



# **NATIONAL OPERATIONAL GUIDELINES FOR VIRAL LOAD TESTING**



**National AIDS Control Programme  
Care Support and Treatment Services**



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





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## **PREFACE**

More than three decades have elapsed since the first case of HIV/AIDS was identified in India. During this period, our country has implemented a series of strategic plans and interventions towards preventing spread of HIV/AIDS.

The National Program is evolving from targeted viral load testing to routine viral load monitoring. This significant development will help us to move towards 90-90-90 targets set under National Health Policy 2017. Moreover it will add to quality treatment monitoring for PLHIV receiving ART in the country.

These "National Operational Guidelines for Viral Load Testing" covers the operational aspects of Viral Load testing at the ART centre and Viral Load testing lab along with the timing, algorithm and clinical interpretation of results of routine monitoring tests for PLHIV.

I thank all the national experts and contributors for their hard work in producing these operational guidelines for Viral Load testing.

  
(Dr. R. S. Gupta)



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## Abbreviations

AIDS	Acquired Immuno Deficiency Syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral (drug)
CD4	T-lymphocyte CD4+
COE	Centre of Excellence
CSC	Care and Support Centre
EDTA	Ethylenediaminetetraacetic Acid
HIV	Human Immunodeficiency Virus
IMS	Inventory Management System
LT	Lab Technician
MO	Medical Officer
NABL	National Accreditation Board for Testing and Calibration Laboratories
NACO	National AIDS Control Organization
OI	Opportunistic Infection
PCOE	Pediatric Centre of Excellence
PCR	Polymerase Chain Reaction
PLHIV	People living with HIV
PPP	Public Private Partnership
RNA	Ribonucleic Acid
SACEP	State AIDS Clinical Expert Panel
SACS	State AIDS Control Society
SMO	Senior Medical Officer
TO	Technical Officer
TRRF	Test Requisition cum Result Form
VL	Viral Load
WB	Whole Blood
WHO	World Health Organization



## Executive Summary

The national programme provides antiretroviral therapy (ART) to all people living with HIV (PLHIV). The goal of ART is to ensure viral suppression in patients for as long as possible. Thus, it is important to monitor the viral load in a patient's body to measure the success of ART. The national programme used targeted viral load testing to confirm suspected treatment failure owing to clinical or immunological failure of the patient.

The national programme is now moving from targeted viral load to routine viral load testing for all patients on ART. This will help in earlier and more accurate detection of treatment failure. Additionally, it will also help the programme to quantify the third 90 goal. A viral load test will be conducted for all patients at 6 and 12 months after initiation of ART. All second/third line patients will be tested every 6 months and the first line patients will be tested annually after 12 months of ART initiation.

These guidelines provide extensive details on how routine viral load testing needs to be implemented at the facility level. It includes information on frequency and interpretation of monitoring tests, sample collection, storage and transportation, receiving results, adherence counselling, and recording and reporting requirements. It also outlines the key roles and responsibilities of each stakeholder (ART centre, VL testing lab, SACEP and NACO) along with the turnaround time of viral load testing at the facility level.

## 1. Introduction

Currently, the overall HIV adult prevalence in India is 0.26%<sup>1</sup> with an estimated ~2.1 million<sup>1</sup> PLHIV. Out of these, ~1.26 million<sup>2</sup> PLHIV are registered in the National AIDS Control Programme (NACP) and ~1.13<sup>2</sup> million are on antiretroviral therapy (ART). India is committed to achieving the global 90-90-90 target by 2020 viz. 90% of people living with HIV would know their HIV status, 90% of people who know their HIV status will receive treatment and 90% of people on treatment would have suppressed viral load to minimize HIV transmission. Thus, it is important that viral load testing is scaled up in the country to monitor the viral load suppression.

One of the important goals of ART is to ensure suppression of viral load for as long as possible. This is to ensure reduction in HIV transmission and improvement in the quality of life of patients on ART. In order to achieve this goal, it is important for the programme to use the best available monitoring tests to assess the viral load suppression in the patients on ART.

The national programme introduced targeted viral load testing in 2010 for the suspected cases to confirm treatment failure before switching the patient to appropriate regimen. It was using immunological (CD4) and clinical monitoring to monitor response to ARV drugs. However, numerous studies have demonstrated the poor predictive value of the WHO immunological criteria for identifying treatment failure and have shown delayed detection of treatment failure leading to accumulation of HIV drug resistance. The 2013 WHO consolidated antiretroviral guidelines recommended viral load testing six months after initiating ART and then annually for people receiving ART. Those with true treatment failure would then be switched to second-line or third line ART.

The objective of introducing routine viral load monitoring in the national programme is to provide early and accurate indication of treatment failure, and assess need to switch the treatment regimen. This will help in reducing the accumulation of drug resistance mutations and improve patient outcomes. Additionally, it will help in the quantification of third 90 goal.

The purpose of these guidelines is to discuss the operational plan for implementation of viral load testing for routine monitoring of patients on ART in the national programme.

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<sup>1</sup> India HIV Estimations 2015

<sup>2</sup> Monthly Progress Report Aug'17

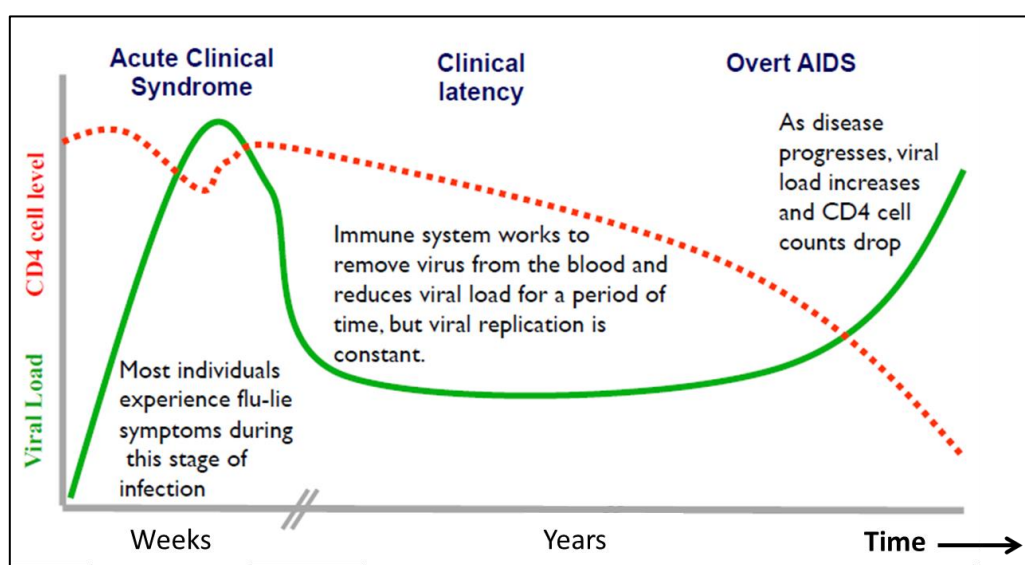
## 2. Viral Load Testing in the National Programme

### 2.1 Role of Viral Load Testing

Monitoring of individuals receiving ART is important to ensure success of treatment and determine whether ART regimens should be switched in case of treatment failure. Earlier, immunological (CD4) and clinical monitoring were used to monitor response to ARV drugs. However, viral load is recognized as the gold standard for monitoring of ART response.

Plasma HIV-1 viral load refers to the number of viral particles found in each millilitre of blood. Higher the HIV-1 viral particles in the blood, faster the CD4 cell depletion and faster the progression towards AIDS. Viral Load is the earliest indicator of viral reproduction. The drop in CD4 occurs after viral load has been elevated for some time.

