwide network of HIV-1 Viral Load testing facilities.

1.2 Monitoring of HIV-1 positive individuals

WHO recommends various tests for the monitoring of the HIV infected individuals (Table 1).

Table 1: Summary of WHO recommended laboratory parameters for monitoring HIV infected individuals

Monitoring	Laboratory tests
Virological	▸ HIV-1 Viral load* ▸ Resistance to antiretroviral drugs
Immunological and Haematological	▸ Total lymphocyte count ▸ CD4+ T lymphocyte count
Opportunistic infections (OI)	▸ Reactivation of TB ▸ Occurrence of new OI ▸ Recurrence of treated OI ▸ Antimicrobial susceptibility of bacterial pathogen
Adverse drug reactions	▸ Liver and kidney function tests ▸ Haematological parameters

*Currently there are no guidelines FDA /CE approved kits for monitoring of HIV-2 infected individuals and in-house assays are being used, if required.

The CD4+ T lymphocyte count is the most important laboratory indicator of immune function in HIV-infected individuals. WHO has evolved on the level of CD4+ T cell counts for the initiation of ART from 200cells/µl in 2003 to 500 cells/µl - by - year 2013. Subsequently, WHO adopted the test and treat policy in the year 2015. Hence, periodic tests are necessary to evaluate all patients for virological and immunological failure. Among the different monitoring tests used, CD4+ T lymphocyte cell count is the strongest predictor of subsequent disease progression and survival according to findings from clinical trials and cohort studies. It is the key factor in determining the need to initiate opportunistic infection (OI) prophylaxis and also diagnosis of immunological failure. However, several studies have shown that people living with HIV-1 on ART with routine viral load monitoring had better health outcomes than people monitored with CD4+ T lymphocyte count testing alone. This also includes lower rates of loss to follow-up and death. Viral load testing is more likely to detect treatment failure early. This will enable an opportunity to undergo improved adherence support to conserve the first-line regimens and in case there are no adherence problems, it will help in the prompt switching of ART to the second and third-line treatment regimens. The 2013 WHO consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection recommends viral load testing as the preferred monitoring tool for diagnosing and confirming the failure of antiretroviral therapy (ART). In accordance with these guidelines, many countries are investing in viral load testing to monitor PLHIV receiving ART. Successful models have been developed in countries such as South Africa and Thailand having case and geographical diversity like India. In South Africa monitoring of HIV-1 infected individuals on ART with viral load showed encouraging results with 75% of PLHIV receiving a VL testing in the year 2015. Amongst these 78% were virally suppressed. This data has helped the programme managers to strategise the priorities for implementing the treatment programme.

Currently in India PLHIV on ART are monitored with CD4+ T lymphocyte count every 6 months. When the individuals show immunological failure during follow up, viral load test is carried out as targeted viral load testing. This is based on the advice of SACEP (State AIDS Control Expert Panel) and these individuals are referred to COEs (Centre of Excellence) for onward management. The Viral Load testing is carried out in one of the existing 10 VL testing laboratories. However, the important challenges of instituting a HIV-1 Viral Load testing in a country like India as a national programme are the exorbitantly high cost for the establishment of monitoring facility, issues with sample collection, its transportation and quality of the results generated. In addition, there is a requirement of a well-established laboratory infrastructure, lab network and well-trained human resources across the country.

1.3 HIV-1 Viral Load testing expansion plan

In order to efficiently and successfully expand the VL testing generally a phased scale-up is recommended as depicted in Figure 1. This will enable programmatic and operational data to be collected in meticulous way and optimise national expansion.

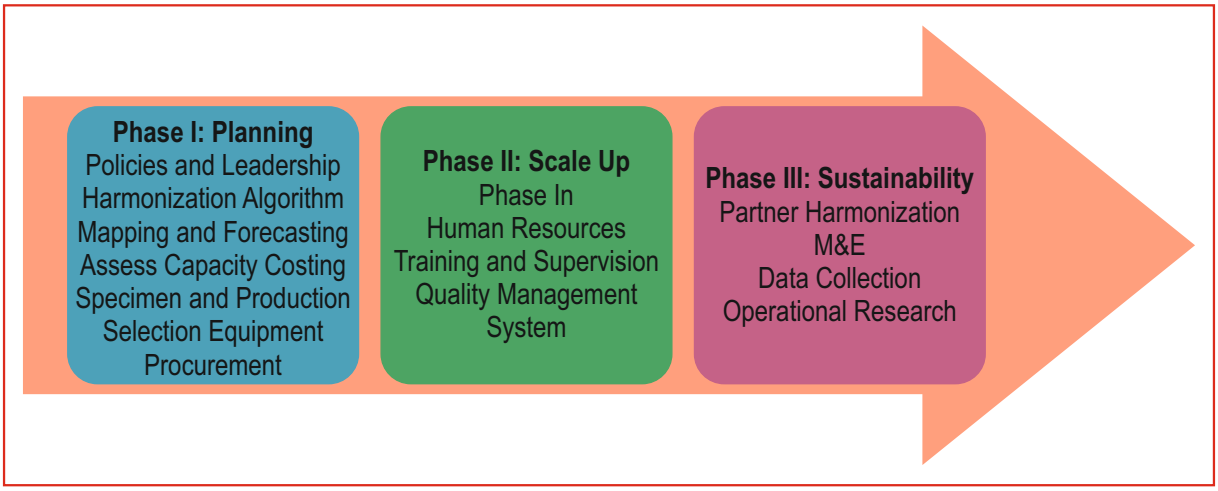


Figure 1: Phased scale-up of HIV Viral Load testing facility as recommended by WHO (2013).

Accordingly, NACO plans to scale up the reach of routine HIV-1 Viral Load testing in a phased manner. Currently there are 10 laboratories in the country carrying out VL testing and NACO has planned to start 80 VL testing facilities mapped based on patient load, HIV prevalence and sample transportation considerations with appropriate geographical distribution across the country to enable a well-connected network with the existing ART & ICTC centres. By doing this NACO is aiming to enhance the total number of PLHIV accessing HIV-1 Viral Load testing from current 14000 to more than a million PLHIV per annum.

1.4 Algorithms for the monitoring of HIV-1 infected individuals

As a part of the GOI's test and treat policy, NACO formulated algorithms on the routine viral load and CD4 testing.

1.4.1 Timing for HIV-1 Viral Load test

Patient Group	Timing for HIV-1 viral load test*
First line patients	▸ For **existing patients***, testing should be done once every 12 months after initiation on ART ▸ For **new patients**, VL test should be conducted at 6 months and 12 months from the date of ART initiation in first year, and once every 12 months thereafter
Second/third line patients	▸ For **existing patients***, testing should be done once every 6 months after initiation on ART ▸ For **new patients**, VL testing should start at 6 months after initiation of ART and conducted every 6 months thereafter

*For existing patients, the first viral load test should be done on their next monthly visit to the ART Centre

1.4.2 Timing for CD4 test:

Patient Group	Timing for CD4 test*
New patients	▸ CD4 to be done every 6 months
Existing patients (on ART for at least 12 months)	▸ CD4 testing at Baseline during ART initiation ▸ CD4 to be done every 6 months thereafter

1.4.3 Timing chart of CD4 and routine viral load tests

(A) For all patients on first line ART

Type of Test	Time since initiation of ART (in months)							
	0	6	12	18	24	30	36	...
Routine Viral Load Test	✗	✓	✓	✗	✓	✗	✓	...
CD4 Test*	✓	✓	✓	✓	✓	✓	✓	...

*CD4 monitoring can be stopped for any patient (except in children aged <5 yrs), if CD4 count is greater than 350 cells/cmm and viral load is less than 1000 copies/mL (when both tests are conducted at the same time)

(B) For patients on second/third line ART

Type of Test	Time since initiation of ART (in months)							
	0	6	12	18	24	30	36	...
Routine Viral Load Test	At SACEP Assessment	✓	✓	✓	✓	✓	✓	...
CD4 Test*	✓	✓	✓	✓	✓	✓	✓	...

*CD4 monitoring can be stopped for any patient (except in children aged <5 yrs.), if CD4 count is greater than 350 cells/cmm and viral load is less than 1000 copies/mL (when both tests are conducted at the same time)

Please note the following -

- A baseline CD4 count on ART initiation is necessary for determining immunological failure in future and understanding presence of Opportunistic Infections (OIs)

- Timing of CD4 and VL testing should be synced, so that the same sample collected from a patient can be used for both CD4 and VL testing. For existing patients (on ART for at least 12 months), the next CD4 test should be done such that it gets synchronized with a VL test
- CD4 monitoring should be re-started for any patient if (a) the patient has suspected treatment failure i.e. virological failure (≥ 1000 copies/mL) or suspected clinical failure, or if the patient has undergone a switch in regimen
- The treating clinician can request for a CD4 or viral load test when deemed necessary for clinical management at any point in time
- All HIV-2 patients should be monitored through CD4 test only

1.4.4 Routine HIV-1 Viral Load Testing Algorithm

The routine HIV-1 Viral Load Testing Algorithm (Fig 2). It is important to note that adherence for a month is defined as total pills consumed by patient divided by total pills given to the patient in the given month.

Important: Adherence should be ≥ 95% for each of the last 3 months to be referred to SACEP directly in case VL ≥ 1000 copies/mL

E-referral to SACEP means that the ART centre should refer the patient to SACEP through an email with the following information:

- HIV-1 Viral Load test details
- Latest CD4 test details
- Clinical records
- Treatment adherence details

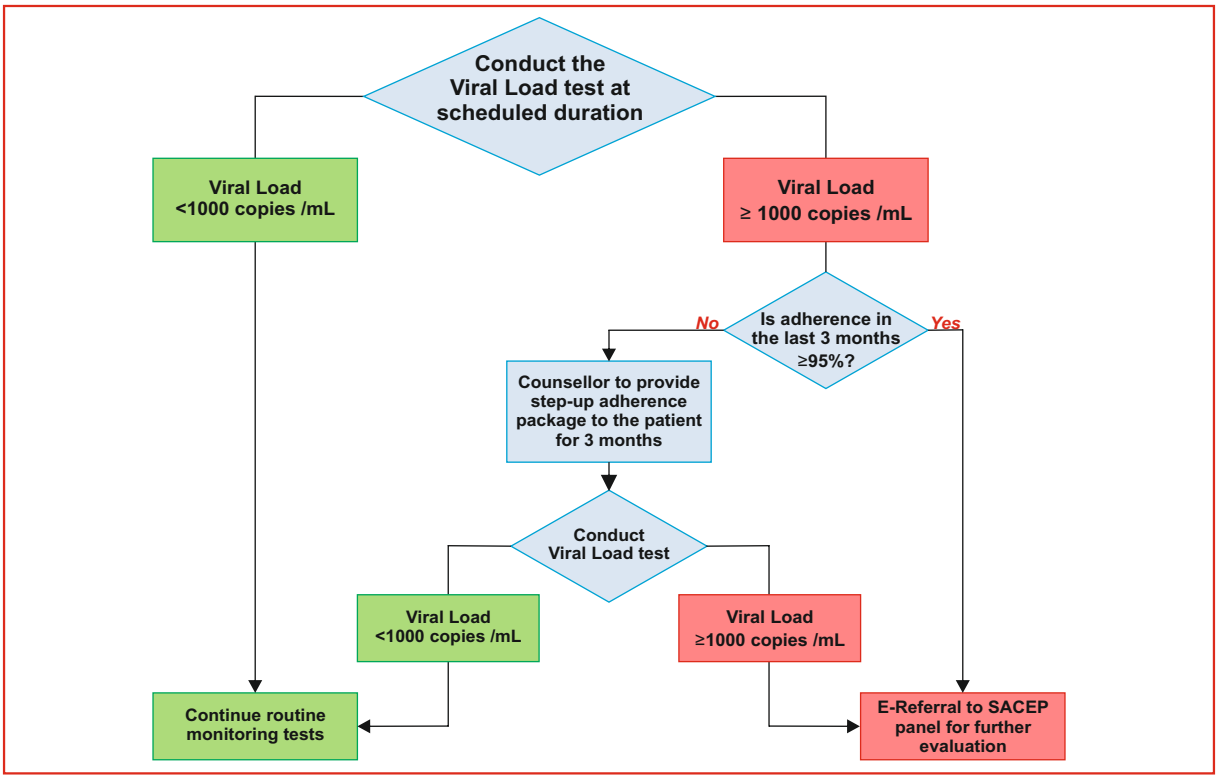


Figure 2: Flowchart for Routine Viral Load Algorithm

SACEP should refer to latest ART guidelines to decide the regimen to which the patient should be switched. ART centre should receive the decision by SACEP over email within eight days of email referral by ART centre.

Design for a Molecular Laboratory 2

Establishing a molecular laboratory involves planning of space and design. Two major components of the laboratory design are safety and ease of operation. The molecular techniques involved in polymerase chain reaction (PCR)-based assays are highly prone for contamination.The design of the laboratory should be such that it prevents cross-contamination of samples as well as contamination from amplified products. Setting up of a molecular laboratory for monitoring of HIV-I viral load requires similar stringent standards of precautions as recommended for any PCR laboratory.

2.1 INFRASTRUCTURE (LABORATORY DESIGN)

2.1.1. Pre-Analytical

Sample collection area

Sample collection facility must be easily accessible for patients or couriers agencies for receiving outstation samples. Signage and directions in local languages must be placed prominently. Separate areas must be allocated for waiting and sample collection.The waiting area should be ventilated and provide basic amenities, such as safe drinking water. Sample collection facility should have adequate space; proper lighting and chairs with armrest to facilitate blood collection. Proper biomedical waste disposal containers with biohazard symbol, colour coded bags and puncture proof containers for the disposal of needles and sharps, must be available in the area. Other ancillary objects required in the sample collection area are hand washing facility with running water supply, liquid soap in dispensers, hand sanitizers, eye wash, spill containment kit and first aid kit.

Designated area for sample receiving and handling

The laboratory should have designated space for sample receiving and processing. Facility for preliminary sample preparation (plasma separation) and storage will also require equipment like centrifuge and refrigerator for sample storage until transferred to the PCR laboratory. Samples are received, sorted and entered into the Laboratory Information System/viral load register in this area.

The samples once received are stored in refrigerator at 2-8°C for maximum five days or at -70°C deep freezer depending on the expected time for processing. (Refer to manufacturer's instructions depending on the HIV-I Viral Load platform used for testing)

2.1.2. Analytical

A molecular laboratory should have functional areas independent of each other, so as to minimize contamination. The major areas are (i) pre-amplification area (ii) amplification area and (iii) post-amplification area. With real-time PCR platforms currently used for HIV-I Viral Load

testing, the steps are combined and performed in a single area and are closed systems. Since cross-contamination in closed systems is minimal, physical separation of areas may not be required as the sample preparation, amplification and post amplification procedures can be carried out in a single area, but in designated space within the laboratory. A single important criterion is that the work flow should be unidirectional to avoid contamination or cross contamination (between samples). Minimum 200 square feet of space is required for the same.

A. Pre-amplification Area: Functionally this area is further divided into two areas

1. **Reagent preparation:** One area is the master mix room and this should have appropriate space with a laminar flow/PCR work station. There should be adequate storage space for reagents and other disposable lab ware/ consumables (pipettes with aerosol barrier tips, storage vials for aliquoting reagents required for the procedure, etc.). There must be space for keeping dedicated laboratory coats, disposable gloves and foot wear and these should be worn at all times while working in the laboratory.

2. **Sample preparation and extraction area.** Sample preparation and extraction area is designed to process and aliquot samples, including positive and negative controls, master mix aliquot to be added to the samples, should be brought from the reagent preparation area into this area prior to sample processing so that one does not have to go back to the reagent preparation area. There should be a bio safety cabinet level II for processing the samples and adequate storage space for dedicated pipettes, vials etc. There should be adequate space for an automated sample extractor. Colour coded biomedical waste containers must be available for discarding materials within easy reach of the technical staff.

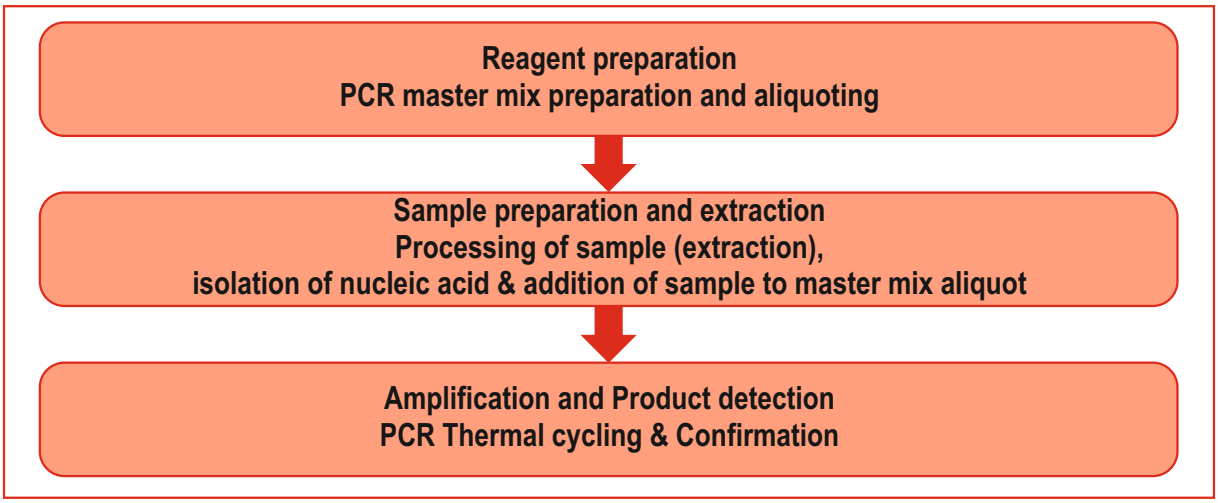
B. Amplification and product detection: RT-PCR and product visualisation equipment are kept in this room. Laboratory personnel accessing these facilities must remove their personal protective gown and gloves before leaving this area.

Unidirectional workflow

A unidirectional workflow should be observed to reduce the chances for contamination. No material, supplies or equipment from one room should be taken to the other room, or from one area to another if the work is done in a single room. The clean area must be free of amplicon at all times, to ensure this, there should be no movement back and forth from the sample processing area to the reagent preparation area.

To ensure minimal movement between areas during the running of molecular assays, it is optimal to have dedicated storage (freezer, refrigerator, pipettes, and consumables) for each area. This can also be ensured by colour coding of pipettes, laboratory coats and reagent bottles. Furthermore, before starting the assay one must check that sufficient reagents and consumables required for the run are available.

