National Guidelines on Second-line and Alternative First-line ART For Adults and Adolescents May 2013

NACO

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

Table of Contents

Chapter

Annexure

Acronyms and Abbreviations

3TC	Lamivudine
ABC	Abacavir
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral
ATV	Atazanavir
AZT/ZDV	Zidovudine
bPI	Boosted PI
CD4 count	CD4+ T-Lymphocyte
COE	Centers of Excellence
d4T	Stavudine
ddI	Didanosine
EC	Enteric Coated
EFV	Efavirenz
FDC	Fixed-Dose Combination
FTC	Emtricitabine
Hb	Hemoglobin
HIV	Human Immunodeficiency Virus
IDV	Indinavir
LPV	Lopinavir
NACEP	National AIDS Clinical Expert Panel
NFV	Nelfinavir
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NVP	Nevirapine
PI	Protease Inhibitor
PLHIV	People Living with HIV
/r	Low-Dose Ritonavir
RTV	Ritonavir
SACEP	State AIDS Clinical Expert Panel
SQV	Saquinavir
TDF	Tenofovir Disoproxil Fumarate
VL	Viral Load

Introduction and Preamble

The National ART programme launched on 1st April 2004 in eight government hospitals in six high prevalence states has since been scaled up to 400 ART centres where in a total of around 16,00,000 patients have been registered in HIV care and nearly 6,00,000 patients are currently on ART. The national programme provides free First line, alternate First line and Second line antiretroviral drugs to adults and children as per their eligibility.

A Technical Resource Group (TRG) on ART has been constituted at NACO that meets regularly to update and formulate the technical guidelines for First line and Second line ART. All ART centers in the country have access to Second line ART but for initiation of Second line ART around 41 centers have been identified. These include 10 Centres of Excellence, 7 Pediatric Centres of Excellence and 20 ART Plus centers. This is to ensure that only those who have confirmed failure to First line ART are initiated on Second line treatment thereby minimizing unnecessary switches. Besides this, a special training of health care providers at all ART centers is done on monitoring patients initiated on antiretroviral therapy along with regulatory mechanisms to minimize the chances of resistance due to irrational prescriptions. In order to ensure high quality of care, to ensure that only eligible patients are started on Second line ART and maintain uniformity in treatment, State Clinical Expert Panels (SACEP) have been constituted at all CoEs and ART plus centers where Second line ART is initiated. Every patient that is referred to these centers with a suspected failure to First line ART is First reviewed by SACEP and, if the suspected treatment failure is confirmed by Viral load testing, then eligible patients are initiated on Second line ART after adequate preparedness. After the patients become stable on Second line ART, they are transferred back to their referring ART centre. They continue to get their Second line drugs at their own ART center thereafter. Also complicated cases that need further review and opinion are referred to National AIDS Clinical Expert Panel (NACEP) at NACO.

The initial roll out of Second line ART was done at 2 Centers of Excellence: Government Hospital of Thoracic Medicine (GHTM), Tambaram and Sir JJ Hospital, Mumbai. During the pilot phase, only those patients who were on ART at these centers for at least 6 months were considered for Second line ART. The experience gained at these two centers during the pilot phase was used for further modification and refinement of protocols. Currently, Second line ART is initiated at 10 Centers of Excellence, 7 Pediatric Centers of Excellence and 20 ART plus centers. All ART centers in the country are linked to these CoE/ART plus centers and a well-defined referral procedure is in place.

Section I: TECHNICAL GUIDELINES

1.1 First LINE & Second LINE ART REGIMEN:

The working definition of First and Second Line ART Regimen is as follows:

First-line ART:

First-line ART is the initial regimen prescribed for an ART naïve patient when the patient fulfills national clinical and laboratory criteria to be initiated on ART.

(Current NACO treatment guidelines for First-line ART recommend three drug combination therapy from two classes of ARV drugs for initial treatment i.e. 2 NRTI + 1 NNRTI)

Second-line ART:

Second-line ART is the subsequent regimen used in sequence immediately after First-line therapy has **failed**.

(Current NACO treatment guidelines recommend use of Ritonavir-boosted protease inhibitors (bPIs) supported by two agents from the NRTI class, of which at least one should be new.

1.2 SUBSTITUTION VS. SWITCH

Change of ARVs prescribed should be carefully distinguished between substituting a drug within a given regimen and switching an entire ART regimen:

- **Treatment Failure** refers to the loss of antiviral efficacy to current regimen and it triggers the **SWITCH** of the entire regimen from First to Second line. It is identified by clinical and/or immunological and confirmed by the virological criteria.
- Single drug replacement of individual ARV (usually within the same class) for **toxicity**, drug-drug interactions, or intolerance refers to **SUBSTITUTION** of individual drug and which does not indicate a Second line regimen is being used.

The following ART regimens have been designated as "**National ART regimen**" by National AIDS Control Organization. (Table 1)

Table 1: **NACO Approved ART regimen**

Regimen	ARV Drug Combinations	Indications
Regimen I	Zidovudine + Lamivudine + Nevirapine	First line Regimen for patients with Hb ≥9 gm/dl and not on concomitant ATT
Regimen I (a)	Tenofovir + Lamivudine + Nevirapine	First line Regimen for patients with Hb<9 gm/dl and not on concomitant ATT
Regimen II	Zidovudine + Lamivudine + Efavirenz	First line Regimen for patients with Hb ≥9 gm/dl and on concomitant ATT
Regimen II (a)	Tenofovir + Lamivudine + Efavirenz	First line Regimen for patients with Hb<9 gm/dl and on concomitant ATT First line for all patients with Hepatitis B and /or Hepatitis C co-infection First line Regimen for pregnant women, with no exposure to sd-NVP in the past
Regimen III	Zidovudine + Lamivudine + Atazanavir/Ritonavir	Regimen for patients on AZT Containing First line regimen, who develop toxicity to both NVP and EFV Also Second line regimen for those who are on TDF containing First line regimen if Hb ≥ 9 gm/dl
Regimen III (a)	Zidovudine + Lamivudine + Lopinavir/Ritonavir	For patients of Regimen III who develop severe Atazanavir toxicity First line regimen for patients with HIV-2 infection with Hb ≥ 9 gm/dl
Regimen IV	Tenofovir + Lamivudine+ Atazanavir/Ritonavir	Second line regimen for those who are on AZT/d4T containing regimen in the First line Also for patients on TDF containing First line regimen who develop toxicity to both NVP and EFV
Regimen IV (a)	Tenofovir + Lamivudine+ Lopinavir/Ritonavir	For patients on Regimen IV who develop severe Atazanavir toxicity First line Regimen for patient with HIV 2 infection with Hb < 9 gm/dl First line Regimen for all women exposed to sd-NVP in the past
Regimen V	Stavudine+ Lamivudine+ Atazanavir/Ritonavir	Second line for those who are on TDF containing regimen in the First line if Hb< 9 gm/dl
Regimen V (a)	Stavudine+ Lamivudine+ Lopinavir/Ritonavir	For patients on Regimen V who develop severe Atazanavir toxicity

- Patients on EFV due to concomitant ATT need to be substituted with NVP two weeks after completion of ATT or in the next clinic visit. No lead in dose for NVP required in such patients.
- If NVP is discontinued for > 2 weeks, while re-starting NVP, one has to use a lead-in dose for 2 weeks.
- Patients on AZT based regimen to be substituted with TDF, if they develop anaemia.
- For patients with TDF toxicity, use AZT containing regimen if not anaemic, d4T if anaemic (e-approval from SACEP required for d4T as supply of d4T is limited).
- If AZT, d4T and TDF cannot be used, refer to SACEP at CoE only. For established 3TC toxicity, refer to SACEP at CoE only. SACEP can take a decision to provide alternative drug and inform NACEP or can ask NACEP, if opinion is required.
- Patients who have developed AZT induced anaemia in the past should not be re challenged with AZT again.
- Patients who developed severe NVP hypersensitivity (SJS and TEN) in the past should not be re-challenged with NVP again.

1.3 ALTERNATIVE ARV DRUGS FOR INTOLERANCE TO ZDV/TDF AND NVP/EFV: SUBSTITUTION

Substitution of ARV drugs for reasons of intolerance or toxicity or drug-drug interactions may be needed in following cases:

Intolerance to both ZDV and TDF: in this case, d4T based treatment may be an alternative (e-approval from SACEP required for d4T as supply of d4T is limited).

Intolerance to both NVP and EFV: in this case, ATV/r as a substitution ARV will be provided upon review and approval by the SACEP.

As per NACO ART Guidelines for Adults and Adolescents, as a general principle, mild toxicities (grade 1 and 2), do not require discontinuation of ART or drug substitution. Symptomatic treatment may be given. Moderate or severe toxicities (grade 3 and 4) may require substitution of the drug with another ARV of the same class. (Table 2)

Table 2: Major Toxicities caused by First-line ARVs Regimen and recommended drug substitutions

Regimen	Toxicity	Drug substitution
ZDV/3TC/ NVP	ZDV related persistent GI intolerance or severe hematological toxicity NVP related severe hepatotoxicity NVP related severe rash (but not life-threatening) NVP-related life threatening rash (Stevens Johnson Syndrome, angio -edema)	Substitute with TDF Substitute with EFV (except in pregnancy. In this situation, switch to LPV/r) Substitute with bPIs
ZDV/3TC/EFV	ZDV related persistent GI intolerance or severe hematological toxicity EFV related persistent CNS toxicity	Substitute with TDF Substitute with NVP, if not on concomitant ATT or there is no contraindication to NVP use. bPI (ATV/r) is recommended in such circumstances
TDF +3TC+NVP/ EFV	Usually well tolerated Minor: weakness and lack of energy, headache, diarrhea, nausea, vomiting and intestinal gas TDF can reduce bone mineral density Renal dysfunction (Check urine for routine/microscopic , blood urea, creatinine every 6 months) Renal dysfunction is usually mild, asymptomatic Reverses when stop medication Acute renal failure (rare): reduce dose when renal failure or if severe toxicity, substitute	Symptomatic management of minor side effects Vitamin D3, Bisphosphonates, and Calcium supplements may be used in patients with osteoporosis as per NACO lab monitoring protocol Substitute with AZT, if Hb≥ 9 gm/dl; otherwise, substitute with d4T following review & e-approval by SACEP
ATV	Indirect hyperbilirubinaemia, clinical jaundice, prolonged PR interval- First degree, symptomatic AV block in some patients, hyperglycemia, fat mal distribution, possible increased bleeding episodes in people with haemophilia, nephrolithiasis	Seek SACEP/NACEP guidance on substitution with LPV/r.

Notes:

- *The general principle is that single drug substitution for toxicity should be made within the same ARV class e.g. substitution of TDF with AZT or d4T (for renal toxicity or anemia) or EFV with NVP (for CNS toxicity or in pregnancy).*
- *d4T should not be a part of HAART for ART naive patients. Patients who are on d4T, substitution should be done with AZT or TDF depending on Hb. Substituting d4T may not reverse lipodystrophy but may slow its progression.*
- *If a life-threatening toxicity occurs, all ART should be stopped until the toxicity has resolved and a revised regimen commenced when the patient has recovered.*

The 'triple NRTI regimen' has been shown to be inferior in its outcome and is not used in the National programme

Table 3: Substituting with alternative First Line ARV drugs

First line ARV causing the toxicity	Alternative substitute	Remarks
(a) **Intolerance to both TDF and AZT** Patient should have been tried on ZDV **and** TDF with **documented intolerance** to both. d4T+3TC will be provided after review and e-approval by the SACEP		
TDF + 3TC	d4T + 3TC	Continue the same NNRTI (either NVP or EFV)
ZDV + 3TC		

(b) **For intolerance to both NVP and EFV**

Patient should have been tried on both NVP **and** EFV (except for if history of Steven Johnson Syndrome, exfoliative dermatitis, erythema multiform or toxic epidermal necrolysis is present) and **documented** as not tolerating, before requiring substitution for the NNRTI component.

NVP or EFV	ATV/r	Continue with the same NRTI backbone i.e. ZDV/3TC or TDF/3TC if no problems

Essentially this moves the patient to the PI-based regimen. Counsel for good adherence. If this regimen fails, there is no other optimal alternative regimen at present in the National programme.

These patients should be referred to the SACEP for review, then COE shall manage and provide ATV/r as substitution for intolerance to NNRTI.

(c) **Intolerance to TDF, d4T and AZT**

Refer such cases to SACEP at CoE for review and ABC based ARV, if required

See Annex I: Severity grading of clinical and laboratory toxicities of ARVs

1.4 MONITORING PATIENTS ON First LINE ART FOR FAILURE

Good adherence is the key to maintaining the First line ART for longer duration.

Good adherence is required for Second line ART to ensure viral suppression and increase survival.

The principles of monitoring patient on First line ART are:

- **Clinical monitoring and staging** at each visit as per NACO guidelines.
    - Do clinical staging at each visit: use the T staging for clinical events. (see Table 4 below)
- **Immunological monitoring:** Ensuring the routine monitoring lab tests are done eg. CD4 count every 6 months (or even earlier depending on CD4 value/clinical judgment).

Adherence support and monitoring to ensure >95% adherence:

- Check for progress of improvement at each visit and weight.
- Screen for TB: ask for symptoms and signs of TB e.g. fever, weight loss, night sweats, haemoptysis, lymph nodes in neck etc.
- Determine if Cotrimoxazole is required or not, based on CD4 counts and WHO Clinical staging.

Development of a new or recurrent WHO Clinical stage 4 condition while on ART for at least 6 months is considered functional evidence of the **progression of HIV disease**. However, certain WHO stage 3 conditions like pulmonary TB and severe bacterial infections may signify treatment failure whereas certain WHO stage 4 conditions like TB lymphadenitis, uncomplicated pleural TB, esophageal candidiasis and recurrent bacterial pneumonias may not indicate treatment failure which needs to be taken into consideration. If the patient doesn't improve following institution of appropriate treatment for these stage 4 conditions, they should be promptly referred to SACEP. (see below)

Table 4: Clinical staging events to guide decision making on switching

New or recurrent event on ART[a]	Recommendations	Additional Management Options
Asymptomatic (T1)	Do Not switch regimen	• Maintain schedule follow-up visits, including CD4 monitoring (if available) • Continue to offer adherence support
Stage 2 event (T2)	Do Not switch regimen[b]	• Treat and dmanage staging event • Access and offer adherence support • Check if on treatment for at least six months • Assess continuation of reintroduction of OI prophylaxis • Schedule earlier visit for clinical review and consider CD-4 (if available)[c]

New or recurrent event on ART[a]	Recommendations	Additional Management Options
Stage 3 event (T3)	Consider switching regimen[bd]	• Treat and manage staging event and monitor response • Assess and offer adherence support • Check if on treatment for at least six months • Check CD4 cell count (if available)[cd] • Assess continuation of reintroduction of OI prophylaxis
Stage 4 event (T4)	Switching regimen[be]	• Treat and manage staging event and monitor response • Check if on treatment for at least six months • Assess continuation of reintroduction of OI prophylaxis • Check CD4 cell count (if available)[c] • Assess and other adherence support

a Refers to clinical stages while on ART for at least six months (termed T1, T2, T3, T4).

b Differentiation of opportunistic infections from immune reconstitution inflammatory syndrome is necessary.

c Treat and manage the staging event before measuring CD4 cell count.

d Certain WHO clinical stage 3 conditions (e.gpulmonary TB, severe bacterial infections) may be indicators of tratment failure and thus require consideration of Second-line therapy; response to appropriate therapy should be used to evaluate the need for switching of therapy.

e Some WHO clinical stage 4 conditions (lymph node TB, uncomplicated TB pleural disease, oesophageal candidiasis, recurrent bacterial pneumonia) may not be indicators of treatment failure and thus do not require consideration of Second-line-therapy, response to appropriate antimicrobial therapy should be used to evaluate the need to switch therapy.

TB can occur at any CD4 level and does not necessarily indicate ART failure. The response to TB therapy should be used to evaluate the need to switch ARV drugs. In the case of pulmonary TB and some types of extrapulmonary TB (e.g simple lymph node TB or uncomplicated pleural disease), the response to TB therapy is often good and the decision to switch ARV drugs can be postponed and monitoring can be stepped up. This also applies if severe and/or recurrent bacterial infections (as stage 3 or 4 events) or oesophageal candidiasis respond well to therapy.

1.5 IDENTIFYING TREATMENT FAILURE

High index of suspicion is required

Look for the following evidence of suspected treatment failure among patients on First line ART (en sure patient has been on First line ART for at least 6 months):

- New OIs/recurrence/clinical events after 6 months on First line ART(after ruling out IRIS).
- Progressive CD4count decline.
- Slow/no clinical improvement over 6-12 months, associated with stationary CD4, despite good adherence.
- Clinical deterioration in spite of good adherence to therapy.

** Even though one is less likely to develop IRIS after 6 months of therapy.*

The NACO definitions of ART failure are listed in table 5 below:

Table 5: Definitions of Clinical, Immunological, and Virological treatment failure for First-line regimen.

Clinical failure (i)	**New or recurrent WHO stage 4 condition, after at least 6 months on ART (ii, iii)**
Immunological failure	Fall of CD4 count to pre- therapy baseline (or below) 50% fall from the on- treatment peak value (if known) Persistent CD4 levels below 100 cells/mm^3 (iii, iV)
Virological failure	Plasma viral load > 5,000 copies/ml^3 (vi)

Notes:

i. Current event must be differentiated from IRIS.

ii. Certain WHO clinical stage 3 conditions (eg pulmonary TB, severe bacterial infections) may indicate treatment failure and thus require Second line therapy to be considered.

iii. Some WHO stage 4 conditions (lymph node TB, uncomplicated TB pleural disease, oesophageal candidiasis, recurrent bacterial pneumonia) may not indicate treatment failure and thus Second line therapy need not be considered.

iv. Some experts consider persistent CD4 counts of below 50 cells/mm^3 after 12 months of ART to be more appropriate.

v. Switch of treatment should be done only when the viral count is more than 5,000 cells/mm^3.

At the ART center, **suspicion of treatment failure** depends on good clinical assessment backed up by use of regular CD4 counts. Before labeling a person as having **'failure'** – ensure that the following has been done:

- Patient had a reasonable trial of First line ART for (at least 6 months)
- Assess adherence and support patient to improve this (reinforce)
- Screen and treat intercurrent OIs, exclude IRIS
- Provide Cotrimoxazole based on CD4 or clinical stage immediately
- If TB is present: assess if this is re infection or IRIS or a new infection. If the response to TB therapy is good, then the decision to switch therapy can be postponed and the patient re-evaluated again.
- CD4 count (most recent) latest by a month after treating the intercurrent illness, if any.

The Second line ART will be initiated only at CoE/pCoE/ART plus centers after SACEP review and patients will be transferred back to their referring center after 6 months viral load testing (except those having an OI at 6th month or toxicity to ART drugs).

In order to minimize the unnecessary referral to SACEP, the referring ART centre should follow the following guidelines before referring patients to SACEP:-

1) It is important to differentiate between complications of HIV disease and ARV toxicities as these may present with similar signs and symptoms.

2) The inter-current illnesses like Hepatitis-A, Malaria, etc. must be kept in mind as they may also lead to symptoms similar to ARV drugs toxicities.

3) Toxicities due to other drugs used concurrently like Cotrimoxazole, anti-TB drugs, other antibiotics, herbal or other medications etc. must be ruled out before the toxicities are thought to be due to ARV.

4) As a general principle, mild toxicities do not require discontinuation of ART or substitution with alternative First line drugs. Symptomatic treatment may be given if toxicity is mild and patient monitored closely.