**Figure 1: Natural course of HIV in an untreated patient**



In the 2013 consolidated guidelines, WHO recommends viral load as the preferred approach for monitoring PLHIV on ART over immunological (CD4) and clinical monitoring<sup>3</sup>. This is because viral load provides an early and more accurate indication of treatment failure and the need to switch to second-line drugs. This helps reduce the accumulation of drug-resistance mutations and improve clinical outcomes of the PHLIV on ART. Measuring viral load can also help distinguish between treatment failure and non-adherence.

### 2.2 Intended use of viral load tests in the programme

The national programme plans to use the viral load tests for two purposes:

**Routine viral load monitoring:** This type of viral load test is done at specific regular intervals to detect change in viral load for patients on ART. The result of this test can trigger step-up adherence support and/or switching ART regimen

**Targeted viral load monitoring:** This test is used for confirmation of treatment failure

<sup>3</sup> WHO Consolidated ARV guidelines, June 2013

### **2.3 Eligibility Criteria**

All HIV-1 and HIV-1&2 co-infected patients who are registered under the programme and have been on ART for at least 6 months are eligible for viral load testing.

## 3. Viral Load Test Details

### 3.1 Sample Type

The viral load testing will be done on a 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 5 days at 2° - 8°C. The transportation of plasma samples requires a cold chain.

### 3.2 Available Technologies and Platforms

Viral load assays measure the amount of HIV-1 RNA copies in the collected plasma sample. During treatment, the decay of viral load 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 details on these technologies are available in NACO's **National Guidelines for HIV-1 Viral Load Laboratory Testing**.

## 4. ART Treatment Monitoring Algorithm

The purpose of introducing viral load testing in the country is to provide early and accurate indication of treatment failure, and assess need to switch the treatment regimen. Currently programme is using clinical and immunological criteria to identify suspected cases of failures and conducts viral load test for these cases to confirm virological failure (targeted VL testing). With the introduction of routine viral load testing, the programme will be able to identify cases of virological failure much earlier.

The national programme plans to use the viral load testing for both routine and targeted viral load testing -

- Patients will be tested using routine viral load monitoring tests to identify virological failure
- In cases of suspected clinical or immunological failure, targeted viral load monitoring will be used to confirm treatment failure

A viral load assay estimates the number of viral load copies in the HIV infected individuals. This estimation is critical in monitoring patients' response to ART and progression towards AIDS. In context of viral load testing it is important to take into consideration both the absolute count and the change in viral load from previous test. A consistently high viral load may indicate failure on a given treatment regimen.

The national programme is currently using a cut off of less than 1000 copies/mL to determine if the patient is responding to ART since several clinical and epidemiological studies (listed in Annexure 9) reviewed by WHO have shown that the risk of HIV transmission is very low at viral load lower than 1000 copies/mL. Patients with viral load greater than 1000 copies/mL may require adherence counseling and subsequent switch to a second/ third line treatment in case of failure of current treatment regimen.

Viral Load tests should be used, together with CD4 counts, to get a complete picture about how the immune system is fighting the Human Immunodeficiency Virus. As HIV reproduces within the body, the viral load increases and HIV destroys the CD4 cells and thus lowers the amount of cells present. The goal of ART treatment is to keep viral load low and CD4 cell count high.

The laboratory test used for measuring CD4 count is CD4 T-cell enumeration. For detailed reading on CD4 enumeration test refer to *National Guidelines for the Enumeration of CD4, NACO (2015)*.

### 4.1 Routine Viral Load and CD4 Testing Algorithm

**Table 1: Timing for Routine Viral Load Test**

Patient Group	Timing for Routine Viral Load test
<b>First line patients</b>	<ul style="list-style-type: none"> <li>▪ For <b>existing patients*</b>, testing should be done <u>once every 12 months</u> after initiation on ART</li> <li>▪ For <b>new patients</b>, VL test should be conducted at <u>6 months and 12 months</u> from the date of ART initiation in first year, and <u>once every 12 months</u> thereafter</li> </ul>
<b>Second/third line patients</b>	<ul style="list-style-type: none"> <li>▪ For <b>existing patients*</b>, testing should be done <u>once every 6 months</u> after initiation on ART</li> </ul>

	<ul style="list-style-type: none"> <li>For <b>new patients</b>, VL testing should <u>start at 6 months after initiation of ART</u> and conducted <u>every 6 months</u> thereafter</li> </ul>
--	--

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

**Table 2: Timing for CD4 test**

Patient Group	Timing for CD4 test
<b>Existing patients</b>	<ul style="list-style-type: none"> <li>CD4 to be conducted <u>every 6 months</u></li> </ul>
<b>New patients</b>	<ul style="list-style-type: none"> <li>CD4 testing at <u>Baseline</u> during ART initiation</li> <li>CD4 to be conducted <u>every 6 months</u> thereafter</li> </ul>

**Table 3.1: Timing chart of Routine Viral Load and CD4 Tests: First line patients**

Type of Test	Time since initiation of ART (in months)							
	0	6	12	18	24	30	36	...
<b>Routine Viral Load Test</b>	X	√	√	X	√	X	√	...
<b>CD4 Test*</b>	√	√	√	√	√	√	√	...

**Table 3.2: Timing chart of Routine Viral Load and CD4 Tests: Second/ third line patients**

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

*\*CD4 monitoring can be stopped for any patient (except in children aged < 5 years) if CD4 count is greater than 350 cells/mm<sup>3</sup> and viral load is less than 1000 copies/mL (when both tests are conducted at the same time).*