Unidirectional workflow in a PCR laboratory is shown below:



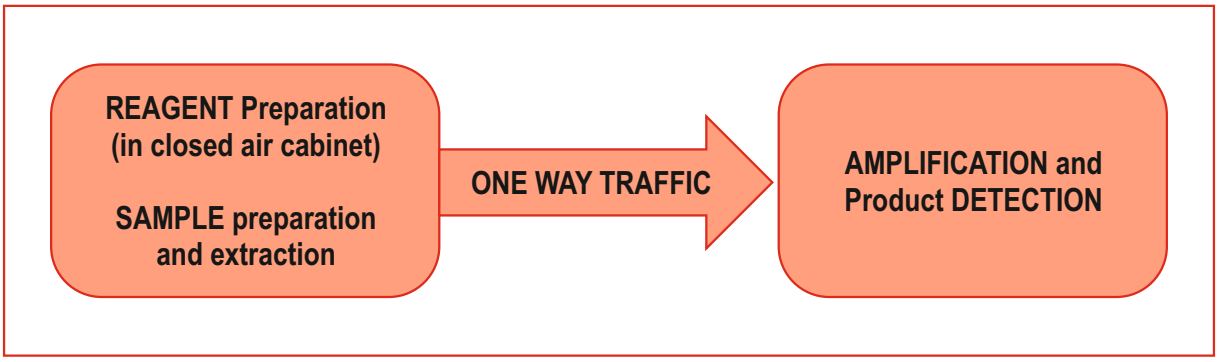


Figure 3: Two room option for a HIV-1 Viral Load testing laboratory

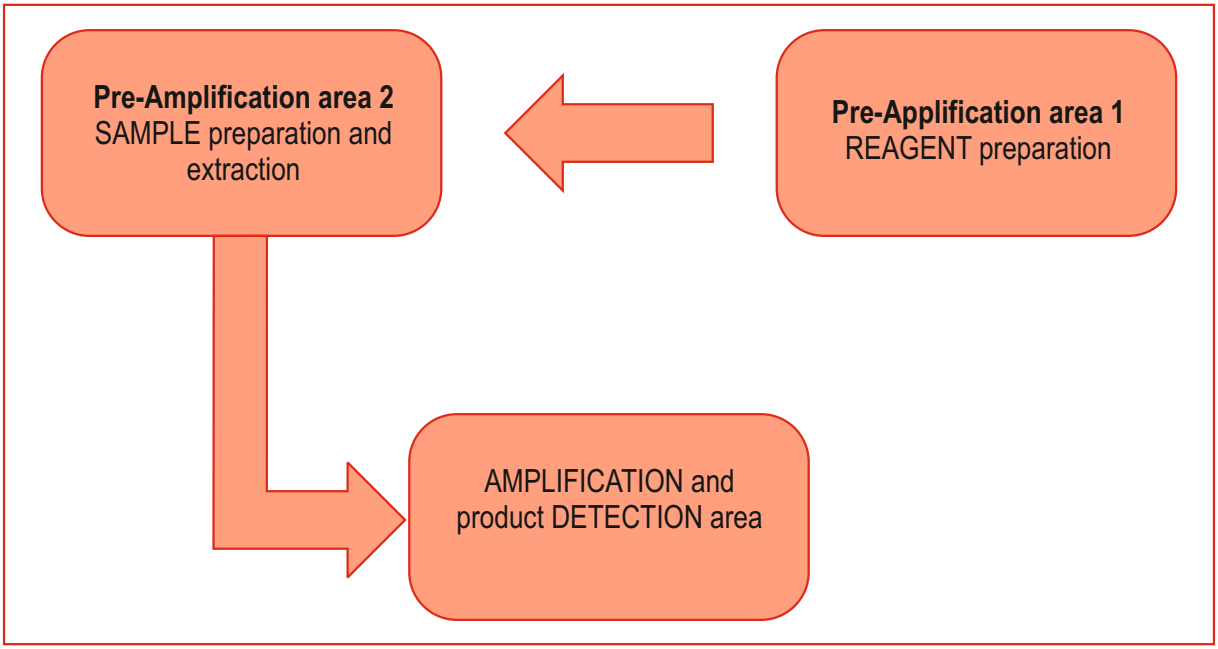


Figure 4: Three room option for a HIV-1 Viral Load testing laboratory

2.1.3 Post Analytical

The laboratory should have adequate space for documentation, reporting of results and storage of samples. This will require access to the internet / data and information needed to provide services to the client / patient with the help of LIMS.

2.2 PHYSICAL SET-UP

Laboratory Material Storage

The laboratory should have adequate storage space and space to accommodate

- Dedicated refrigerators and deep freezers for storage of samples and reagents to ensure that there is adequate separation of samples from reagents
- Secured cupboards for storage of confidential records and patient's reports

Laboratory Infrastructural Facility

- Temperature / humidity monitoring of the rooms
- Wash basins for hand wash in dirty and clean areas
- Provision for eye wash
- Spill containment kits
- First aid kits
- Rest rooms at close quarters for laboratory staff, if possible
- Fire safety measures (smoke detectors/ CO_2 extinguisher / water sprinklers)

Equipment

The laboratory should have adequate space for the installation of core and non-core equipment and instruments. Equipment should be suitably located in the laboratory so as to allow accessibility and sequential utilization thus minimizing the need for frequent movement of samples or reagents.

Power supply

The laboratory should have adequate lighting, power plugs and uninterrupted power supply to ensure that adequate electrical service is available. The use of exposed cables should be minimum; wires should not criss-cross across the room as this may pose a danger to the staff. All equipment, computers, air-conditioning, peripherals and communication devices should be supported with a power back-up to ensure uninterrupted services.

HIV-1 Viral Load Technologies 3

3.1 Introduction

Monitoring of HIV-1 Viral Load has become the standard of care for assessing the response to the ART in HIV-1 infected individuals and for detection of treatment failure. HIV-1 Viral Load tests are nucleic acid tests (NAT) which quantify the amount of HIV-1 nucleic acid in the plasma of infected individuals. The commercially available tests are run on 'closed' platforms, which are based on various molecular techniques.

3.2 Techniques

Viral Load assays measure the amount of HIV-1 RNA copies in the collected plasma sample. During treatment, the decay of VL in tissues typically corresponds with virological responses in plasma, making blood plasma a useful sentinel for virological response in general.

To measure the amount of HIV-1 RNA in plasma, different technologies are available under the national programme. The commonly used tests for HIV-1 viral load estimation are FDA approved and/ or, CE marked and/or WHO pre-qualified, and approved by national bodies like DCGI. These includes*:

Target amplification assays

- Real time PCR (qPCR)
  - COBAS TaqMan HIV-1 Test (Roche)
  - RealTime HIV-1 Amplification (Abbott)
  - VERSANT HIV-1 RNA 1.5 Assay (kPCR) (Siemens)
  - Xpert HIV-1 Viral Load Assay (Cepheid)
- Nucleic Acid Sequence Based Amplification Assays (NASBA) with real time detection
  - NucliSENS EasyQ HIV-1 v2.0 (BioMerieux)
- Transcription-mediated amplification (TMA)
  - Aptima HIV-1 Quant Assay (Hologic)
- Signal amplification
- Branched DNA (bDNA) assay
  - VERSANT HIV-1 RNA3.0 assay (Siemens)

Disclaimer:** ***These are not the only tests available for HIV-1 Viral Load estimation

All these methods are used to measure HIV-1 nucleic acid in plasma samples. Dried blood spot protocols have also been developed for some of the newer assays (e.g. NucliSENS EasyQ HIV-1 v2.0, COBAS TaqMan HIV-1, Real Time HIV-1), though only NucliSENS EasyQ HIV-1 v 2.0 has FDA-approval for VL using DBS. This could be useful in resource limited settings, where viral load assessment may be limited to specific centres and samples may have to be transported.
The principle and performance characteristics of some of these tests are summarised in Table 2. The plasma Viral Load assays already in use at NACO designated centres are the Roche COBAS TaqMan HIV-1 and the Abbott RealTime HIV-1

3.3 Real- time PCR

Real time PCR can detect the amplified DNA during the process of amplification, in real time, rather than at the end of the process, using specifically designed and labelled fluorescent probes. In their native state, these probes adopt a folded structure, positioning the quencher next to the fluorescent dye. In this condition, most of the fluorescence of the dye is absorbed by the neighbouring quencher, minimizing the emitted fluorescence. When amplicons are generated, fluorescent dye-labelled probes uncoil as they hybridize to the amplicons, separating the fluorescent dye from the quencher, thereby increasing fluorescence. The strength of fluorescence is proportional to the original amount of HIV-1 RNA in the sample. This ensures more accurate and precise quantification of nucleic acid.

3.3.1 COBAS AmpliPrep/COBAS TaqMan HIV-1 Test (Roche)

The COBAS TaqMan HIV-1 assay was the first commercial real time PCR-based test for VL . RNA extraction from plasma is automated (Cobas AmpliPrep), with a high throughput (extraction run size of 48 or 96). The targeted viral genome is a highly conserved region of the gag gene. Quantification of HIV-1 RNA is made using a second target sequence, the HIV-1 Quantitation Standard (QS), a known concentration of which is added to each test sample. The QS amplicons have the same length and base composition as HIV-1 target amplicons. Detection of the QS binding region has been modified to discriminate it from the target. The use of dual-labelled fluorescent probes (the proprietary TaqMan probes) allows the real-time detection of the accumulated PCR products, by monitoring the emission intensity of fluorescent reporter dyes released during the amplification process. The amplification of HIV-1 RNA and the QS are measured independently at different wavelengths. This process is repeated for a designated number of cycles, each one effectively increasing the emission intensity of the individual reporter dyes, allowing a separate recognition of HIV-1 and the QS. The exponential growth decay in the curve of the PCR amplification directly correlates with the baseline amount of genetic material. The test is able to quantify HIV-1 RNA over a dynamic range of 48-10^7 copies/ml.

Disclaimer: ***Lower limit of detection may vary with different versions or batches.***

3.3.2 RealTime HIV-1 Assay (Abbott)

The Abbott Real Time HIV-1 assay on the m2000 instrument has the option of manual nucleic acid extraction, as well as automated extraction (batch-size of 96 or 24, with m2000sp and m24sp, respectively). The Real Time HIV-1 assay amplifies a highly conserved 172- nucleotide region of the part of the pol gene that codes for the integrase. An Internal Control (IC), unrelated to the HIV-1 target, is incorporated for each assay. Exponential amplification of the product is achieved through repeated temperature cycling as in PCR. Both targets, HIV-1 and IC, are amplified simultaneously in the same reaction. The products hybridize with HIV cDNA and IC-specific probes, labelled with different fluorescent dyes (molecular beacon probes, similar to the proprietary TaqMan probes described in the previous method). The amplification cycle at which a specific fluorescent signal is detected is proportional to the amount of HIV-1 RNA present in the original sample. The assay has a lower limit of detection of 40 HIV-1 RNA copies/ml for 1 mL, 75 copies/ml for 0.5 ml and 150 copies/ml for 0.2 ml volume of plasma sample. The

upper limit of quantification is 10 million copies/ml. This assay can quantify all HIV-1 variants, including groups M, N and O.

3.3.3. VERSANT HIV-1 RNA 1.5 (kPCR) (Siemens)

VERSANT HIV-1 RNA is another automated amplification method based on reverse transcription and kinetic PCR (kPCR) or real time PCR technology. The RNA is first extracted in an extraction module. In the amplification module, purified RNA is eluted and added to a PCR plate containing an HIV-1 primer/ probe and enzyme mix. The reverse transcriptase PCR step targets a highly conserved region of the HIV-1 pol gene. The dNTPs include uracil instead of thymidine to minimize amplicon contamination and allowing amplicon destruction by uracil DNA glycosylase added to the enzyme mix. Both HIV-1 RNA and internal control RNA are reverse transcribed to make cDNA, then simultaneously amplified and detected using separate dual-labelled fluorescent probes specific for HIV-1 and control amplicons. The test requires 0.5 ml of plasma sample. The linear dynamic range of the assay is between 37 and 1.1×10^7 HIV-1 RNA copies/ ml.

3.3.4 Xpert HIV-1 Viral Load (Cepheid)

The Xpert HIV-1 Viral Load assay combines automated nucleic acid purification, reverse transcription and amplification, followed by real time PCR detection of the 5'-LTR region of the HIV-1 genome, in a single, closed cartridge-based system. High and low controls are included in the cartridge for quantification of HIV-1 RNA and to check for any inadequacies in sample processing or due to inhibitors of amplification. A probe check control verifies reagent rehydration, proper PCR tube filling, integrity and stability of the probe and its label. On a module for four cartridges, 8-12 samples can be tested per working day. The limited throughput can be overcome by the use of multiple modules. The lower limit of detection is 40 copies/ ml of plasma.

3.3.5 Nucleic acid sequence-based amplification (NASBA) assay with real-time detection NucliSENS EasyQ HIV-1 v2.0 (BioMerieux)

The NASBA assay selectively and directly amplifies HIV-1 RNA isothermally at 41°C, without a PCR, in a one-step sandwich hybridization procedure using two oligonucleotide primers, three enzymes (simultaneous activities of avian RT, ribonuclease H and bacteriophage T7 RNA polymerase), nucleoside triphosphates and the appropriate buffers. In this viral load test, the HIV-1 is lysed and HIV-1 RNA is extracted. Nucleic acid extraction is automated with the EasyMAG or EasyQ systems (batches of 24 or 48, respectively). Nucleic acid amplification then occurs using specific primers derived from a well conserved region of the gag gene. This isothermal cycle is repeated resulting in exponential amplification (1 million to 1 billion-fold).

The generated amplicons are then detected as in a real time PCR, using molecular beacons (hairpin probes) designed from the same region, with a fluorescent dye and a quencher at the end. Quantification is done by using fixed amount of calibrator RNAs as a reference. The linear dynamic range of NucliSENS EasyQ HIV-1 v2.0 is from 10 to 10^7 HIV-RNA copies/ml with 1 ml plasma.

3.4 Transcription-mediated amplification (TMA) with real-time detection

3.4.1 Aptima HIV-1 Quant Assay (Hologic)

The Aptima HIV-1 Quant assay involves three main steps, in a single tube on the proprietary Panther automated system. These are target capture (by oligonucleotides complementary to highly conserved regions of the HIV-1 genome), target amplification by TMA and detection of the amplicon by fluorescent labelled probes (similar to real-time PCR detection and quantification).

TMA utilizes two enzymes for isothermal amplification, Moloney Murine Leukemia Virus (MMLV) reverse transcriptase and T7 RNA polymerase. The reverse transcriptase generates a DNA copy (including a promoter sequence for T7 RNA polymerase) of the target. T7 RNA polymerase makes multiple copies of HIV-1 RNA (pol and LTR gene target sites) using this DNA as a template. HIV-1 groups M, N, and O can be amplified.

The test uses an internal control/ calibrator in each sample. The linear dynamic range is from 30 to 10^7 copies/ ml.

3.5 Branched DNA (bDNA) assay

3.5.1 VERSANT HIV-1 RNA 3.0 assay (Siemens)

The Versant HIV-1 RNA 3.0 assay differs from all the above methods in using signal amplification instead of target amplification, to measure viral load. The bDNA assay consists of a series of hybridization procedures followed by an enzyme substrate reaction. In this assay, HIV-1 present in the patient's sample is disrupted to release the viral RNA. The RNA is captured by a set of capture probes (bound by solid phase) while a set of target probes hybridizes both the viral RNA and the preamplifier probes. The amplifier probe hybridizes to the pre-amplifier probe forming a branched DNA (bDNA) complex. The bound bDNA is incubated with an enzyme and then with a chemiluminescence substrate. Light emission is directly related to the amount of HIV-1 RNA present in each sample and the results are recorded as Relative Light Units (RLUs) by the analyzer. The test can quantitate HIV-1 RNA over the range of 75-500,000 HIV-1 RNA copies/ml. Plasma samples containing Group M Subtypes A - G have been validated for use in the assay.

3.6 Other viral markers

3.6.1 ExaVir Load assay (Cavidi)

In the ExaVir Load (Cavidi) reverse transcriptase (RT) assay, the RT enzyme is separated from the virus particle using a solid-phase extraction manifold and the amount of RT enzyme is quantified using a functional assay whereby the RT incorporates bromodeoxyuridine (BrdU) monophosphate into DNA using a poly (A) template bound to a 96-well plate. BrdU is then quantified spectrophotometrically using anti-BrdU conjugated to alkaline phosphatase, followed by the addition of its substrate. The RT activity in the unknown sample is compared to that of a recombinant RT enzyme standard with a known concentration. The extrapolated result is reported as femtogram (fg) of RT/ml of plasma or as HIV-1 RNA equivalents/ ml based on a conversion factor supplied by the manufacturer. The RT assay (Cavidi) v3.0 has a dynamic range of detection of ~ 200- 600,000 RNA equivalents/ ml. The advantage is that it can detect all groups and subgroups of HIV-1, as well as HIV-2.

Table 2: Principle and performance characteristics of some assays for VL determination

Method	COBAS AmpliPrep/ COBAS TaqMan HIV-1	RealTime HIV-1	NucliSENSEasy Q HIV-1 v2.0	VERSANT HIV-1 RNA 1.5 (kPCR)	*Xpert HIV-1 Viral Load*
Manufacturer	Roche	Abbott	BioMerieux	Siemens	Cepheid
Principle	Target amplification (real time PCR with TaqMan probes)	Target amplification (real time PCR with molecular beacon probes)	Target isothermal amplification (NASBA; with detection in real-time by molecular beacons)	Signal amplification (branched DNA)	Target amplification
Dynamic range (copies/ml)	48-10^7	40-10^7	25-10^7	37- 1.1x 10^7	40-10^7
Specimen type	Plasma in EDTA tube	Plasma in EDTA tube	Plasma in ACD, EDTA, or heparin tube	Plasma in EDTA tube	Plasma in EDTA and ACD tube
Detected subtypes	Group M (A-H; CRF01_AE). Group O	Group M (A-D, F, G; CRF01_AE, CRF02_AG), Group O	Group M (A-G)	Group M (A-H), CRF, Group O	Group M (A-H, AB, AE, AG, J, K,) Group N, Group O
Area of HIV genome selected for amplification	*Gag*	*Integrase region of pol*	*gag*	*Integrase region of pol*	*3' end of the 5' LTR*

4 Specimen Collection and Handling

4.1 Introduction

Plasma remains the gold standard for testing HIV-1 Viral Load with years of data to back up utility of this sample as it can be tested on any platform. It is used to measure the amount of free virus circulating in the blood and requires less processing time during testing phase. Plasma sample can remain stable over long period when stored at appropriate temperatures.