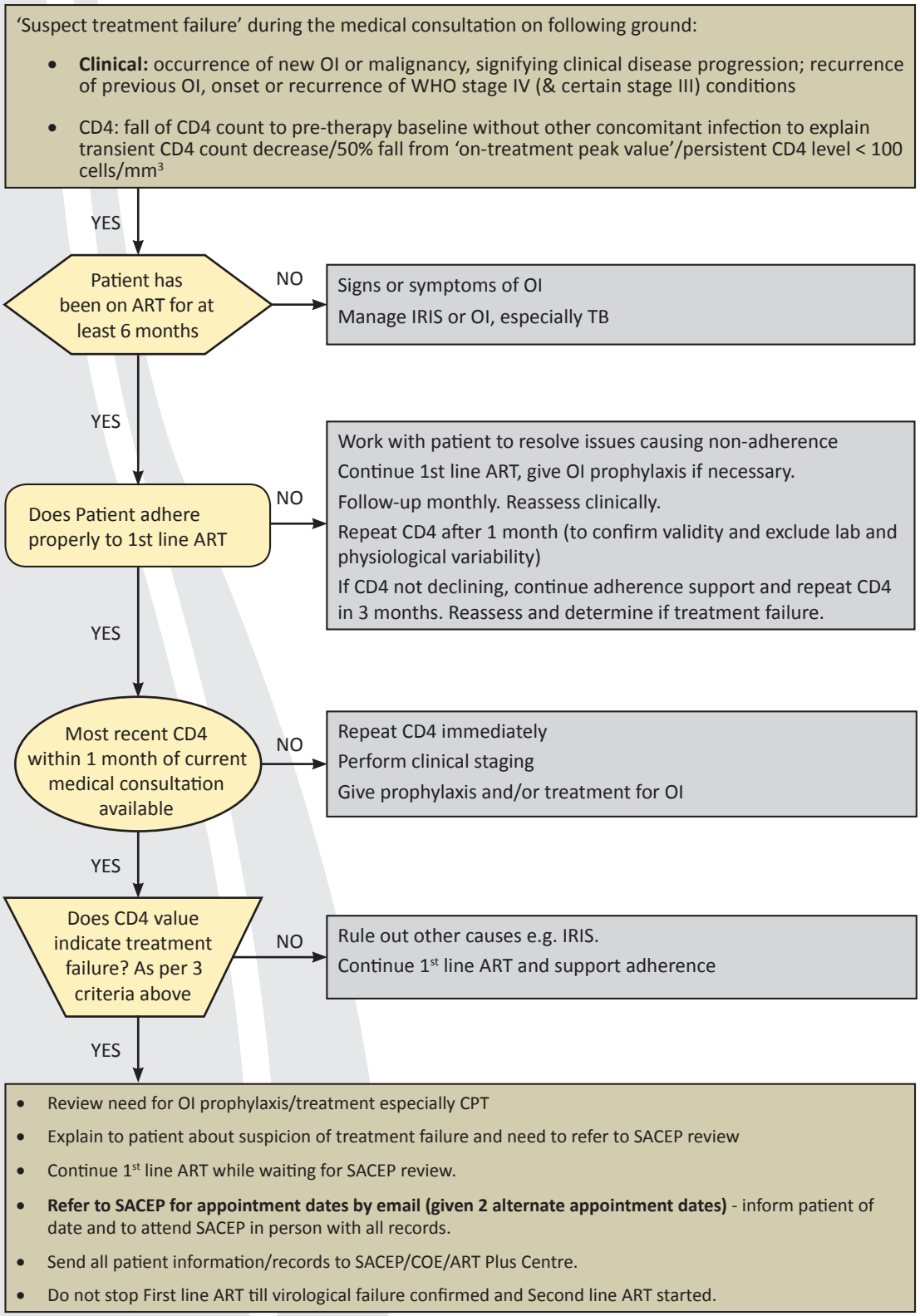
5) Moderate to severe toxicities require substitution with a different drug of same class with a different toxicity profile. A grading of common lab and clinical toxicities is at **Annex I.**

6) Severe life threatening toxicities e.g. Stevens – Johnson syndrome require discontinuation of all ARV drugs until patient is stabilized.

7) History and nature of toxicities to ARV drugs must be clearly documented in the patient record, and where possible objective grading of toxicities should be done according to Annexure I. Photo documentation of skin rash & mucosal lesions, lipodystrophy etc. should be done at the ART Centre and must be sent during SACEP referral.

All ART centers shall use protocol A1.1 listed below before referring patients to their linked CoE/ART plus centre as a case of “suspected treatment failure”.

1.6 PROTOCOL FOR DETERMINING ART FAILURE (PROTOCOL A1.1) AT ART CENTERS



Patients who are referred to SACEP for review should be accompanied with complete details of the history and all records, recent CD4 and other baseline tests, photographs of skin/mucosal lesions, if any. Incomplete records will delay decisions to be taken by SACEP. A referral format is enclosed at **Annex II**

Eligibility for enrollment into Second Line Treatment:

All patients who require Second line ART shall be reviewed by SACEP at COE/pCoE/ ART Plus centres as per laid down referral procedure, irrespective of the fact whether they started treatment in private sector or NACO centres.

The SACEP review will be based on the referral from the ART centre providing First line ART to the patient suspected of treatment failure.

Each COE/pCoE/ART Plus Centre will have defined ART centres linked to it and patients from these centres *only will be reviewed by a particular CoE/pCoE/ART plus center after proper referral.*

When a patient is suspected to have treatment failure, the ART centre must follow the NACO protocol 1.1) before referring to the SACEP. This is to ensure that appropriate referrals for 'suspect treatment failure' are made:

- Laboratory tests including Hb, LFT, RFT and CD4 and other symptom-directed testing should be done immediately if suspected of treatment failure. There should not a big gap between these tests and SACEP referral
- Most recent CD4 count (within 1 month) of the current medical consultation should be available
- In the meanwhile, the patient is to be counseled for 100% adherence in a few sessions (and/or be optionally linked to the nearest Care and support Centre for adherence support)
- Ensure use of Cotrimoxazole prophylaxis if CD4 < 250 cell/mm3 or based on WHO Clinical staging
- Continue with the 1st line ART regimen during this time.
- If OI or any intercurrent illness is present, treat for the specific OIs. If the OI treatment is not available in the institution then these patients can be referred to the nearest Government hospital or COE/pCoE/ART Plus Centre for further management.
- Counsel and support adherence during this period of OI treatment as some patients may not adhere to taking ART when they feel ill.

- For suspected TB, refer to RNTCP services for treatment and monitor the response to TB therapy. If the response to TB therapy is good, the patient should be evaluated again for suspicion of treatment failure. Repeat CD4 counts 2 weeks after the OI has been treated, and for TB – after the intensive phase (i.e. 8 weeks of ATT completed). Review the patient for suspected treatment failure as per protocol.

If protocol 1.1 points towards a suspected treatment failure, following steps need to be taken:

- The referring ART centre will inform and counsel the patient on the findings of 'suspected treatment failure', support psychosocial needs, counsel to continue 1st line 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 for COE/pCoE/ART Plus Centres.

- **During counseling/while referring, never tell the patient that his First line has failed until viral load report is available as failed. Counsel about suspicion of failure and that he is being referred for opinion and confirmation.**

- The ART center will send the request form 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/ART Plus Centre by e-mail.

- The CoE/ART plus centre will enter the patient details in the SACEP register and review the referral form with other clinical details.

- After review of patient records by the SACEP coordinator/research fellow/SMO, if the SACEP at CoE/pCoE/ART plus centre considers that the patient should be reviewed by SACEP then two alternative appointment dates are given by e-mail to the referring centre who will inform the patient.

- After review and other necessary investigations by the SACEP, the CoE/pCoE/ART plus centre will send the feedback form (lower half of the referral form) to the referring centre and inform them about the final decision of the SACEP.

- If the patient is to be started on 2nd line ART, the CoE/pCoE/ART plus centre will ask the referring centre to transfer out the patient to that particular CoE/pCoE/ART plus centre and patient may be sent back before 6 month (may be at 3 month) to Nodal ART Centre on request, if clinically stable and improving

1.7 MANAGEMENT PROTOCOL BASED ON SACEP DECISION AND VIRAL LOAD TEST RESULTS

Once patient has been referred to SACEP for evaluation according to the technical protocol (A1.2), the SACEP will review the patient with his record and document its decision, which can be

- Provide 2nd line ART
- Not eligible for 2nd line ART
- Continue First line, reinforce adherence and re-evaluate
- Evaluate for HIV 2

After the decision of the SACEP to provide 2nd line ART to the patient, the COE staff will ensure the necessary work to prepare the patient for initiation of Second line ART.

The SACEP coordinator/Data Manager shall communicate the decision of the SACEP to the referring ART centre for their reference, and ART centre to 'transfer out' the patient to COE/ pCoE/ART plus Centre. (See M&E section). The center shall ensure baseline laboratory and clinical screening is done and recorded before initiation of Second line ART. As a part of Treatment preparedness, the patient should undergo a minimum of 3 counseling sessions. Treatment supporter should ideally be present. Consent form for 2nd line ART initiation should be signed by the patient – **ANNEX III**

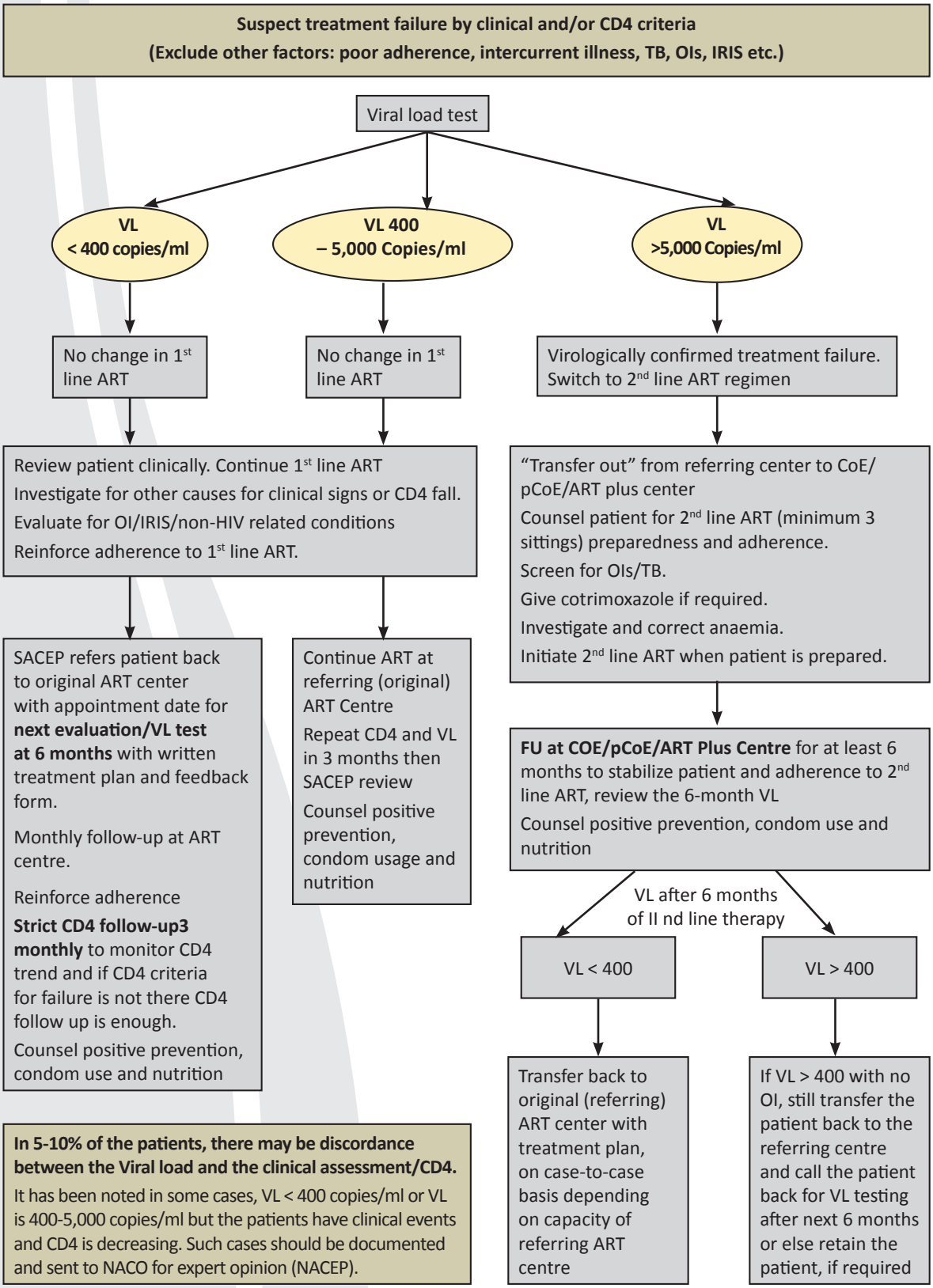
The CoE/pCoE/ART plus center shall also ensure linkage with NGO/CBO/FBO/positive network/Care and Support Centre/ICTC for outreach and community/home based care

Only the **Programme Director/nodal officer of the COE/pCoE/ART plus Centre are authorized to prescribe Second-line ARVs** after approval of SACEP. The decision to switch from First line to Second line therapy resides on the decision of the SACEP which will meet every Tuesday (or the designated day of the centre), to review the case history, order the Viral load testing and approve initiation of Second line ART for treatment failure and use of alternative regimens.

Details in the reporting and recording formats should be completed by the COE/ART plus Centre staff so that good documentation is present. This will enable the COE/ART plus Centre and the national programme to learn from the national rollout of Second line ART.

Routine drug resistance testing is not part of the national protocol for Second-line ART, however it may be done for research/surveillance and monitoring purposes. Blood samples on **dried blood spots (DBS)** shall be collected and stored for use at a later date for drug resistance genotyping. (See Laboratory guidelines section below)

Protocol A1.2: SACEP Management according to viral load results



1.8 INITIATING THE STANDARDIZED NACO SECOND-LINE REGIMEN

After the SACEP has approved Second line ART for the patient, the clinical management shall be the responsibility of the COE/pCoE/ART Plus Centre and the patient will be 'Transferred out' from the referring ART centre to the COE/pCoE/ART Plus Centre.

The objective of the Second-line is:

- To prolong survival of the PLHIV

The NACO standard Second line regimen (TDF + 3TC + ATV/r) (for those on AZT/d4T in First line regimen) aims to achieve viral suppression for as long as possible, so that survival can be prolonged.

Under the public health settings in India, the objective of the Second-line is to prolong survival of the PLHIV rather than a complete viral suppression as in developed countries where multiple Second line options and salvage regimen are available and early switching is the norm. The NACO technical resource group on ART had extensive discussions on the regimen which is optimum for the national setting and on 'early' vs 'late' switching as well. Since we had only one Second line regimen with no salvage regimen, it was decided to go for late switching to give the benefit of First line ART for as long as possible.

Table 6: NACO Second Line Regimen:

ARV drugs for 2nd line	Dosage	Dosing schedule
TDF + 3TC	Fixed dose combination of Tenofovir 300 mg + Lamivudine 300 mg once daily in tablet form	Please advise the patients to consume all the three pills simultaneously after meal (preferably dinner). (Keep the drug interactions in mind)
ATV/r	Tab. Atazanavir 300mg, Tab. Ritonavir 100 mg Each tab to be taken once daily simultaneously	

The major side effects of Second line ART are described below in Table 7.

Table 7: Side effects related to the NACO Second Line Regimen:

ARV drug	Side effect/toxicity	Management
TDF	**Usually well tolerated** Minor: weakness and lack of energy, headache, diarrhea, nausea, vomiting and intestinal gas. More serious side effects include liver or kidney failure and pancreas disease. TDF can reduce bone mineral density	Symptomatic management of minor side effects Monitor Liver and Renal function test as per NACO lab monitoring protocol Calcium supplements may be used in patients with osteoporosis
ATV*	Apart from the PI-class specific side-effects like hyperglycemia, fat maldistribution, hyperlipidaemia (especially with Ritonavir boosting), increased bleeding episodes in hemophiliacs etc. the unique side-effects of Atazanavir include indirect hyperbilirubinaemia (producing yellow discoloration of eyes), skin rash and nephrolithiasis (rare). **Unique drug interaction involving Atazanavir:** In addition to all the drug interactions involving PI class, Atazanavir has significant drug interaction with antacids, H2 Receptor antagonists and proton pump inhibitors*	The patients need to be counselled that they may appear to be jaundiced with yellow eyes but they should not be afraid as it will be a cosmetic problem only (like Gilbert disease). It should not be taken as hepatotoxicity. However, LFT has to be done should someone appears to have jaundice. Also, they should be advised to consume plenty of water. Antacid: Give Atazanavir at least 2 hours before or 1 hour after antacids or any buffered medications.
LPV/r	Side effects include abdominal pain, abnormal stools or bowl movements, diarrhea, feeling weak/tired, headache and nausea. In addition, patients taking Lopinavir should be monitored for possible liver problems. People taking the drug who have liver disease, such as hepatitis B or hepatitis C, may experience a worsening of their liver condition. A small number of patients have experienced severe liver problems.	Symptomatic management of minor side effects. Supportive counseling and use of other drugs to manage GI effects should be done. These symptoms improve after a few weeks. Monitor LFTs, Lipid profile and blood sugar regularly
3TC	**Usually well tolerated** Side effects may include cough, diarrhea, dizziness, headache, loss of appetite, mild stomach cramps or pain and trouble sleeping. More serious side effects include burning, tingling, or pain in the hands, arms, feet, or legs; chills; ear, nose, or throat problems; fever; muscle aches; nausea; pale skin; severe stomach pain; skin rash; unusual tiredness or weakness; vomiting; and yellow eyes or skin.	Symptomatic management of minor side effects

CAUTION : (WE SHOULD BE CAUTIOUS IN RECOMMENDING THE USE OF H2RA & PPIs WITH ATV/r AS MOST OF OUR PATIENTS ARE ARV-EXPERIENCED; THIS IS A BLACK BOX WARNING FOR ATV; ONLY SYSTEMIC ANTACIDS CAN BE CO-PRESCRIBED)

Side-effects of Atazanavir: Apart from the PI-class specific side-effects like hyperglycaemia, fat maldistribution, hyperlipidaemia (especially with Ritonavir boosting); the unique side-effects of Atazanavir include **indirect hyperbilirubinaemia (producing yellow discolouration of eyes),** skin rash, prolongation of PR interval and nephrolithiasis.

1.9 DRUG INTERACTION:

Avoid concurrent use of the following drugs:

Astemizole, Cisapride, Fluticasone, Indinavir, Lovastatin, Simvastatin, Midazolam, Terfenadineetc

Unique drug interaction involving Atazanavir:

In addition to all the drug interactions involving PI class, Atazanavir has significant drug interaction with antacids, H2 Receptor antagonists and proton pump inhibitors.

S No	Patient on following drugs concomitantly	Recommendation/points to remember for ATV
1	Antacids	Give ATV at least 2 hours before or 1 hour after antacids or buffered medications
2	H2 Receptor Antagonist	1. H2 receptor antagonist dose should not exceed a dose equivalent to Famotidine 40 mg BID in ART-naïve patients or 20 mg BID in ART-experienced patients. 2. Give ATV 300 mg + RTV 100 mg simultaneously with and/or >10 hours after the H2 receptor antagonist. ***Example:*** *If a PLHIV on Zidovudine + Lamivudine + Atazanavir/Ritonavir (Regimen IV) requires to be treated with Famotidine 20 mg BID or Ranitidine 150 mg BID, S/he should be instructed to take Tab. Famotidine/Ranitidine with Zidovudine + Lamivudine at 8.00 AM and in evening give ZL and ATV/r at 8 p.m. and ensure that there is a gap of at least 2 hours before or 1 hour after Famotidine/Ranitidine evening dose.*

S No	Patient on following drugs concomitantly	Recommendation/points to remember for ATV
3	Proton pump Inhibitor	1. **H2 receptor antagonist is not recommended with** Tenofovir + Lamivudine + Atazanavir/Ritonavir 2. PPIs should not exceed the dose of Omeprazole 20 mg daily or equivalent dose of Esomeprazole 20mg/Pantoprazole 40 mg/Rabeprazole 20 mg in PI-naïve patients, along with Ritonavir boosted Atazanavir. PPIs should be administered at least 12 hours prior to Atazanavir/Ritonavir. 3. **PPIs are not recommended in PI-experienced patients**. ***Example:*** *If a PLHIV is on Tenofovir + Lamivudine + Atazanavir/Ritonavir requires to be treated with PPI, S/he should be instructed to take Tab. Omeprazole 20 mg/Esomeprazole 20mg/Pantoprazole 40 mg/Rabeprazole 20 mg OD at 8 AM and Tenofovir + Lamivudine + Atazanavir/Ritonavir after 8 PM.*

a. *Prolongation of P-R and Q-Tc interval in the ECG can occur. So PR interval needs to be monitored in patients with known conduction defects or with concurrent use of other drugs that alter conduction abnormalities (like diltiazem, clarithromycin, cisapride, ketoconazole etc.). However, routine ECG before starting Atazanavir based ART is not mandatory.*

b. *Atazanavir induced urolithiasis is also reported; presumably due to precipitation of the drug resulting in crystalluria in a manner analogus to Indinavir.*

c. *If the serum total Bilirubin is > 7 mg/dl or the Child-Pugh Score is ≥ 7, then ATV/Ritonavir cannot be used and the details of such cases should be mailed to National AIDS Clinical Expert Panel (NACEP).*

Counseling issues:

There is a need for enhanced counseling of the PLHIV on these regimens particularly unique side effects of Atazanavir/Ritonavir.