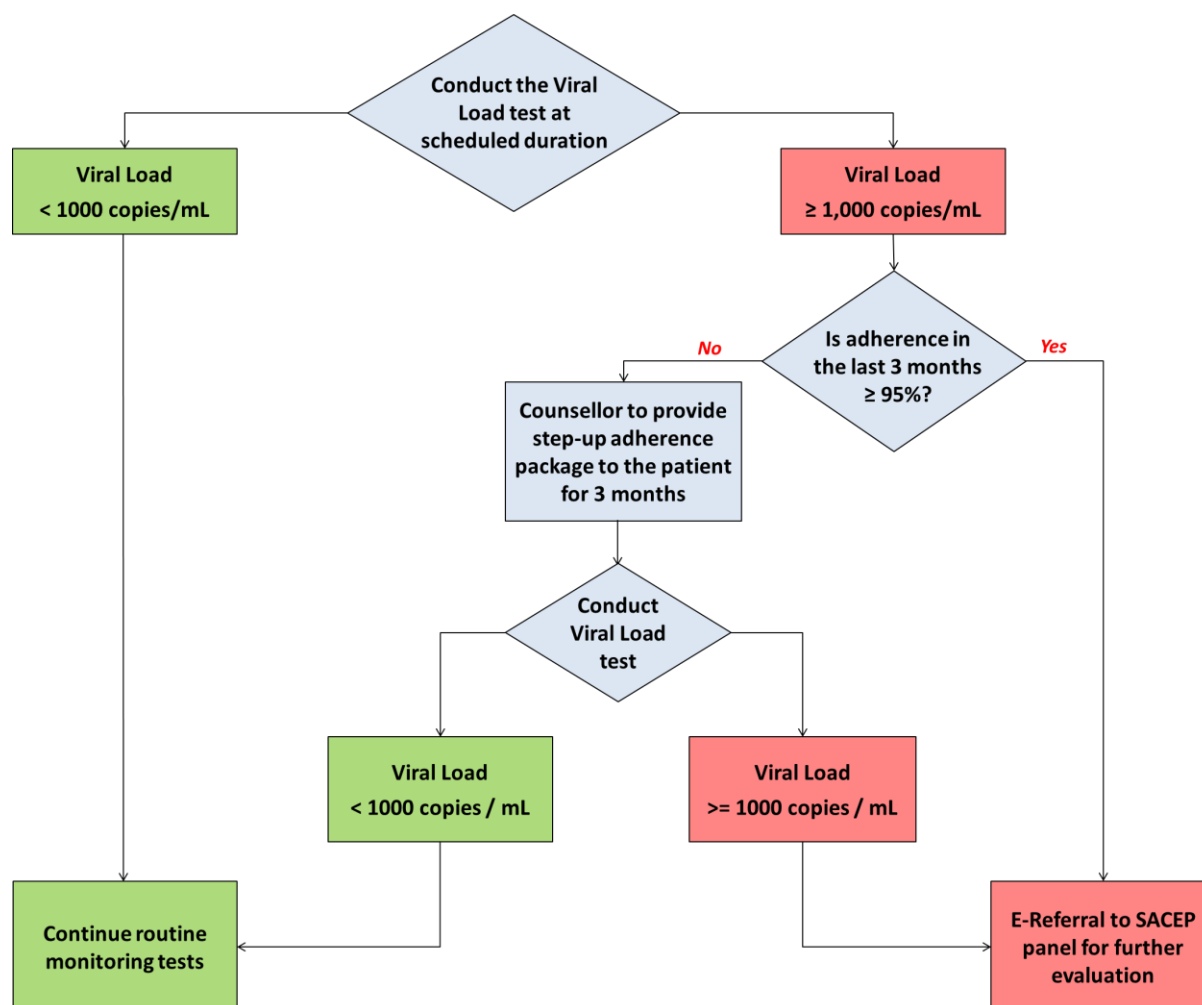
Please note the following -

- 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 viral load testing should be synced so that the same sample collected from a patient can be used for both CD4 and viral load 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 viral load test
- CD4 monitoring should be re-started for any patient if (a) the patient has suspected treatment failure i.e. virological failure ( $\geq 1000$  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

#### 4.1.1 Routine Viral Load Testing Algorithm

Figure 2: Flowchart for Routine Viral Load Algorithm



Please note the following -

- Adherence for a month is defined as (Total pills dispensed during last dispensation – total pills returned by the patient) divided by the number of days since last dispensation
- Adherence should be  $\geq 95\%$  for **each of the last 3 months** to be referred to SACEP directly in case VL  $\geq 1000$  copies/mL
- E-Referral to SACEP means that the ART centre should refer the patient to SACEP through an email with the following information:
  - *Viral Load test details*
  - *Latest CD4 test details*
  - *Clinical records*
  - *Treatment adherence details*



- SACEP should refer to latest National ART Guidelines to decide the regimen to which the patient should be switched in case the patient is failing a regimen, and share the result over email and phone within 8 days of receiving all necessary details from ART centre
- ART centre should receive the decision by SACEP within 8 days of e-referral, and should follow up actively in case the decision is not received

#### 4.1.2 Patient Management Based on Monitoring Test Results

##### Routine Viral load and CD4 test conducted at the SAME TIME:

Virological Failure*	Immunological Failure**	Opportunistic Infection	Action
Yes	Yes	Yes	<ul style="list-style-type: none"> <li>▪ Monitor the patient closely</li> <li>▪ Initiate treatment for OI and conduct viral load test after OI is under control</li> <li>▪ Follow routine VL algorithm after VL test conducted post OI control</li> </ul>
Yes	Yes	No	<ul style="list-style-type: none"> <li>▪ Follow routine VL algorithm</li> </ul>
Yes	No	Yes	<ul style="list-style-type: none"> <li>▪ Monitor the patient closely</li> <li>▪ Initiate treatment for OI and conduct viral load test after OI is under control</li> <li>▪ Follow routine VL algorithm after VL test conducted post OI control</li> </ul>
Yes	No	No	<ul style="list-style-type: none"> <li>▪ Follow routine VL algorithm</li> </ul>
No	Yes	Yes	<ul style="list-style-type: none"> <li>▪ Rule out HIV-2 and Hepatitis</li> <li>▪ Initiate treatment for OI and conduct CD4 test after OI is under control</li> <li>▪ Continue viral load as per scheduled frequency</li> </ul>
No	Yes	No	<ul style="list-style-type: none"> <li>▪ Rule out HIV-2 and Hepatitis</li> <li>▪ Monitor the patient and conduct another CD4 test after 3 months</li> <li>▪ Based on CD4 result after 3 months, clinician can decide on the next viral load test as per his/her discretion</li> </ul>
No	No	Yes	<ul style="list-style-type: none"> <li>▪ Initiate treatment for OI and continue monitoring tests as per scheduled frequency</li> </ul>
No	No	No	<ul style="list-style-type: none"> <li>▪ <b>Continue monitoring tests as per scheduled frequency</b></li> </ul>

\*Defined as viral load  $\geq 1000$  copies/mL

\*\*Defined as (a) Fall of CD4 count to pre- therapy baseline (or below) OR (b) 50% fall from the on-treatment peak value OR (c) persistent CD4 levels below 100 cells/mm<sup>3</sup>

**Viral load test conducted after CD4 monitoring has been stopped:**

- Follow Routine Viral Load Algorithm. *Clinician can recommend additional CD4 test at any point to monitor patient's health as per his/her discretion*

**CD4 test conducted between two consecutive Routine VL tests (where CD4 monitoring has not been stopped):**

- In absence of immunological failure, continue monitoring tests as per scheduled frequency
- In case of immunological failure, check for Opportunistic Infections (OIs):
  - If OI is **present**, initiate treatment for OI and conduct viral load test after OI is under control. Follow routine VL algorithm after VL test conducted post OI control
  - If OI is **not present**, conduct viral load test and follow routine VL algorithm after VL test

Please note that clinician can recommend additional Viral Load test at any point to monitor patient's health as per his/her discretion.

**4.2 Factors to consider while interpreting VL testing results**

The HIV-1 VL 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).

RNA assays used to measure VL are perhaps most heavily relied upon in the medical management of people diagnosed with AIDS and in people who test positive on the HIV-1 antibody tests. As many important clinical decisions are based on these tests, the highest standards of sensitivity and specificity are recommended. There are however some concerns with the viral load tests as given below:

- Intra-assay and biologic variability may affect the findings
- The viral load test results can be unreliable if the body is fighting an infection, or if the patient has just received an immunization with live vaccines. Blood should not be taken for a viral load test within four weeks of any diagnosed infection or immunization with live vaccines. Temporary increases in viral load have been seen in these instances.

## 5. Adherence Counselling

The most common cause of failure of ART is poor adherence to treatment. Adherence should be assessed and routinely reinforced by everyone in the ART team (physicians, counsellors, nurses, pharmacists, peer educators, NGO workers, etc.) at each of the patient's visits to the ART centre.

***Please refer to Section A 15: Adherence to ART in NACO's ART Guidelines for HIV Infected Adults and Adolescents 2013 for more details on adherence counselling***

### **5.1 Step-Up Adherence in Patients with Non-Adherence or Suspected Treatment Failure**

Step-up adherence counselling is important in understanding possible reasons of non-adherence in a patient and then providing guidance to form an adherence plan.

The counsellors should try to review factors like psychological, behavioural, emotional, and socio-economic that may lead to non-adherence in a patient and provide customized counselling with the objective of improving adherence to treatment.

A minimum of three sessions are recommended for step-up adherence counselling but additional sessions can be conducted as needed (See Table 4 for details of each session). If adherence of patient is found to be adequate, a repeat viral load test is conducted three months after suspected treatment failure point to assess the benefits of step up adherence counselling.