Sample integrity is the cornerstone of a quality VL test result. To protect sample integrity, they must be properly collected in the correct type of tube, stored at the correct temperature and transported within the proper time-frame at appropriate temperature and packaging to the testing laboratory. Coordination between the ART center and the HIV-1 Viral Load testing laboratory is important to ensure timely pick-up, transportation in prescribed conditions, proper delivery of samples, processing of sample and timely delivery of quality reports.

4.2 Sample collection by venepuncture

All registered individuals on ART who are scheduled for VL testing should be referred by the Medical Officer to the technician at the ARTC for sample collection with filled in triplicate carbon copies of the Test Requisition and Result Form (TRRF). On receiving the patient, the laboratory technician shall verify the TRRF, confirm the identity of the patient by Unique ART Number and at least one other identifier such as name, age, gender etc. Unique VL test ID (17 digit) is generated by the laboratory technician at the ART centre at the time of blood collection. Prior to the collection of the sample by venepuncture, the procedure should be explained to the patient. The blood collection tube should be labelled with 17 digit number/ any of two identifiers and the date and time of collection using cryo labels.

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

Standard precautions should be strictly followed and blood sample should be collected wearing powder free gloves. 6 ml of whole blood sample should be collected in a K2 EDTA evacuated tube using an eclipse needle (usually 23G) while 3 ml blood should be drawn from infants less than one year. In children and adults with thin fragile veins where it may be difficult to draw blood with vacutainer alternative blood collection devices should be used. For the complete procedure of blood collection refer to National Guidelines for Enumeration of CD4, NACO (2015).

Important: Heparin containing tubes must not be used when collecting blood samples for viral load testing because heparin has been shown to inhibit PCR.

The EDTA tubes should be gently inverted 8-10 times to ensure proper mixing of whole blood and EDTA to prevent clotting. The tubes are kept upright at room temperature (15-30°C). The biomedical waste generated while blood collection should be disposed into appropriate color coded bins.

Following sample collection the date and time of sample collection should be entered in the TRRF. This information should be also entered manually and digitally respectively in the register and IMS. Completed TRRF in triplicate should accompany the sample throughout handling and transport.

4.3 Sample Type

The Viral Load testing will be done on plasma sample. Whole blood will be collected preferably by phlebotomy (Process of collecting blood by puncturing a vein). Plasma will be extracted by centrifugation process. Plasma samples can be stored up to five days at 2 - 8°C.

4.4 Separation of plasma

Plasma should be separated from whole blood within six hours of sample collection. The whole blood cannot be frozen for later use for viral load testing. The sample tubes should be centrifuged at 2000-2500 rpm for 10-15 minutes. Suitable plasma samples are clear and have a slight yellow tint with defined buffy coat and red blood cell layers (as shown in Figure 4 a). Haemolysed plasma samples appear bright pink to red in color after centrifugation (as shown in Figure 4 b). Haemolysis occurs if the sample is shaken too vigorously due to the lysis of the red blood cells. Clotting can occur if the EDTA tube is not mixed immediately or properly by inversion after blood collection (as shown in Figure 4 c). Lipemic plasma that appears white and thick occurs (as shown in Figure 4 d) when there is an elevated amount of fat in the blood. Samples that are visibly contaminated can have extra layers of unidentifiable cells or debris (as shown in Figure 4 e). Contamination in plasma samples could be due to the improper EDTA collection tube and collecting devices. However, if plasma is separated from whole blood within 6 hours of collection, chances of contamination is rare.

Following centrifugation, maximum amount of clear straw colored plasma should be separated using a sterile Pasteur pipette and transferred into sterile 5 ml polypropylene tubes with O ring screw cap labelled with patient details using cryo labels. The separated plasma samples can be kept upright in a plastic box with ice packs or stored in a refrigerator maintained at 2-8°C for a maximum of 5 days.

Appropriate Sample	Haemolysed Plasma Sample	Clotted Sample	Lipemic Sample	Contaminated Sample
				
(a)	(b)	(c)	(d)	(e)

Figure 4: Characteristics of plasma following centrifugation

Specimen Transportation and Storage 5

5.1 Storage

Plasma can be stored at 2-8°C for a maximum of 5 days and can be transported with frozen ice packs. For long term storage, as it contains RNA, the ideal temperature is -70°C or lower. Once plasma is frozen, it must be always transported frozen to avoid freeze thaw cycles.

Table 3: Recommendations for time of transportation and storage at various conditions for plasma and whole-blood samples for HIV-1 viral load testing

Temperature	15–30°C (room temperature)	2-8°C	–70°C or lower
Whole Blood	6 hours	-	-
Plasma	-	5 days	5 years

5.2 Packaging Viral Load Samples for Transportation

If the sample collection centre & viral load testing laboratory are located in the same premises or nearby, the plasma sample can be transported within 2-3 hours in the sample transportation box with ice packs (the samples and the ice pack should be in different container).

When the samples have to be transported over long distance through courier, triple package system mentioned below should be used to maintain biosafety and integrity of the sample. During packaging it is important to record the temperature inside the box once the ice packs are kept and before sealing. Sample transport begins at the ART centre where packaging takes place and ends at the testing laboratory where samples are received and subsequently tested. The samples must be properly packaged according to all safety guidelines (IATA) and ice packs used must be frozen. TRRF must be filled out, checked and signed. If the samples have to be transported from an ART centre which is far away from the Viral load testing laboratory, the samples will be transported with the TRRF in duplicate and ensure delivery to the testing lab at 2 - 8°C within 24 hours of sample collection.

When frozen plasma samples are to be transported in case of sending the samples to referral laboratory for quality assurance purpose, all the above precautions and packaging should be carried out. In addition instead of frozen ice packs always dry ice should be used. This is to avoid freeze thaws that can affect the level of RNA in the plasma.

5.3 Triple Packaging System

All packaging includes 3 layers (Fig 5):

a) Primary Receptacles

- Tube containing sample for Viral Load testing
- The tube must be watertight and leak proof

- Must be appropriately labelled as to content
- Wrapped in enough absorbent material to absorb all liquid in case of breakage or leakage

b) 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. Often the second layer of packaging has a rack or similar item to keep samples from moving around too much

c) 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 cooler. The outside of the 3rd container should remain clean so as to be easily handled without any need for PPE
- HIV positive blood sample is belong to "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
- The proper shipping name "BIOLOGICAL SUBSTANCE, CATEGORY B" should be mentioned adjacent to the diamond shape

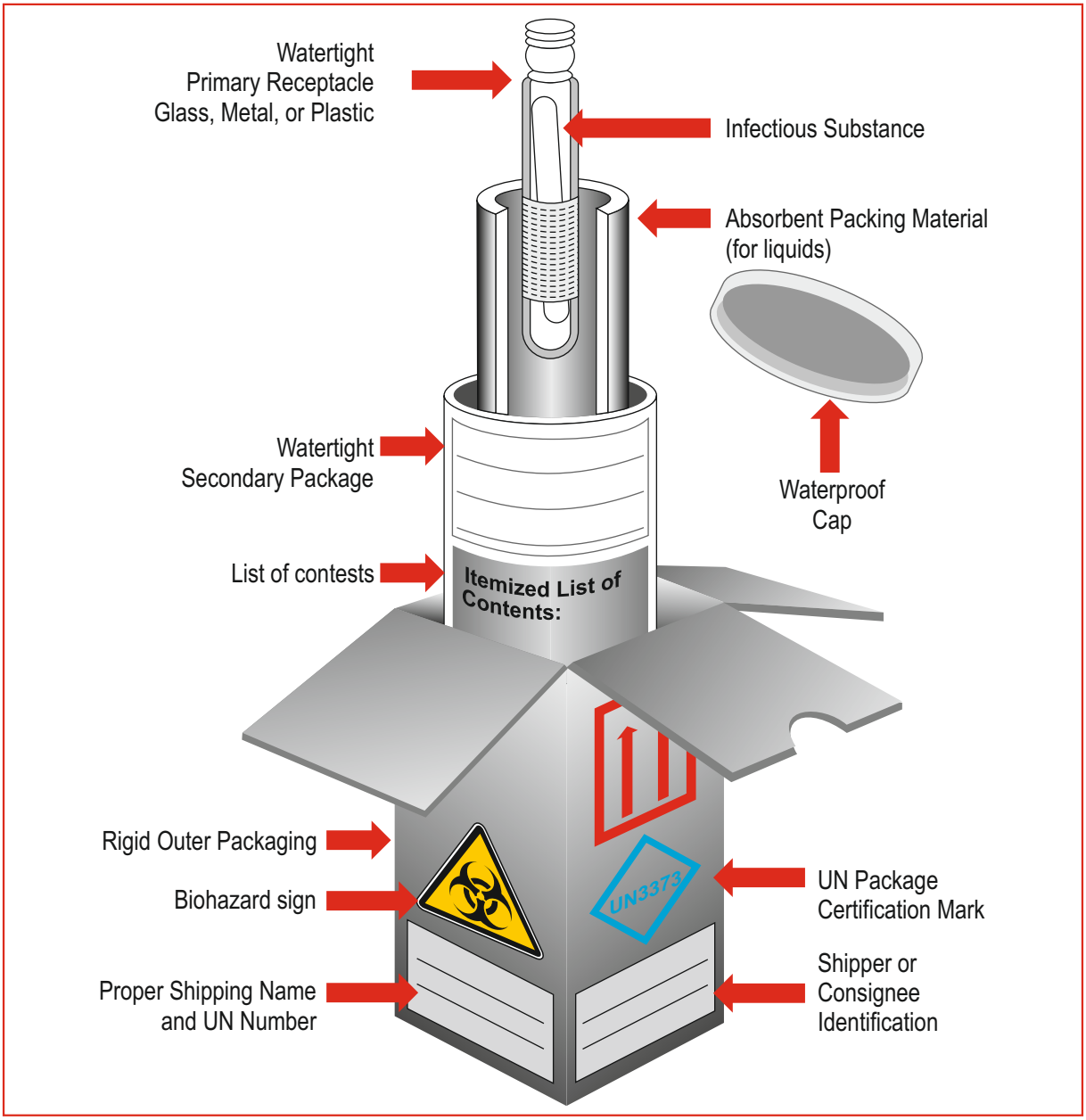


Figure 5: Diagrammatic representation of a triple package system for the transportation of "Category B" infectious substances

Figure 6: The marking used for the transportation of infectious substances category B

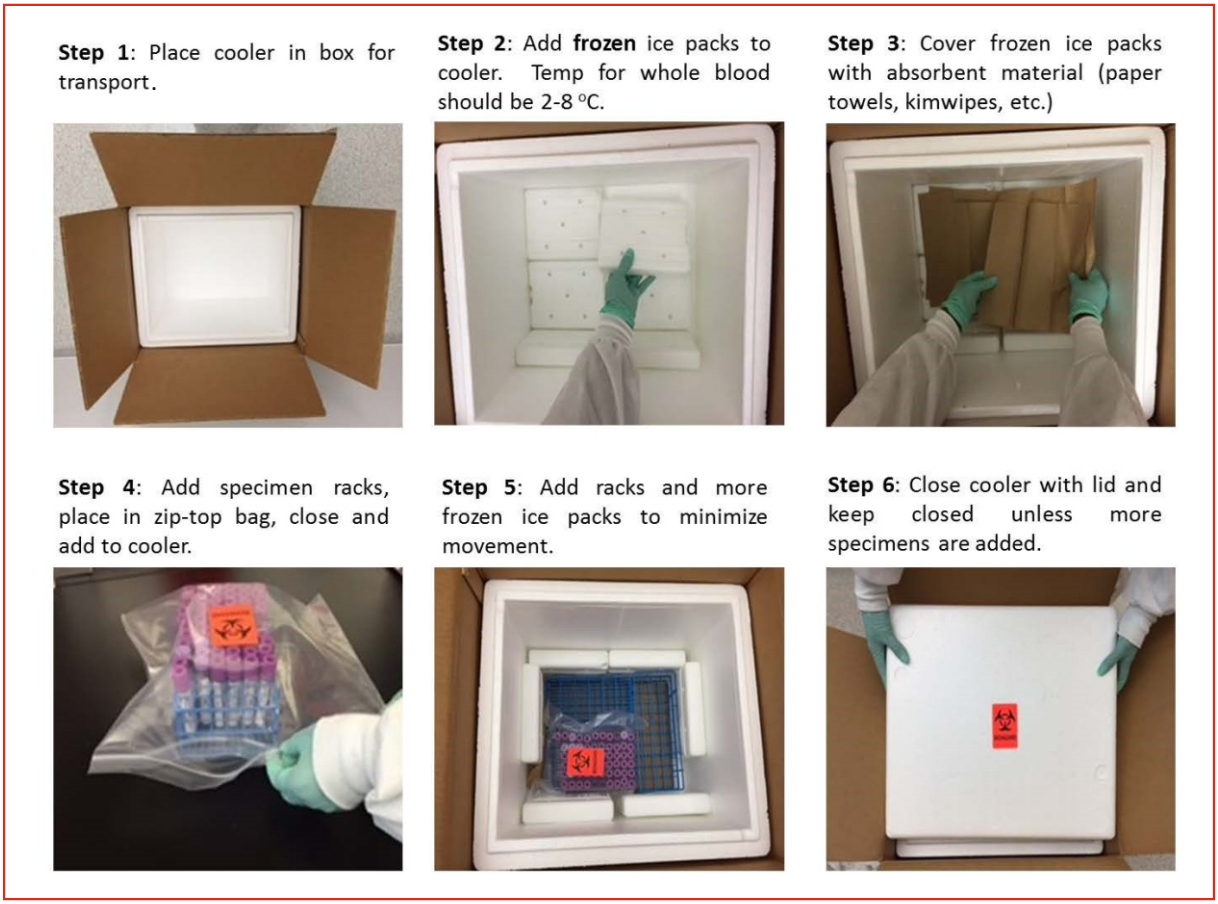


Figure 7: Job aid for sample packaging

5.4 Reception of Plasma samples at Viral Load testing lab

- Viral Load testing laboratory will receive the sample and viral load TRRF in triplicate
- Each sample should be divided into two aliquots which should be properly labelled. One aliquot should be stored between -70°C or lower and the other aliquot at (a) 2-8°C if it can be tested within five days of collection or (b) between -70°C or lower if delay of more than five days is anticipated
- As soon as the samples are received by the viral load testing laboratory, they should be verified by the LT for proper transportation, integrity and completeness of the TRRF. The condition of the samples, date and time of receipt should be recorded. While opening the transportation box, the temperature should be recorded
- The LT must verify the details on the sample and the TRRF received from the ART centre. In case of any discrepancy or missing information, the lab must immediately inform the ART centre by phone or email and resolve it
- The issue and resolution must be recorded in the VL register present at the laboratory under the "Remarks" section
- The LT is supposed to evaluate if the sample is received in good condition. In case the sample is not received in good condition, the ART centre must be informed immediately by phone and email.
- If the samples are rejected In case of sample rejection, LT is supposed to send the filled TRRF to the ART centre asking for a repeat sample for the patient immediately

- The LT must move forward with the testing of sample only if (a) all the details of the sample and TRRF are complete and verified (b) the sample is received in good condition

Appropriate samples

- Sample properly labelled
- Sample tube integrity maintained, no leakage
- Sample label matched with request form
- Adequate volume
- Clear plasma
- Transport temperature 2-8°C

Inappropriate Samples

- Haemolysed sample
- Grossly lipemic samples
- Samples subjected to repeated freezing and thawing
- Visibly contaminated samples
- Inadequate volume
- Leaking tubes
- Improperly labelled sample
- Label not matching with request form
- Plasma samples stored or transported at temperature > 8°C
- Samples from HIV-2 infected individuals

5.5 Turn Around Time for Viral Load testing

The ART centre and testing laboratory should try to adhere to the turnaround time of each step for Viral Load testing as given below:

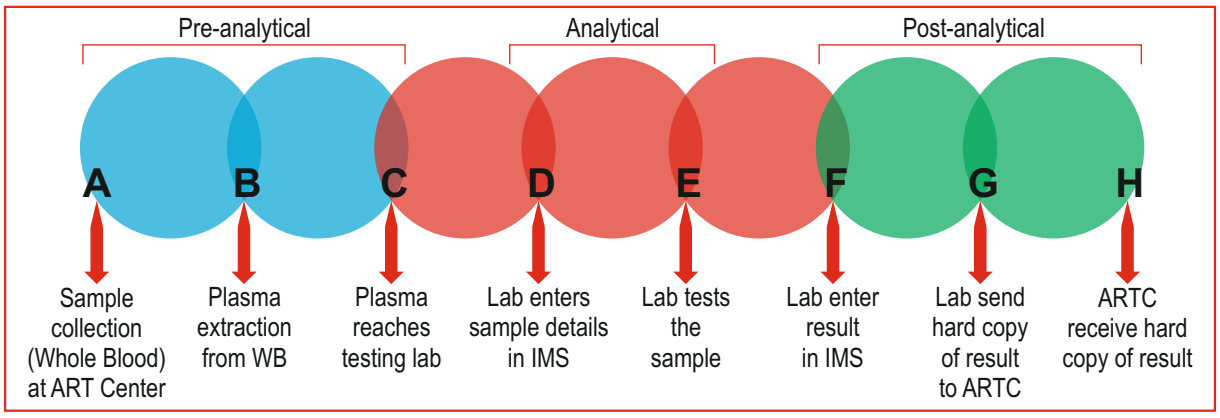


Figure 8: Operational steps in Viral Load testing

TAT between steps:

- A to B: Whole blood should be processed for plasma separation within six hours of sample collection
- A to C: Plasma should be transported at 2-8°C and should reach the testing laboratory within 24 hours of sample collection

- C to D: The sample related details should be entered into IMS within 24 hours of receiving the sample at the lab
- B to E: The lab should test the plasma sample preferably within five days after separation from whole blood when kept at 2-8°C and within two weeks when kept at the temperature specified in the kit insert
- E to H: The signed hard copy of the results should reach the ART centre within 72 hours of testing of the sample
- A to H: The Turnaround Time (TAT) for reporting of results to the ARTC is 14 days from the time of sample collection

Equipment for HIV-1 Viral Load testing 6

6.1 Viral Load Testing Equipment

All the Viral Load testing technologies which are WHO prequalified and/ or CE/ approved by US FDA are based on closed systems. These are described in detail in Chapter 3. The maintenance of such equipment should be performed only by the manufacturer.

6.2 Ancillary Equipment

6.2.1 Centrifuge

Centrifuge is required for sample preparation before the RT PCR can be performed. It is used for separation of plasma if whole blood is received from the collection centre. The whole blood sample is to be centrifuged at 2000-2500 rpm for 10-15 minutes to separate plasma, which is further processed for testing.