So, the patients need to be counseled that they may appear to be jaundiced with yellow eyes but they should not be afraid as it is only a cosmetic problem. It should not be taken as hepatotoxicity. However, LFT has to be done should someone appears to have jaundice. Also, they should be advised to consume plenty of water.

As we are using the ATV and RTV as separate pills, during counseling we need to impress upon the patients to take both the pills together as our patients are going to take ATV, RTV along with TDF, if they miss RTV, then there will be decreased ATV blood level and it may lead to treatment failure. So during the counseling session, these points need to be stressed adequately. They should also be counselled that ATV/r should always be taken on a full stomach as otherwise the absorption of ATV goes down.

All the Nodal Officers, SMOs & MOs are requested to sensitize the counselors, pharmacists, nurse and other ART Centre staff on use of Atazanavir/Ritonavir.

Important consideration in pregnancy

Ritonavir booster Lopinavir (not ATV) is to be used for pregnant women exposed to sd- NVP in the past

1.10 SECOND-LINE ART AND TB TREATMENT

Tuberculosis is the most commonly detected serious opportunistic infection among PLHIVs in India. While tuberculosis has to be treated appropriately and on priority, in the context of Second-line ART drug-drug interactions must to be considered. Rifampicin alters the metabolism of Protease Inhibitors, including Lopinavir, Atazanavir and Ritonavir, and reduces effectiveness of standard doses. However, Rifamycin-class drugs are highly efficacious in treatment of tuberculosis. Annex V

Another Rifamycin, **Rifabutin**, can be administered in the presence of PI-containing Second line ART regimen without compromising the efficacy of ART or Anti TB treatment. Therefore NACP and RNTCP have recommended the substitution of Rifabutin for Rifampicin for the duration of TB treatment. In the presence of the boosting drug like Ritonavir (PI), Rifabutin metabolism is altered, and less Rifabutin is needed than would be without Ritonavir. Therefore, the dosage of Rifabutin during the administration of Second line regimen containing LPV/r shall be 150 mg thrice weekly for all patients, weighing >30 kg weight. The remainder of the TB treatment regimens, including dosing and duration, remain unchanged as per RNTCP guidelines.

As with all anti-TB treatment, **supervised treatment under DOTS is required.** The patients' DOTS provider should be informed and counseled regarding the substitution using Rifabutin, by the treating medical officer.

Rifabutin dose: 150 mg OD, three times a week

Some of the common side effects of Rifabutin include orange- brown discoloration of skin, tears, saliva, sweat, urine, and stools and will stop when you finish taking Rifabutin. The ART centre SMO/MO should be contacted in case of h/o chest pain, muscle aches, severe headache, fatigue, sore throat, flu-like symptoms, vision changes, unusual bruising or bleeding, nausea/vomiting, yellowing of the skin or eyes.

Initiation of 2nd line ART in patient already on anti-TB treatment

If a patient is already on anti-TB treatment, and needs to be initiated on Second-line ART, then **substitute Rifabutin for Rifampicin within the RNTCP regimen for 2 weeks prior to initiation of Second-line ART**. This is to allow hepatic metabolism (induced by Rifampicin) to normalize prior to initiation of PI-containing regimens. While the patient is counseled and prepared for initiation of 2nd line regimen, the patient should still be given the 1st line ART regimen. There is no need to increase the dose of EFV with Rifabutin containing ATT

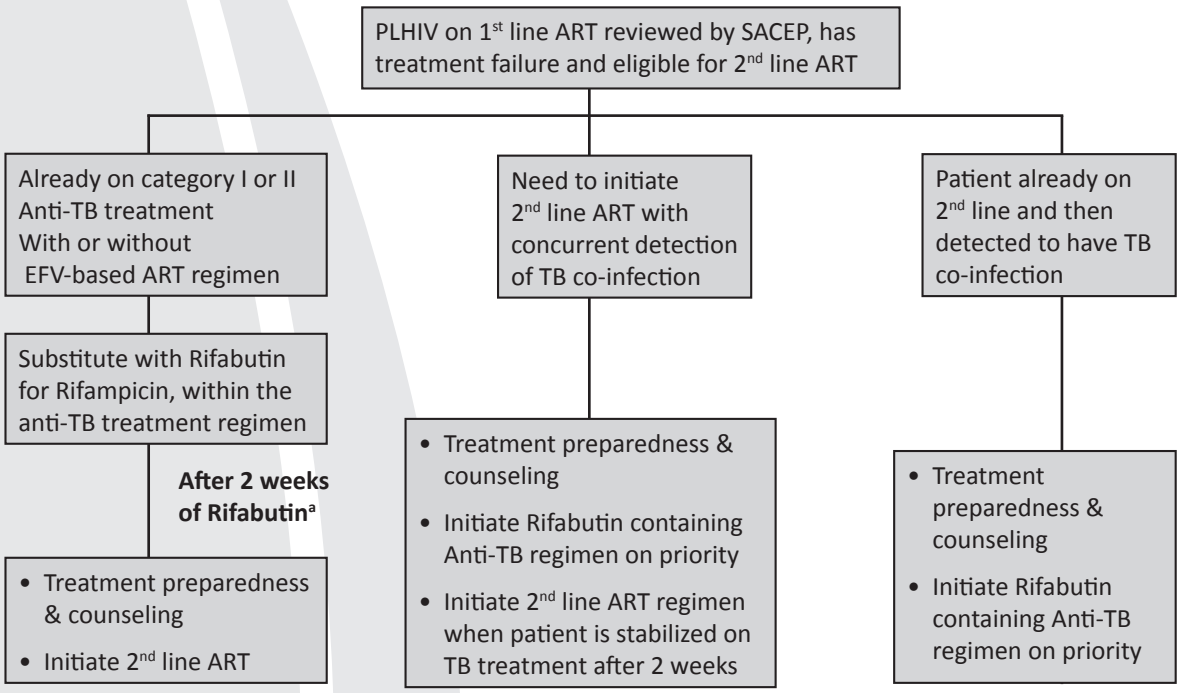
RNTCP recording and reporting: TB treatment categorization does not change with the use of Rifabutin, which is a simple substitution for Rifampicin. The substitution of Rifabutin for Rifampicin should be noted on the TB treatment card, and in the TB register "Remarks" column.

Initiation of Anti-TB treatment in patients already on 2nd line ART

If the patient is already on 2nd line ART (Atazanavir/Ritonavir containing treatment protocol), and detected to have TB, **subsitute Rifabutin for Rifamipicin** within the category I or II anti-TB regimen from the start of TB treatment.

In regards to the availabilty of Rifabutin at the DOTS centres a joint letter has been issued by DDG (TB) and DDG (NACO) dated, 19th Feb 2013. The letter is enclosed at Annex X.

Initiation of Second line ART/Anti TB Treatment in the presence of TB co-infection (Protocol A 1. 3)



Note:

a The 2 week period allows hepatic function to normalize after induction of P450 cytochrome enzymes by Rifampicin

b Patient to be counseled well for both anti-TB and 2nd line ART. Pill burden is high. If patient is not started on 2nd line ART immediately, then continue 1st line regimen till patient is switched to switch to 2nd line ART occurs.

1.11 LABORATORY MONITORING OF PATIENTS ON SECOND LINE REGIMEN

The protocol for laboratory monitoring of patients on Second line ART is described in table 9 below

Table 9: Protocol for laboratory monitoring of patients on Second line ART

Tests	Base-line 0	3	6	12	18	24
Hb, CBC	✓	✓	✓	✓	**Then annually**	
LFT	✓	✓	✓	✓		
Renal function test	✓	✓	✓	✓	**Then every 6 -monthly**	
Fasting blood sugar	✓			✓	**Then annually**	
Fasting lipid profile	✓			✓		
Viral Load (VL)	✓		✓	**Then no more VL unless indicated in protocol A1.2**		
CD4	✓					
More frequently if required.						

Note: Resistance testing is for operational research/surveillance/monitoring only, under current national programme.

A baseline and annual checkup of fasting blood sugar and lipid profile is recommended. This is to understand the baseline and monitor the possible morbidities/side effects of PIs. However, the national programme will not be providing statins or fibrates for treatment of dyslipidemia.

1.12 ADHERENCE AND SECOND-LINE ART

NACO 2008 tools to support counseling and treatment adherence for all patients and especially for patients who have difficulty with their First line ART and those taking Second line ART:

1. Patient counseling diary (individual)
2. Use the Visual Analogue Scale (VAS) to help understand drug adherence
3. Patient education information leaflets for their prescribed regimens
4. Patient pill-taking monthly calendar

Long term adherence continues to be challenge to PLHIV taking lifelong ART. The most common reason for HIV drug resistance is due to non-adherence and missed doses. Adherence approaching 100% is required for optimal viral suppression both in First line and Second line ART.

The experience of the national programme to date is that in general, socio-demographic characteristics such as age, sex, social class, marital status or personality traits, race, religion and educational levels are poor predictors of adherence. Patients' beliefs, knowledge and expectations (sometimes shared by friends and community), strongly influence medical decision-making and willingness to begin and then adhere to prescribed treatments. Adherence is found to be greater when the person perceives the need for treatment, believes the treatment will be helpful and understands the purpose of the medications. Attitudes of friends, trust in physicians and confidence in one's own ability to follow the agreed-upon treatments are also associated with adherence. Lack of belief in the efficacy of treatment may lead to either treatment refusal, or inadequate adherence once initiated.

It is for this reason that the **initial detailed counseling by the doctor** about combination therapy is crucial for later success. Simply telling the patient that it's time to begin antiviral therapy, writing out prescriptions and handing them to a patient who asks no questions and expresses no opinions is likely to lead to adherence problems and treatment failure. Occasionally, well-meaning physicians urge reluctant patients to start treatment, despite the patients' reservations. The more assertive patient may state that he or she is not ready to start, but others may acquiesce to a regimen that they consider of doubtful value or not compatible with their life circumstances. **Do not start Second line ART if the patient is not ready to comply with adherence and follow up schedule.**

HIV/AIDS is associated with **neurocognitive problems** such as memory loss. This will cause adherence problems. Some patients may have trouble sorting their pills for the day or the week, while others may have trouble keeping track of the time, or may simply forget. Even among asymptomatic patients, memory problems are present. When memory is a significant problem, prescription of complex medical regimens is unlikely to succeed unless social (e.g., family member) or institutional (e.g., home attendant) resources are regularly available to help with the scheduling and taking of medications.

Psychiatric disorders also may constitute barriers to adherence. Even mild conditions such as depressed mood, as elicited on self-report rating scales, may be associated with medication non-adherence, either because of impaired concentration, which is one of the criteria for diagnosing mood disorders, or because of feelings of hopelessness and despair. Among those with chronic and severe psychiatric disorders, noncompliance with psychotropic medication often contributes to relapse. When substance abuse is also present in HIV-infected patients with severe mental illness, many of whom live alone in unstable housing, the probability of effective management of combination therapy is poor.

Two-way communication between the clinical team and patient is critical to the success of adherence. **The patient has to believe that combination therapy will make a profound difference in extending life, or else the regimen's burdens will outweigh the perceived rewards.** Initiating combination therapy is not usually regarded as an immediate need. If it takes extra time, or additional visits, for the clinical team to convince the patient that combination therapy should be initiated now, or for the patient to convince the doctor that his or her current life circumstances are simply not conducive to starting now, then extra time must be provided. However, there are a few urgent situations when immediate initiation may be considered imperative. One example is a patient with an essentially untreatable condition such as PML who is rapidly getting sicker. In these cases, ART can lead to life-saving remission. In less urgent situations, treatment can be safely put off while doctor-patient discussions continue.

At the outset, before even beginning combination therapy, it is helpful to **review anticipated problems and barriers to adherence, which then permits the patient to work out solutions on his or her own or with assistance.** For this purpose, some providers give their patients 1-2 weeks of cotrimoxazole and to use this time to reinforce adherence. Which doses are problematic? What are the circumstances? What is the patient thinking when errors occur? What is the patient's attitude about mistakes? Does he consider a fifteen-minute delay a catastrophe signifying irremediable failure? Alternatively, what do they think about missing a weekend's worth of "medications"? Such rehearsal is often extremely helpful in anticipating and correcting potential pitfalls.

Some patients find it helpful to have a **written treatment plan** that shows the name of the medication, time of each dose, number of pills or capsules per dose and meal restrictions, if any, along with a telephone number to call with questions and for the next appointment date. Both doctor and patient should keep a copy of the plan for review at the next visit. Other techniques for promoting adherence include identifying daily activities that can be linked to pill-taking (e.g., a regular TV show), keeping a medication diary or log (preprinted forms can be prepared), preparing pills for the week at fixed times (e.g., Sunday evening), and otherwise relating pill-taking to the normal rhythms of daily life. Planning ahead for changes in routine or for weekends can forestall lapses at such times.

Mechanical aids are often useful. These range from pill boxes with dividers in which medications can be sorted by the week and time of day to timers, alarms, beepers that can be set to ring when it is time to take pills, to signs and checklists posted on refrigerators.

Social assistance can make a major difference, especially at the beginning of the regimen. Some people have a **family member/treatment supporter** who agrees to provide reminders every time medication is scheduled. Children remind their mothers; sometimes mothers remind their adult children.

Over time, initial enthusiasm can dwindle as the incessant demands of scheduling persist. As people feel better and return to work, new problems arise. Among these are maintaining confidentiality, arranging schedules to accommodate pill-taking, frequent trips to the bathroom (if taking medication that requires a high liquid intake), occasional days with significant side effects such as diarrhoea, the constant reminder of illness and simply the ongoing burden of the regimen. It is important to know in more detail about the hurdles, questions and worries that arise over time with ART, and accordingly to develop **individual interventions** to maintain adherence once therapy is established.

For patients who have difficulties with adherence to First line ART or who are suspected of treatment failure, or who will start Second line ART, **the objectives of adherence counseling are:**

- To improve the adherence to medications so that ART is successful
- To encourage self management of medications
- To foster **honest communication** between provider and patient
- To respect patient choices and decision-making related to their HIV related medical care

Patients don't want to disappoint their health care provider. The challenge is: how do you make it okay to say 'no' to doctors/counselors/nurses; or make it okay to say 'I can't do this'

Section II: OPERATIONAL GUIDELINES

2.1 STATE AIDS CLINICAL EXPERT PANEL (SACEP)

a. State AIDS Clinical Expert Panels (SACEP) have been constituted at all COEs and ART plus centre to review the patients referred to them with need for alternative First line and Second line ART. Each COE/ART plus centre has ART centres linked to it and patient from these centers only will be reviewed by a particular COE/ART plus centre.

b. The SACEP will review the patient and his/her records and will approve the COE/ ART plus to provide PI based Alternate First line drugs if the patient is found to have severe toxicities to the First line ART or Second line ART as per clinical protocols.

c. Once the decision is made by SACEP to provide alternative First line ART to the patient reviewed, patients who are recommended for PI based alternate First line are 'transferred out' to the CoE/ART plus centre from the referring centre.

d. The patient shall be started alternative First/Second line ART only after adherence counseling has been done and patient has been assessed to be prepared for 100% adherence to the regimen.

e. Patients who have not been found eligible for Second line ART after SACEP review a detailed follow up plan should be given to the referring ART centre in the reply back form.

State AIDS Clinical Expert Panel (SACEP) have been established at each centre of excellence/pCoE/ART plus centre. It consists of:

1. Program Director/Deputy Program Director of COE/pCoE, Nodal Officer of ART plus centre
2. One more ART expert (preferably not from the same ART centre) - Panel to be formed by NACO
3. Regional Coordinator/Joint Director (CST)/Consultant (CST) at SACS/DPM.

In addition to the above, there would be observers from central level regularly for monitoring purposes.

The Terms of reference of SACEP/are:

- Review and decide on all cases referred by the referring ART centre for PI based alternate First line/Second-line ART provision – both for eligibility for Viral load testing and starting Second line or alternative First-line ART regimen
- Meet at the COE/ART plus centre to review cases every Tuesday (or a prefixed day) (next working day in case of a holiday). This is to ensure that there is no delay in review and processing of the case referred for review of suspected treatment failure. A maximum of 15-20 patients shall be reviewed at each meeting (old and new). However if there are too few patients, the meeting may be deferred to the next week. If the number of patients on next week is again too small, the meeting still should be held to avoid delay. It should be ensured that there is minimum time taken for review of patients and provision of drugs. All efforts should be made to ensure that this period does not exceed four weeks.
- Mentoring and ensure high quality case management of the PLHA on Second-line ART by the referring ART centre
- Document the registration and monitor progress of all patients suspected of treatment failure sent for SACEP/review

The SACEP will follow the technical protocols as laid out by NACO in section I of these guidelines.

However for all technical issues, the ART plus centres can refer difficult cases to SACEP of their CoE in their region. For patients requiring any further technical/operational clarification, COE/ART plus centres can refer cases to NACEP (National AIDS Clinical Expert Panel) at Secondline2008@gmail.com and drbbrewari@yahoo.com. The functioning of NACEP will be coordinated by Dr B B Rewari, NPO (ART). This panel can have representatives from TRG, institutions or independent experts that would be deemed most appropriate for the query under consideration. Hence, the composition of the NACEP will be dynamic and its composition will vary depending on the type of technical query. This will ensure that most appropriate response and guidance is provided for the query by the variety of experts and expertise on various areas under treatment and care for HIV.

Referral to SACEP

Once a patient on ART is suspected to have toxicity to any ARV or is suspected to have treatment failure, the ART centre SMO/MO should follow the steps mentioned below:

Step 1	• The referring ART centre should go through section on "Suspecting drug toxicities among patients on First line therapy"/Suspected treatment failure (Protocol A1.1). • Go through the toxicities grading in ART guidelines. • Referring ART center will send photocopies of patient's records and photo documentation for type of toxicities to SACEP coordinator at CoE/ART plus centre together with Referral/Reply Form **(Annex II)** and confirm that SACEP coordinator has received it.
Step 2	SACEP coordinator/COE research associate will First enter the patient details in the SACEP register and then get the patients history reviewed and if eligible give appointment date/time (2 alternative dates) to the referring centre by e-mail. Before giving appointment the CoE/ART plus centre may request for any other patient treatment related documents.
Step 3	The referring ART center will then communicate the date/time to patient by phone/personal contact by ORW
Step 4	Only after the SACEP has reviewed the patient and recommended initiation of Second line/Alternate First line ART (PI based), in its reply form, will the patient be 'transferred out' to the CoE/ART plus centre.

Note:

For alternate First line ART: only those patients would be 'transferred out' to CoE/ART plus centre who are being initiated on PI based alternate First line ART. For other patients for whom substitution is being done with in NNRTI or NRTI class of drugs no 'transfer out' is required and treatment can be changed at that ART centre itself after SACEP recommendations. SACEP review is not required for TDF based regimen. However e SACEP approval shall be required for d4T based regimen as only limited quantities of d4T shall be procured in future

For Second line ART: all patients starting Second line ART need to be 'transferred out' to CoE/ART plus centres.