It is preferred that all counselling sessions are taken by the same counsellor to ensure consistency, continuity and proper documentation of issue resolution. These sessions can be conducted when the patient visits the ART centre to collect his/her medication.

<b>Table 4- Step-Up Adherence Counselling Sessions: Overview</b>	
<p><b>Session 1</b> (Just after receiving the test results indicating suspected treatment failure)</p>	<ul style="list-style-type: none"> <li>▪ Review patient's understanding of viral load and discuss possible reasons for high viral load</li> <li>▪ Assess possible barriers to adherence:               <ul style="list-style-type: none"> <li>○ Knowledge of medication – dosage and timing</li> <li>○ Motivation to take medicines</li> <li>○ Patient response to side effects (if any)</li> <li>○ Mental health (check for depression and other reasons)</li> <li>○ Discuss patient's support systems</li> </ul> </li> <li>▪ Check patient's history with referral services such as support groups, medical clinics, etc. and evaluate their response to such services</li> <li>▪ Support patient in developing an adherence plan which addresses the identified issues</li> <li>▪ Check the patient treatment adherence (both pill adherence and appointment adherence) on IMS to create a baseline</li> </ul>
<p><b>Session 2</b> (15 days or 1 month – as deemed fit by the counsellor at ART centre after session 1)</p>	<ul style="list-style-type: none"> <li>▪ Review patient adherence between first and second session, and discuss any emerging issues or gaps</li> <li>▪ Follow up on any referral services which the patient undertook post the first session</li> <li>▪ Support patient in modifying the adherence plan to tackle the identified issues</li> <li>▪ Check the patient treatment adherence again, and record it in the</li> </ul>

	white card on regular basis
<b>Session 3</b> (15 days or 1 month as deemed fit by the counsellor at ART centre after session 2)	<ul style="list-style-type: none"> <li>▪ Review patient adherence between second and third session and discuss any emerging issues or gaps</li> <li>▪ Support patient in modifying the adherence plan to tackle the identified issues</li> <li>▪ Decide next course of action based on adherence: <ul style="list-style-type: none"> <li>○ If adherence is good, plan a repeat VL test post three months of good adherence.</li> <li>○ If adherence is not adequate, plan further sessions with the patient before repeating viral load test. Explain the importance of adhering to the treatment and risk of treatment failure due to non-adherence</li> </ul> </li> </ul>
Session after repeat viral load test	<ul style="list-style-type: none"> <li>▪ Discuss results of the repeat viral load test</li> <li>▪ Decide next steps based on repeat viral load test results: <ul style="list-style-type: none"> <li>○ If VL &lt; 1000 copies/mL, appreciate the patient for his/her success and suggest continuation of current regimen; repeat VL as per the scheduled frequency of the patient</li> <li>○ If VL ≥ 1000 copies/mL, prepare the patient for a change in regimen</li> </ul> </li> </ul>

### Adherence Support Systems

Support systems play a pivotal role in helping the patient being adherent. They need to be adaptable to patient needs. Patients like children, adolescents, patients with mental disorder and substance users need special attention. Following are some innovative adherence support systems than can be used to improve the patient's adherence:

- **Case Manager**

Patients needing step-up adherence counselling can be assigned a case manager. The case manager can be senior counsellor of ART centre. The case manager can take the sessions listed in table 4 above and also coordinate with other support systems to ensure best practices by the patient.

- **Treatment Support/Daily Witnessed Ingestion**

For patients on step-up adherence counselling, patient can also have treatment support (somebody watching in person when patient is taking its medicine every day) to ensure good adherence during the three month period of enhanced adherence counselling. Treatment support can be provided by caregivers, guardians or family members.

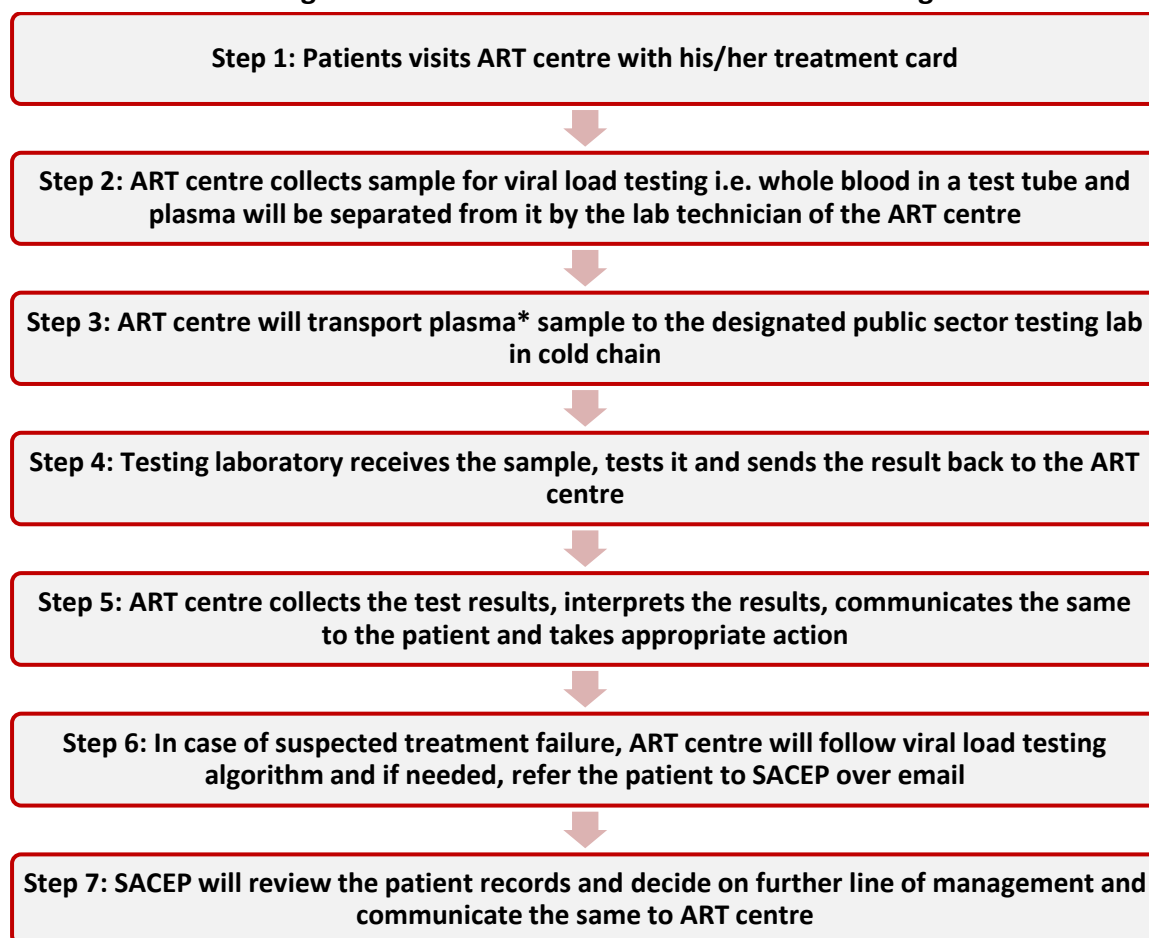
- **Support Groups**

Support groups through care and support centres (CSCs) can be made so that patients can put it collective efforts to overcome adherence challenges. Community support groups with linkage to facility can also be formed to support patients with adherence challenges.