6.2.2 Vortex mixer

Vortex mixture is used to mix the reagents, calibrator and Internal Controls of the Viral Load assay kit. The reagents, calibrator and Internal Controls are stored in -20°C. Once thawed after removing from the freezer, these must be vortexed before use.

6.2.3 Micropipettes

Micropipettes ensure quick aliquoting and dispensing precise amount of sample/reagent/calibrator/Internal Controls. Micropipettes with the following variable volume ranges are required for the Viral Load assay:

i. 100-1000µl
ii. 20-200µl

(Ref: Annex 4.4 National Guidelines on QMS in HIV Testing Laboratory, 2015)

6.2.4 Biological safety cabinets (BSCs)

A class II biosafety cabinet is required for sample preparation and nucleic acid extraction. The Class II (Types A1, A2, B1 and B2) BSCs provide personnel, environmental and product protection since it uses High Efficiency Particulate Air (HEPA) filters in the exhaust and supply systems.

Airflow is drawn into the front grille of the cabinet, providing personnel protection. In addition, the downward flow of HEPA-filtered air provides product protection by minimizing the chance of cross contamination across the work surface of the cabinet. Because cabinet exhaust air is passed through a certified HEPA filter, it is particulate-free (environmental protection), and may be re-circulated to the laboratory (Type A1 and A2 BSCs) or discharged from the building via a canopy or "thimble" connected to the building exhaust. Exhaust air from Types B1 and B2 BSCs must be discharged directly to the outdoors via a hard connection.

The cabinet blower should be operated at least four minutes before beginning work to allow the cabinet to "purge." This purge will remove any suspended particulates in the cabinet. The work surface, the interior walls (except the supply filter diffuser), and the interior surface of the window should be wiped with 70% ethanol (EtOH) or 1:100 dilution of household bleach (i.e., 0.05% sodium hypochlorite), to meet the requirements of the particular activity. When bleach is used, a second wiping with sterile water is needed to remove the residual chlorine which may corrode stainless steel surfaces. Similarly, the surfaces of all materials and containers placed into the cabinet should be wiped with 70% ethanol to reduce the introduction of contaminants to the cabinet environment.

Important: A BSC must be routinely inspected and tested by training personnel following strict protocols to verify that it is working properly

The following parameters need to be verified at the time of installation and, as a minimum, annually thereafter. This process is referred to as certification of the cabinet and should be performed annually.

Table 4: Field Performance Tests Applied to Biological Safety Cabinet

Test Performed for	Class II
Cabinet integrity	Required for proper certification if the cabinet is new, has been moved or panels have been removed for maintenance (A1 only)
HEPA filter leak	Required during certification
Down flow velocity	Required during certification
Face velocity	Required during certification
Negative pressure / ventilation rate	Not applicable
Airflow smoke patterns	Required during certification
Alarms and Interlocks	If present, encouraged for electrical safety
Electrical Safety	
Electrical leakage, etc.	Optional, at the discretion of the user; Encouraged for electrical safety
Ground fault interrupter	Encouraged for electrical safety
Other	
Lighting intensity	Optional; at the discretion of the user
UV intensity	If present; optional, at the discretion of the user
Noise level	Optional; at the discretion of the user
Vibration	At the discretion of the user

6.2.5 Deep Freezer -20°C

A-20°C deep freezer is required to store some of the kits and reagents as per the manufacturer's instructions.

6.2.6 Deep Freezer -70°C or lower

A-70°C deep freezer or lower is required for long term storage of plasma samples aliquots. Specimen storage is required for QA, retesting and as per NABL requirements.

6.2.7 Refrigerator

A refrigerator is required to store plasma sample for short term storage up to five days. The samples are to be stored at 2-8°C till the assay is completed, verified and report is generated.

6.3 Equipment Management

A good equipment management programme is necessary to ensure accurate, reliable and timely testing and to maintain a high level of laboratory performance.

A reliable and continuous power supply ensures proper functioning of the equipment and providing uninterrupted services.

Refer to National Guidelines for Quality Management Systems in HIV Testing Laboratories (2015) and manufactures instructions for requirements on equipment installation, validation, daily equipment maintenance, calibration and documentation.

7 Quality Control and Quality Assurance

A laboratory should have Quality Management System (QMS) in place to ensure that Viral Load testing is reliable and accurate. A comprehensive QMS comprises an on-going cycle of quality assessment and improving the process and programme at all functional levels, including the organizational and individual levels, equipment and inventory management, the use of quality control material, data management and documentation, occurrence management, sample management, safety and waste management. QMS should incorporate national standards for testing and training of staff involved in testing services and laboratory management. External quality assurance strategies should be developed to adequately ensure that the quality of Viral Load testing is maintained. As Viral Load testing is scaled up, strengthening the laboratory network and quality assurance systems becomes of paramount importance.

QMS in the testing laboratory comprises of Quality Control (QC) of the test procedure and the Quality Assurance (QA) of the testing system. In a VL testing laboratory, QC verifies that test results are valid by assessing the reliability of three aspects of the testing process: the reliability of test reagents, the integrity of instrumentation and ability of the tester to perform the test accurately while QA is an evaluation of testing process by an impartial outside source.

7.1. Quality Control (QC) materials

Ideally in HIV-1 Viral Load testing three levels of control as provided by the manufacturer are required to be incorporated in every run.

Control material provided with the assay includes (i) control sample with a low copy number (ii) with a high copy number and (iii) no copy sample. This ensures that the laboratory is capable of detecting the entire range of values of the analyte in the clinical samples.

QC must be acceptable (in control) before testing and reporting of results. Any test results obtained when QC is unacceptable or not performed are invalid and must be repeated. The person performing testing must evaluate QC results and accordingly determine validity of the test run.

7.2. L J chart

Regular testing of quality control samples creates a QC database that the laboratory uses to validate the test system. Validation occurs by comparing daily QC results to a laboratory-defined range of QC values. The lab-defined range is calculated from QC data collected from testing of low copy and high number controls in HIV-1 Viral Load assay.

Mean and Standard deviation is commonly used for defining the control limits in Levey-Jennings (LJ) charts. The LJ chart is used to plot, successive (run-to-run or day-to-day) quality control

values. For Viral Load assay the QC decision limit for a LJ chart should be +2SD. An analytical run should not be rejected if a single QC value is outside the ±2s QC limits but within the ±3s QC limits. Approximately 4.5% of all valid QC values will fall somewhere between ±2 and ±3 standard deviation limits.

The laboratory needs to document that QC materials are assayed and that the QC results have been inspected to assure the quality of the analytical run. This documentation is accomplished by maintaining a QC Log and using the LJ chart on a regular basis. When the results are plotted, an assessment can be made about the quality of the run. The technician performing the test should look for systematic error and random error. The QC log can be maintained on a computer or on paper. LJ chart should also be reviewed for trends and shifts. Actions should be taken to resolve any situation which is identified as "out-of-control" or unacceptable after root cause analysis. (Refer the QMS guidelines for Westgard multi rules and interpretations).

7.3 Coefficient of Variation

The coefficient of variation [CV] is the ratio of the standard deviation to the mean and is expressed as a percentage. The % CV allows making easier comparisons of the overall precision. Since standard deviation typically increases as the concentration of the analyte increases, the % CV can be regarded as a statistical equalizer.

The values obtained with the low copy number sample provided by the kit manufacturer as one of the controls in the assay can be used to determine the % CV. Usually, the % CV is calculated on a monthly basis, on 20 or more number of data points. The formula used to calculate % CV is as follows: SD/Mean X 100.

7.4 Co-efficient of Variation Ratio

Although accuracy of test results is paramount in the Viral Load testing laboratory, precision is just as important. One way a laboratory can determine whether the precision of a specific test is acceptable is to compare its precision to that of another laboratory performing the same test on the same instrument using the same reagents (laboratory peer group). The Apex Lab and Regional Reference VL labs in the network may participate in this exercise.

7.5 Kit Lot verification

The Viral Load laboratory must validate the accuracy and reliability of each new lot / batch of the kit before testing is permitted. Ideally 3 samples with known HIV-1 copy number tested earlier by the laboratory and those that fall in the no copy, low copy and high copy range are included in the verification process. The difference between observed and expected value should be within +/- 0.5 logs.

7.6 Analyte calibration and verification by use of control material

Calibration is the process of testing and adjusting the instrument or test system read out to establish a correlation between the instrument's measurement of the substance being tested and

the actual concentration of the substance. Calibration verification means testing materials of known concentration in the same manner as patient samples to assure that the test system is accurately measuring samples throughout the reportable range.

Analyte calibration and verification is done as specified in the test system's instructions and whenever any of the following occur:

- All of the reagents used for a test procedure are changed to new lot numbers, unless the laboratory can demonstrate that changing reagent lot numbers does not affect the range used to report patient test results and control values are not adversely affected by reagent lot number changes.
- There is major preventive maintenance or replacement of critical parts that may influence the test's performance. This includes when the laboratory sends a test system to the manufacturer for repairs.The laboratory must check the calibration of a repaired test system before resuming patient testing and reporting results.
- Control materials reflect an unusual trend or shift, or are outside of the laboratory's acceptable limits, and other means of assessing and correcting unacceptable control values fail to identify and correct the problem.
- The laboratory has determined that the test system's reportable range for patient test results should be checked more frequently A variety of materials with known concentrations may be used to verify calibration, for example, commercially available standards or calibration materials, proficiency testing samples with known results, control materials with known values, or patient samples with known values.

After each calibration event QC samples are analysed to check whether the test system is providing accurate results throughout the reportable range, three levels should be tested– High copy, low copy (at the medical decision) level and no copy cannot be used for internal QC purposes.

7.7 Inter Laboratory Comparison

External Quality Assessment (EQA) is an important tool in the quality management of the Viral Load laboratory. EQA complements internal quality control to help assure that patient results are valid.

7.7.1 External Quality Assessment (EQA)

External Quality Assessment (EQA) is an evaluation of the testing process by an impartial outside source/agency, is a way to evaluate how well testing is being performed and whether it is being performed reliably. Proficiency Testing (PT) can help to identify existing or potential problems. Moreover, information gathered can provide an educational tool to improve performance.The laboratory must participate in a PT program.Any incorrect PT results must be investigated and the source of the error must be determined.

Proficiency Testing (PT)

Proficiency testing programs are intended to measure bias or inaccuracy in test results.

- PT samples are tested in the same manner as client samples
- PT results are documented on daily test logs and the score sheet supplied with the samples
- PT results should be submitted within the time frame required by the PT agency
- Corrective action is required whenever an incorrect result is obtained on a PT sample
- All testing personnel need to review the PT scoring and sign an acknowledgement of review

Some of the international HIV-1 viral load test PT providers are:

(i) Centers for Disease Control and Prevention (CDC),Atlanta

(ii) Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP), Australia

(iii) College of American Pathologists (CAP), USA

(iv) National HIV Reference Laboratory (NRL),Australia

7.7.2 Alternative Assessment Process (AAP)

If formal EQA programmes/PT are not available, alternate methods are necessary to assess quality of the laboratory performance. These assessment methods can also be used to supplement formal EQA to address pre and post- analytical factors.The laboratory should have a system for validating the accuracy of its test results at least twice a year.

In the programme, an inter-laboratory comparison (ILC) by re-testing of split samples can be initiated with an accredited Regional ReferenceVL laboratory with comparative test method and equipment.

An ILC will consist of re-testing of previously tested patient samples.The samples selected for this purpose should be stable for the period of the activity in given temperature conditions. An ILC will be performed once in every six months. If ILC is performed as an additional activity, it should complement the PT cycle and should not overlap.The lab will randomly select 1 % of total patient samples tested from the first week of the month in which the ILC is initiated. For the purpose of ILC, select not more than five samples that cover the dynamic range - high, mid and low.The ILC samples are sent to the reference laboratory for analysis.The samples for ILC will be blinded to the reference laboratory.

The ILC results will be evaluated by the Apex Laboratory. A copy of the ILC report will be sent to both the reference and the participating laboratory.The participating laboratory will review the results for acceptability and will troubleshoot in case of unsatisfactory results.

Acceptance criteria:There should not be a more than 0.5 log difference between the reference laboratory and the testing laboratory.

8 Troubleshooting of Common Problems/Errors

HIV-1 Viral Load testing measures the amount of HIV-1 RNA in the clinical samples and reports how many copies of the virus are present. Plasma Viral Load serves as an invaluable marker for monitoring the progression of HIV-1 infection and the efficacy of ART in HIV infected individuals. It is a quantitative measurement of HIV-1 RNA copies in an infected patient and is quantified by molecular assay involving various steps such as RNA extraction and amplification procedures.

The nature and frequency of issues, problems, occurrences and/or errors while performing an assay on any HIV-1 VL may vary from platform to platform and from laboratory to laboratory. Ironically, the problems or errors can happen even with the most experienced hands. Therefore, it is important not only to be aware and have some knowledge of these potential problems and errors but also to have the knowhow to document, analyse and trouble shoot them. Though, in most instances troubleshooting is simply a logic problem and like any skill, improves with practice and experience, it is always wise to learn from your past errors and their remedial measures or corrective actions for handling such issues in the future.

Error minimization and prevention of its recurrence is central to the concept of quality management system. The prerequisites to prevent and minimize the problems or errors during Viral Load assay includes apt laboratory design, appropriate and adequate sample collection, good laboratory practices including pipetting technique and path of workflow, strict implementation of quality management system and laboratory safety protocols in the Viral Load laboratory. You are required to refer, adopt and implement all of these essentials which have been described in other chapters of the guidelines. Here it is pertinent to mention that you are also required to follow every step of kit/manufacturer instructions for a successful run. However, implementing these quality components still does not guarantee an error free laboratory but most definitely by following these essentials meticulously, the system detects errors and helps prevent them from reoccurring.

As with any technique, it is critical that all of the processes of the HIV-1 VL assay are fully understood so that data are reliable and any problems can be addressed with confidence. When troubleshooting, it is important to be open to any possible sources of error, however insignificant they may seem, to explore each potential problem independently (E.g. if two assays are failing, try to treat each separately and not assume that there is a single reason), to recognize the value of your time and to be pragmatic about getting the assays working.

Keeping these in mind, this chapter has been designed to deal with errors and occurrences during the conduct of viral load assay. These issues, errors/error codes do require their documentation, Root Cause Analysis (RCA), immediate action, Corrective Action and Preventive Action (CAPA). Examples of some of these problems and errors commonly faced in the VL laboratory along with their corrective actions have also been summarised.

Important: As all the viral load platforms in-use, at present, are closed systems, if there is any error NOT due to operating error or due to lack of Good Laboratory Practices (GLP), one should immediately seek assistance from the manufacturer specifically for problems related to the VL analyser, its calibration, for hardware/software issues and for issues related to the kit

8.1 Common Potential Sources of Occurrence (Errors)

8.1.1 Operator Error

Unfortunately there are endless possibilities for operator error with even more creative mistakes being developed as the established ones are solved. More often than not, the sources of these errors remain unidentified. The first step in any troubleshooting procedure is to check the protocol and then repeat the assay. Checking the protocol and even asking the lab in-charge to review the workflow of that particular run is important. It is pertinent to know that even the best hands are vulnerable to simple errors.

Molecular tests require highly skilled and well trained staff. To achieve this, the Viral Load laboratory staff must be trained and then deemed competent prior to starting testing in the laboratory. Furthermore, the laboratory in-charge should assess the competency of the staff on an on-going basis using either external or internal quality control exercises.

8.1.2 Sample collection

The type of collection device used for collection of HIV-1 Viral Load samples is very important. Some collection devices are coated with a substance that can result in inhibition of the molecular assay. For example, some anti-coagulants such as heparin result in inhibition of the PCR based molecular assay. Long and cumbersome methods are required to remove the heparin prior to starting the assay. Therefore the preferred method of collection of clinical sample for viral load testing is in an evacuated EDTA coated tube.

Important: To avoid any contamination always wear powder-free nitrile gloves before handling the tubes containing blood or plasma.

8.1.3 Template

The effect of template quality in sample may have an effect on performance of the assay. Template quality encompasses consideration of quantity, integrity and the presence of inhibitors. It is critical to prevent RNA degradation by RNases. Sources of RNase are: skin, hair, general laboratory glassware and contaminated solutions. Also, the quantity of extracted RNA that is added to reaction mix must be within the scope of the protocol and this should be the same for all reactions. When troubleshooting a sample which is yielding a higher than expected value of upper limit of detection, follow the instructions by the manufacturer in the kit insert and repeat the assay.

8.1.4 Master Mix

Mistakes or problems with the master mix may be the source of a catastrophic failure of

amplification in all samples and positive controls. Before starting the assay, ensure that adequate amount of master mix is available in the vial provided with the kit.

8.1.5 Instrument Failure

Instrument faults can have an insidious onset and can, therefore, be difficult to diagnose. Always ensure that all operators of instruments are fully trained and initially supervised. Instrument failure can result in no amplification or distorted data. Therefore, the periodic preventive check-up of the machine by the service engineer is absolutely necessary. To prevent expensive repair costs, the VL platform must be under maintenance contract and the manufacturer must immediately be contacted to resolve the instrument failure or its malfunctioning issues.

Other potential sources of errors could arise when the laboratory deviates from the assay protocol and instructions provided by the manufacturer.

8.1.6 Root cause analysis (RCA)

An immediate action to address the error is followed by the root cause analysis which is important to monitor problems and make appropriate changes to prevent them from recurring in the future. The nature of the error whether random or systematic and linked to samples, procedural/technical issues, laboratory equipment, quality assurance and laboratory safety should be monitored regularly/periodically. Once the cause has been identified, a corrective action is determined and preventive action is undertaken.

8.1.7 Corrective action

Failures are an indicator of potential problems in the system and should be dealt at priority. Corrective action is an improvement measure to a laboratory's processes undertaken to eliminate causes of errors or other undesirable situations. It also serves as an educational process by identifying areas of deficiency in the knowledge and facilitating correction thereof through supervised feedback sessions. All corrective actions must be documented and filed in a designated place (such as a corrective action file), once it has been reviewed and signed by the laboratory in-charge.