2.2 ELIGIBILITY AND CRITERIA FOR PROVISION OF SECOND LINE ART

All patients who require Second line ART shall be reviewed by SACEP as per laid down referral procedure, irrespective of the fact whether they started treatment in private sector or NACO centres

The SACEP review will be based on the referral from the ART centre providing First line ART to the patient suspected of treatment failure. *Each COE/ART Plus Centre will have defined ART centres linked to it and patients* from these centres *only will be reviewed by a particular COE/ART Plus Centre.*

Criteria for provision of Second line ART:

- Referred by the NACO ART centre following the NACO protocol on determining treatment failure (section 1.6)
- Reviewed by the SACEP and determined to be medically eligible as per the NACO protocol for Second line provision
- Has been assessed by counselor/nurse/doctor for adherence and is prepared for Second line treatment
- Should have family support/treatment supporter, and linked to NGO/CBO/CSC/ICTC for outreach/community/home based care services and monitoring of adherence

Furthermore, the patient to be provided Second line ART shall need to sign a consent form for informed consent **(ANNEX III)** for home visits/followed at the community by link worker/NGO/positive network/ICTC which will support the adherence at community level for Second line provision.

The SACEP coordinator at COE/Data Manager at ART Plus Centre shall coordinate the meeting of the SACEP to be held at the COE/ART Plus Centre meeting room. The COE/ART Plus Centre will request the patient to be physically present for the review panel. The SMO at ART centre should also be involved in the meeting.

2.3 PROTOCOL FOR SACEP REVIEW

Step 1	Follow Protocol A1.1 for 'Suspected treatment failure' and then e-mail the details (brief ART history, clinical stage and serial CD4 values, reasons for referrals) of patient to CoE/pCoE/ ART plus centre to get appointment dates
Step 2	CoE SACEP coordinator/ART plus centre's data manager will enter patient details into SACEP register (1 SL+AL) CoE SACEP coordinator/CoE research associate/ART plus centre's data manager will review patient history details sent by centre and give appointment date (two alternatives) and time to the referring centre by e-mail for review by SACEP
Step 3	The referring ART centre will then communicate the date/time to patient by phone and personal contact by ORW/DLN volunteer

Step 4	Referring ART centre will send photocopies of patient records together with Referral/Reply Form VI and confirm that CoE SACEP coordinator/ART plus centre's data manager has received it. CoE SACEP coordinator/ART plus centre's data manager prepares weekly SACEP meeting formats (2 SL)
Step 5	The SACEP will review the case notes only in the presence of the patient and guardian
Step 6	The SACEP will order a Viral load test according to Protocol A 1.2. Blood sample to be taken to the linked Viral Load Lab will be processed according to Laboratory guidelines section
Step 7	The SACEP will review the Viral load results in next meeting and decide on management of the patients and will document one of the following decisions: • Provide Second line ART • Not eligible for Second line ART • Re-evaluate/others
Step 8	Once the decision is made by the SACEP to provide Second line ART to the patient reviewed, the clinical management of the patient will be done at the CoE/pCoE/ART plus centre itself. Only the Program Director or Deputy Program Director of CoE/pCoE and/or Nodal officer of ART plus centre prescribe Second line ART
Step 9	The CoE SACEP coordinator/ART plus centre's data manager shall inform the referring ART centre for the following actions: • 'Transfer-out' the patient in the M&E formats, to CoE/pCoE/ART plus centre, • Send the completed reply form to the referring ART centre
Step 10	The patient must undergo 3 counseling sessions (minimum) to ensure treatment preparedness at the CoE/pCoE/ART plus centre
Step 11	The follow up visits shall be monthly at the CoE/pCoE/ART plus centre
Step 12	After 6-months of Second line ART, the follow up Viral load test has to be performed. The result will be reviewed by CoE/pCoE/ART plus centre. The patients who have suppressed viral loads with clinical stability may be transferred back to the referring ART centre, after ascertaining patent's willingness
Step 13	CoE SACEP coordinator/pCoE/ART plus centre's data manager to send SACEP Monthly report to NACO by e-mail by 4th of every month (Secondline2008@gmail.com) under over all supervision of Program Director/Deputy Program Director &/or Nodal officer

The COE Program Director/Deputy Program Director &/or Nodal officer of ART Plus Centre will be the physician responsible; backed up by the COE/ART Plus ART centre staff to ensure high quality of care for the patient on 2nd line ART. Prescription of the 2nd line ART shall be done only by the Program Director or Deputy Program Director of CoE/pCoE and/or Nodal officer of ART plus centre.

The patient who is confirmed treatment failure and is to be started 2nd line may be admitted at the COE for treatment of any OI or for reinforcing adherence, if required. The COE ART

team shall ensure that the patient on 2nd line is linked to a NGO/CBO/ICTC/CSC for care and support as well as the positive network for other support. Condom use, nutrition advice and positive prevention are to be emphasized.

Details in the reporting and recording formats should be completed by the COE staff so that good documentation is present.

2.4 TOR OF ADDITIONAL MANPOWER: SACEP COORDINATOR

For carrying out these activities, each COE shall be provided with an additional manpower in form of a SACEP coordinator. The TORs for **SACEP Coordinator are**:

1. Screen and review all records and communications of the referrals made to the SACEP
2. Maintaining SACEP-schedule diary, schedule and communicate appointment dates of patients to the referring centres
3. Organize SACEP meetings and coordinate with members of the SACEP
4. Ensure laboratory test results of patients attending are available for SACEP meetings
5. Coordinate with pharmacist for patient drug transfers
6. Ensure follow-up of patients attending SACEP
7. Be responsible for patients' registration, maintaining all forms and registers related to SACEP
8. Prepare and send SACEP reports to SACS and NACO.
9. Coordinated activities of in the region linked to the CoE
10. Be responsible for receiving and sending communications from and to the linked ART Centres
11. Be responsible for all data entries, maintaining and updating all records, registers and files pertaining to the CoE
12. Assist the Program Director and the Deputy Program Director of CoE in receiving and sending all communications related to the CoE
13. Work in the ART centre and perform the duties of Data Manager, if and when required.

14. Responsible for procurements, maintaining accounts, audits, handling contingency petty cash of the CoE

15. Assist the training and mentoring coordinator in communications and maintaining records

16. Perform any other job as assigned by the Program Director/Deputy Program Director of the CoE.

For SACEP meetings at the ART plus centres, the coordinator shall be one of the data managers at the centre and no additional staff shall be provided presently.

Section III: M & E TOOLS

The soft copies of revised M&E tools have been sent to all COE and ART plus centres with instructions on how to use them and only those are to be used. The formats are enclosed at Annex IX.

The monitoring plan for Second-line ART focuses on two main aspects of National ART Programme namely – Clinical Monitoring of the Patients and Programme Performance. The system developed; tries to build on the existing monitoring systems existing at ART centres. Refinements are added in current tools to record details of Second line and additional tools are developed for critical areas of monitoring.

These tools would allow:

- Clinicians to effectively monitor the patient on 2nd line clinically, and
- The ART programme to monitor the progress in implementation, identify problems, refine and adapt the implementation strategies; assess the effectiveness of the interventions.

3.1 INSTRUCTIONS FOR MONITORING/RECORDS KEEPING FOR SECOND LINE ART

1. **When the patient is referred from any ART centre to COE for evaluation, the patient is NOT 'Transferred Out' and would continue to receive the First line drugs from referring ART Centre till recommended otherwise.** SACEP/COE (/ART Plus Centre) will inform the referring centre once decision is made if patient is to be transferred out or not.
2. The patient once recommended for 2nd line; is then transferred out (T/O) to CoE/ ART plus centre. The normal procedure of T/O is followed. The records are thus transferred as per the usual procedure, if not already done.
3. At the ART centre of COE/ART Plus centres, the current ART enrollment register is used for recording the clinical details of the patient on Second-line by filling the information on switch
4. In the same ART enrolment register **a post fix to the ART registration** – S (Second line) can be indicated in the First blank column before "ART date of start" and "A" for alternative First line and registration number are given in a sequential order in continuation to the patients on First line ART.

The summary of Second line and alternative First line formats is as below:

Monitoring and Evaluation tools for Second line and Alternative First line ART (Adults and Children) (To be maintained at CoE/pCoE/ART plus centres)			
Tool/Abbreviations		**M & E Tool- formats/Registers**	**Directions**
1	RRF	**SACEP Referral and Reply form**	To be generated at ART Centre when referring the patient. The reply portion to be sent back to referring centre by SACEP after the evaluation of the patient. (computer printed form only)
	1 SL*+ AL**	**SACEP register** (for all patients being referred to SACEP)	To be maintained at CoE/pCoE/ART plus Centre by SACEP coordinator/ data manager (Hard bound printed register to be supplied by the SACS). There should be no more than six rows per page in order to ensure that there is adequate space for writing the details.
2	2 AL	**SACEP Meeting format (alternative First line)** This format should be prepared before every SACEP meeting for all patients (children and adults) to be reviewed in that particular meeting for consultation for Alternative First line ART	To be prepared before every SACEP meeting; given to all members during the meeting; and maintained at all CoE/pCoE/ART plus centres (in file ring binder and soft copy)
	2 SL	**SACEP Meeting format (Second line)** This format should be prepared before every SACEP meeting for all patients (children and adults) to be reviewed in that particular meeting for suspected treatment failure.	To be prepared before every SACEP meeting, given to all members during the meeting and maintained at all CoE/pCoE/ART plus centres (in file ring binder and soft copy)
3	3 AL+ SL	**Monthly reporting format (Second line and alternative First line)** Combined monthly reporting format for Second line and alternative First line ART for adults and children	To be submitted to NACO by email by 4th of every month at Secondline2008@gmail.com.

Tools to be maintained at all CoE/pCoE/ART plus centres (in ring binder file and/or soft copy):			
4	4 AL	**Line list (alternative First line)** Cumulative list of patients ever initiated on alternative First line ART	To be maintained at all CoE/pCoE/ ART plus Centres in one continuous excel sheet (soft copy only)
	4 SL	**Line list (Second line)** Cumulative list of patients ever initiated on Second line ART	To be maintained at all CoE/pCoE/ ART plus centres in one continuous excel sheet (soft copy only). To be maintained at referring ART centre also after transfer back of the patient after 6 months.
5	5 SL	**Centre wise (linked centre information) break up for PLHIV initiated on Second line ART**	Linked Centre wise cumulative information about PLHIV. To be maintained at all CoE/pCoE/ART plus centres in one continuous excel sheet (soft copy only)
6	CF	**Consent form for Second line ART**	To be obtained from each patient by SACEP coordinator/data manager when starting Second line ART (computer print out, signed form to be kept in ring binder form)
Note: **Out of this only SACEP register is to be printed as hard bound register as per printing instructions sent to SACS**. Rest are to be used as soft copies and print out of soft copies to be kept at CoE/pCoE/ART plus centres in separate ring binder files year-wise.			
* SL- Second line ART **AL- Alternative First line ART			

<u>All Second line treatment related formats are enclosed at Annexure IX.</u>

1. **SACEP Register (1 SL + AL)** would be maintained by all CoE and ART Plus centers that conduct SACEP meetings. The SACEP register is a clinic administration tool which helps to track patients from referral to outcomes, provide information for weekly and monthly reporting to NACO.

   - The SACEP coordinator (COE)/data manager (ART Plus Centre) is responsible for maintaining and updating the register
   - To fill in the register, please note that:
     - It has to be printed in the form of a register and all patients who are referred to CoE/ART plus centre for SACEP; should be entered in this register

- Patient is to be entered only ONCE in SACEP/register with details in same row. No multiple entries for same patient should exist (unique) but if the patient was not found eligible for review or after review it was decided by the SACEP that it is a wrong referral then a new SACEP number would be given the next time the patient is referred to SACEP.

2. **SACEP Meeting format (2 SL & 2 AL)** records the details of every meeting and is to be maintained at COE/ART plus centre

3. **Second line monthly ART centre report (3SL + AL) -** this report is to be sent on a monthly basis to NACO by all CoE's and ART plus centres. This report should be generated after compilation of line lists from all the linked ART centres.

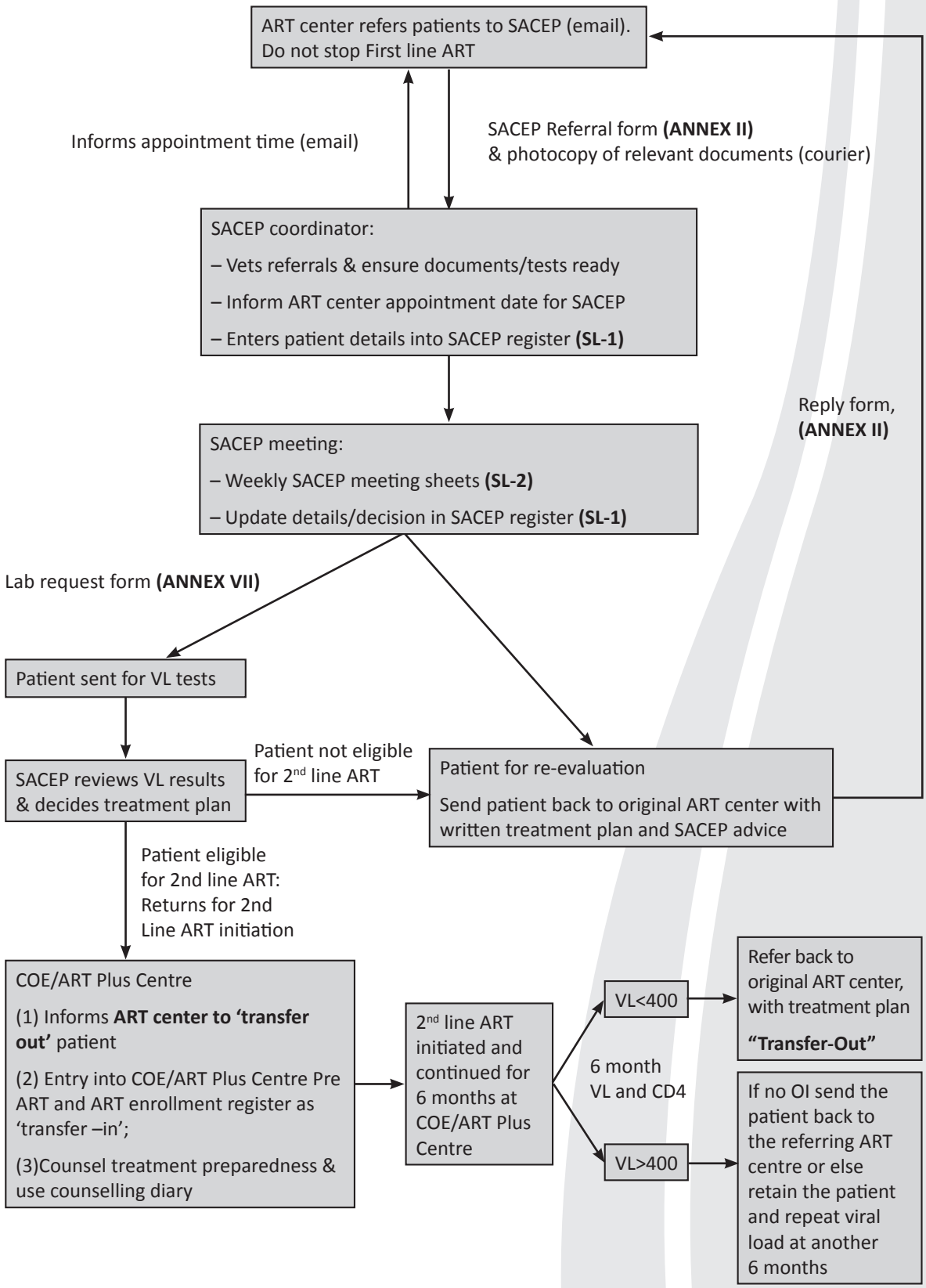
   All COE/ART plus centre will sent monthly report for alternative First line and Second line combined formats for adults and children to NACO at **Secondline2008@gmail.com latest by 4th of every month** in prescribed format.

4. **Line Lists (4SL/4 AL):** All ART centres should maintain a list of patients on Second line and Alternative First line ART in prescribed format and send it to their linked CoE/ART plus centre, so that Second line monthly ART centre report can be generated.

5. **Centre wise information (5SL)** - linked centre wise detailed information on patients on Second line should be available with all CoEs and ART plus centres.

Note: Electronic copies (excel) are available for the above formats from NACO. Contact Secondline2008@gmail.com

DATA FLOW


ART center refers patients to SACEP (email). Do not stop First line ART
Informs appointment time (email)
SACEP Referral form (ANNEX II) & photocopy of relevant documents (courier)
SACEP coordinator:
– Vets referrals & ensure documents/tests ready
– Inform ART center appointment date for SACEP
– Enters patient details into SACEP register (SL-1)
Reply form, (ANNEX II)
SACEP meeting:
– Weekly SACEP meeting sheets (SL-2)
– Update details/decision in SACEP register (SL-1)
Lab request form (ANNEX VII)
Patient sent for VL tests
SACEP reviews VL results & decides treatment plan
Patient not eligible for 2nd line ART
Patient for re-evaluation
Send patient back to original ART center with written treatment plan and SACEP advice
Patient eligible for 2nd line ART: Returns for 2nd Line ART initiation
COE/ART Plus Centre
(1) Informs ART center to 'transfer out' patient
(2) Entry into COE/ART Plus Centre Pre ART and ART enrollment register as 'transfer –in';
(3)Counsel treatment preparedness & use counselling diary
2nd line ART initiated and continued for 6 months at COE/ART Plus Centre
6 month VL and CD4
VL<400
Refer back to original ART center, with treatment plan
"Transfer-Out"
VL>400
If no OI send the patient back to the referring ART centre or else retain the patient and repeat viral load at another 6 months

Section IV: LABORATORY GUIDELINES

4.1 INTRODUCTION:

HIV infection has become a pandemic in the last 20 years. The dynamics of the HIV/AIDS epidemic in India will have a major impact on the overall disease burden of HIV in the Asia-Pacific region and the world. National estimates indicate that India has 2.1 to 2.09 million people living with HIV infection (2011). The overall average adult prevalence in India is 0.27%.

The viral load assay (estimation of copies of HIV-1 ribonucleic acid (RNA) in plasma of infected individuals) is critical in monitoring patients' response to ART (Antiretroviral therapy) and progression towards AIDS. Hence, HIV-1 viral load assay would be one of the key parameters for assessment of patients with suspected treatment failure on First line ART (who may be requiring Second line ART). HIV-1 viral load assay results will help to initiate Second line ART at designated centers as per NACO guidelines.

Viral load assays quantify the amount of HIV-1 RNA circulating in the blood of an infected individual. Although total quantification includes cell-free virus, virus in infected cells in all compartments of the body as well as integrated provirus, the easiest measurement of viral load is of cell-free virus in an individual's plasma. Currently there is no clinical indication for viral load testing of tissues. HIV-1 plasma viral load (PVL) level is being successfully used to predict time to progression to AIDS and to assess efficacy of ART. During treatment, the decay of viral load in tissues typically corresponds with virologic responses in plasma, making blood plasma a useful sentinel for virologic response in general.

4.2 INTENDED USE OF HIV-1 PVL ASSAY IN NACO'S SECOND LINE ART INITIATIVE:

The HIV-1 PVL assay will be performed in HIV infected individuals that fail First line ART at NACO designated ART sites. The results of PVL assay will be used to decide the initiation of Second line ART.

The PVL assay will be performed:

- Before starting Second line ART to get the reference value to decide on further course of action. A PVL measurement will be performed on patients referred by SACEP. The decision on whether to switch ART or not will be made based on the viral load detected as detailed below:

  - **PVL < 400copies/ml:** No change in 1st line ART

- **PVL 400-5,000 copies /ml:** Repeat PVL after 3 months with stringent monitoring of patient for adherence to First line ART regimen
- **PVL >5000 copies/ml:** Start patient on Second line ART. Repeat PVL after 6 months to assess suppression in viral load
- Repeat PVL assay at 12 months if 6 months PVL is more than 400

- **Repeat PVL on the same patient must be done at the same laboratory with the same technique/procedure, using the same platform**

**** This must be especially noted for laboratories which are initially linked to other laboratories and likely to have their own viral load assay set ups in the near future***

If treatment failure is suspected based on immunological (Second confirmation of CD4 T cell levels) and/or clinical criteria, the ART centre must follow the NACO protocol for management as detailed in pages 19 (protocol A1.1).