## 6. Roles and Responsibilities for Viral Load Testing

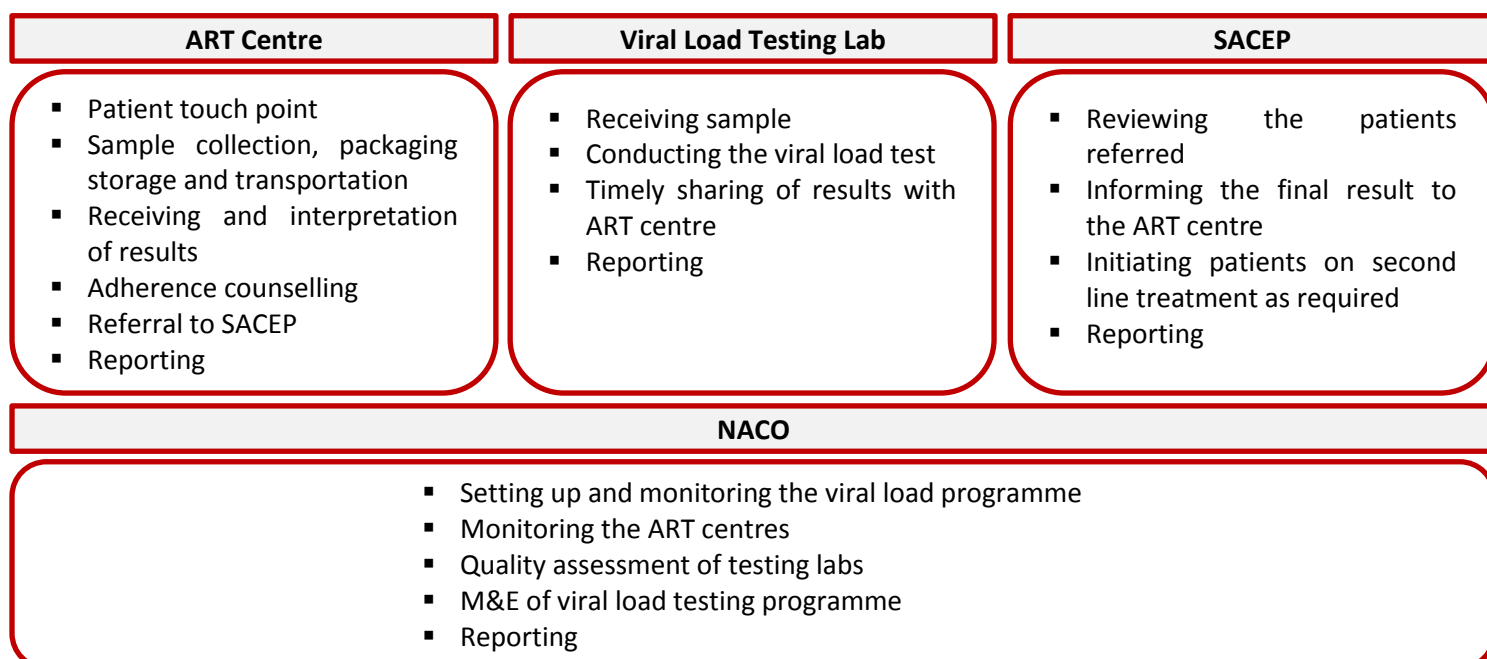
This section highlights key responsibilities of each key stakeholder involved in viral load testing

**Figure 3: Flowchart for Routine Viral Load Testing**



*\*in case plasma separation facility is not available at ART centre then whole blood may be sent for viral load testing. Please refer to annexure 3 for sample transportation and storage of whole blood*

**Figure 4: Summary of Roles and Responsibility for Viral Load Testing**



## **6.1 ART Centre**

### **6.1.1 Sample collection, packaging, storage and transportation**

- ART centre employees should be aware of the dates on which a patient is scheduled for his/her routine or targeted viral load test. As soon as the patient arrives for the collection of his/her medication, the ART centre counsellor should remind the individual to get the viral load test done
- Patients at Link ART centres (LACs) should visit their nodal ART centre for viral load testing
- ART centre counsellor should explain the importance of viral load testing to the patient if he/she is unaware about the same
- Senior Medical Officer / Medical Officer will decide on eligibility of viral load test and should fill viral load test requisition cum result form (TRRF) in triplicate as present in annexure 4.
- The lab technician is supposed to keep the ART centre copy of TRRF with him/her and send the remaining two copies to the viral load testing laboratory
- The programme recommends that the patient should only go to his/her ART centre for viral load testing. However, in a case where the ART centre does not have sample collection facility, the ART centre can refer the patient to another ART centre where the patient will travel with his/her green card and the referred ART centre will follow the steps below
- Lab technician should assign a Unique Viral Load Test ID to this sample after referring to annexure 1
- ART centre lab technician should ensure that all the necessary material required for sample collection is present and that the TRRF is filled before the sample collection process is started
- The patient should be asked to come back to collect his/her reports on his/her next scheduled monthly visit to ART centre
- Lab technician should draw sample from the patient after referring to annexure 2 for sample collection and sample handling
  - The patient's name and unique viral load test ID should be pasted on the test tube used for collection of WB sample
- Lab technician should check the name and other details of the patient on the TRRF and sample tube before proceeding to next steps
- The collected sample should be stored and packaged as per instructions in annexure 3 for sample transportation and storage
  - The WB sample would have to be processed and plasma should be extracted by the ART centre. Refer to annexure 2 for sample collection and sample handling
- The sample should be transported as per instructions provided in annexure 3 for sample transportation and storage
  - The extracted plasma should be transported to the designated viral load testing laboratory within the given temperature and time limitations as defined by the programme
    - Whole blood should be processed for plasma separation within 4-6 hours of blood-draw when kept/transported at 2-25°C. Plasma should

be transported at 2-8°C and should reach the testing laboratory within 24 hours of sample collection

- ART centre lab technician should enter the sample details in the viral load register (Annexure 5) at ART centres
- ART centre data manager should enter sample details in the IMS as explained in Annexure 7

### 6.1.2 Receiving & interpreting the results

- ART centre will receive patient copy of the viral load TRRF from the viral load laboratory.
- Once the hard copy of test results is received by the medical officer, he/she should enter the 'Date of receiving hard copy of results' in the viral load register.
- The results and remarks from the patient copy of TRRF should be copied onto the ART centre copy of TRRF, white card and viral load register present at the ART centre
- If, by any chance, there is any discrepancy between information on IMS and physical TRRF, or if the results could not be obtained from the laboratory for some reason, the ART centre medical officer should immediately contact the viral load laboratory for verification of information and resolution of any such issue.
  - This issue and resolution undertaken should be noted in the "Remarks" section of the viral load register present at the ART centres
- Interpretation of the results: Refer to viral load monitoring algorithm present in Chapter 4 for more details on the interpretation of the result.
  - In case the viral load is less than 1000 copies/mL, then ART centre medical officer should provide the report and date of next viral load test to the patient whenever he/she visits the ART centre again.
  - In case the viral load is greater than 1000 copies/ mL, then ART centre medical officer should reach out to the patient immediately (either by calling the patient directly or through an outreach worker) and follow the next steps as outlined in the viral load monitoring algorithm.
- ART centre medical officer should enter the current viral load results (Viral Load and date of testing) in the patient's white card also. The viral load needs to be entered in the patient's white card under the "12. Investigations" section. While entering the numerical value of the Viral Load, the viral load test number should also be entered in the white card.
- The 'Date of next viral load test' should be entered under the "13. Patient Follow-up" section of the white card and also in patient's green book.
- ART centre medical officer should enter the 'Date of next viral load test' in viral load register

### 6.1.3 Referral to SACEP

- ART centre MO needs to refer the patients suspected of failure to SACEP (CoE/PCoE/ART plus centre) linked to the ART centre
  - Referral to SACEP should be on an email with all supporting clinical documents and the decision from SACEP should be obtained within 8 days of referral

- The referring ART centre should inform and counsel the patient on the findings of 'suspected treatment failure', support psychosocial needs, counsel to continue ongoing line of ART until otherwise advised by the ART SMO/MO; and with the informed consent of the patient for shared confidentiality initiate the process for referral to the SACEP in COE/PCoE/ART plus centres.
- ART centre should counsel the patient about suspicion of treatment failure and that he/she is being referred for opinion and confirmation.
- The ART centres should send the referral details as per the formats in the latest Operational Guidelines for ART centres with the filled details together with a confirmed contact phone of the patient; and photocopies of the case sheets/patient treatment record to the COE/PCoE/ART plus centre by e-mail.