Examples of troubleshooting for common problems on Cobas TaqMan 48 (Roche) Analyzer

Pre Amplification Problems:

S No.	Problem	Root Cause Analysis	Corrective Action
1	Spillage of samples during the centrifugation	Broken lids of the Lysis rack/ Filter rack and /or Elution rack	Check all the racks and their lids before us If broken lids detected, ask the supplier to replace the defective racks with new ones having intact lids
2	After the extraction if MMX vial supplied with the kit is found empty, further process is stalled	MMX vial received in the kit was empty	After receiving the Kit, check all the reagents supplied with the kit If found empty, ask the supplier to replace the MMX vial with a new one of the same batch or replace the entire kit

Problems during transfer of K-carrier to the analyser:

S No.	Problem	Root Cause Analysis	Corrective Action
1	Falling down and mixing of the K- Tubes during the transfer of K-carrier /loading of Taqman 48 Analyser	Loose Grip of K- carrier with K- carrier transfer tool	Double check the locking system between K-carrier & K- carrier transfer tool before loading in the Cobas TaqMan 48 Analyser

Problems during amplification:

S No.	Error code	Root Cause Analysis	Corrective Action
1	TB083100207 Monitor Sensor TCB short circuit	Room temperature not maintained	Maintain the room temperature between 20-22°C
2	Thermal cycler hardware error	K-Tube cap inside the moving path of the thermal cycler	Check all the caps of the K-Tubes before inserting the K-Tubes in the Thermal cycler

Post amplification Problems:

S No.	Error code	Interpretation	Root Cause Analysis	Corrective Action
1	QS_INVALID	QS outside the range	Viral Load in the sample is much higher than the upper limit of detection	Follow the kit instructions, dilute the sample 1:100 with HIV-1 negative human EDTA plasma, repeat the test & multiply the result with dilution factor
2	PRECHECK	Software detected error at one or more built in checkpoints at the time of validation of result	Multiple causes	Refer to the manufacturer and /or Repeat the test
3	Q BLTOO High	Base line intercept too high/ fluorescence level too high	Pipetting Error or Bubbles in the K- Tube	Repeat testing
4	NC Invalid	Invalid result or a valid result other than TND	Pipetting error/cross-contamination	Repeat testing
5	LPC Invalid	Low positive controls out of range	Pipetting error/ cross contamination	Repeat testing
6	HPC Invalid	High positive controls out of range	Pipetting error/ cross contamination	Repeat testing

Examples of troubleshooting on Abbott RealTime platform

S No.	Error code	Root Cause Analysis	Corrective action
1	Error code 4450 in single or multiple samples	Fluorescence too low/calibration problem	Contact the manufacturer for re-calibration and then repeat the whole run
2	High positive control coming negative	Calibration problem	Contact the manufacturer for re-calibration and then repeat the whole run
3	Error code 4447	Insufficient level of assay reference dye	Master mix volume inadequate. Visually verify the levels in the plate before starting every run
4	Error code 3130 and 3131	Sample volume low	Ask for repeat sample with adequate volume
5	Error codes 4442, 4433, 4434, 4436	Calibration problem	Contact the manufacturer to re-calibrate and then repeat the whole run
6	Error code 4457	Internal control failure	Sample inhibitory/inappropriate, ask for appropriate repeat sample
7	Error code 3153	Insufficient volume	Sample insufficient, ask for repeat sample with adequate volume
8	Error code 4438	NC Invalid	Pipetting error/cross contamination. Repeat testing Repeat the testing for this sample
9	Error code 3119	Clot or bubble in the sample	Check plate well to ensure all sample and master mix were well dispensed
10	Error code 4455	Unable to process result due to software error	Repeat the testing

Examples of troubleshooting on GeneXpert

S No.	Error	Root Cause Analysis	Corrective action
1	2096/2097 Assay specific termination errors	Sample volume not adequate	Verify that the user is properly pipetting sample into the sample chamber. If the error occurred on one patient test only, then inquire if repeat testing on the same sample gave a valid result or not If the error occurred multiple times, then inquire if it happened on multiple samples, on different modules and by different users
2	5007 Probe check; 5011 Signal Loss detected in the amplification curve for analyte	Are different samples giving the same error? Did the sample have any blood/mucous/cloudy looking? Was the sample processed correctly? Does failure occur in one module only?	May have to recollect or request alternative testing View if probe check values for all targets are zero or very low across all targets. If yes, cartridge related issue. If failure for the lot is above package insert claims, replace failed tests Monitor to see if error reproduces in the module. If error re-occurs in module, replace module and replace for lost tests

Interpretation of Results 9

Viral Load test is useful for early and accurate detection of treatment failure. This chapter provides details on the interpretation of Viral Load test results along with recommended TAT of HIV-1 Viral Load testing at laboratory.

9.1 Use of HIV-1 Viral Load Test

HIV-1 plasma Viral Load refers to the number of viral particles found in each millilitre of blood. Thus, this test is used for the following purposes:

- Prognosis: predicts disease progression in the body. High HIV-1 Viral Load indicates a fast progression of the disease in body and vice versa.
- Prevention: predicts the risk of transmission of virus from person to person. High HIV-1 viral load indicates a high risk of transmission and vice versa.
- Therapy management: demonstrates the effectiveness of ART for a person. It varies from patient to patient. A high HIV-1 Viral Load might indicate a need to switch the regimen.

9.2 Laboratory Interpretation of HIV-1 Viral Load Results

The HIV-1 Viral Load is influenced by many factors. Thus, the interpretation of absolute concentration of virus measurement is not straightforward. However, one important issue that needs to be considered is whether measured change in Viral Load actually reflects a biological event, or whether it is within the variability limit of the assay (viral blip). Table 5 explains the results obtained through a Viral Load test

Table 5: Results obtained through a Viral Load test

Result	Interpretation
Not Detected (ND)	Target not detected (TND)*
< Lower Limit of Quantification (LLOQ)**	Target is detected but cannot be quantified since it is less than lower limit of quantification (LLOQ)
≥ LLOQ and ≤ ULQ (Absolute value in number and log will be displayed)	Target is detected and quantified
> Upper Limit of Quantification (ULOQ)**	Target is detected but cannot be quantified since it is greater than ULOQ

* A specimen with a result of "Target not detected (TND)" cannot be presumed to be negative for HIV-1 RNA.

** Values of LLQ and ULQ depend on the equipment, sample volume and the protocol used. For exact LLQ and ULQ values, the package insert of the assay may be referred.

There is a considerable variation in the results of various types of assays used in quantification of the same sample but if performed proficiently, a commercial assay shows reproducibility within approximately 0.5 log value, which implies difference of ~3 times from the previous result.

Therefore, an increase or decrease in viral load by more than 0.5 log value, may be considered to represent true biological events.

9.3 Turnaround Time for Testing Lab

HIV-1 Viral Load 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 receiving sample at testing lab and dispatch of result from testing lab, is kept to a minimum.

Important: The TAT for any testing lab should not exceed two weeks. Any delay in testing should be reported to the ART Centre and NACO with a written notice

Laboratory Safety 10

10.1 Introduction

The laboratory can be a potential source of physical, chemical and biological safety hazards e.g. fire, breakage of glassware, sharps, spillages, pressure equipment and gas cylinders, extremes of heat and cold and radiation. Awareness of the laboratory risks, as well as the knowledge of the means needed to prevent infection in a laboratory setting, has dictated the need for safety as an important part of the laboratory function and management. This is especially true in a molecular biology set up where protection of both the work and worker is equally important. However, with proper laboratory design, adherence to biosafety procedures and safety programme these hazards can be prevented. The laboratory work space and services must be maintained in such a way that various tasks can be performed without compromising the quality of work or the safety of laboratory staff, other health care personnel, patients, visitors, the community or environment. All laboratories must comply with the national and state regulations.

Laboratory facilities are designated as Biosafety levels 1 to 4 based on a composite of the design features, construction, containment facilities, equipment, practices and operational procedures required for working with agents from the various risk groups. HIV-1 Viral Load testing laboratories can be classified as Risk Group 2 (moderate individual risk, low community risk) and require at least Biosafety Level 2 facility.

10.2 Laboratory Safety Management

10.2.1 Job responsibilities in relation to laboratory safety

1. Laboratory in-charge

- To ensure the development and adoption of a biosafety management plan and a safety or operations manual, required infrastructure, training, medical evaluation and vaccination (For job responsibilities refer to Chapter 11).

2. Designated safety officer

- The laboratory in charge should designate a specifically-trained person as Lab-Safety Officer, who in turn will be responsible to conduct biosafety, biosecurity and technical compliance consultations.
- To carry out periodic internal biosafety audits on technical methods, procedures and protocols, biological agents, materials and equipment *(For safety check list refer to Annexure 7.2 National Guidelines on Quality Management Systems in HIV Testing Laboratories)*.
- To ensure that regular training in laboratory safety is provided and to evaluate technical staff for competency.
- To ensure that laboratory personnel read the safety or operations manual and follow standard practices and procedures.
- To discuss violation of biosafety protocols or procedures with the appropriate persons.
- To ensure appropriate decontamination following spills or other incidents involving infectious material(s).

- To ensure proper waste management.
- To conduct and record weekly inspections to ensure that the laboratory is safe and in a good condition.
- To inspect and test fire extinguishers and eye-wash stations and to replenish first aid kit when necessary.
- To organize periodic (at least 6 months) fire drills and laboratory evacuation procedures.
- To ensure that the Material Safety Data Sheets (MSDS) are available in the respective laboratory sections and that the staff have read and understood them.
- To ensure all incidents are documented and attended to immediately.

10.2.2 Safety Policies

The following safety policies must be in place to ensure the continued safety of laboratory staff and any authorized individual who may enter the laboratory:

1. Standard Precautions Policy - This policy defines all human biologic samples as potentially infectious and addresses topics of consideration when dealing with potentially infectious samples and handling contaminated objects.
2. Chemical Hygiene Policy - This policy addresses aspects of safe chemical handling, including storage, utilization and disposal of chemicals and availability of MSDS.
3. Waste Management Policy - This policy details appropriate measures to take when disposing of waste materials to ensure continued human and environmental health.
4. General Safety Policies - These policies address less specific topics as they relate to laboratory safety, such as fire and electrical safety.

10.2.3 Safety Training

Training should include information about standard precautions, infection control, chemical and physical safety, how to use Personal Protective Equipment (PPE), how to dispose of hazardous waste, and what to do in case of emergencies. Documentation related to the completion of safety training and competency evaluation must be maintained. Safety training must be completed before any laboratory personnel begins working in the laboratory and on a regular basis thereafter.

10.2.4 Infrastructure

1. Laboratory Design - Laboratory should be planned and designed in such a way that it prevents cross contamination of samples and contamination from amplified products. For details refer to Chapter 2 on Laboratory Design for a Molecular Laboratory.
2. Safety Equipment - The following safety equipment/items must be in the laboratory to ensure the continued safety of laboratory staff and any authorized individual who may enter the laboratory:

- First Aid Box - All staff must familiarize themselves with the contents of the first aid kit and learn how to use them (List of laboratory safety kit contents Annexure-1)
- Site map shows locations of fire exits and extinguishers, evacuation routes, emergency showers, eye wash stations and emergency exit signage

- Spill management kits (List of laboratory safety kit contents Annexure-1)
- Sharps containers
- Bio-safety cabinet
- Eye wash station
- Emergency shower
- Fire extinguishers
- Post-Exposure Prophylaxis (PEP)

3. Equipment safety - Laboratory equipment like autoclaves, centrifuges, and bio safety cabinets are a significant source of potential injury to laboratory staff. Therefore training in specific safety procedures and precautions are important. While using BSCs it is important to install it in appropriate place, it should be operated only by a trained person, and maintained, serviced and calibrated regularly. Centrifuges may create microbiological hazards when they are used due to aerosol, splashing or tube breakage. This can be prevented by using sealable buckets (safety cups) or sealed rotors. Or it is recommended to open buckets or rotors after aerosols have settled (at least 30 minutes) or in a biological safety cabinet. Also, regular maintenance and yearly calibration of the centrifuge is recommended. For details refer to WHO Laboratory Biosafety Manual, third edition.
4. Cleanliness and Housekeeping - Good housekeeping is essential to ensure a clean, safe and pleasant work environment. It provides work areas devoid of physical hazards, prevents the accumulation of materials (from current and past examinations/activities) that constitute a hazard to laboratory personnel and prevents the creation of aerosols of hazardous materials as a result of the procedures used.
5. Archiving and storage spaces - Space must be allocated for the archiving of data in a safe and secure environment that is accessible only to authorized personnel.

10.3 Safety Manual

The Laboratory In charge/Safety Officer is responsible for ensuring that a laboratory-specific safety manual is developed, adopted, annually reviewed and accessible to all laboratory personnel. All laboratory employees must read this manual and the Safety Officer must maintain records of personnel who have read it. The manual should be reviewed and updated annually and whenever procedures or policies change. *(For details refer to chapter 7 National Guidelines on Quality Management Systems in HIV Testing Laboratories)*.

10.4 Biosafety

Biosafety is the application of knowledge, techniques and equipment to prevent personal, laboratory, and environmental exposure to potentially infectious agents or biohazards. A fundamental objective of any biosafety program is the containment of potentially harmful agents. The term "containment" is used in describing safe methods, facilities and equipment for managing infectious materials in the laboratory environment where they are being handled or maintained. The precautions mentioned below are also essential in a PCR laboratory:

10.4.1 Access

a. Only authorized persons should be allowed to enter the laboratory working areas.
b. Laboratory doors should be kept closed.
c. Children should not be authorized or allowed to enter laboratory working areas.
d. Appropriate signage should be posted in the laboratory at appropriate places.

S No.	Description	Signage
1	A "BIOHAZARD SYMBOL" should be pasted on the outer surface of the package containing samples.	
2	"MOBILE PHONE NOT ALLOWED" should be pasted inside the laboratory.	
3	"EMERGENCY EXIT" should be pasted on the surface of the emergency door.	
4	"NO EATING OR DRINKING" should be pasted inside the laboratory.	
5	"NO SMOKING" should be pasted inside the laboratory.	
6	"NO ENTRY FOR UNAUTHORISED PERSONNEL" should be pasted on the surface of the laboratory door.	

Figure 9: Signage for use in the laboratory

10.4.2 Hand Hygiene

Keeping hands clean is one of the most important steps we can take to avoid infection or contamination.Always wash hands before and after removing gloves.

Important: Gloves are not a substitute for hand washing.
Hand hygiene is important: Before patient contact, Before aseptic task, After body fluid exposure risk,After patient contact,After contact with patient surroundings

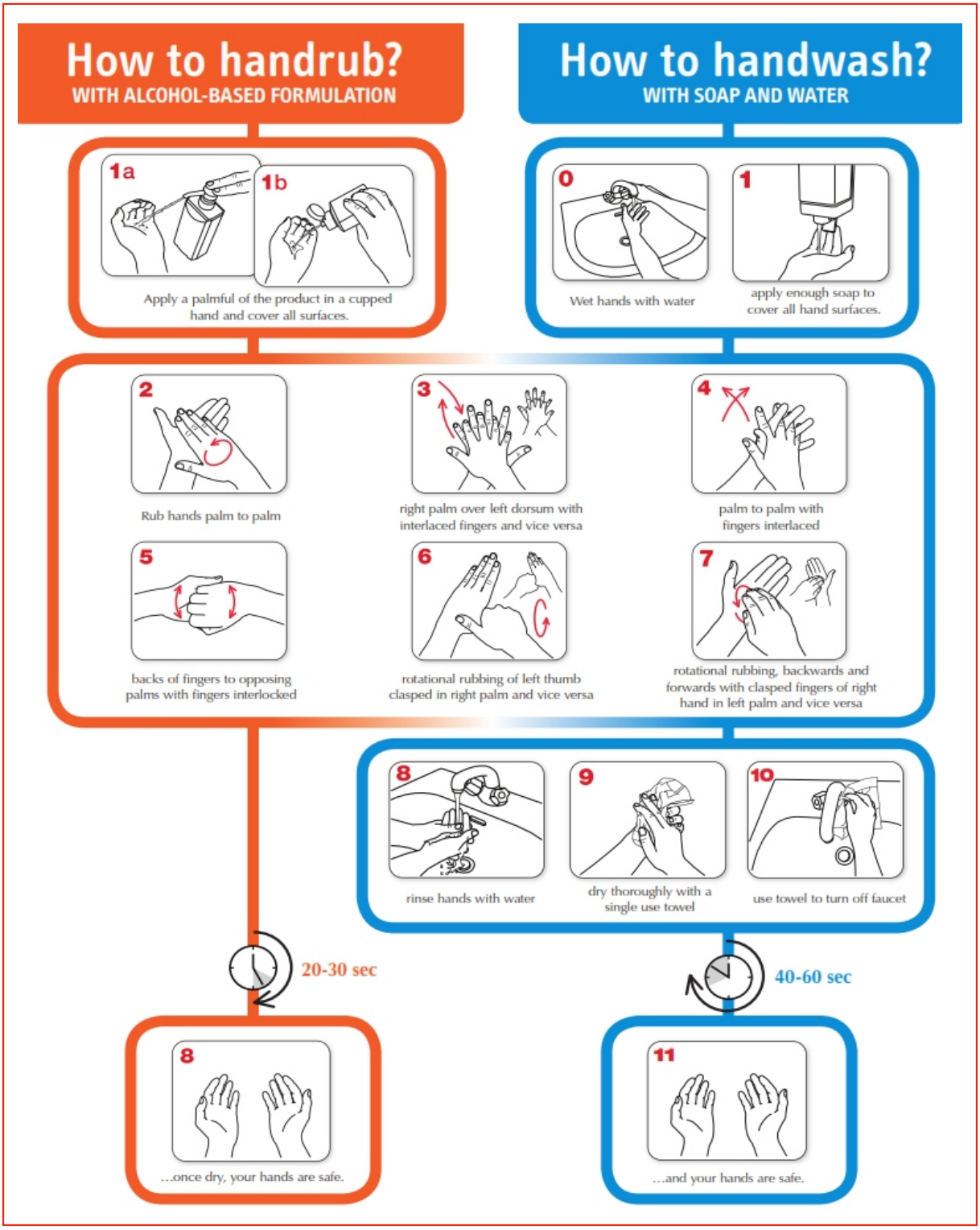


Figure 10: Steps of hand hygiene

10.4.3 Personal Protective Equipment (PPE)

The type of PPE used will vary based on the level of protection required and must be based on an appropriate risk assessment. PPE usage includes:

a. Laboratory coveralls, gowns or uniforms must be worn at all times for work in the laboratory.

They should be changed frequently especially when moving from one work area to another. These should be removed while walking out of the laboratory after finishing the work. These should never be carried in the eating or meeting areas.

b. Appropriate well fitting, disposable powder free gloves must be worn for all procedures that may involve direct or accidental contact with blood, body fluids and other potentially infectious materials. Gloves should be removed after use and must be changed if visibly contaminated with blood/breached (soiled, punctured or torn). Hands must be washed upon removal of gloves and gloves should never be reused. Gloves should be discarded appropriately before moving out of the laboratory at the finish of day's work and should never be taken to non-working, eating and meeting areas.
c. Safety glasses, face shields (visors) or other protective devices must be worn when it is necessary to protect the eyes and face from splashes, impacting objects and sources of artificial ultraviolet radiation.
d. It is prohibited to wear protective laboratory clothing/gloves outside the laboratory, e.g. in canteens, coffee rooms, offices, libraries, staff rooms and toilets.
e. Covered footwear must be worn in laboratories.
f. All skin cuts, scratches or other breaks must be covered with water-proof dressing.