The ART centre will suspect treatment failure in patients who are on First line ART for at least 6 months and have:

- A new OI (clinical failure)
- Slow rise of CD4 cells or failure of CD4 cells to rise after 6 -12 months of treatment
- Decline in CD4 cells by 50% after an initial rise, as per NACO/WHO guidelines

Perform CD4 cell estimation immediately in cases of suspected clinical failure and repeat after 4 weeks for confirmation of failure as per protocol A1.1

- Simultaneously undertake the following:
    - Evaluate patient for ART adherence
    - Rule out presence of OIs like tuberculosis, oesophageal candidiasis, etc.
    - Ensure patient has been on cotrimoxazole prophylaxis.
    - Treat and review OIs if present.
    - The ART centre to send the request form with complete patient details along with the confirmed contact phone number to the nodal officer.

The nodal officer refers such a patient to State AIDS Clinical Expert Panel (SACEP) at COEs and/or ART plus Centres for decision on HIV-1 viral load testing.

Eligibility

All HIV positive patients showing signs of failure on First line ART will be eligible for review by SACEP following the proper referral procedures from the ART centres:

State-wise list of CoEs and ART plus centres

State	COE*/ART Plus centre
Tamilnadu	Government General Chest Hospital (GGCH), Tambaram* Government Mohan Kumaramangalam Medical College, Salem Madurai Medical College, Madurai Tirunelveli Medical College, Tirunelveli
Maharashtra	Grant Govt. Medical college and Sir JJ Hospital, Mumbai* Govt. Medical College, Aurangabad Government Medical College, Sangli, Maharashtra B. J. Medical College & Sassoon Hospital, Pune Govt. Medical College, Nagpur
Gujarat	B. J. Medical College (BJMC), Ahmadabad* Govt. Medical College, Majura Gate, Surat Pandit Din dayalUpadhyay Hospital, Rajkot
Karnataka	Bowring and Lady Curzon Hospital, Bangalore* Karnataka Institute of Medical Sciences, Hubli, Karnataka VijayanagaraInstitute of Medical Sciences, Bellary, Karnataka District Hospital, Gulbarga, Karnataka District Hospital, Udupi, Karnataka
Andhra Pradesh	Gandhi Hospital and Medical College, Hyderabad* Government General Hospital, Vijayawada, Andhra Pradesh Rajiv Gandhi Institute of Medical Sciences, Kadappa, Andhra Pradesh King George Hospital, Visakhapatnam, Andhra Pradesh
West Bengal	School of Tropical Medicine (STM), Kolkata*
New Delhi	Maulana Azad Medical College (MAMC), New Delhi*
Kerala	Govt. Medical College, Trichur
Assam	Medical College and Hospital, Guwahati
Manipur	Regional Institute of Medical Sciences (RIMS), Imphal*
Bihar	RajendraMemorial Research Institute of Medical Sciences, Patna
Madhya Pradesh	Gandhi Medical College, Bhopal, Madhya Pradesh
Rajesthan	SawaiManSingh Medical College and Hospital, Jaipur
Uttar Pradesh	King George Medical College, Lucknow
Mizoram	Civil Hospital, Aizwa
Punjab	Government Medical College, Amritsar
Panjab/Haryana	Post Graduate Institute of Medical Education and Research (PGI), Chandigarh*
Uttar Pradesh	Institute of Medical Sciences (BHU), Varanasi*
Uttaranchal	Doon Hospital, Dehradun
Jammu & Kashmir	Government Medical College, Jammu
Orissa	Shrirama Chandra BhanjMedical College, Cuttack

13 more ART plus centres have been sanctioned so as to have at least one ART plus centre in each state to provide Second line ART

NOTE: Blood is not to be drawn for a viral load test within four weeks of any infection or immunization.

Protocol for the review panel

The SACEP will review the case notes along with the patient.

The panel will then order a viral load test if required. Specimens (whole blood and DBS) will be collected from the patient at the COE on a designated day and sent to the identified viral load lab as detailed below in the section on specimen collection, storage and transport. The SACEP will meet on the following designated day (which if happens to be a holiday then on the next working day) to review the results of viral load assay and, based on the result of PVL, decide on further management of the case.

4.3 TECHNIQUES OF HIV – 1 PVL ASSAY:

Viral load assays measure the amount of HIV-1 RNA in the collected plasma specimen. HIV-1 RNA is responsible for HIV replication. The amount of HIV-1 RNA in plasma can be measured by the following different techniques for measurement of HIV-1 RNA.

- **Quantitative PCR (polymerase chain reaction)** is the most frequently used test. For PCR the viral RNA is extracted; an enzyme converts the extracted RNA to DNA (cDNA); this DNA is then multiplied many folds by the help of an enzyme polymerase; the product is detected through changes in the intensity/color of certain chemical markers. This process makes the detection of viral RNA easier. The original number of RNA copies would be then quantified based on the final numbers of cDNA obtained. Test results are reported as HIV-1 RNA copies/ml.

- **The bDNA (branched DNA)** is a fairly frequently used test. It makes use of signal amplification (light emitting material). This material binds with the HIV particles. The amount of light is measured and converted to a viral count.

- **NASBA (Nucleic Acid Sequence Based Amplification Assay)** is an *in vitro* nucleic acid amplification test for quantification of amplified HIV-1 RNA which is measured by means of electrochemiluminescence.

The PCR test results are often different from the bDNA results for the same specimen. Because the tests are different, only one kind of test (PCR or bDNA) should be used to measure a person's viral load over time.

Viral load assay results are reported as copies of HIV-1 RNA in one milliliter of blood. The best viral load assay result in patients on ART is "undetectable" viral load. This does not mean that there is no virus in the blood; it just means that the level is not enough to be detected through the test used. "Undetectable" level (No of copies/ml of plasma) will depend on the sensitivity of the assay system used.

Some relevant terminologies

- **Sensitivity:** Sensitivity is defined as the lowest viral load level that can be detected in the specimen 95 percent of the time. The statistical method of assessing sensitivity is generally considered to be the standard to determine the quantitative "limit of detection" (LoD) for quantitative HIV-1 RNA assays

- **Precision:** The precision or reproducibility of an assay is defined by its ability to obtain the same value when tested repeatedly. In viral loads, precision is measured by the ability to detect "fold changes" in the levels of the viral loads.

- **Dynamic Range:** The dynamic range of an assay is defined as the quantitative range over which the results are reliably reported.

4.4 PLASMA VIRAL LOAD ASSAYS CURRENTLY IN USE IN NACO'S SECOND-LINE ROLL OUT:

4.4.1 AMPLICOR HIV-1 MONITOR TEST, VERSION 1.5

The Amplicor HIV-1 Monitor Test, version 1.5 (v1.5) is an *in vitro* nucleic acid amplification test for the quantification of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma. The Amplicor HIV-1 Test, version 1.5 uses PCR technology to achieve maximum sensitivity and dynamic range for the quantitative detection of HIV-1 RNA in EDTA anti-coagulated plasma.

The Amplicor HIV-1 Monitor version 1.5 (v1.5) is programmed on, and approved for use on Applied Biosystems Gene-Amp PCR system 9600/9700 thermal cycler. In Amplicor HIV-1 monitor the specimen preparation is manual, the amplification is automated on the ABI 9600/9700 and detection is by manual ELISA or automated ELISA reader.

The test can quantitate HIV-1 RNA over the range of 50-750,000 copies /ml by using a combination of two specimen preparation procedures, the Standard (dynamic range 400-750,000 copies /ml) and Ultra Sensitive (dynamic range 50-1,00,000 copies/ml) procedures. Test results less than 400 are below the lower limit of detection of the Standard test. If quantitative results are desired for such specimens, original plasma specimens should be retested using the Ultra sensitive specimen preparation procedure

This test is based on five major processes: specimen preparation, reverse transcription of target RNA to generate complementary DNA (cDNA); PCR amplification of target cDNA using HIV-1 specific complementary primers; hybridization of the amplified products to oligonucleotide probes specific to the target(s); and detection of the probe bound amplified products by colorimetric determination.

The Amplicor HIV-1 Monitor Test, v1.5 can be used with either of two specimen preparation procedures, the Standard procedure or the Ultra Sensitive procedure. In the Standard specimen preparation procedure, HIV-1 RNA is isolated directly from plasma by lysis of virus particles with a chaotropic agent followed by precipitation of the RNA with alcohol. With the Ultra Sensitive specimen preparation procedure, HIV-1 viral particles in plasma

are concentrated by high speed centrifugation, followed by lysis of the virus particles with a chaotropic agent and precipitation of the HIV-1 RNA with alcohol. A known number of quantitation standard RNA molecules are introduced into each specimen with the lysis reagent. The HIV-1 Quantitation Standard is carried through the specimen preparation, reverse transcription, amplification and detection steps and is used for the quantitation of HIV-1 RNA in the test specimen.

4.4.2 COBASAMPLICOR HIV-1 MONITOR™ TEST, VERSION 1.5

The CobasAmplicor HIV-1 Monitor Test, version 1.5 (v1.5) is an *in vitro* nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma on the CobasAmplicor™ analyzer.

In CobasAmplicor the specimen preparation is manual and the amplification and detection steps are automated.

The test can quantitate HIV-1 RNA over the range of 50-750,000 copies/mL by using a combination of two specimen preparation procedures, the Standard (dynamic range 400-750,000 copies/ml) and Ultra Sensitive (dynamic range 50-100,000 copies/ml) procedures. Test results less than 400 are below the lower limit of detection of the Standard test. If quantitative results are desired for such specimens, original plasma specimens should be retested using the Ultra sensitive specimen preparation procedure.

This test is based on five major processes: specimen preparation; reverse transcription of target RNA to generate complementary DNA (cDNA); PCR amplification of target cDNA using HIV-1 specific complementary primers; hybridization of the amplified products to oligonucleotide probes specific to the target(s); and detection of the probe bound amplified products by colorimetric determination.

The CobasAmplicor HIV-1 Monitor Test, v1.5 can be used with either of two specimen preparation procedures, the Standard procedure or the Ultra Sensitive procedure. In the Standard specimen preparation procedure, HIV-1 RNA is isolated directly from plasma by lysis of virus particles with a chaotropic agent followed by precipitation of the RNA with alcohol. With the Ultra Sensitive specimen preparation procedure, HIV-1 viral particles in plasma are concentrated by high speed centrifugation, followed by lysis of the virus particles with a chaotropic agent and precipitation of the HIV-1 RNA with alcohol. A known number of quantitation standard RNA molecules are introduced into each specimen with the lysis reagent. The HIV-1 Quantitation Standard is carried through the specimen preparation, reverse transcription, amplification and detection steps and is used for the quantitation of HIV-1 RNA in the test specimen.

4.4.3 COBAS TAQMAN HIV-1 TEST

The COBAS TaqMan HIV-1 Test is an *in vitro* nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma with EDTA, using the High Pure System Viral Nucleic Acid Kit for manual specimen preparation and the COBAS

TaqMan analyzer for automated amplification and detection. The test can quantitate HIV-1 RNA over the range of 47 - 10,000,000 copies/ml

The COBAS TaqMan HIV-1 Test utilizes real time PCR technology to achieve maximum sensitivity and dynamic range for the quantitative detection of HIV-1 RNA in EDTA anti-coagulated plasma. The use of dual-labeled fluorescent probes provides for real-time detection of PCR product accumulation by monitoring of the emission intensity of fluorescent reporter dyes released during the amplification process. The COBAS TaqMan HIV-1 Test accurately provides quantitative results over a very wide dynamic range since the monitoring of amplicon is performed during the exponential phase of amplification. The higher the HIV-1 titer of a specimen, the earlier the fluorescence of the reporter dye of the HIV-1 probe rises above the baseline fluorescence level.

The COBAS TaqMan HIV-1 Test is based on four processes: Specimen preparation to obtain HIV-1 RNA; Automated reverse transcription of the target RNA to generate complementary DNA (cDNA); Simultaneous PCR amplification of target cDNA using HIV-1 specific complementary primers; and detection of cleaved dual fluorescent dye-labeled oligonucleotide detection probes.

The COBAS TaqMan HIV-1 Test processes EDTA containing plasma specimens and isolates HIV-1 RNA through a generic manual specimen preparation (in case ampliprep is not available) based on nucleic acid binding to glass fibers. The HIV-1 virus particles are lysed by incubation at an elevated temperature with a protease and chaotropic lysis/binding buffer that releases nucleic acids and protects the released HIV-1 RNA from RNAs in plasma. A known number of HIV-1 'Quantitation Standard Armored' RNA molecules are introduced into each specimen along with the lysis reagent. Subsequently, isopropanol is added to the lysis mixture which is then centrifuged through a column with a glass fiber insert. During centrifugation, the HIV-1 RNA and HIV-1 Quantitation Standard RNA are bound to the surface of the glass fiber filter. Unbound substances, such as salts, proteins and other cellular impurities, are removed by centrifugation. The adsorbed nucleic acids are washed and eluted with an aqueous solution. The disposables allow for a parallel processing of 12 specimens or multiples thereof. The processed specimen, containing HIV-1 RNA and HIV-1 Quantitation Standard RNA, is added to the amplification/detection mixture. The HIV-1 target RNA and the HIV-1 Quantitation Standard RNA are then reverse transcribed, amplified and detected on the COBAS TaqMan - analyzer using the amplification and detection reagents provided in the test kit.

4.5 SPECIMEN COLLECTION, STORAGE, AND TRANSPORTATION:

4.5.1 COLLECTION DAYS AND TIMINGS

- Specimen collection is to be done between 11.00 A.M to 1:00 PM on SACEP day as decided by the centre.

- In case the specimen collection day is a holiday, specimen collection is to be posted on an alternate day with prior arrangement with the receiving laboratory.
- Do not collect specimen if next day to SACEP is a holiday (as specimens have to be processed for HIV-1 PVL by the receiving laboratory within 24 hours of collection)
- Previous arrangement with testing centre to be made in case the specimen is to be collected on a day not scheduled for the purpose (Collection and transport of specimen)

4.5.2 SPECIMEN COLLECTION

- Standard work precautions are to be followed stringently
- Page 1 of the VL-1 form (as per **Annex VII**) is to be filled mandatorily in **duplicate**/ photocopy. (Specimens accompanied with incomplete forms will be rejected)
- Confirm information on VL-1 form (patient's name, registration/accession number, test needed, date and time of collection, and physician's/clinic's name, etc) mandatorily before collection of specimens
- Sterile EDTA (lavender top) evacuated blood collection tubes are to be used.
- The blood collection tubes are to be **labeled** (cryolabel) with patient's name, registration/ accession number, test needed, date and time of collection, and physician's/clinic's name. The information on the form should match the information on the specimen collection tube
- 4ml blood is to be collected and placed in prescribed sterile tubes using EDTA (lavender top) as the anticoagulant. *Do not collect blood in heparin vials*. (The choice of anticoagulant used in blood collection tubes can significantly alter viral load results, by affecting either the virion decay rate *ex vivo* or the detection by the assay type used. Plasma treated with sodium heparin is not appropriate for PCR assays because heparin is a potent inhibitor of PCR)

In case the linked HIV-1 PVL laboratory is in a different city, Dried Blood Spot (DBS) Preparation and Storage (as per Section 4.13) and then plasma separation (as per below) are to be performed by the ART Centre itself.

In case the linked laboratory is in the same city or same hospital, DBS Preparation and Storage (as per Section 4.13) and then plasma separation (as per below) are to be performed by the PVL laboratory within 6 hours of receipt of specimen.

4.5.3 PACKAGING AND TRANSPORTATION

- All specimens will be transported by hand by lab technicians of the centre of excellence

- The specimen is to be packaged carefully to protect from breakage, and leakage and insulated to protect from extreme temperature. Cool packs are to be used to maintain temperature of 2-8^{0}C. Ensure whole blood does not freeze during transportation.
- For packaging, the tube containing the specimen is placed in a leak proof container (e.g. a sealed plastic bag).
- The cool packs are to be placed around and the package is to be placed inside a puncture proof container with sufficient material to absorb all the contents in case the tube breaks or leaks.
- Cap the container tightly.
- Place the VL-1 form in an envelope and fasten securely to the outside of the container.
- A biohazard label should be pasted on the visible outer surface of the package containing the specimens.
- In case the laboratory is located in the same city: The Lab technician of the ART centre should transport the specimen with the VL-1 form in duplicate and ensure delivery to the testing lab of the whole blood specimen at 2-8^{o}C within 3 hours of collection (i.e. by 2.00 P.M. on Monday/Tuesday afternoon).
- In case the laboratory is located in a different the city: The Lab technician of the ART centre should transport the ***plasma*** specimen (First having prepared, packaged and stored DBS; and then separated the plasma at the ART centre) with the VL-1 form in duplicate and ensure delivery to the testing lab of the plasma specimen at 2-8^{o}C within 24 hours after collection (i.e. by 10AM. on Tuesday/Wednesday).
- The technician from the centre of excellence carrying the specimens must participate with the technicians at the PVL laboratory in the processes of estimation of HIV-1 PVL in order to learn the techniques involved.

The DBS samples will be collected and stored as per section 4.13. It is recommended that the DBS samples be stored at 2-8^{o}C for 20 days and then be couriered to NARI, Pune along with the consent forms. It is important to note that the consent form must be sent to the lab making DBS in case of intercity linkages for sending to NARI, Pune. The details on the envelope may be as follows:

For Second Line roll out

Dr R Paranjape
National AIDS Research Institute (NARI)
73 G Block, MIDC
Bhosari, Pune-411026
Maharashtra, India

4.5.4 RECEIVING SPECIMEN AT THE HIV-1 PVL TESTING LABORATORY

Receiving lab to identify the specimen properly. If there is discrepancy in the test requisition form versus the labeled tube, *DO NOT PROCEED*. Take corrective action to ensure that the patient's name and number on the request form are correct. In case of any confusion check back with the collection site.

- Receiving lab to check and thereby ensure at the time of specimen receipt that the temperature of the specimen never exceeded 8°C and the whole blood specimen was not frozen, during storage at the collection site and transport to the testing site. Leakage is also checked for.
- In case, of any doubt send back the specimen and VL-1 form back to the COE, duly signed by lab in-charge, with the COE technician. Another specimen must be collected for PVL.
- The receipt of the specimens to be duly documented on both the copies of the VL-1.
- In case specimen is rejected, one copy of the VL-1 form with signatures of lab in charge to be sent back with the center of excellence technician by hand on the same day.
- Receiving lab to record the time/date of specimen receipt.
- **Do not freeze whole blood.** Do not store whole blood for more than 6 hours after collection-even at temperature range of 2-25° C. Plasma at the receiving lab has to be separated from the whole blood within 2 hours of specimen receipt, within 6 hours of specimen collection (i.e. by 4.00 P.M. on the same day Monday/Tuesday).

4.5.5 PROCESSING OF THE WHOLE BLOOD SPECIMEN IN THE RECEIVING LAB:

- After preparation of DBS, the remaining whole blood specimen is be centrifuged for separation of plasma as detailed below and processed further for estimation of HIV-1 PVL as per the instructions of the manufacturer of the kit being used.

- Separation of plasma from whole blood:
    - Centrifuge whole blood at 800 -1600 x g for 10 minutes at room temperature.
    - Remove the plasma and recentrifuge at 800 x g for another 10 minutes.
    - Aliquot and store 800-900µl of plasma in a sterile 2 ml polypropylene screw-capped tube.
    - In case of inadvertent delay, plasma specimen to be separated and stored at 2-8°C overnight and transported to the testing site next morning at 2-8°C for performance of the test on the same day (to be processed for PVL within 24 hours after collection).
    - Plasma specimen is to be kept at 2-8°C till processed.