#### **6.1.4 Collection days and timings**

Ideally, all ART centres are required to collect samples from 9:00 A.M. to 1.00 P.M. from Monday to Saturday, except on public holidays. Change in collection days are subject to the approval of the head of institute

### **6.2 Viral Load Testing Laboratory**

#### **6.2.1 Receiving and Testing the Sample**

- The viral load testing lab will receive the sample and viral load TRRF (lab and patient copy)
  - The lab should test the plasma sample preferably within 5 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
- The Lab technician should match the details present on the sample and the TRRF received from the ART centre. In case of any discrepancy, the lab should immediately inform the ART centre by phone or email and resolve it
  - The issue and resolution should be recorded in the viral load register present at the laboratory under the "Remarks" section
- The lab technician 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 should be informed immediately by phone and email.
  - The lab technician is supposed to send the filled TRRF to the ART centre asking for a repeat sample for the patient immediately
- The sample related details should be entered into IMS/emailed within 24 hours of receiving the sample
- The lab technician must move forward with the testing of sample only if (a) all the details of the sample are present and complete on the sample and TRRF and (b) the sample is received in good condition
- The lab technician is supposed to perform viral load PCR test on the sample as per the protocol mentioned in the kit insert of the selected viral load testing platform
  - The Turnaround Time for reporting of results to the ARTC is 14 days from the time of sample collection. Any delay in testing should be reported to the ART Centre and NACO with a written notice



- In case the result of a given sample is “Inconclusive” due to any technical reasons, the viral load testing laboratory should inform relevant ART centre immediately by phone and email, and ask for a repeat sample
  - The lab technician is supposed to send the filled TRRF to the ART centre asking for a repeat sample for the patient immediately
  - The lab In charge should provide information on number of sample whose result was inconclusive to NACO every month on the email ID – [labservices2.naco@gmail.com](mailto:labservices2.naco@gmail.com)

### **6.2.2 Sharing the Viral Load Test Results**

- The signed hard copy of the results should reach the ART centre within 72 hours of testing of the sample. The report must be signed by an authorized signatory as per NABL requirements
- The lab technician and lab in charge should ensure that details entered in IMS and those present on the TRRF is exactly same. In case of any discrepancy, the lab technician must resolve the issue and inform the ART centre immediately via phone and email.
  - If any results on IMS need modification, the lab should write an email to ART Centre, Lab Services Division (NACO), Care, Support and Treatment Division (NACO), and SACS (of the state to which the ART centre belongs) requesting for a modification of VL test result on IMS
- In case of PPP scale up, a well-defined communication mechanism should be devised by the testing lab (including methods like telephone, online, call centre etc.) to ensure proper and prompt communication between NACO staff, ART Centres and viral load testing laboratory
- The viral load laboratory should store the samples tested for an year at the temperature specified in the kit insert
- The testing lab should have a backup plan in case of breakdown

### **6.3 State AIDS Clinical Expert Panel (SACEP)**

Please refer to the latest Operational Guidelines for ART centres for the roles and responsibilities of State AIDS Clinical Expert Panels (SACEP)

### **6.4 Reporting Requirements**

This section summarizes the responsibilities of each ART centre, viral load testing laboratory and SACEP for reporting and capturing key data elements:

#### **6.4.1 ART Centre**

- Completed TRRF and viral load register for all patients whose samples have been collected
- Completed entry in IMS for all those whose samples have been collected
- Stickers with name of the patient and Unique Viral load test ID should be present on all plasma samples
- The ART centre TRRF copy should have the final viral load of the patient
- The patient copy should be provided to the patient on his/her next visit
- The viral load should be present on the white and green card of the patient

- Referral for all the patients who have been referred
- Reply for all those who have been evaluated by SACEP

#### **6.4.2 Viral Load Testing Laboratory**

- Completed Viral load TRRF for all the samples received at the laboratory
- Completed details in IMS for all the sample received at the laboratory
- Completed details in viral load testing register at laboratory

#### **6.4.3 SACEP**

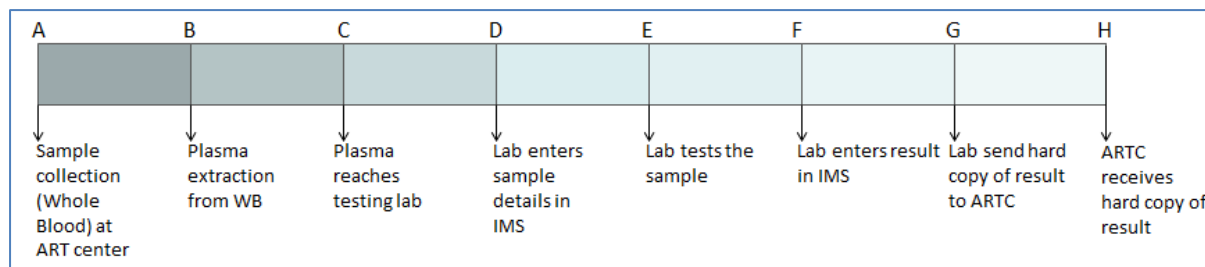
- SACEP Registers and line lists
- Meeting formats
- Reply for all the case who have been evaluated by SACEP
- Referral for all the referred cases

*\*All formats as per the latest Operational Guidelines for ART centres*

## 7. Turnaround Time (TAT) 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 5: Operational steps in Viral Load testing**



### TAT between steps:

- **A to B:** Whole blood should be processed for plasma separation within **6 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 **5 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

# ANNEXURES

### ANNEXURE 1: UNIQUE VIRAL LOAD TEST ID

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

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

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

For instance –

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

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

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

In the TRRF, the unique viral load ID is followed by the reason (coded) of viral load testing in parenthesis. The codes for reasons of viral load testing are:

- 'G' for Routine Testing
- 'T' for Targeted Testing
- 'R' for Repeat Testing

Thus, in the above example, if the sample of the second viral load test of the patient was rejected by the lab and thus a repeat sample was sent, then the unique viral load test to be entered in TRRF will be **ART-MH-PNA-01 – 00876 – 02 (R)**

## ANNEXURE 2: SAMPLE COLLECTION AND SAMPLE HANDLING

### **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. Sample can remain stable over long period when stored at appropriate temperature.

Sample integrity is the cornerstone of a quality viral load test result. To protect sample integrity, they must be properly collected in the correct type of tube, stored at the correct temperature, properly processed and within the proper timeframe, transported in the appropriate temperature and packaging to the testing laboratory. Coordination between the ART centre and the HIV-1 viral load testing laboratory is important to ensure timely pickup, proper delivery of samples, processing of sample and timely delivery of quality reports.