10.4.4. Good Laboratory Practices

a. Standard precautions must be practiced with all samples at all times.
b. Long hair must be pinned up or covered.
c. Eating, drinking, smoking, applying cosmetics and handling contact lenses must be prohibited in the laboratory working areas.
d. Storing human foods or drinks anywhere in the laboratory working areas must be prohibited.
e. Pipetting by mouth must be strictly forbidden.
f. Materials must not be placed in the mouth.
g. All technical procedures should be performed in a way that minimizes the formation. of aerosols and droplets (e.g., avoiding vigorous pipetting; briefly centrifuging tubes with samples, extracted DNA and especially amplicon prior to opening the caps)
h. All spills, accidents and overt or potential exposures to infectious materials must be reported to the safety officer. A written record of such accidents and incidents should be maintained.
i. Written documents that are expected to be removed from the laboratory need to be protected from contamination while in the laboratory.
j. All contaminated materials, samples and cultures must be decontaminated before disposal or cleaning for reuse.
k. Packing and transportation must follow applicable national and/or international regulations.
l. Biosafety laminar flow cabinets (Class II) should be used to ensure safety and to prevent cross-contamination.
m. Barrier tips or positive displacement pipettes should be used for dispensing samples.

10.5 Biomedical waste management

An HIV-1 Viral Load testing laboratory must establish a waste management plan. Categories of waste generated by the laboratory should be identified. For each category of waste generated, applicability of national, state and local regulations, including how that category of waste will be segregated, packaged, labelled / colour-coded, stored, transported, and tracked within the laboratory, outside the laboratory, and outside the facility to comply with the applicable regulations should be determined. All the objects or materials should be effectively decontaminated or disinfected by an approved procedure before disposal. It should be packaged in an approved manner for immediate on-site treatment or transfer to another facility with treatment facility as per the Bio-Medical Waste Management Rules, 2016, Ministry of Forest, Environment and Climate change, Government of India.

Important: Individual laboratories must comply with their own Institution guidelines and colour codes for biomedical waste management.

Table 6: Container and colour coding for disposal of biomedical waste in a HIV-1 Viral Load testing laboratory

S.No.	Colour coding	Type of container	Type of waste	Treatment / Disposal option at VL testing lab
1	**Yellow**	Autoclave safe plastic bags or containers	Soiled waste – items contaminated with blood, body fluids Microbiology & biotechnology waste; Non sharps non plastic solid waste	Decontaminate at site before disposal and final treatment as per the respective Institute policy
2	**Red**	Non chlorinated plastic bags or containers	Contaminated recyclable plastic wastes	Decontaminate at site before disposal and final treatment as per the respective Institute policy
3	**White**	Puncture proof, leak proof, tamper proof containers	Waste sharps including metals	Decontaminate at site before disposal and final treatment as per the respective Institute policy

(All other types of biomedical waste, if generated should be discarded as per the Institute policy)

10.6 Disposal of liquid waste

Non-infectious: Chemical waste should first be neutralized with appropriate reagents and then flushed into conventional sewer system.

Infectious: Liquid waste is treated with equal quantity of 2% sodium hypochlorite solution to obtain a final concentration of 1% or autoclaved for decontamination. (Refer to Annexure-1 for preparation of sodium hypochlorite solution)

10.7 Management of sharps

Policies for the handling of sharps must be developed and implemented. Needles/sharps must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Sharps must never be passed directly by hand from one person to another but always in a tray or container. They should be disposed only in the appropriate container as per the policy.

10.8 Spill management

Blood spillage may occur because a laboratory sample breaks or because there is excessive bleeding during phlebotomy. In this situation, clean up the spillage and record the incident, using the following procedure.

a. Evacuate the contaminated area
b. Wear a pair of non-sterile gloves preferably heavy duty ones
c. Mark the contaminated area with a chalk or pen
d. Use a pair of forceps or tongs or a pan and brush to sweep up as much of the broken glass (or container) as possible. Do not pick up pieces with your hands (even with gloves)
e. Discard the broken glass in a sharps container. If this is not possible due to the size of the broken glass, wrap the glass or container in several layers of paper and discard it carefully in a separate container. Do not place it in the regular waste container
f. Use disposable paper towels / absorbent material (gauze pieces, cotton, blotting paper, etc.) to absorb as much of the sample as possible
g. Saturate the area again with 1% sodium hypochlorite (which should be prepared daily) from the periphery to the centre. Wait for 15-20 minutes
h. Discard the absorbent material and wipe the area clean with a disinfectant
i. Clean and disinfect the forceps/tongs/ brush and pan
j. Remove gloves and discard them
k. Wash hands carefully with soap and water and dry thoroughly with single-use towels
l. Record the incident in the incident book if a sample was lost, or persons were exposed to blood and body fluids

10.9 Post-Exposure Prophylaxis

In case of exposure perform appropriate first aid and report to the medical officer and start PEP preferably with in 2-6 hours, if required after assessment of infection risk.

10.10 Chemical, Fire and Electrical Safety

10.10.1 Chemical Safety

a. All laboratory personnel should have thorough working knowledge of hazardous chemicals with which they work.
b. All the hazardous substances in the work place must be identified and clearly marked with label stating health risks such as whether carcinogen, mutagen or teratogen and hazard class, whether corrosive, poisonous, flammable or oxidising.
c. Each laboratory should have chemical hygiene plans which include guidelines for proper labelling of the containers, Safety Data Sheets (SDS) and other chemical hazard information

available from chemical manufacturers and/or suppliers, and written chemical safety training programme. These should be accessible in laboratories where these chemicals are used, e.g. as part of a safety or operations manual.

d. Appropriate charts should be displayed in a prominent position in the laboratory.
e. Only amounts of chemicals necessary for daily use should be stored in the laboratory.
f. Chemical spill kit (Annexure-1) should be made available in the laboratory and necessary training should be given to the staff regarding the action to be taken in the event of a significant chemical skill.

Common chemicals used in laboratory

a. **Chlorine-** Wear utility gloves protective clothing, masks and goggles while preparing Chlorine solution
b. **Alcohol-** Always keep the container tightly closed and away from ignition source

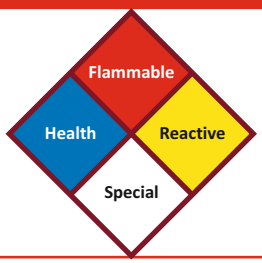
Label	Hazard Type		White symbols		Hazard Level	
	blue	health	OX	oxidizer	4	extreme
	red	fire	ACID	acid	3	serious
	yellow	reactivity	ALK	Alkali	2	moderate
	white	other	COR	corrosive	1	slight
		information	W–	water reactive	0	no or minima

Figure 11: Universally recognised labels used for hazardous chemicals

Steps in Chemical spill management

a. Notify the Safety Officer.
b. Evacuate non-essential personnel from the area.
c. Attend to persons who may have been exposed.
d. Establish what material has been spilt to know what personal protective measures should be followed and to understand the specific information related to spill containment and clean-up which is available in the MSDS.
e. If the spilled material is flammable, extinguish all open flames, turn off gas in the room and adjacent areas, open windows (if possible), and do not operate any electrical switches or appliances.
f. Avoid breathing vapour from spilled material.
g. Establish exhaust ventilation if it is safe to do so.
h. Secure the necessary items from the chemical spill kit (Annexure-1) to clean up the spill.

I. Absorb free liquids with an appropriate absorbent, neutralise residues and decontaminate the area. At the completion of the spill clean-up, all absorbent or contaminated material should be placed in sealed containers, labelled and disposed of as contaminated waste.

10.10.2 Fire Safety

Fire evacuation plan should be displayed in the laboratory showing the nearest fire escape route. Exit way should always remain clear of obstructions. Staff should be trained to use extinguisher. Fire-fighting equipment should be placed near room doors and at strategic points in corridors and hallways. This equipment may include hoses, sand buckets and fire extinguishers for all types of fire. Fire extinguishers should be regularly inspected and maintained and their shelf-life kept up to date.

Table 7: Types and uses of fire extinguishers

Type / Fire / Extinguisher	CLASS A Combustible materials e.g. paper & wood)	CLASS B Flammable liquids (e.g. paint& petrol)	CLASS C Flammable gasses (e.g. butane and methane)	CLASS D Flammable matals (e.g. lithium & potassium	Electrical Electrical equipment (e.g. computers & generators)	CLASS F Deep fat fryers (e.g. chip pans)	Comments
Water	✓	✗	✗	✗	✗	✗	Do not use on liquid or electric fires
Foam	✓	✓	✗	✗	✗	✗	Not suited to domestic use
Dry Powder	✓	✓	✓	✓	✓	✗	Can be used safely up to 1000 volts
CO2	✗	✓	✗	✗	✓	✗	Safe on both high and low voltage
Wet Chemical	✓	✗	✗	✗	✗	✓	Use on extremely high temperatures

All laboratory personnel must learn how to operate a portable fire extinguisher. Remember the acronym "PASS" when using the extinguisher:

P: Pull and twist the locking pin to break the seal.
A: Aim low and point the nozzle at the base of the fire.
S: Squeeze the handle to release the extinguishing agent.
S: Sweep from side to side until the fire is out.

10.10.3 Electrical safety

All electrical installations and equipment are to be inspected and tested at least annually, including earthing / grounding systems. Miniature Circuit-breakers (MCB) and Earth Leakage Circuit Breaker (ELCB) should be installed. MCB does not protect people; they are intended to protect wiring from being overloaded with electrical current and hence to prevent fires. ELCB is intended to protect people from electric shock.

All laboratory electrical equipment should be earthed/grounded, through three-pin plugs. Laboratory should have a dedicated socket for each equipment. Avoid extension cords and multipoint sockets. All laboratory electrical equipment and wiring should conform to national electrical safety standards and codes. Staff should know how to cut off the electrical supply to the laboratory in the event of an emergency. Electrical circuits should not be overloaded, do not create electrical hazards in wet and damp areas, replace frayed cords promptly and properly coil up loose cords. Only carbon dioxide or dry chemical fire extinguishers should be used for electrical fires.

10.11 Biosecurity

"Laboratory biosecurity" refers to institutional and personal security measures designed to prevent the loss, theft, misuse, diversion or intentional or unintentional release of pathogens, toxins or sensitive data. In HIV-1 Viral Load testing laboratory it refers primarily to loss of samples, data, reports or breach of confidentiality. Effective biosafety practices are the very foundation of laboratory biosecurity activities. A specific laboratory biosecurity programme must be prepared after bio risk assessment and implemented for the laboratory according to the requirements of the laboratory, and the local conditions. Laboratory biosecurity training, distinct from laboratory biosafety training, should be provided to all personnel. *(For details refer to Chapter 7 National Guidelines on Quality Management Systems in HIV Testing Laboratories)*.

10.12 Biological risk assessment

Biological risk assessment is a subjective process requiring consideration of many hazardous characteristics of agents and procedures with judgements based often on incomplete information. There is no standard approach for conducting a biological risk assessment; but the following five step approach gives structure to the risk assessment process:

1. Identify agent hazards and perform an initial assessment of risk.
2. Identify laboratory procedure hazards.
3. Make a determination of the appropriate biosafety level and select additional precautions indicated by the risk assessment.
4. Evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment.
5. Review the risk assessment with a biosafety professional or subject matter expert.

11 Viral Load Laboratory Network

NACO is implementing targeted HIV Viral Load testing in the programme through 10 Viral Load Testing Centres in the government run facilities across the country and around 20,000 tests are done annually (NACO 2017). Aligned with the WHO guidelines, GoI has laid down a policy to provide Viral Load test to all the PLHIV on ART. Under support from GFATM, 70 additional Viral Load testing laboratories will be setup under NACO. These Viral Load labs were identified based on patient load at connecting ART Centres, HIV prevalence and sample transportation consideration.

All existing Viral Load Laboratories will be designated as "Regional Reference Laboratories" (RRL). The public sector HIV-1 VL laboratory network will have a three tier system with NARI as the Apex Laboratory which will also be a RRL. The other RRLs besides NARI will be at tier two level of the network and the new Viral Load Laboratories will be at the third tier.

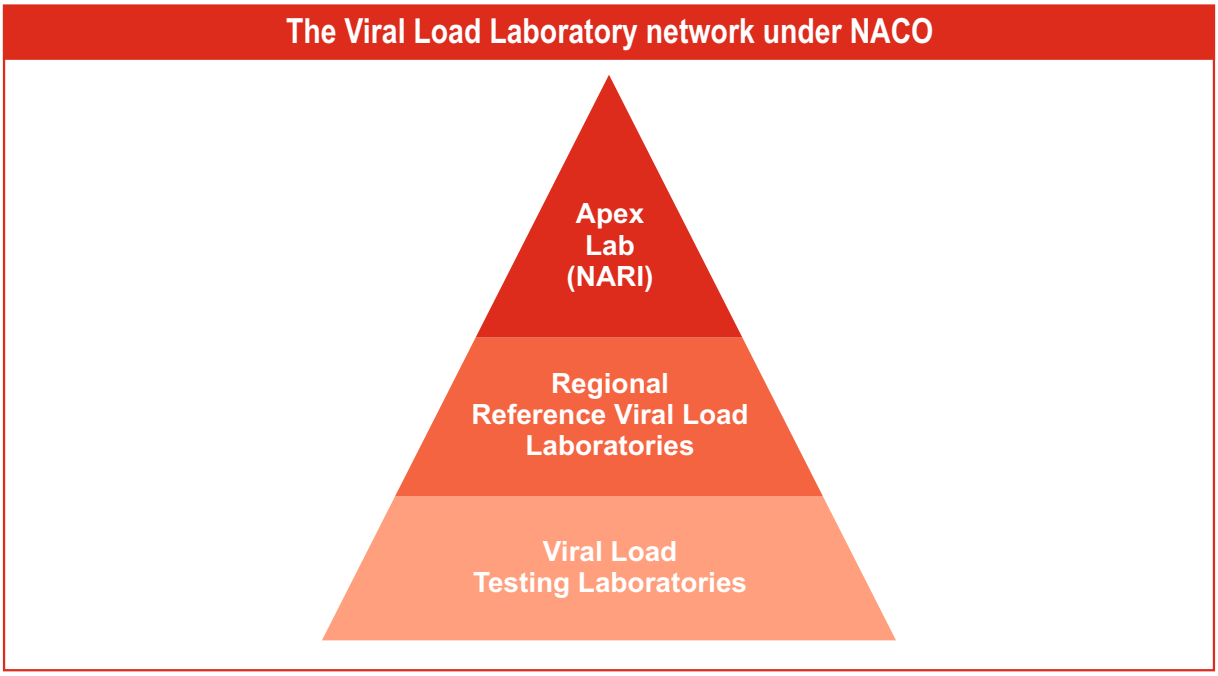


Figure 12: The three tiers of Viral Load Laboratories network

11.1 Apex Laboratory

NARI as an Apex laboratory will provide overall leadership to the HIV-1 VL testing laboratory network and will be responsible for mentoring, training and monitoring performance of all RRLs.

11.2 Regional Reference Laboratories (RRLs)

The Regional Reference Laboratories will be responsible for HIV-1 VL testing as per National Guidelines. Additionally, they will be responsible for mentoring, monitoring, training and capacity building of the HIV-1 VL testing laboratories in their regions. They will also support training and capacity building of LTs of ART centres.

Table 8: Functions of Apex Lab, RRL and VL testing laboratories

Level	Functions
Tier 1: Apex Laboratory	▸ To provide leadership to the viral load testing laboratory network ▸ Mentoring and monitoring performance of RRLs ▸ Training and capacity building of RRLs ▸ Conduct proficiency testing (PT/ EQA) for Viral Load testing laboratories and resolve discordance ▸ Perform HIV-1 VL testing as per national guidelines ▸ Resolve discordant results
Tier 2: Regional Reference VL Laboratories (RRL)	▸ Perform HIV-1 VL testing as per national guidelines ▸ Mentoring and monitoring of HIV-1 VL testing laboratories ▸ Training and capacity building of HIV-1 VL testing laboratories ▸ Training and capacity building of lab technicians at the ART centre ▸ Implementing proficiency testing (EQA) for HIV-1 VL testing laboratories ▸ Reporting of results to ART centres/SACEPs ▸ Data management through LIMS
Tier 3: VL testing laboratories	▸ Perform HIV-1 VL testing as per national guidelines ▸ Participate in proficiency testing and quality control activities ▸ Participate in training activities conducted by RRLs ▸ Reporting of results to RRL, ART centres/SACEPs ▸ Data management through LIMS

NACO also has plans to roll out monitoring treatment by VL testing engaging private sector on a turnkey model. NACO will provide guidance and directions to the private labs on requisite quality parameters and processes. It will also monitor the private labs through periodic review of reports and supervisory visits.

11.3 TORs of V L Laboratory Network staff

To perform the above defined activities, Apex Laboratory is supported by one TO, one LT and one Laboratory Coordinator from NACO. The RRLs (except NARI) and VL laboratories are supported by one TO and one LT from NACO. Each VL laboratory will be supported by Department of Microbiology/Virology (Lab Director and designated Viral Load Lab in-charge / Quality Manager). The administrative and financial link of the VL labs will exist with NACO, Apex Lab and the SACS. The organogram showing the details is depicted below.

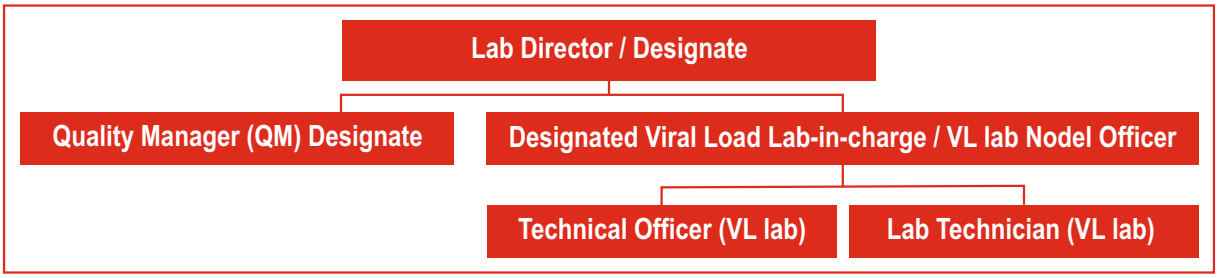


Figure 13: Organogram of Viral Load Testing Laboratories (Viral Load Lab In charge may act as the QM or may designate function to TO)

11.4 Qualification norms for Laboratory Director/Head (Designated)

The qualifications of the Laboratory Director shall be same as given in NABL-112, Clause 5.1 Table 2) with five years' experience. Laboratory Director/Designate shall have the overall responsibility of Technical / Advisory / Scientific operations of the laboratory.