- Receiving lab must process the specimen within 24 hours after specimen collection.
- The plasma specimen must be brought to ambient temperature before performing the test as per the manufacturer's protocol.

o Perform PVL assay as per manufacturers protocol on the available platform

4.6 FACTORS TO BE CONSIDERED WHILE INTERPRETING THE VIRAL LOAD RESULTS:

The HIV-1 PVL quantification assay is influenced by many factors. Thus the interpretation of absolute viral concentration measurement results is not straightforward. One important issue to consider is whether measured change in viral load actually reflects a biological event, or whether the change is within the variability limit of the assay. Repeat tests of the same blood specimen can give results that vary by a factor of 3. This means that a meaningful change would be a drop to **less than 1/3** or an increase to **more than 3 times** the previous viral load result. For example, a change from 200,000 to 600,000 is within the normal variability of the test. A drop from 50,000 to 10,000 would be significant. However, the most important change in a patient responding well/optimally to ART is reaching an undetectable viral load level.

There is a considerable variation in the results of various types of assays used in quantification of the same specimen but if performed proficiently, a commercial assay shows reproducibility within approximately 0.2-0.5 $\log_{10}$, (varying in different regions of the assays' dynamic range). Daily variation in viral loads among clinically stable patients is minimal at approximately 0.4 $\log_{10}$. Therefore a change in viral load, greater than 0.5 $\log_{10}$ RNA copies/ml (approximately 3-fold), exceeds assay and diurnal variations, and may be considered to represent true biological events, while changes of less than 0.5 $\log_{10}$ cannot be distinguished whether these are from random variability or a biological event. It is important to note that in the low end of the dynamic range, assay variability has greater impact on interpretation of absolute viral load change.

How are the changes in viral load measured?

Viral load changes are often described as "log" changes. This refers to scientific notation, which uses powers of 10. For example, a 2-log drop is a drop of 10^2 or 100 times. A drop from 60,000 to 600 would be a 2-log drop. Small changes of 10, 20, 30 copies are often not considered to be a significant change in viral load and can reflect normal viral "blips," not a change in treatment response.

What do the numbers mean?

It is not known how long a HIV positive patient would stay healthy with any particular viral load. All that is known so far is that lower PVL is better and seems to mean a longer, healthier life. The viral load should drop to reach less than 50 copies within 6 months of ART. Even when the HIV-1 viral load in a HIV positive is undetectable, the HIV virus can be passed on

to another person, although the risk is lower. There is no "safe" level of viral load.

4.7 LIMITATION OF VIRAL LOAD ASSAYS:

RNA assays used to measure PVL 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. Blood should not be taken for a viral load test within four weeks of any infection or immunization. Temporary increases in viral load have been seen in these instances. Also, the physician must review the patient's adherence to the ART regimen and should postpone testing if recent doses of ART have been missed that may cause rapid replication of HIV, *in vivo*. Such patients may already be experiencing viral rebound and their ART therapy could be incorrectly judged to be failing.
- A drawback to PVL testing is the high cost of assays and requirement of technical expertise.

4.8 STANDARDIZATION OF HIV-1 PLASMA VIRAL LOAD REPORTING:

Laboratory reports of viral load assays should be standardized, accurate and adequate for patient treatment and public health monitoring of the HIV infection and acquired immunodeficiency syndrome (AIDS) epidemic. To ensure test report comparability among laboratories, standard testing and reporting methods are needed; moreover, standardized results are needed for early detection of treatment failure and early access to patient care.

Required items to report

- The laboratory should completely fill out the report found in Annex VII (a to d), as per testing platform.
- It should be duly signed in the VL-1 reporting format (See Annex VII (a to d))
- It should be sent from the lab to the ART centre by courier by Saturday to reach the ART centre latest by Monday. The courier will be paid from the contingency grant.
- A copy of the report should be e-mailed to NACO by Saturday at drbbrewari@yahoo.com and labservices.naco@gmail.com

4.9 QUALITY ASSURANCE FOR HIV-1 PLASMA VIRAL LOAD TESTING:

Viral load testing is an integral part of the management of HIV disease. It is absolutely imperative to ensure the accuracy, precision and reproducibility of the test results by adhering to stringent Quality Assurance and Quality Control measures at all times.

Quality Assurance is defined as a set of planned and systematic activities to ensure adequate confidence that requirements for quality will be met.

Internal Quality Control refers to those measures that must be taken to ensure that the test is working, the technical aspects of the test procedure have been met/followed and the results produced are valid within the limitation of the test system used. Laboratories performing HIV-1 Viral load quantification need to use external quality control specimens in addition to the controls contained in test kits for validation of the result.

It is recommended that a high positive control, low positive control and a negative control that come with the test kits be included with each run. The copy number per ml for each positive result should fall within the range of values indicated in the package insert. The negative control should give a less than the lower detection limit result. If controls are not as expected, the run is not valid and is to be repeated. These controls should meet the prescribed regulatory requirements for such controls. It is good to have traceability to international reference standards. Use of above test controls, that are used to validate a test run and to quantitate HIV-I RNA, would however not validate the analytic testing process which may include testing problems related to pipetting, inadequate incubation or washing or variability in kit lot sensitivity.

4.9.1 INVALID TEST RUNS

When invalid positive or negative results are obtained on running internal controls, the run is declared invalid and the entire test procedure for all the specimens has to be repeated in another run by processing another aliquot of the original plasma specimens.

Flags and comments may be generated by the analyzer during the run. The operator must check the run printout(s) for flags and comments to verify that the run is valid.

With the exception of instrument failures subsequent to the denaturation of amplicons, an instrument failure during a test run as indicated by the system error messages also constitutes an invalid test run.

4.9.2 INTERNAL QUALITY CONTROL (ASSAY CONTROLS-PART OF THE SYSTEM)

- Controls are supplied by the manufacturer and are to be processed like patient specimens. A low positive, high positive and a negative control are supplied with the kit. Run controls to check accuracy and reproducibility.
- All quality control failures must be logged and corrective action completed before specimen analysis takes place.

- All reagents used must be logged onto log sheets with the date opened, expiry date and signature. Reagents discarded due to contamination, spillage, etc. must be logged in the appropriate log sheets.
- Reagents must be stored as recommended by the manufacturer and daily temperature logs should be maintained.
- Results should be entered onto Levy-Jennings plots to monitor any trends, shifts or bias in results.

4.9.3 EXTERNAL QUALITY ASSESSMENT

Every testing facility must be able to demonstrate and document its competence in performing the tests. External quality assessment (EQA) is an evaluation by an outside agency of the performance of a laboratory on specially supplied small panels of well characterized specimens. The objective is to achieve between-laboratory and between-method comparability.

4.9.4 OBJECTIVES OF EQAS

- The primary objective is to continually improve and maintain the high standard of the laboratory's performance,
- To continually institute a formal monitoring and evaluation programme,
- To continually promote the concept of quality assurance, quality control, and quality assessment in the laboratory,
- To assess the quality of service of the participating laboratory,
- To identify problems and take appropriate interventions for corrective actions,
- To encourage the implementation of good laboratory practices, and
- To provide teaching and training programs.

Participation in external quality assessment (EQA) is not a substitute to day to day internal quality control practices. EQA is designed to assess the integrity of the entire lab testing process. Even when all precautions have been taken to achieve accuracy and precision in the laboratory, errors may arise which may be detected by objective external assessment. The principle is that the assessed material (values are known) is sent from an international, national or regional centre to a large number of participating laboratories at regular designated intervals. The results produced by the participating laboratories are assessed for accuracy, precision and reproducibility in a confidential manner by the lab conducting EQA. The labs producing inaccurate results are revamped through training and troubleshooting.

The EQAS essentially contains the following components:

- Filling up questionnaire to understand the laboratory capabilities and additional requirements
- Training for viral load estimation procedure and participation in EQAS
- Processing of the specimens received under EQAS following the routine procedure of viral load estimation
- Submission of report to the EQA conducting laboratory
- Doing analysis of results, assessment of performance and trouble shooting

4.9.5 EQA FOR HIV-1 PLASMA VIRAL LOAD TESTING

The NACO designated laboratories that will initiate/perform HIV-1 PVL testing for Second line roll out are to participate in EQA being conducted by the centre listed below for the time being:

RCPA (Royal College of Pathologists Australasia) Quality Assurance Programme

RCPA Quality Assurance Programmes Pvt Ltd was established by the Royal College of Pathologists of Australasia in 1988 and is providing the EQA services to over 40 countries and 4,000 programs around the world. RCPA Quality Assurance Program Pvt Limited is a NATA (National Associated Testing Authority, Australia) accredited Proficiency Testing provider for HIV-I RNA Viral load and complies with the requirements of ILAC G13. The details for EQA are given at Annex- VIII

4.10 OPERATIONAL PLAN FOR HIV-1 VIRAL LOAD TESTING AND EQAS

It is proposed that HIV-1 viral load testing facilities for the National HIV/AIDS care and treatment programme would initially be set up at select centers. The NACO labs that have been identified to initiate and perform HIV-1 PVL testing for specimens being sent by the NACO identified Second line centres are:

- Kasturba Hospital for Infectious Diseases, Mumbai
- Tuberculosis Research Centre (TRC), Chennai
- Institute of Human Behavior and Allied Sciences (IHBAS), New Delhi
- National Institute of Cholera and Enteric Diseases (NICED), Kolkata
- Gandhi Hospital and Medical College, Hyderabad

- St John's Hospital, Bangalore
- Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh
- Institute of Medical Sciences (BHU), Varanasi
- Biramjee Jeejeebhoy Medical College (BJMC), Ahmedabad
- Regional Institute of Medical Sciences (RIMS), Imphal and
- National AIDS Research Institute (NARI), Pune

NACO has created the following interim linkages until all the designated HIV-1 PVL testing centres become operational:

The above linked centres, both the COEs and the testing labs, should coordinate their collection days and times so that viral load specimens that are being sent from different centres to the same lab can be coordinated to be processed together on the same day, ensuring the efficient use of viral load reagents and laboratory time.

It is proposed to identify the eligible patients after a detailed examination by the SACEP. The SACEP meeting will be held on Monday/Tuesday (or any designated day) morning between 9:00AM to 11:00AM depending on the convenience of the institution. Eligible candidates will be subjected to HIV-1 PVL testing before SACEP takes the decision to initiate Second line ART.

Blood specimen (4ml) will be collected in an EDTA vacuum evacuated tube immediately after SACEP meeting and processed as given below:

- In case the PVL testing lab is in the same city as the COE: whole blood specimen is to be transported to the PVL testing lab at 2-8^{o}C within a maximum of 6 hours after collection
- In case the PVL testing lab is in a different city as the COE: the laboratory technician at the COE will prepare, package, and store the DBS (Section 4.13) and separate the plasma (as detailed above) within 2 hours after specimen collection. The plasma specimen will be stored at 2-8^{o}C until transported by the COE technician. The specimen will be hand carried by the COE technician from COE to the PVL testing laboratory as detailed above. The COE must ensure that the lab technician carrying the specimens must leave the COE that same day.
- Frequency and processing of specimen and transport details will be as detailed in Table 1
- Once the specimens have been received at the PVL testing lab, estimation of HIV-1 PVL must be done within 24 hours after the specimen was collected.
- The PVL testing lab must courier back the test reports to the corresponding COE by the weekend. Cost of the same will be met from contingency grant.

To ensure the quality of the testing on a daily basis and to ensure that all labs meet

international testing standards, these centers would participate in EQAS for HIV-1 viral load testing. An attempt is being made to provide EQAS specimens to all the NACO identified testing labs for HIV-1 PVL testing. All labs must participate and provide necessary cooperation for implementing the same.

The details of the EQAS process flow have been annexed at the end of the laboratory guidelines for VL testing.

4.11 PRECAUTIONS AND SPECIFIC INSTRUCTIONS FOR REAGENTS AND EQUIPMENTS:

4.11.1 GENERAL

- Viral load assay is for *in vitro measurement of plasma HIV-1 RNA copies and is not for* diagnostic use
- Treat all specimens as potentially infectious. Adhere to Standard Work Precautions when performing the assay.
- Only personnel trained in handling infectious material should perform this procedure
- Screw capped tubes must be used for processing specimens and controls to prevent splashing and potential cross-contamination of specimens or controls***. Do not use snap-cap tubes*****.** Handle all specimens or controls in accordance with the Good Lab Practices in order to prevent cross-contamination
- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents
- Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills of these reagents occur, dilute with water before wiping dry.
- Avoid contaminating gloves while manipulating specimens
- Specimens and controls should always be prepared in the laminar flow -failure to do so may result in specimen contamination
- Handle and manipulate specimens in a Class II Biological Safety Cabinet
- Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite and follow by wiping down the surface with 70% ethanol.
- Any deviations from the specified procedures and guidelines may affect optimal assay performance.

- This test is for use with human plasma collected in EDTA anticoagulants only. ***Heparin has been shown to inhibit PCR and must not be used with this procedure.***

4.11.2 REAGENTS STORAGE AND USE

- Store reagents strictly as per manufacturers specific reagent storage recommendations
- Visually inspect each reagent bottle before use to ensure that there are no signs of leakage and or abnormal colour.
- Do not use a kit after expiry date. DO NOT interchange, mix or combine reagents from kits with different master lot numbers. Ensure that all reagents used are of the same master lot of reagents.
- Add all reagents using a pipette capable of accurately delivering specified volumes.
- Regularly calibrate pipettes for accurate delivery and maintain logs
- Avoid microbial and ribonuclease contamination of reagents when removing aliquots from reagent bottles. The use of sterile disposable pipettes and RNase-free pipette tips is recommended.
- Do not freeze reagents or controls
- Do not pool reagents from different lots or from different bottles of the same lot.

4.11.3 EQUIPMENT

- Perform manufacturer recommended maintenance and calibration of all equipment, including pipettes to ensure proper functioning.

4.11.4 WORK AREAS

To minimize the possibility of lab areas becoming contaminated with the amplicon, the lab area should be separated into several distinct areas organized around the pre-amplification (separate reagent and specimen preparation areas) and post-amplification (Amplification and Detection) areas. Personnel should use proper anti-contamination safeguards when moving between areas.

- Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Pre-Amplification Area and moving to the Post-Amplification (Amplification and Detection) Area.
- Pipettes and other supplies should be dedicated to a specific area. Specimens, equipment and reagents should not be returned to the area where a previous step was being performed and should not be used for other activities or moved between areas

- Pre-amplification activities must begin with reagent preparation and proceed to specimen preparation.
- Supplies and equipment must be dedicated to each pre-amplification activity and not used for other activities or moved between areas. Equipment and supplies used for reagent preparation must not be used for specimen preparation activities or for pipetting or processing amplified DNA or other sources of target DNA
- Gloves must be worn separately in each area and must be changed before leaving that area.
- The pre-amplification area should have a template free area for preparation of reagents and an amplicon free area for specimen and control preparation
- Post-amplification supplies and equipment must remain in the Post-Amplification Area at all times
- The post-amplification area should have analyzer(s) and Data station(s) with additional area for preparing Working Amplification and Detection reagents.

4.12 TROUBLE SHOOTING:

- While performing the viral loads in the lab, there will be occasions when things may go wrong. The problems could occur because of mechanical, chemical or human error. Staff should be trained to recognize when there is a problem and how to correct them so that the final patient results sent out are not affected. Please follow the manufacturer's instructions for troubleshooting

Note: Adhere to standard work precautions and PEP as per NACO HIV testing. Keep eye splashers, body showers and supply of running tap water within vicinity of the working lab

4.13 DBS COLLECTION FOR ALL PATIENTS UNDERGOING VIRAL LOAD TESTING

The national programme will collect DBS samples from all patients who are sent for Viral load. The consent form is as annex IV (integrated consent for 2nd line ART as well as for collection of blood for storage)

The purpose of this collection and storage of blood on Dried Blood Spots (DBS) from patients undergoing evaluation for Second-line ART in India, is to subsequently conduct HIV genotypic resistance testing on these specimens to evaluate the patterns of HIV drug resistance that have developed among patients who have developed treatment failure to the standard First-line ART regimen. Clinical and laboratory data will be abstracted from patient records to further analyze and correlate HIVDR findings with virologic, immunologic, and clinical outcomes of patients receiving Second-line treatment. Overall, the findings of these analyses can be used to guide the expansion of ART services, and specifically Second-line ART services, in India.

The objective of the analysis will be:

1. To determine the patterns of HIV-DR that are present among patients who have developed treatment failure to the standard First-line regimen,

2. To determine if HIV-DR patterns at therapy switch have an impact on virologic suppression at 6 months and/or 12 months among patients who receive Second-line therapy, and

3. To evaluate the clinical, immunologic, and survival outcomes of patients who developed failure to First-line ART at 12 and 24 months. This analysis can be stratified by HIV RNA at the time of initial Second-line evaluation (<400 copies/mL, 401-5,000 copies/mL, >5,000 copies/mL)

4.13.1 SPECIMEN COLLECTION, PROCESSING, AND STORAGE:

Blood is routinely collected from patients at the time of evaluation for Second-line ART at the SACEP (COE) for HIV RNA, chemistry panel, liver function tests, and complete blood count. At this time, a dried blood specimen (DBS) will also be collected.

Specimen collection:

DBS should only be made from patient's blood tubes that have been specifically labelled or marked as eligible. Before the DBS is made, the DBS-ID and ART-ID for the participant should be written on the filter paper card. Anti-coagulated blood should be spotted onto filter paper within 24 hours of collection. The filter paper should be handled only at the edge; the areas that will be used to collect specimens should not be touched. A filter should only be spotted with the blood of a single patient.

For recently collected, fresh whole blood, invert the blood collection tube 2-3 times to mix the whole blood. Carefully open the blood collection tube. Use a pipette (with disposable tip) to aspirate 100 µl of whole blood and apply it to the centre of one pre-printed circle to fully saturate the circle.Repeat this procedure to fill each circle on the card with blood. For each specimen at least **four** saturated circles should be obtained. Opening of the tubes and pipetting should be performed following standard laboratory biosafety precautions.

Specimen drying:

Avoid touching the part of the card with the blood spot. . Dry all specimen cards at least 4 hours at ambient temperature in a suspended horizontal position. Depending on the climate it might be necessary to allow spots to dry over night. Do not use oven to fasten the drying time of the cards. When dry, the spots should be a uniform dark brown. The appearance should be similar to that of a dried bloodstain and no areas of red coloration should be seen.

Specimen packaging:

Make sure the DBS or DPS are completely dry before packing. Packaging of each DBS card into a separate gas-impermeable zip-lock bag with 2-3 desiccant packs (to remove any residual moisture from the cards) per bag and a humidity indicator card (to indicate the relative humidity inside the bag) is recommended. When packing, make sure that the humidity indicator cards are faced outside. Place the front of the humidity indicator card facing outside so that the markings are clearly visible. Press the bag with both hands to squeeze out the air from the bag and then seal it. Place 5-10 of the above small bags into a large plastic bag. In the large plastic bag, also place a printed manifest with specimen information. Plastic or foil bags used for storage must be gas impermeable to keep the contents of the bag humidity-free. Bags available from grocery stores or other outlets that do not sell scientific supplies are inadequate.

Humidity indicator cards and desiccant packs have a color indicator which changes from blue to pink as humidity increases. All humidity indicator and desiccants should be replaced with fresh material before they have all changed to a pink colour. To ensure proper packaging of the DBS cards, the humidity indicator card should be examined once a week if the sample is kept at room temperature. Before placing desiccant packs into a zip-lock bag with DBS, make sure desiccant packs have remained dry during storage (indicator card should show blue color). When an indicator is beginning to change, it is time to change the humidity indicator and desiccant packs. Desiccant packs can become moist after use with DBS, but also after storage in a humid environment. Store desiccant packs with humidity indicator cards to evaluate whether their moisture level has become too high. Humidity cards and desiccant packs can be re-used. Moist humidity indicator cards and desiccant packs should be dried in a 65°C oven over night until the colour indicator returns to blue. Remove from the oven and store in a sealed bag with a humidity indicator card until reuse or until they once again need to be dried in the oven.