### **Sample collection by venepuncture**

All registered individuals on ART who are scheduled for viral load testing should be referred to the technician at the ART centre for sample collection with filled in triplicate carbon copies of the test requisition and result form (TRRF) by the Medical Officer. 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 viral load 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 patient. The blood collection tube should be labeled with any of two identifiers like patient name, ID number and the date & time of collection using cryo labels. Standard precautions should be strictly followed. For any molecular tests like HIV-1 viral load, blood sample should be collected wearing powder free gloves. Six mL of whole blood sample should be collected in a K2 EDTA vacutainer by the vacuum evacuation method using an eclipse needle (usually 23G). Three mL of 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. Heparin containing tubes must not be used when collecting samples for viral load testing because heparin has been shown to inhibit PCR. For the complete procedure of blood collection, refer to the most recent "National Guidelines for Enumeration of CD4".

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 i.e., 15 – 30°C. The biomedical waste generated while collection should be disposed into appropriate color coded bins.

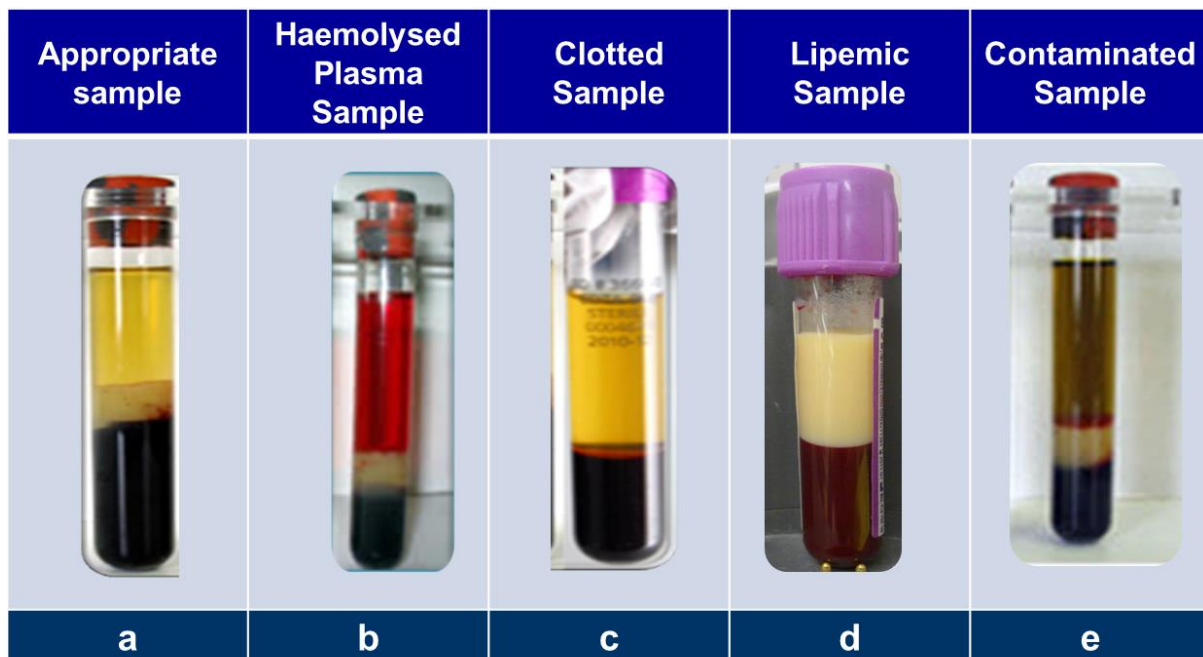
Following sample collection the date and time of sample collection should be entered in the TRRF. This information should be also entered manually and digitally in the register and in the IMS respectively.

**Separation of plasma:**

Plasma should be separated from whole blood within 6 hours of sample collection. The whole blood can never 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 6a). Haemolysed plasma samples appear bright pink to red in color after centrifugation (as shown in figure 6b). 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 6c). Lipemic plasma occurs when there is an elevated amount of fat in the blood and the plasma is white and thick (as shown in figure 6d). Samples that are visibly contaminated can have extra layers of unidentifiable cells or debris (as shown in figure 6e). Contaminated samples could be caused due to the improper EDTA collection tube and collecting devices. However, if the separation is going to happen within 6 hours, chance of contamination is very 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.

**Figure 6: Different characteristics of plasma following centrifugation**



### ANNEXURE 3: SAMPLE TRANSPORTATION AND STORAGE

#### Storage

Plasma can be stored at 2-8°C for maximum up to 5 days and can be transported with frozen ice packs. For long term storage, as it contains RNA, the ideal temperature is -70°C to -80°C. Once plasma is frozen, it must always be transported frozen to avoid freeze thaw cycles.

**Table 5: 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

#### 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 triplicate and ensure delivery to the testing lab at 2 to 8°C within 24 hours of sample collection.

#### Triple Packaging System

All triple packaging includes 3 layers.

##### a) Primary Receptacles

- Tube containing sample for viral load testing
- The tube must be watertight and leak proof
- Must be appropriately labelled
- Wrapped in enough absorbent material to absorb all liquid in case of breakage or leakage

##### 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

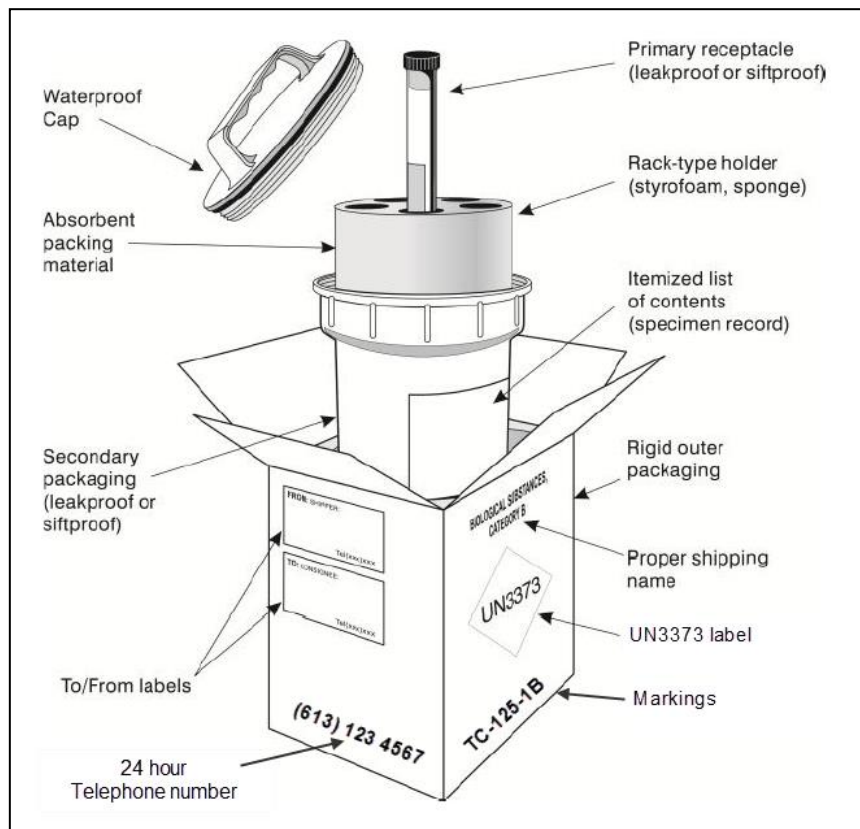


layer of packaging has a rack or similar item to keep samples from moving around too much.