The Laboratory Director/Designate shall also fulfil the other requirements of ISO 15189. In the case of a laboratory where there are more than one person designated as Laboratory Director, one of them should be available to ensure that she/he is responsible for overall operations. In a hospital setting or in a large or very large laboratory, each department/discipline may have a separate head. However, one of them, represented as Laboratory Director should be available at all times for consultation.

Job responsibility of Laboratory Director/Designate

a) To provide effective leadership of the laboratory, in accordance with the institutional assignment of responsibilities
b) To design, approve, implement and maintain the quality management system
c) To ensures that the necessary human and material resources(qualified and competent staff, material, equipment) as well as the necessary information are available to enable effective operation and control of the processes of the quality management system
d) To ensure the provision of clinical advice with respect to the choice of examinations, use of the service and clinically relevant interpretation of examination results
e) To select and monitor laboratory suppliers in coordination with the stores department
f) To select referral laboratories and monitor the quality of their services in coordination with the QM and technical-in-charges
g) To provide professional development programme for laboratory staff and opportunities to participate in scientific and other activities
h) To address any complaint, request or suggestion from staff and/or users of laboratory services in co-ordination with the respective section head
i) To ensure the development, adoption and implementation of a biosafety management plan
j) To ensure safety of the staff, environment and samples
k) To design and implement a contingency plan to ensure that essential services are available during emergency situations
l) To ensure data management with focus on data security, archival and retrieval
m) To ensure internal and external communication

11.5 Qualification of the Quality Manager/Designate

The Quality Manager/Designate should be trained in "Four-days Training in Internal Auditor & Quality Management Systems (IA & QMS) as per ISO 15189'. QM should be a full-time staff and can be delegated with additional responsibilities.

Job responsibility of the Quality Manager/Designate

a) To ensure that processes needed for the quality management system are established, implemented and maintained.

b) To report to Laboratory Director.
c) To ensure the awareness of users' needs and requirements throughout the laboratory organization through continuous training programmes.
d) Coordinate with the management and HOD in developing the laboratory QMS.
e) To oversee the compliance and implementation of the requirements of the QMS.
f) Report directly to the level of laboratory management at which decisions are made on the laboratory policies and resources.
g) To ensure there are procedures to control all documents and information that forms quality documentation.
h) To coordinate with HOD in regular calibration of equipment and maintain records.
I) To conduct the scheduled general training programmes.
j) To control documents and records.
k) To review the Non Conformities log and ensuring compliance of CAPA initiated.
l) To plan organize and carry out internal audits of all elements of the system, both managerial and technical.
m) To address the customer feedback/complaints in coordination with the HOD and Laboratory Director.
n) To conduct MRM as per the QMS.
o) Ensuring that the NABL logo usage is in accordance with NABL guidelines.
p) Interacting with the accreditation bodies such as NABL and providing interface to technical personnel.
q) Verify the compliance of the technical section of the training programmes.
r) Impart training as and when required in quality systems, in house calibration of equipment.
s) Assist Technical Advisor and take up additional responsibility as and when required.
t) Implement a contingency plan to ensure that essential services are available during emergency situations.
u) To ensure safety in the laboratory with regard to biosafety, fire, chemical and electrical safety with a robust safety plan and periodic safety audits.
v) To ensure safety training of all staff and periodic safety drills.
w) To ensure that the MSDS are available in the respective laboratory sections and that the staff have read and understood them.
x) To ensure all accidents/incidents are attended immediately in consultation with respective authorities and documented.

11.6 Qualification of the Technical Officer

Qualification of the TO will be as per the latest NACO guideline (specific to Viral Load lab).

Job responsibility of the Technical Officer

a) To monitor work related to PT/ EQA, including all measures in implementing QMS, RCA and CAPA in case of NC and ensure accreditation of HIV-1 VL in the region in a defined period.
b) To ensure that the laboratory reports are issued within the defined TAT.
c) To conduct HIV-1 VL testing.
d) To implement a safe laboratory environment in compliance with good Laboratory practice and applicable regulations.

e) Supervision of testing done in the laboratory and conducting training programmes.
f) To prepare SOPs, Quality Manual, Safety Manual and Primary Sample Collection Manual and ensure all the documentation for NABL.
g) To support maintenance and Calibration of all Equipment in the Lab.
h) To assist lab in-charge in data compilation and analysis.
i) To ensure dispatch of HIV-1 VL test reports, consolidated reports and monthly reports to RRL & NACO.
j) To address any complaint, request or suggestion from users of laboratory services relevant to the section in coordination with the QM and Laboratory Director.
k) Monitoring and mentoring visits to ARTC from where samples are collected for VL testing and VL laboratories within the region Ensure good staff morale.

11.7 Qualification of the HIV-1 VL Laboratory Technician

Qualification of the LT will be as per the latest NACO guideline (specific to Viral Load lab)

Job responsibility of the VL- LT

a) To carry out work as per the laid down standard operation procedures.
b) To maintain work area clean and follow biosafety guidelines.
c) To receive samples, verify the test requested.
d) To maintain and handle equipment as authorized by the Section Head / Lab in-charge.
e) To process the sample and do the analysis as per the examination procedures.
f) To perform preventive maintenance of equipment on daily, weekly and monthly basis.
g) Daily updating of the relevant records.
h) To support PT/ EQA and participate in EQA training programmes.
I) To send reports to TO and HIV-1 VL Laboratory in-charge.

11.8 Monitoring and Mentoring

The monitoring and mentoring cascade will start with the Apex lab which will provide leadership for mentoring and monitoring of the HIV-1 VL laboratory network through the RRLs. Similarly the RRLs will mentor and monitor the activities of the VL testing Labs. The mentoring visits by Apex lab for RRLs and for RRLs visit to the HIV-1 VL Testing laboratories will be detailed in the plan of each institution. Mentoring and monitoring will also include training and capacity building and implementing Proficiency Testing (PT/ EQA).

During the supervisory visits, 'Checklist to Review Quality Management Systems at Viral Load Testing Laboratories' should be utilized for the baseline audit and monitoring progress in quality parameters. The monitoring and mentoring activities for the VL testing Labs are depicted in Fig 14.

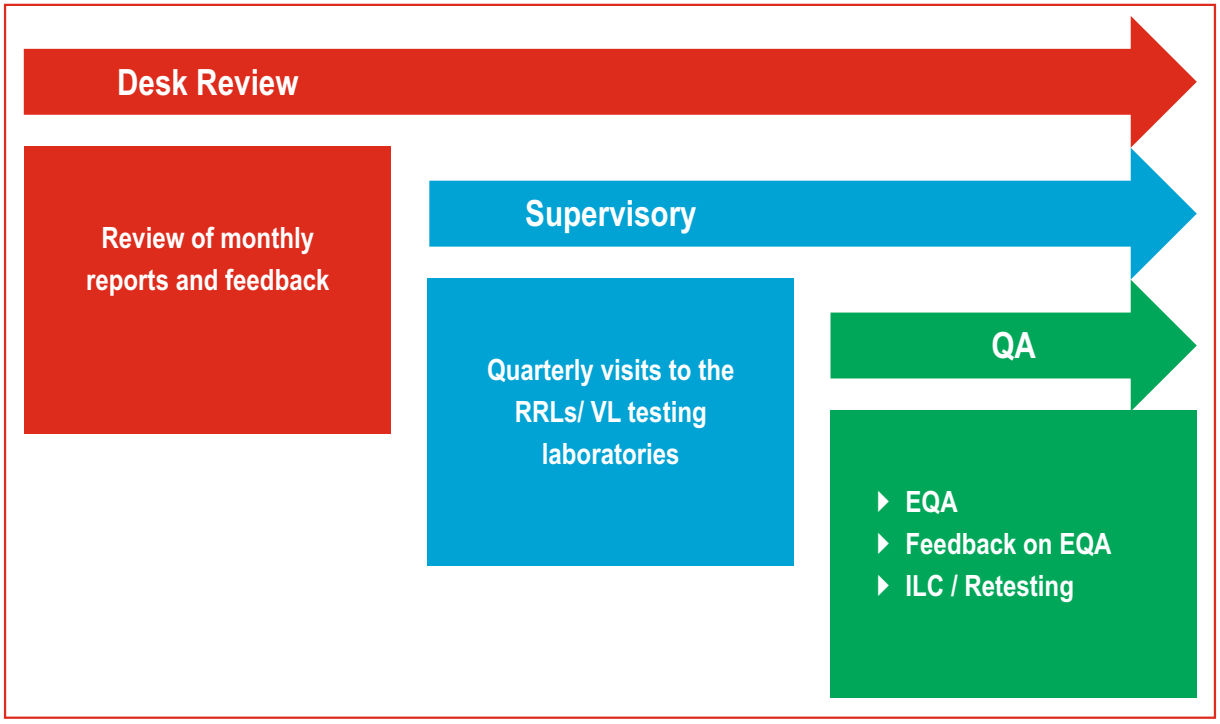


Figure 14: Monitoring and mentoring cascade for VL labs

11.9 Qualification norms for authorized signatories (as per NABL-112)

As per NABL-112, qualification of M.D. (Microbiology) is required for Microbiology and Serology, Flow Cytometry, Molecular Biology, Clinical Pathology, routine Haematology and routine Biochemistry (Reference: Annexure1 as per NABL- 112, Table-2, Page 17).

11.10 Training Need

The laboratory shall ensure that all technical staff (TO and LT) are given appropriate training before they are assigned the responsibility of operating new equipment or performing a new test.

All technical staff needs to undergo training at NACO-designated centres as per the training curriculum recommended by NACO. These trainings are organized by NACO/Apex Lab/RRLs. Participation of training shall be documented by certificates/emails/letter and produced at the time of review.

Trainings required by staff include:

- Induction training for all newly recruited staff
- Training to perform VL assays and other relevant techniques
- Biosafety training
- Refresher training periodically to upgrade their knowledge and skills. The refresher training is a training programme designed for the old or existing technical staff of the organization, with a purpose to train them with the new skills, methods and processes required to improve their performance on the jobs. Refresher training are conducted by NACO/Apex Lab/RRLs.
- Training of staff of linked collection centres will be conducted periodically to upgrade their knowledge and skill.

12 Inventory Management and Record Keeping

Inventory Management involves a series of activities to ensure the continuous supply of commodities from the point of manufacture to the point of care.

Inventory Management provides programme managers with the data to determine what type of commodities, where and when they are needed, in what quantity and condition, how to maintain an appropriate stock level of all commodities to avoid shortages, over supply, emergency rationing of commodities and associated products to avoid disruption in service delivery.

12.1 Objectives

- To implement a system for inventory management for HIV-1 VL testing
- Leverage available resources (Human Resources, Finance, External Stakeholders, Public Relations/communication and Technology) for Inventory Management
- Procurement and distribution of quality assured laboratory products, in adequate quantity, in time to support the HIV-1 VL testing activity
- Monitor the optimum utilization of materials in a cost effective way and report
- To identify roles and responsibilities of NACO, SACS and HIV-1 VL testing laboratories in management of commodities supply

12.2 Supply Chain Network and Supply Chain Management (SCM), of HIV-1 Viral Load Assay Commodities (Equipment, Kits and Consumables)

Supply Chain Management (SCM), is the monitoring of materials and information as they are transferred through well laid-out processes from manufacturer to supplier to NACO and then to VL testing laboratories.

Steps of Supply Chain Management

Step-1	Development of plan or strategy to meet customer needs
Step-2	Analysis of various factors involved in supply chain systems to develop suitable strategies to meet Customer requirements
Step-3	Integrate several functions like sourcing, procurement, production scheduling, order processing, inventory management, transportation and manufacturing – at different organisational levels for better performance results.
Step-4	Receive orders/indents and plan delivery of stocks/goods
Step-5	Manage problems associated with return management and reverse logistics within the firm and across key components of the supply chain

Procurements of equipment, kits and consumables for VL test are done centrally by NACO and supplied to Viral Load testing laboratories.

12.3 Storage of V L kits and consumables

The purpose of a storage and distribution system is to ensure the physical integrity and safety of commodities. VL kits and consumables should be stored in accordance to manufacturer's instructions. While planning for an appropriate storage space, the following factors are essential:

Protection from unauthorised access

The store supplies will be stocked in a secured stock room with a limited access to supplies to only one or two persons, ensuring that at least one person is always available to distribute supplies.

Protection from heat, light, moisture/rain, dust, pests and fire

- Storage facilities (warehouses, storage rooms) must be clean, secure, and adequate
- The storage facilities must be temperature controlled and monitored
- The store room must be having regular pest control
- The store room must be moisture-free with the walls and floors free of debris
- The inflammable material must be stored separately in a well-ventilated area, away from open flames and electrical appliances
- Implement fire prevention measures. Keep fire safety equipment available, accessible and functional

Organise the stockroom so that all the supplies can be easily and quickly located and identified

- Store kits and consumables with the cartons arranged with arrows pointing up and with identification labels, expiry dates and manufacturing dates clearly marked and visible.
- Use the FEFO (First Expiry-First Out) rule, i.e. the, supplies that are likely to expire first are to be taken out first.
- In order to ensure FEFO, kits and drugs which are going to expire earlier should always be kept ahead of those which are going to expire later.
- Expired material should never be stored along with supplies being used.
- The damaged commodities are to be removed from the inventory immediately and disposed of using Biomedical Waste (BMW) management guidelines.

Maintenance of records:

- Maintain accurate and up-to-date records so that sufficient stock is available at all levels and there is no stock of expired commodities.
- Carry out physical verification at least twice in a year, match with the records and review with data triangulation in registers/records and reports every quarter.

12.4 Monitoring of stock

Monitoring of logistics is important to ensure uninterrupted supply as it:

a. Reduces over stocking:Avoids wastage of scarce resources leading to expiry.
b. Prevents stock-outs:Avoids delay in testing, treatment or management.

Monitoring is done through a central system at NACO ensuring adequacy of supplies to VL testing laboratories. Distribution systems must be dependable and the following actions should be completed at the time of receipt when the material is distributed:

a. Verify the products received: type and quantity.
b. Conduct visual inspection: any signs of damage, expiry dates, etc. for QA.
c. Complete and sign transaction records or vouchers.
d. Store the products appropriately.
e. Update stock records.

12.5 Commodities for Viral Load testing facility

Laboratory commodities can be classified into three categories: reagents, consumables, and durables.

Reagents are chemicals and biological agents that are used in laboratory testing for detecting or measuring an analyte (the substance being measured or determined). The reagents vary widely in cost, stability, cold or cool chain requirements, availability, and the hazards associated with each variant. Reagents can be further subcategorized into liquid and solid reagents.

Consumables are the disposable items that are used while performing a test and should not be reused. Consumables can include items such as bleach, alcohol, and gloves, used in all service areas and are classified as general laboratory consumables.

Durables are items that can be reused for multiple tests. They include items such as glassware that can be washed, sterilized, and reused. This type of commodity also includes equipment and instruments used for testing.

Generally, reagents and consumables are commodities that are routinely reordered and managed. Durables are ordered as and when needed.

12.5.1 List of Commodities For HIV-1 Viral Load Assay

REAGENTS	CONSUMABLES	DURABLES
HIV Viral load assay kits ▸ Extraction kits ▸ Amplification kits ▸ Calibrator kits ▸ Control kits	▸ Distilled water ▸ Hypo solution ▸ Saline ▸ Room fresheners ▸ Kim wipes ▸ Tissue rolls ▸ Powder free gloves ▸ Micro tips(filtered) ▸ Stationary ▸ Marking pens ▸ Labels ▸ Syringes ▸ Information posters ▸ Report formats ▸ TRFs ▸ Registers ▸ Cryo vials	▸ Equipment platform ▸ Centrifuge ▸ Refrigerators(4-8/-20,-70) ▸ Water bath ▸ Autoclave ▸ Cyclomixer ▸ PCR Station ▸ Pipettes (100-10000 µl,20 µl - 200 µl,50 µl,) ▸ Colour coded Biomedical waste bins Biomedical waste bags(red, blue, yellow ,white) ▸ Discard jars ▸ Computers ▸ Almirahs ▸ Lab coats(colour coded) ▸ Lockable cupboards/shelves for patient records and supplies ▸ Sink with running water ▸ Tables, chairs and stools for staff and patients ▸ Safe drinking water facility

12.5.2 List of Commodities For HIV Viral Load Assay and their Storage Information

S No.	Name of the article	Storage Temperature
1	Plasma sample	Eambient /Room temperature/ 2-8^{o}C-70^{o}C (Kit insert can be referred for more details)
2	Extraction kit	Ambient /Room temperature
3	Amplification kit	-20^{o}C (Kit insert can be referred for more details)
4	Calibrator kits	-20^{o}C (Kit insert can be referred for more details)
5	EDTA Vacutainers (6 mL) with needles	Ambient /Room temperature
6	Cryo vials2 ml	Ambient /Room temperature
7	Filter tips	Ambient /Room temperature
8	Gloves	Ambient /Room temperature
9	Biomedical waste colour coded bags (Y,R,B)	Ambient /Room temperature
10	Micro centrifuge tubes	Ambient /Room temperature
11	Ethanol	Ambient /Room temperature
12	Distilled Water	Ambient /Room temperature
13	Filter tips	Ambient /Room temperature
14	Kim wipes	Ambient /Room temperature
15	Tissue rolls	Ambient /Room temperature
16	Powder free Gloves	Ambient /Room temperature
17	Hypo solution	Ambient /Room temperature

12.6 Inventory Registers

Stock register for kits/ consumables atVL testing laboratories

Guidelines for filling stock register

- Maintain a separate sheet for each item and track stocks on day-to-day basis;
- The Lab in charge /technician must fill in and maintain the Stock Register at the VL laboratories
- At the end of day, tally the stock with records of the stock available in the stock.

Frequency of filling Stock Register: On a day-to-day basis, as and when kits are received/ consumed as per tests done

Note:

1. The facility must have supply of kits and consumables for at least three months;
2. There should be a buffer stock for each item and an indent/ request must be put up whenever supply reaches less than one quarter of supply;
3. Follow policy of FEFO (First Expiry-First Out).