Specimen storage:

For short-term storage (preferably two weeks maximum, but no more than 30 days) at the collecting sites, DBS can be kept in the gas impermeable zip-lock bags with desiccants and stored at room temperature. DBS held at room temperature should be stored in a box or container so that direct light will not damage them. DBS should be examined frequently (e.g., weekly) to evaluate whether the 30% circle in the humidity indicator card has changed to a pink color; when it does, the desiccants must be changed immediately.

DBS can be kept at room temperature or at 4°C *only* for short term storage (<30 days). DBS should not be frozen at the collection site unless definite arrangements can be guaranteed to maintain them in a frozen state until they reach the genotyping laboratory. In settings where constant refrigeration may not be possible because of frequent electricity outages, or where high humidity is likely within the available refrigerator/freezer, it is preferable to hold the DBS at room temperature. If possible, DBS should not remain at a collecting site with limited storage conditions for more than 7 days before being transported to a laboratory with a constant electricity supply and a refrigerator or freezer in which the humidity has

been evaluated and confirmed as suitable for long-term storage of DBS.

For long term storage (>30 days), DBS should be transported to a central facility where there is a constant electricity supply in a freezer at **20°C or below** that has been evaluated and confirmed as suitable for long term storage of DBS. If frozen, DBS should only be taken out of cold storage when they are being transported to a reference laboratory or tested.

Specimen Transport

DBS should be transported to the regional or national genotyping laboratory using the quickest and reliable arrangements. Unless humidity at the blood draw site is substantially higher than in the processing laboratory, and provided suitable storage boxes are available at the site to keep DBS from light and contamination, no special arrangements need be made to transport DBS more often than weekly.

For specimens that have been stored at room temperature: The desiccants in the specimens bags should be changed before transport for DBS specimens that have been stored for longer than 7 days at the collection site. This should be done even if the indicator remains blue. Reseal the bag and transport specimens by the fastest means using courier service or through the postal system (preferably with expedited service and a guaranteed delivery) at room temperature.

For specimens that have been stored at 4ºC: Remove the bagged specimens from the refrigerator and allow them to reach room temperature before opening the bag. Once the sealed bag has equilibrated, open it and remove the old desiccants. Add fresh desiccants and reseal the bag. Transport the bag by the fastest means. If a cooler is available for transport this will protect specimens from short periods of high temperature.

For specimens have been frozen at -20ºC or -70ºC: These specimens should be transported on dry ice or liquid nitrogen. Thawing of frozen DBS specimens should be avoided if possible. A cooler is not sufficient to maintain them in a frozen state.

All DBS specimens should be logged into the survey system (whether it is a notebook or a computer software package). The log should include notes on specimen quality and packaging.

The logbook should include a record of eligible specimens for which there is no DBS material available to be sent to the genotyping laboratory, and the reason. An acknowledgement or notification system should be set up involving the survey coordinator, the transport system, and the receiving genotyping laboratory, to ensure all DBS are delivered promptly to genotyping lab and arrive in good condition. Either email or fax notification using the shipping manifests may be used for this purpose. DBS should be re-examined for packaging and specimen quality on arrival in the genotyping laboratory and recorded in the genotyping laboratory.

Every ART centre shall be linked to a VL lab for which instructions will be given by NACO to send the DBS filter paper to the corresponding HIV drug resistance genotyping national reference labs.

ANNEXURE

ANNEX I:

SEVERITY GRADING OF SELECTED CLINICAL AND LABORATORY TOXICITIES

(source: Division of AIDS, National Institute of Allergy and Infectious Diseases, USA – Modifies,)

For abnormalities NOT found elsewhere in the toxicity table use the scale to estimate grades of toxicity.

GRADE 1 Transient or mild discomfort; no limitation of activity; no medical intervention/ therapy required.

GRADE 2 Mild to moderate limitation of activity; some assistance may be needed; no or minimal medical intervention/therapy required.

GRADE 3 Marked limitation of activity; some assistance usually required; medical intervention/therapy required; hospitalization possible.

GRADE 4 Extreme limitation of activity; significant assistance required; significant medical intervention/therapy required; hospitalization or hospice care.

HAEMATOLOGY	GRADE 1	GRADE 2	GRADE 3	GRADE 4
Haemoglobin	8.0 – 9.4 g/dl OR 80-94 g/l OR 4.93 – 5.83 mmol/l	7.0 – 7.9 g/dl OR 70-79 g/l OR 4.31 – 4.92 mmol/l	6.5 – 6.9 g/dl OR 65 - 69 g/l OR 4.03 – 4.30 mmol/l	<6.5 g/dl OR <65 g/l OR <4.03 mmol/l
Absolute neutrophil count	1000 – 1500/ mm^3 OR 1.0 – 1.5/G/1*	750 – 999/mm^3 OR 0.75 – .99/ G/1*	500 – 749/mm^3 OR 0.5 – 0.749/ G/1*	<500/mm^3 OR <0.5/G/1*
Platelets	75000 – 99000/ mm^3 OR 75 – 99/G/1*	50000 – 74999/ mm^3 OR 50 – 74.9/G/1*	20000 – 49999/ mm^3 OR 20 – 49.9/G/1*	<20000/mm^3 OR <20/G/1*

CHEMISTRIES	GRADE 1	GRADE 2	GRADE 3	GRADE 4
SODIUM				
Hyponatraemia	130 – 135 meq/l OR 130 – 135 mmol/l	123 – 129 meq/l OR 123 – 129 mmol/l	116 – 122 meq/l OR 116 – 122 mmol/l	<166 meq/l OR <116 mmol/l
Hypernatraemia	146 – 150 meq/l OR 146 – 150 mmol/l	151 – 157 meq/l OR 151 – 157 mmol/l	158 – 165 meq/l OR 158 – 165 mmol/l	>165 meq/l OR >165 mmol/l
POTASSIUM				
Hyperkalaemia	5.6 – 6.0 meq/l OR 5.6 – 6.0 mmol/l	6.1 – 6.5 meq/l OR 6.1 – 6.5 mmol/l	6.6 – 7.0 meq/l OR 6.6 – 7.0 mmol/l	>7.0 meq/l OR >7.0 mmol/l
Hypernatraemia	3.0 – 3.4 meq/l OR 3.0 – 3.4 mmol/l	2.5 – 2.9 meq/l OR 2.5 – 2.9 mmol/l	2.0 – 2.4 meq/l OR 2.0 – 2.4 mmol/l	<2.0 meq/l OR <2.0 mmol/l
BILIRUBIN				
Hyperbilirubin-armia	>1.0 – 1.5 x ULN	>1.5 – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 x ULN
GLUCOSE				
Hypoglycaemia	55 – 64 mg/dl OR 3.01 – 3.55 mmol/l	40 – 54 mg/dl OR 2.19 – 3.00 mmol/l	30 – 39 mg/dl OR 1.67 – 2.18 mmol/l	<30 mg/dl OR <1.67 mmol/l
Hyperglycaemia (nonfasting and no prior diabetes)	116 – 160 mg/dl OR 6.44 – 8.90 mmol/l	161 – 250 mg/dl OR 8.91 – 13.88 mmol/l	251 – 500 mg/dl OR 13.89 – 27.76 mmol/l	<500 mg/dl OR <27.76 mmol/l
Triglycerides	200 – 399 mg/dl OR 2.25 – 4.51 mmol/l	400 – 750 mg/dl OR 4.52 – 8.47 mmol/l	751 – 1200 mg/dl OR 8.48 – 13.55 mmol/l	<1200 mg/dl OR <13.55 mmol/l
creatinine	>1.0 – 1.5 x ULN	>1.5 – 3.0 x ULN	>3.0 – 6.0 x ULN	>6.0 x ULN
TRANSAMINASES				
AST (SGOT)	1.25 – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 10.0 x ULN	>10.0 x ULN
ALT (SGPT)	1.25 – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 10.0 x ULN	>10.0 x ULN
GGT	1.25 – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 10.0 x ULN	>10.0 x ULN
Alkaline Phosphatase	1.25 – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 10.0 x ULN	>10.0 x ULN
Pancreatic enzymes				
Amylase	>1.0 – 1.5 x ULN	>1.5 – 2.0 x ULN	>2.0– 5.0 x ULN	>5.0 x ULN
Pancreatic amylase	>1.0 – 1.5 x ULN	>1.5 – 2.0 x ULN	>2.0– 5.0 x ULN	>5.0 x ULN

CHEMISTRIES	GRADE 1	GRADE 2	GRADE 3	GRADE 4
TRANSAMINASES				
Lipase	>1.0 – 1.5 x ULN	>1.5 – 2.0 x ULN	>2.0– 5.0 x ULN	>5.0 x ULN
Lactate	<2.0 x ULN without acidosis	>2.0 x ULN without acidosis	Increased lactate with pH <7.3 without life- threatening consequences	Increased lactate with pH <7.3 with life- threatening consequences
GASTRO- INTESTINAL	**GRADE 1**	**GRADE 2**	**GRADE 3**	**GRADE 4**
Nausea	Mild OR transient; reasonable intake maintained	Moderate discomfort OR intake decreased for <3 days	Severe discomfort OR minimal intake for ≥3 days	Hospitalization required
Vomiting	Mild OR transient; 2 – 3 episodes per day OR mild vomiting lasting < 1 week	Moderate OR persistent; 4 – 5 episodes per day OR vomiting lasting ≥1 week	Severe vomiting of all foods/ fluids in 24 hours OR orthostatic hypotension OR intravenous Rx required	Hypotensive shock OR hospitalization for intravenous Rx required
Diarrhoea	Mild OR transient; 3-4 loose stools per day OR mild diarrhea lasting < 1 week	Moderate OR persistent; 5 - 7 loose stools per day OR diarrhea lasting ≥ 1 week	Bloody diarrhea OR orthostatic hypotension OR >7 loose stools/ day OR intravenous Rx required	Hypotensive shocks OR hospitalization required
RESPIRATORY	**GRADE 1**	**GRADE 2**	**GRADE 3**	**GRADE 4**
Dyspnoea	Dyspnoea on exertion	Dyspnoea with normal activity	Dyspnoea at rest	Dyspnoea requiring O^2 therapy
URINALYSIS	**GRADE 1**	**GRADE 2**	**GRADE 3**	**GRADE 4**
Proteinuria				
Spot urine	1+	2+ or 3+	4+	Nephrotic syndrome
24 – hours urine	200 mg to 1 g loss/day OR <0.3% OR <3 g/l	1 g to 2 g loss/ day OR 0.3% to 1.0% OR 3 g to 10 g/l	2 g to 3.5 g loss/ day OR >1.0% OR >10 g/l	Nephrotic syndrome OR >3.5 g loss/day
Gross haematuria	Microscopic only	Gross, no clots	Gross plus clots	Obstructive

MISCELLANEOUS	GRADE 1	GRADE 2	GRADE 3	GRADE 4
Fever (oral, >12 hours	37.7 – 38.5 °C OR 100.0 – 101.5 °F	38.6 – 39.5 °C OR 101.6 – 102.9 °F	39.6 – 40.5 °C OR 103 – 105 °F	>40.5 °C OR >105 °F for ≥ continuous hours
Headache	Mild; no Rx required	Moderate OR non-narcotic analgesia Rx	Servere OR responds to initial narcotic Rx	Intracable
Rash hypersesnitivity	Erythema, pruitus	Diffuse maculopapular rash OR dry desquamation	Vasiculation OR moist desquamation OR ulceration	ANY ONE OF: muscous membrane involvement, suspected Stevens-Johnson (TEN), erythema multiforme, exfoliative dermatistis
Fatigue	Normal activity reduce by <25%	Normal activity reduce by <25 – 50%	Normal activity reduce by >50%: cannot work	Unable to care for self

ANNEX II:

Request/Reply form to SACEP for review of patients suspected of treatment failure (to be sent with patient records)

RRF: Request form to SACEP at COE /pCoE/ART plus Centre for Review

Dear Dr ……. **Referral date…….**

Centre of Excellence /ART plus Centre ……

I would like to refer this patient for review by the SACEP for

☐ **Alternative First-line ARV ART drugs**

☐ **Suspect Treatment Failure**

☐ **Others (specify)…….**

Name…….. Age-……
Sex……

Name of the Care giver ……

Address & Phone no. ……

……

……

ART centre name & phone no. ……

Contact person at ART centre&mobile no.

……

Name & Contact no. of Linked NGO/CCC /DLN:

The following are attached with this request form:

- Photocopy of the Patient treatment record(White Card)
- Photocopy of all lab tests including CD4
- Photocopy of all other relevant material
- Photo documentation of toxicity (If available)
- Address proof with photo

The following sections summarize the patient antiretroviral therapy history:

A: Summary of the case history of the patient (pre-ART; ART; suspected treatment failure/suspected ARV toxicity)

……

B. Summary of adherence history and other psycho-social issues

……

C. Summary of relevant laboratory tests including CD4 (of last 1 month) /viral load (if available)

……

Name and Signature of Nodal Officer of referring

ART centre with contact number & email

Reply from SACEP at CoE/pCoE/ART plus Centre to Referring ART centre (After review)

Dear Dr …… Date……..

ART centre……..

Patient name……Gender/Age……..

Address…….. SACEP registration no. ……

Referred for……on Date…….. ……

Findings/investigation results:……

……

……

Treatment/follow-up Plan:……

……

Name and Signature of Nodal Officer

COE/ART plus with contact number & e-mail

ANNEX III:

CF Consent form for patients starting Second line ART – at COE

Consent form for patients starting Second line ART

I, (name)……, (address) …… CONSENT to share all information pertaining to my health and HIV/AIDS status with the service providers who will be part of the management of my condition.

And

I AGREE to receive the Second line antiretroviral therapy.

I fully understand the information that has been provided by the health care staff in the following:

- That Second line ART is not an emergency and thus will be started as per the decision of the doctor .I shall attend the ART centre as per appointment for timely initiation of ART and regular follow up
- That receiving Second line ART involves shared confidentiality with other service providers such as CBO/NGO/CCC/positive network who may conduct outreach and home-based care activities at home
- That Second line ART requires 100% adherence to drugsand I shall abide by the same.
- That there is no third line ART available in the programme
- That I understand the side effects of Second line ART
- That I shall not stop the drugs on my own and will return to the centre if there is any problem
- That the national programme shall collect and store my blood to find out if the ART medicines are working against the HIV at a later date (Drug resistance testing etc). This will not affect my current treatment. This will help the doctors to improve the care and treatment of all patients undergoing treatment at this centre, and possibly at other centres in the country.

…… ……

Signature of witness Signature of patient with date

(Doctor/nurse/counselor)

(This should be translated in local language &/or explained for patient understanding before taking patients signature)

ANNEX IV:

Drug information on Second line ARVs

LPV/r drug-drug interactions

Class	Protease inhibitor (PI)
NACO Formulation	Heat stable tablet: 200 mg LPV + 50 mg RTV
Contraindication	LPV/r is contraindicated in patients with known hypersensitivity to LPV or RTV
Safety in pregnancy	No data on LPV/r in pregnant women. LPV/r should not be used during pregnancy and breastfeeding.
Precautions	Hepatic impairment – avoid if severe renal impairment, pregnancy; breastfeeding.
Food	Should be taken with food
Interactions	If ddI or antacids are administered, they should be taken at least 1 hour apart LPV should not be taken with these drugs: amiodarone, astemizole, cisapride, ergotamine and similar alkaloids, flecanide, garlic supplements, lovastatin,midazolam, pimozide, propadenone, rifamipicin, simvastatin, St John's wort, terfenadine and triazolam Rifamipicin should not be used in combination with LPV/r because co-administration may cause large decreases in LPV concentrations. LPV levels are increased by delavirdine and Ritonavir (RTV) LPV levels are decreased by amprenavir, carbamazepine, dexamethasone, Efavirenz, ketoconazole, nevirapine, Phenobarbital, St John's wort, phenytoin, rifamipicin and TDF. LPV increases the levels of amiodaron, amprenavir, atorvastatin, bepridil, calcium channel blockers, clarithromycin, ketoconazole, indinavir, itraconazole, lidocaine (systemic), quinidine, rifabutin, saquinavir, sildenafil and TDF. LPV decreases the levels of amprenavir, atovaquone and methadone. LPV has potential interactions with anticonvulsants, statins, oral contraceptives, tricyclic antidepressants, oral anticoagulants and immunosuppressants.

Adverse effects	GI-related: diarrhoea, nausea, vomiting, colitis, abdominal discomfort, asthenia, headache, insomnia, rash. Less frequently: drug mouth, hepatic dysfunction, pancreatitis, dyspepsia, dysphagia, oesophagitis, influenza-like syndrome, appetite changes, hypertension, palpitations, thrombophlebitis, vascultitis, chest pain, dyspnoea, agitation, anxiety, ataxia, hypertonia, confusion, depression, dizziness, dyskinesia, parasthesia, peripheral neuritis, somnolence; Cushing syndrome, hypothyroidism, sexual dysfunction, anaemia, leucopenia, dehydration, oedema, lactic acidosis; arthralgia, myalgia, abnormal vision, otitis media, taste disturbances, tinnitus, acne, alopecia, drug skin, pruritis, skin discoloration, nail disorders, sweating; Lipodystrophy and metabolic effects, raised bilirubin and lowered sodium,low platelet and low neutrophil counts also reported in children.
Storage	Room temperature (below 30 degrees)

Lamivudine (3TC)

Class	Nucleoside reverse transcriptase inhibitor (NsRTI)
NACO Formulation	Combined as fixed dose combination as TDF/3TC at dose of 300 mg once a day
Contraindication	Known sensitivity to 3TC
Safety in pregnancy	Limited data available on safety of 3TC in human pregnancy
Precautions	Renal impairment Hepatic impairment: potentially life-threatening lactic acidosis and severe hepatomegaly reported, caution in liver disease. Recurrent hepatitis may occur in patients with chronic hepatitis B infection on discontinuation of 3TC
Food	Can be taken with or without food
Interactions	Rare
Adverse effects	Nausea, vomiting, diarrhoea, abdominal pain, cough, headache, fatigue, insomnia, malaise, fever, rash, alopecia, muscle disorders, nasal symptoms; peripheral neuropathy reported; rarely pancreatitis; neutropenia, anaemia, thrombocytopenia; lactic acidosis/ hepatic steatosis; raised liver enzymes and serum amylase
Storage	Room temperature (15-30 degrees C)

ATV/r drug-drug interactions

Class	Protease inhibitor (PI)
NACO Formulation	Atazanavir 300mg and ritonavir 100mg
Contraindication	ATV/r is contraindicated in patients with known hypersensitivity to ATV or RTV
Safety in pregnancy	No data on ATV/r in pregnant women. However, its efficacy in pregnancy is less than LPV/r.
Precautions	Hepatic impairment – avoid if severe renal impairment.
Food	Should be taken with food
Interactions	In case of regimen IV/IVa 1. H2 receptor antagonist dose should not exceed a dose equivalent to Famotidine 40 mg BID in ART-naïve patients or 20 mg BID in ART-experienced patients. 2. Give ATV 300 mg + RTV 100 mg simultaneously with and/or >10 hours after the H2 receptor antagonist. ***Example:*** *If a PLHIV on Zidovudine + Lamivudine + Atazanavir/Ritonavir (Regimen IV) requires to be treated with Famotidine 20 mg BID or Ranitidine 150 mg BID, S/he should be instructed to take Tab. Famotidine/Ranitidine with Zidovudine + Lamivudine at 8.00 AM and in evening give ZL and ATV/r at 8 p.m. and ensure that there is a gap of at least 2 hours before or 1 hour after Famotidine/Ranitidine evening dose.* **In case of Second line Regimen V (Tenofovir + Lamivudine + Atazanavir/Ritonavir):** **1.** PPIs should not exceed the dose of Omeprazole 20 mg daily or equivalent dose of Esomeprazole 20mg/Pantoprazole 40 mg/Rabeprazole 20 mg in PI-naïve patients, along with Ritonavir boosted Atazanavir. PPIs should be administered at least 12 hours prior to Atazanavir/Ritonavir. **2. PPIs are not recommended in PI-experienced patients**. ***Example:*** *If a PLHIV is on Tenofovir + Lamivudine + Atazanavir/Ritonavir requires to be treated with PPI, S/he should be instructed to take Tab. Omeprazole 20 mg/Esomeprazole 20mg/Pantoprazole 40 mg/Rabeprazole 20 mg OD at 8 AM and Tenofovir + Lamivudine + Atazanavir/Ritonavir after 8 PM***.** **H2 receptor antagonists are not recommended with** Tenofovir + Lamivudine + Atazanavir/Ritonavir combination.