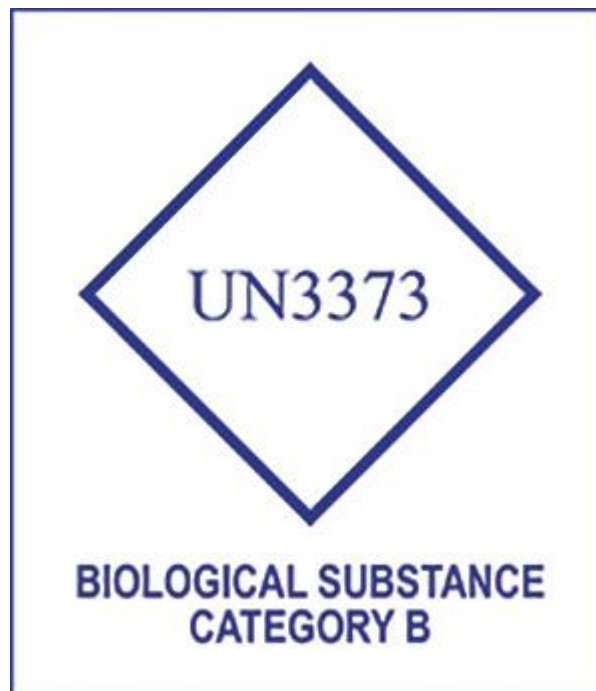
**c) Outer Packaging**

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







**Figure 7: Diagrammatic representation of a triple package system for the transportation of infectious substances category B.**



**Figure 8: The marking used for the transportation of infectious substances category B**



**Figure 9: Job-aid for sample packaging**

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

**Reception of Plasma samples at viral load testing lab:**

- The viral load testing laboratory will receive the sample and two copies of the viral load TRRF
- Each sample should be divided into 2 aliquots which should be properly labelled. One aliquot should be stored between -70°C or lower and the other aliquot at 2-8°C if it can be tested within 5 days of collection or between -70°C or lower if delay of more than 5 days is anticipated
- As soon as the samples are received by the viral load testing Lab, they are verified by the LT for proper transport, 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 viral load 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<sup>0</sup> 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<sup>0</sup> C
- Samples from HIV-2 infected individuals

## ANNEXURE 4: VIRAL LOAD TRRF FORMAT

LABORATORY TEST REQUISITION CUM RESULT FORM (TRRF) FOR HIV-1 VIRAL LOAD TESTING	
To be filled by ART Centre	
<b>Patient Details</b>	
Unique Viral Load Test ID:	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> ( <input type="text"/> )
	ART centre code / Patient's ART number / Viral load test number
Name: .....	Age: ..... Gender: .....
HIV status: <input type="checkbox"/> HIV-1 <input type="checkbox"/> HIV-2** <input type="checkbox"/> HIV -1&2	
<b>Viral Load Testing Details</b>	
If Repeat Testing, reason: <input type="checkbox"/> Previous Sample Rejected <input type="checkbox"/> Inconclusive Result <input type="checkbox"/> Other.....	
Date of previous viral load test: __/__/----	Result of previous viral load test: .....
Date of sample collection: __/__/----	Time of sample collection: .....
Date of sample dispatch: __/__/----	Date of receiving results: __/__/----
Date of sharing results with the patient: __/__/----	Final advice of medical officer: .....
Authorizing clinician name and signature: .....	
To be filled by Viral Load Laboratory	
Name of the laboratory: .....	
Date of sample receipt: __/__/----	Lab Number: .....
Time of sample receipt: .....	Sample received in proper condition: <input type="checkbox"/> Yes <input type="checkbox"/> No
Date on which sample is tested: __/__/----	Viral load by real time PCR***: ..... (copies/mL)
If no result is given, please specify reason .....	
Date of result dispatch: __/__/----	Platform used: Abbott/Roche/Other(Name).....
Name & signature of lab technician: .....	
Name & signature of laboratory in-charge: .....	
* Code for reason of viral load testing should be entered in parenthesis after the viral load test number: 'G' for Routine Testing, 'T' for Targeted Testing and 'R' for Repeat Testing	
** HIV-2 samples should not be sent for viral load testing	
*** A sample with a result of "Target not detected (TND)" cannot be presumed to be negative for HIV-1 RNA	

## ANNEXURE 5: VIRAL LOAD REGISTER AT ART CENTRES FORMAT

## (A) VIRAL LOAD REGISTER

Name of ART Centre:

Date: \_\_/\_\_/----

ART Centre Code:

Name of ART MO:

S. No.	Name of Patient	Unique Viral Load Test ID (17 digit)	Date of VL sample dispatch (dd/mm/yy)	Date of receiving VL results (dd/mm/yy)	Viral load ('RJ' if the sample was rejected, 'IR' if inconclusive result was obtained)	Date of next viral load test (dd/mm/yy) (to be entered by SMO/MO)	Remarks

## (B) MONTHLY CONSOLIDATED SUMMARY

Name of ART Centre:

Year:

ART Centre Code:

Month:

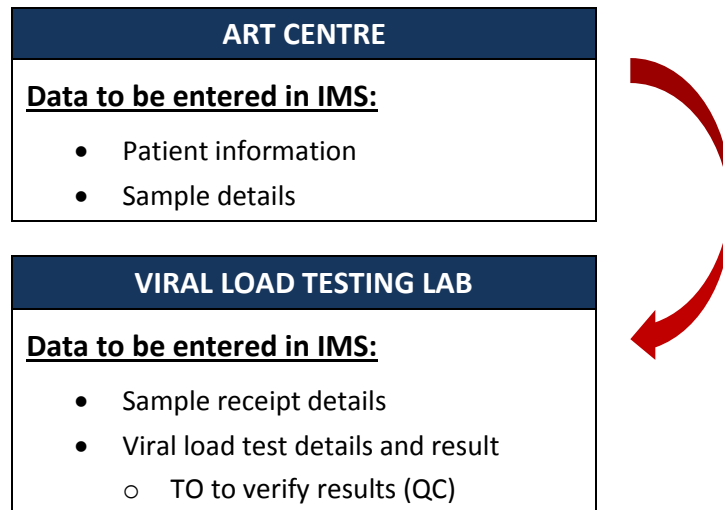
Name of ART MO:

Consolidated Summary of ART Centre		
	Viral load (copies/mL)	
	<1000	≥ 1000
Total		
Name and signature of ART centre staff:		



**ANNEXURE 7: ENTERING RESULTS IN IMS SOFTWARE**

The ART centre is responsible for entering patient and viral load sample details in IMS as per the viral load module in IMS. The testing lab will be responsible for entering sample receipt and testing details along with the final result. The following diagram shows the information flow once the viral load module is rolled-out on IMS:



## ANNEXURE 8: LIST OF EQUIPMENT REQUIRED AT ART CENTRE FOR VIRAL LOAD SAMPLE COLLECTION

Table 6: List of Equipment

PARTICULAR	PURPOSE
Tourniquet	To control venous and arterial circulation for blood withdrawal
Needle holder	To collect sample
Needle with safety mechanism	To collect sample
Needle destroyer	To destroy needles used for sample collection
Personal protective equipment (PPE)	For safety of HCW
Dry cotton swab	To stop blood flow
Spirit/ 70% alcohol	To clean the vein before blood withdrawal
K2 EDTA 6mL blood collection tube	To stop coagulation of blood
Centrifuge	To separate plasma
5 mL storage vials along with a box	To store plasma
Bar code labels/Stickers (as mandated by NACO)	To label the vials
Thermometers/Temperature sensitive strips (as mandated by NACO)	To monitor temperature
Sample transportation box	To store the cryo-vials during transportation
Cool packs	To store sample at ambient temperature
Disposable, sterile Pasteur Pipettes	To separate plasma
Biosafety bags for waste disposal	To dispose bio-waste
VL register	To document VL test
VL TRRF	To document VL test



## ANNEXURE 9: REFERENCES

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