12.7 Reporting

- Provides the mechanism through which personnel collect, organize, manage and report data
- Determines quantity to ensure uninterrupted supply of commodities and plan procurement and shipments
- Calculation of average monthly consumption. Capture the essential data needed to determine consumption pattern for future projections.
- Monitor losses and maintain logistics records

12.8 Roles and Responsibilities

This section highlights key responsibilities of each key stakeholder involved in viral load testing

NACO:

- NACO will be responsible for forecasting and indicating annual quantity of kits and consumables of each laboratory
- Forecasting, indenting and procuring commodities centrally and make them available to Consignees (Labs). NACO's distribution will follow a hub and spoke model where the supplier delivers the quantity required to theViral Load Lab
- Facilitate interstate relocation in case of low stocks, near-expiry kits and consumables, or during natural calamities and conflict situations.

SACS:

- Financial and administrative support to RRLs andViral Load testing labs as defined by NACO.
- Monitor and analyse stock positions and submit the monthly stock report to NACO on time.
- Prevent stock-outs, kits and consumables expiry by timely relocations within the State and, if needed, facilitate outside-the State relocations with official directives from NACO.
- Refer to the revised guidelines sent from time-to-time by NACO.

HIV-1 VL Testing Laboratories:

- Ensure proper receipt of consumables, cross verify exact amount of consumables received against allocated quantity after physical counting of consumables, acknowledge receipt and confirm figures to NACO/Logistic coordinator/Supplier/procurement agency/Regional Coordinator.Any deviation should be highlighted for further action
- Keep accurate record of all consumables received from suppliers/other States
- Safe storage of kits and consumables according to general storage guidelines for laboratory commodities mentioned
- Monitor and analyse stock positions. Submit on monthly stock report to SACS
- Prevent expiry/stock-outs by timely reporting to SACS
- Reconcile stocks by doing a quarterly physical count

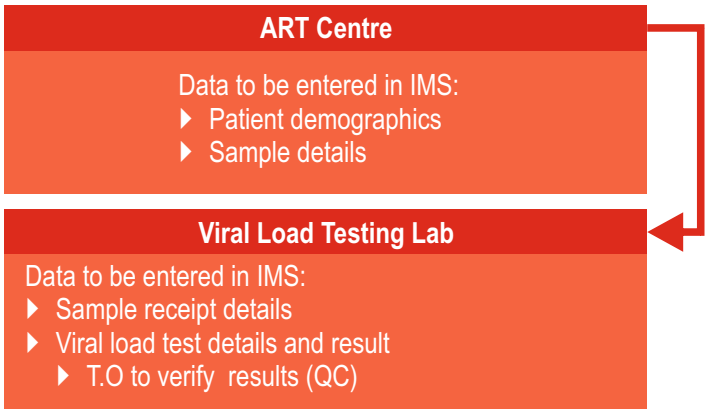
13 Laboratory Information Management

All the viral load laboratories under the programme conducting viral load test are required to enter details of the testing in an online system (whenever applicable). This system captures key information points in the end-to-end process flow of viral load testing across all stakeholders. Additionally, real time online reporting allows automated reporting, thus reducing duplication of efforts and ensuring data validated reports.

13.1 Process Flow in the Information Management System

At ART centre, patients' detailed information is entered into the Inventory Management System (IMS). The ART centre then sends a request to the testing laboratory using IMS which notifies the laboratory about the incoming samples. Each testing laboratory will have a login ID which can be used to access the requests coming from different ART centres and to enter test results once the testing is complete. The following information needs to be filled by the testing laboratory on the system:

- Sample receipt details
- Viral Load test details and results
- Date of result dispatch



13.2 Reporting Requirements of Viral Load Testing Laboratory

The testing laboratory should ensure that all the required fields in the reporting formats are completely and accurately filled. The following are the reporting requirements of viral load testing lab:

- Completed viral load TRRF for all the samples received (Annexure 2, Format 1)
- TRRF must be signed by LT and laboratory-in-charge/authorized signatory
- Completed details in IMS for all the samples
- Completed details in viral load register (Annexure 2, Format 2) for all the samples
- Monthly viral load laboratory reporting format (Annexure 2, Format 3) to be sent to NACO and SACS
- Monthly stock register for viral load kits and reagents (Annexure 2, Format 4) to be sent to SACS
- Register for maintaining the record of consumables (Annexure 2, Format 5)

Laboratory-Clinical Interface 14

14.1 Introduction

Medical laboratory plays crucial role in diagnosing and monitoring patient's illness and preventing complications and ensures quality patient care. One of the recognized barriers for effective healthcare delivery is lack of communication between the stakeholders involved in patient management, specifically the laboratory and clinical staff. The laboratory clinical interface can influence clinicians' request behaviour and treatment interventions.

Diagnostic tests requested by clinicians are performed by laboratory staff. The test results are provided to clinicians for clinical decision making. This requires timely availability of test results to users qualified for switching to second-line therapy within a defined timeframe as poor TAT of viral load result affects patient management. The interaction between the clinical and laboratory staff is essential throughout the workflow of the laboratory.

14.2 Interaction of laboratory and clinical staff across the Viral Load spectrum

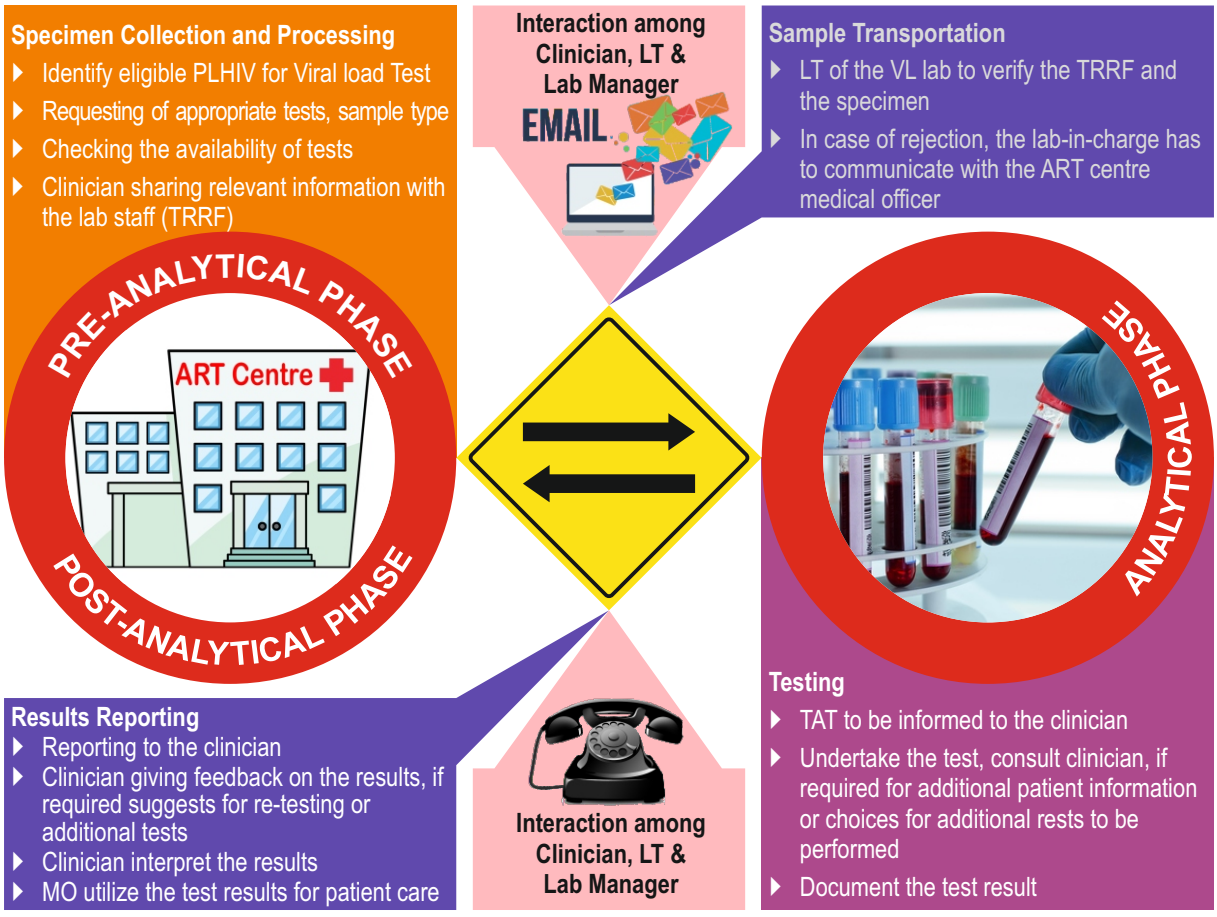
Interaction of the clinical staff at ART centre and VL laboratory staff can be followed across all the three phases of work flow. The following figure depicts processes and interfaces across the VL testing network.

14.3 Role of staff across the spectrum:

Considering the ART centre staff and the VL Laboratory staff, key areas of interface are listed below. The interface can be through a phone/email/direct one to one contact.

Phase		Clinical Staff at ART centre	Lab Staff at ARTC/VL Lab
Pre-Analytical	**Specimen collection and processing**	Medical Officer and the Staff Nurse at ART centre should identify eligible PLHIV for Viral Load test, initiate a duly filled TRRF and request the VL test.	On receipt of the TRRF form, the LT at the ART centre to verify for completeness. In case of discrepancies (transcription error/doubts), a feedback to reach the clinical staff at ART centre
Analytical	**Sample transportation**	The ART centre LT to transport the specimen to VL lab as per the guidelines.	▸ LT of the VL lab to verify the TRRF and the specimen. ▸ In case of rejection, the lab-in-charge has to communicate with the ART centre medical officer

Phase		Clinical Staff at ART centre	Lab Staff at ARTC/VL Lab
Analytical	**Laboratory testing**		▸ The timings of the laboratory and the TAT for the specimens need to be intimated to the clinician. ▸ Laboratory staff, if needed, may seek clarification on the patient's condition in order to interpret the test results or to suggest repeat/additional test
Post Analytical	**Results Reporting**	In case of a clarification (transcription error/doubts), the MO to seek feedback on the results from the laboratory when required	Laboratory staff could seek additional information from the clinician (possible requirement for re-testing or additional test) while reporting results
	Update result and Patient management	MO must utilize the test results for patient decision making and patient to be managed appropriately. In case of further clarification for interpretation of results, the clinician must discuss with VL lab-	Laboratory-in-charge to be part of the SACEP meetings for supporting the clinician to interpret the results and clinical decision making.



14.4 Reference to interface between the laboratory and clinical departments as standard for Accreditation or certification of Laboratories:

Standard accreditation guidelines (ISO 15189, ISO 22869, ISO 9001) recognized the interface as a critical step and hence has included this as a measure of laboratory standard. The following table provides guidelines that refer to the interface between the laboratory and clinical departments:

Maintaining relations with parties outside the laboratory	Monitoring of customer satisfaction / Complaint management
Responsibility for the relationship with any other organization with which the laboratory may be associated (ISO 15189 & ISO 22869)	Policy and procedures for the resolution of complaints or other feedback received from clinicians, patients or other parties (ISO 15189 & ISO 22869)
Relate and function effectively (including contractual arrangements if necessary with the healthcare community (ISO 15189 & ISO 22869)	Monitor information relating to customer perception as whether the organization has met customer requirements (ISO 9001)
	Ensure that customer requirements are determined and meet with the aim of enhancing customer satisfaction (ISO 9001)

14.5 Lab-Clinical Interface Training

Training clinical staff on quality objectives, policies, procedures and appropriate use of laboratory information is critical, as clarity with regard to which tests should be ordered and at what frequency is important. Guidelines need to be tailored to local environments, based on clinical realities and laboratory access and this should be conveyed to facility staff. Staff should receive training on the cost effectiveness of the laboratory test, sample collection, sample handling, storage and accurate data entry.

Communicating results to facilities and patients requires on-going laboratory-clinical interface improvement. Improving results management in the clinics is important, irrespective of how those results are delivered. Initial review of results must be performed by clinical staff, abnormal results flagged for action and communicated to the patient. Laboratories and clinicians need to ensure quality improvement in patient management by adhering to review of the quality indicators on lab and clinical fronts.

Annexure

Annexure 1

List of Laboratory Safety Kit Contents

A. Content of First Aid box

- Instruction sheet giving general guidance
- Individually-wrapped sterile adhesive dressings in a variety of sizes
- Sterile eye-pads with attachment bandages
- Triangular bandages
- Sterile wound coverings
- Safety pins
- Cotton
- Gloves
- Alcohol swabs
- Dettol
- Roller Bandage
- Soframycin
- Burnol
- Band-Aid
- Crocin
- Scissors
- Glucose powder
- An authoritative first-aid manual, e.g. one issued by the International Red Cross.

B. Content of Biological spill containment kit

- Eye shield/Goggle
- Plastic Apron
- Gloves
- Masks
- Cotton/Absorbent material/Paper towels/Rough blotting sheets
- Chalk or marker pen/Signs indicating spill warning for cordoning the area
- Sodium Hypochlorite (4-6%)- 100 ml
- Forceps/Tongs/Plastic scoop and Brush
- Water Bottle (with 100 ml marking)

C. Content of chemical spill containment kit

- Protective clothing – Heavy duty rubber gloves, Overshoes or Rubber boots, Respirators
- Scoops and dustpans
- Forceps
- Mops, clothes and paper towels
- Buckets

- Soda ash (sodium Soda ash (sodium carbonate, Na_2CO_3) or sodium bicarbonate ($NaHCO_3$) for neutralizing acids and corrosive chemicals
- Sand (to cover alkali spills)
- Non-flammable detergent

D. Content of Sodium hypochlorite preparation

Formula for dilution of Stock solution of Sodium hypochlorite to working concentration of Sodium Hypochlorite:

Amount of stock required	=	Working Conc. Required / Stock Conc.	X	Working solution volume required

Water Required = Working solution volume required - Amount Stock Required

E. Preparation of different concentration of Sodium Hypochlorite Solution:

Required strength (Available solution of chlorine)	Stock / commercially available sodium hypochlorite		
	4% (40g/L); dilute	5% (50g/L); dilute	6% (60g/L); dilute
0.1% (1 g/L)	1:39*	1:49	1:59
1% (10 g/L)	1:3	1:4	1:5

* Parts of stock solution: Parts of water

Annexure 2

Format 1: Viral Load TRRF

LABORATORY TEST REQUISITION CUM RESULT FORM (TRRF) FOR HIV-1 VIRAL LOAD TESTING

To be filled by ART Centre

Patient Details

Unique Patient ID for Viral Load: ☐☐☐☐☐☐☐☐☐☐ (ART Centre Code) ☐☐☐☐☐ (Patient's ART No.) ☐☐ (VL Test No.)

Name: _____

Age: _____ Gender: ☐ Male ☐ Female ☐ Other .....

HIV status: ☐ HIV-1 ☐ HIV-2* ☐ HIV-1&2

Viral Load Testing Details

Reason for current viral load testing: ☐ Routine Testing ☐ Targeted Testing ☐ Repeat Testing

If Repeat Testing, Reason: ☐ Previous Sample Rejected ☐ Inconclusive Result ☐ Other

Date of previous viral load test: D D M M Y Y Y Y Result of previous viral load test: .....

Date of specimen collection: D D M M Y Y Y Y Time of specimen collection: H H M M

Date of specimen dispatch: D D M M Y Y Y Y Date of receiving results: D D M M Y Y Y Y

Date of sharing results with patient: D D M M Y Y Y Y Final advice of medical officer: .....

Authorizing clinician name and signature: .....

To be filled by Viral Load Laboratory

Name of the laboratory: .....

Date of specimen receipt: D D M M Y Y Y Y Lab Number: ☐☐☐☐☐☐☐☐☐☐

Time of specimen receipt: H H M M Sample received in proper condition ☐ Yes ☐ No

Date on which specimen is tested: D D M M Y Y Y Y Viral load by real time PCR**: .....(copies/mL)

If no result, please specify reason: .....

Date of result dispatch: D D M M Y Y Y Y Platform used: ☐ Abbott ☐ Roche ☐ Other.....

Name & Signature of lab technician: .....

Name & Signature of lab in-charge: .....

** HIV 2 sample should not be sent for VL Testing*

***A specimen with a result of "Target no detected(NTD)" cannot be presumed to be negative for HIV-1 RNA*

Format 2: Viral Load Laboratory Register

Name of Viral Load Testing Laboratory: ..... Date: D D M M Y Y Y Y

Name of Lab In charge: .....

Name of Lab Technician: .....

Lab Number	Patient Name	Unique Patient ID for Viral Load (17 digit)	Date of receiving specimen (DD/MM/YYYY)	Was the specimen accepted? (Yes/No)	if specimen rejected reason for rejection	Date of testing (DD/MM/YYYY)	Viral Load count ('RJ' if the sample was rejected 'IR' if inconclusive result was obtained)	Date of dispatching results (DD/MM/YYYY)	Remarks

Format 3: Viral Load Laboratory Monthly Reporting Format

NACO VIRAL LOAD Laboratory Monthly Reporting Format										
Year		Month		Laboratory Name						
ART Centre Code	Name of ART Centre	Details of specimen				Viral Load Results				
		Number of specimen received	Number of specimen left for testing last month	Number of specimen tested	Number of specimen left to be tested	Target not detected OR <Lower limit of quantification (LLQ)	LLQ - 1000	1000-Upper limit of quantification (ULQ)	> ULQ	
Total number of specimens tested - Platform 1 Name:										
Stock records	Test type									
	Opening balance (Number of Tests)									
	Number of patients tested									
	Controls									
	Number of tests wasted									
	Total tests used in the month including control and wastage									
	No of tests available with lab at the end of month									
Expiry date of details	Earliest expiry date									
	No of tests expiring on above date									
Total number of samples tested - Platform 2 Name:										
Stock records	Test type									
	Opening balance (Number of Tests)									
	Number of patients tested									
	Controls									
	Number of tests wasted									
	Total tests used in the month including control and wastage									
	No of tests available with lab at the end of month									
Expiry date of details	Earliest expiry date									
	No of tests expiring on above date									
Other comments:										
If any unusual consumption of test like training etc.:										
Date for each month should be entered and forward to AIDS Socities and NACO **by 7th of every month.**										

Format 4: Stock Register for Viral Load Kits and Reagents

Name of Viral Load Testing Laboratory: ..... **Lab ID:** .....

Name of Lab In charge: ..... **Financial year:** .....

Name of Lab Technician: ..... **Month:** .....

S No.	Date	Name of item	Lot No.	Expiry Date	Received Qty.	Opening Balance	Consumption (actual tests)	Wastage	Control	Closing balance	LT Sign	MO Sign	Remarks

Format 5: Consumable Register

Name of Viral Load Testing Laboratory: ..... **Lab ID:** .....

Name of Lab In charge: ..... **Financial year:** .....

Name of Lab Technician: ..... **Month:** .....

S No.	Date	Received from	Received Qty.	Issued Qty.	Balance	LT Sign	MO Sign	Remarks

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