Adverse effects	Apart from the PI-class specific side-effects like hyperglycaemia, fat maldistribution, hyperlipidaemia (especially with Ritonavir boosting). The unique side-effects of Atazanavir include **indirect hyperbilirubinaemia (producing yellow discolouration of eyes),** skin rash, prolongation of PR interval and nephrolithiasis. Prolongation of P-R and Q-Tc interval in the ECG can occur. So PR interval need to be monitored in patients with known conduction defects or with concurrent use of other drugs that alter conduction abnormalities (like diltiazem, clarithromycin, cisapride, ketoconazole etc.). However, routine ECG before starting Atazanavir based ART is not recommended. Atazanavir induced urolithiasis is also reported; presumably due to precipitation of the drug resulting in crystalluria in a manner analogus to Indinavir.
Storage	Room temperature (below 30 degrees)

Rifabutin

Class	Anti-tuberculous agent
NACO Formulation	Tablet 150 mg Concurrent use with ATV/r (no change) , Rifabutin dose 150 mg once daily 3x/wk Rifabutin AUC ↑ by 303%
Contraindication	Allergy to rifampicin
Safety in pregnancy	Limited data in pregnancy. Not teratogenic in rats/rabbits
Precautions	Allergy to rifamipicin
Food	none
Interactions	Rifabutin reduces levels of warfarin, barbiturates, benzodiazepines, beta-blockers, chloramphenicol, clofibrate, oral contraceptives, corticosteroids, cyclosporine, diazepam, dapsone, digitalis, doxycycline, haloperidol, oral hypoglycaemics, ketoconazole, methadone, phenytoin, quinidine, theophylline, trimethoprim, verapamil. Drugs that inihibit cytochrome P450 and prolongs the half life of Rifabutin: PIs and Delavirdine, erythromycin, clarithromycin (56% increase), and azoles (fluconazole, itraconazole, ketoconazole).
Adverse effects	Common: brown-orange discoloration of secretions: urine, tears, saliva, sweat, stool, skin. Infrequent: Rash, GI intolerance, neutropenia. Rare: flu-like illness, hepatitis, hemolysis, headache, thrombocytopenia, myositis. Uveitis is dose-related (usually> 450 mg/day) or with standard 300mg/day combined with drugs that increase rifabutin levels (most PIs, clarithromycin, fluconazole)
Storage	Room temperature (15-30 degrees C)

.

ANNEX V:

Recommendations for Co administering Antiretroviral Drugs with RIFABUTIN – 2007

Non-nucleoside reverse-transcriptase inhibitors			
	Antiretroviral dose change	**Rifabutin dose change**	**Comments**
Efavirenz	No change	___ to 450-600 mg (daily or intermittent)	Rifabutin AUC ¯ by 38%. Effect of Efavirenz + protease inhibitor(s) on Rifabutin concentration has not been studied. Efavirenz should not be used during the 1st trimester of pregnancy.
Nevirapine	No change	No change (300 mg daily or thrice-weekly)	Rifabutin and Nevirapine AUC not significantly changed.
Dual protease inhibitor combinations			
	Antiretroviral dose change	**Rifabutin dose change**	**Comments**
Lopinavir/ ritonavir ä)	No change	___ to 150 mg every other day or 3x/week	Rifabutin AUC by 303%; 25-O-des-acetyl Rifabutin AUC by 47.5 fold.
Ritonavir (any dose) with saquinavir, indinavir, amprenavir, fosamprenavir, atazanavir, tipranavir or darunavir	No change	____ to 150 mg every other day or 3x/week	Rifabutin AUC and 25-O-des-acetyl rifabutin AUC , by varying degrees.

ANNEX VI:

***Treatment education information for Second line ART* patients**

REMEMBER THAT

- If you miss doses (even 2 dose in a month) **FURTHER DRUG RESISTANCE** will develop. This is bad for you as these Second line drugs will stop working.
- Drugs must be taken as prescribed with food, and do not miss any dose
- If you forget a dose, do not take a double dose.
- If you stop taking the ART, you will become ill within months.
- Do not share any of the drugs with your spouse, family or friends.
- If you find it difficult to take your pills, go back to the ART center and discuss this with the doctor and counselor. Ask for support from your treatment supporter, family, friends, NGO and positive network.

Remember : **Adherence is under your control**

Note: The color, shape and size of ARV drugs may be different due to different supplier each year.

It is common to have side effects. They will usually go away in a few weeks. You can ask doctor to give you some medication to help you make it better. If you have side effects, do the following:

If you have	Do the following
Nausea	Take the ARV pills with food.
Diarrhoea	Keep drinking and eating, do not eat spicy food/chilies.
Muscle pain, fatigue	These will go away.

I nausea or diarrhea persists or gets worse, report to the ART center.

Seek care urgently if:

- Yellow eyes with high fever, headache, running nose and body ache.
- Missed periods/possibility of pregnancy.
- Severe abdominal pains.
- Extreme paleness of face, hands or eyes.
- Fatigue and shortness of breath.

Note: Remember to take your cotrimoxazole prophylaxis tablet every day, if the doctor prescribes it.
Always use condom during sex.
Do not stop any drugs by yourself.

Phone:		Call ART centre if you have any questions or problems. (9 AM – 4 PM)
Phone:		After 4 PM, Contact the hospital Emergency number.
Phone:		Call the local positive network number for support.

National AIDS Control Organisation
Ministry of Health & Family Welfare
Government of India, 9th Floor, Chandralok Building
26, Janpath, New Delhi – 110001, India
Tel: 011-23325343, 011-23731774, 011-23731778,
Fax: 011-23731746
E-mail: info@nacoonline.org

ANNEX VII (A) – VIRAL LOAD TEST REQUISITION FORM

Annex VII (a)-Viral Load Test Requisition Form

VL-1 National AIDS Control Organization

Page-1 HIV-1 Plasma Viral Load Test Reauisition Form

Accession Number
For Testing Lab Use Only

To be filled out by the MO/Nurse/Lab technicion

ART Center Information

ART Center I Name : _____

Address : _____

District : _____ State : _____

Telephone # : _____

Patient Section

Patient ID # : _____

Patient Name : _____

Sex : M / F

Age : _____

Previous HIV-1 Plasma Viral Load Test Results from ART Center

Baseline : 6 Months: Other (please Explain):

Relevant Clinical Details

Latest CD4 Count,with date : _____

Earlier HIV-1 PVL Test Performed ? Y / N Date Of Earlier HIV-1 PVL : ____ ____ _____

dd mm YY

Result of Earlier HIV-1 PVL Testing (If Performed) : _____

Manufacturer Of Previous HIV-1 PVL Test : _____ Assay/Kit used : _____

Any infection or immunization in the past 4 weeks ? Y / N

HIV-1 PVL Specimen Collection

Collection Date : _____ _____ _____ Collection Time : _____

Date Month Year

Name Of MO/Nurse?Lab Technician : _____ Signature : _____

Signature Of ART Nodal Officer : _____ Stamp :_____

To Be filled out by the ART lab technician carrying the specimens

For Lab Technician Couriering the Specimen

Name of Lab Technician In-Charge : _____

Name of ART Center : _____

Date Of Specimen Transport to Lab : _____ _____ _____ Time Of Departure : _____

Day Month Year

Signature :

To Be filled out by laboratory - For Laboratory Use Only

Date Specimen Received (dd/mm/yy):_____ Time Received : _____

Specimen received in the acceptable condition : Y / N (Please Circle)

If No,list the state of specimen received :

Unlabeled/Mislabeled/Insufficient/Inappropriate/Invalid/Other

Stamp:

Name Of Lab In-Charge : _____

Signature : _____

ANNEX VII (B) – COBASAMPLICOR REPORTING FORMAT

Annex VII(b) - Cobas Amplicor Reporting Format

VL-1 Lab Name

Page 2 Address (Street ,District,State)

Lab Phone Number

HIV-1 Plasma Viral Load Result Form

Cobas Amplicor

Accession Number/Lab Registration Number : _____

Patient ID Number : _____

Date Specimen Tested(dd/mm/yy):_____Date Of Report (dd/mm/yy):_____

Test Kit Name : Cobas Amplicor HIV-1 Monitor Test ,Version 1 Manufacturer : _____

Version : _____

Result

HIV-1 RNA Copies/ml	
Log-Transformation :	

Notes : HIV-1 Quantization by Cobas Amolicor

Human Immunodeficiency virus(HIV) is the etiologic agent of Acquired immunodefiency Syndrome(AIDS). Quantitative measurements of HIV Viremia in the peripheral blood have shown that higher virus levels may be correlated with increased risk of clinical progression of HIV Disease.

Interpretation : This procedure can detect virions associated HIV-1 RNA plasma at concentrations as 50 RNA copies/ml to 750,000 HIV-1 RNA copies/mL.Low viral load values may occur as "False positives"and have been documented in the plasma of uninfected persons or persons infected with other RNA viruses that resamble HIV(e.g.HTLV).Therefore,caution must be exercised when such a result is obtained on a specimen of a patient not confirmed as being infected with HIV(through EIA,Western Blot,Or HIV DNA Assays).

The minimum reliable and significant change in measurement(as compared to baseline value or previous test value)is a 3-fold(0.5 log) change.

Recommendations:A positive viral load result must always be correlated with clinical hitory and HIV status of the patient.The cobas Amplicor HIV-1 Monitor test 1.5 is not intended to be used as a diagnostic for HIV-1 infection.This test is not intended for HIV-2 Patients. It is recommended that the follow up viral load tests be repeted at the same laboratory with the same technique/procedure in order to caompare changes in subsequent.

Name Of lab in-charge : _____ stamb of lab in-charge :

signature Of lab In-charge : _____

Not valid for medical legal purposes

ANNEX VII (C)– TAQMAN REPORTING FORMAT

Annex VII(c) - Taqman Reporting Format

VL-1 Lab Name

Page 2 Address (Street ,District,State)

Lab Phone Number

HIV-1 Plasma Viral Load Result Form

Cobas Taqman

Accession Number/Lab Registration Number : _____

Patient ID Number : _____

Date Specimen Tested(dd/mm/yy):_____Date Of Report (dd/mm/yy):_____

Test Kit Name : Cobas Taqman HIV-1 Test Manufacturer : _____

Version : _____

Result

HIV-1 RNA Copies/ml	
Log-Transformation :	

Notes : COBAS TaqMan HIV-1 Test

Human Immunodeficiency virus(HIV) is the etiologic agent of Acquired immunodefiency Syndrome(AIDS). Quantitative measurements of HIV Viremia in the peripheral blood have shown that higher virus levels may be correlated with increased risk of clinical progression of HIV Disease.

Interpretation : This procedure can detect virions associated HIV-1 RNA plasma at concentrations low as 47 RNA copies/ml to 10,000,000 HIV-1 RNA copies/mL.Low viral load values may occur as "False positives"and have been documented in the plasma of uninfected persons or persons infected with other RNA viruses that resemble HIV(e.g.HTLV).Therefore,caution must be exercised when such a result is obtained on a specimen of a patient not confirmed as being infected with HIV(through EIA,Western Blot,Or HIV DNA Assays).

The minimum reliable and significant change in measurement(as compared to baseline value / previous test value)is a 3-fold(0.5 log) change.

Recommendations:A positive viral load result must always be correlated with clinical hitory and HIV status of the patient.The cobas Taqman HIV-1 test 1.5 is not intended to be used as a dianostic test for hiv-1 infection.This test is not intended for HIV-2 Patients.

It is recommended that viral load tests be repeted at the same laboratory with the same technique/procedure in order to caompare changes in subsequent viral load counts.

Name Of lab in-charge : _____ stamb of lab in-charge :

signature Of lab In-charge : _____

Not valid for medical legal purposes

ANNEX VII (D) – VIRAL LOAD RESULT FORM, AMPLICOR HIV MONITORING, V1.5

Annex VII(d) - Viral Load Result Form,Amplicor HIV Monitoring,V1.5
VL-1 vvvv
Page 2

Lab Name
Address (Street ,District,State)
Lab Phone Number

HIV-1 Plasma Viral Load Result Form
Amplicor HIV Monitoring,version 1.5

Accession Number/Lab Registration Number : _____

Patient ID Number : _____

Date Specimen Tested(dd/mm/yy):_____Date Of Report (dd/mm/yy):_____

Test Kit Name : Amplicor HIV-1 Monitor Test ,Version 1.5 Manufacturer : _____
Version : _____

Result

HIV-1 RNA Copies/ml	
Log-Transformation :	

Notes : HIV-1 Quantization by amplicor HIV Monitor Test,Version 1.5

Human Immunodeficiency virus(HIV) is the etiologic agent of Acquired immunodefiency Syndrome(AIDS). Quantitative measurements of HIV Viremia in the peripheral blood have shown that higher virus levels may be correlated with increased risk of clinical progression of HIV Disease.

Interpretation : This procedure can detect virions associated HIV-1 RNA plasma at concentrations low as 50 RNA copies/ml to 750,000 HIV-1 RNA copies/mL.Low viral load values may occur as "False positives"and have been documented in the plasma of uninfected persons or persons infected with other RNA viruses that resemble HIV(e.g.HTLV).Therefore,caution must be exercised when such a result is obtained on a specimen of a patient not confirmed as being infected with HIV(through EIA,Western Blot,Or HIV DNA Assays).

The minimum reliable and significant change in measurement(as compared to baseline value / previous test value)is a 3-fold(0.5 log) change.

Recommendations:A positive viral load result must always be correlated with clinical hitory and HIV status of the patient.The Amplicor HIV-1 Monitor test 1.5 is not intended to be used as a dianostic test for hiv-1 infection.This test is not intended for HIV-2 Patients.

It is recommended that follow up viral load tests be repeted at the same laboratory with the same technique/procedure in order to caompare changes in subsequent viral load .

Name Of lab in-charge : _____ stamb of lab in-charge :

signature Of lab In-charge : _____

Not valid for medical legal purposes

ANNEX VIII

Process for External Quality Assessment for the laboratories performing HIV-1 Viral Load Testing

Introduction

Process for External Quality Assessment for the laboratories performing HIV-1 Viral Load Testing will include 4 surveys in a calendar year to the NACO identified centres for performing HIV-1 Viral Load Testing for the Second line initiative.

This will serve as an important tool in the process of continuous quality improvement in these laboratories.

Confidentiality

Participants will be given a Participant Number to be used as a reference in all correspondence. At no time and under no circumstances is the identity of a participating laboratory revealed. Report reviewers who assess results and provide comments/discussions and an educational component for each report are unaware of the identity of participants.

Participating Laboratories:

Name of Laboratory & Contact Person of each participating lab would be provided to RCPA

EQAS coordinating laboratory in India

NACO has designated NARI to be the EQAS coordinating laboratory for VLEQAS in India.

NARI, Pune would take on the responsibility for ensuring that the EQAS programme is successfully and regularly implemented with active participation of testing labs by coordinating, monitoring, supervising, training, and providing troubleshooting support for the VL EQAS runs held at regular quarterly intervals. Roche India would support NARI in this activity and coordinate with RCPA Australia and the participating centers for all related activities including but not limited to the delivery of the EQAS specimens to these labs for proficiency testing four times a year at predetermined schedules. Roche India would also provide coordination training and troubleshooting support to NARI as and when required for the NACO VL labs in this entire activity. NARI would submit formal quarterly reports to NACO on the performance of the NACO VL laboratories in the VLEQAS.

Testing

Participants should test specimens in the same manner as patient specimens.

(1) The specimens should be tested with the laboratory's regular patient workload by personnel who routinely perform the testing in the laboratory.

(2) The participant should test specimens the same number of times that it routinely tests patient specimens.

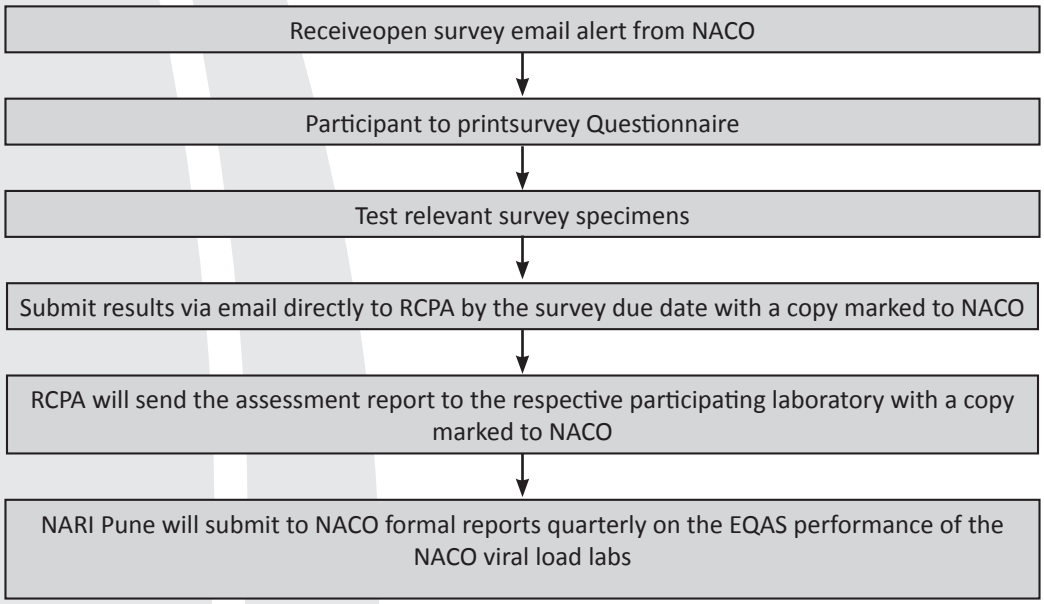
(3) The participant should maintain a copy of all records, including a copy of the completed questionnaire, to record proficiency testing results.

(4) The results sheet should be signed by the analyst and the laboratory manager, documenting that proficiency testing specimens were tested in the same manner as patient specimens, the report reviewed, results discussed and action taken (if appropriate). This documentation should be kept for a minimum of three years from the date of the proficiency testing event.

Specimen Delivery

- The survey panels will be sent to Roche Diagnostics India from RCPA Quality Program Pvt. Limited, Australia in the frozen conditions
- Upon arrival these panels would checked for the shipping conditions by an Authorized person of Roche and supplemented with additional dry ice
- 4 surveys (1 survey per quarter) each consisting of 3 specimens will be sent out to each participating laboratory each cycle of surveys.
- The specimens will be sent out at the start of the survey and delivered by Roche Diagnostics personnel to the individual laboratories to ensure ease of logistics and that the specimen integrity is maintained.
- Upon delivery, the specimens are to be frozen at -70°C or -80°C until the appropriate questionnaire is received via email. It is essential that specimens are frozen immediately upon receipt and stored in a freezer which is *not a frost free instrument* or does not have a defrost cycle.
- Specimens may contain virulent pathogens and must be treated with the same degree of caution as routine diagnostic specimens. Specimens are issued to participants on the understanding that they will be used for quality assurance purposes and that they will be tested by staff trained to handle equivalent clinical specimens.

Survey Process



Survey Questionnaires

- Participants are given 18 days to perform testing and submit survey results.
- Instructions are on each questionnaire explaining what is required. Please contact the NARI if you do not understand any aspect of the questionnaire.
- Feedback from participants is encouraged and a 'Compliments, Concerns, Suggestions' section is in each survey questionnaire, so that participants can immediately provide feedback for specific surveys, as well as the overall Programme.

Disposal of Material

Any part of specimens which is not used by the participant shall be destroyed in the manner required by any law or regulatory agency for the disposal of potentially bio-hazardous waste.

Late Return of Completed Questionnaires (Results)

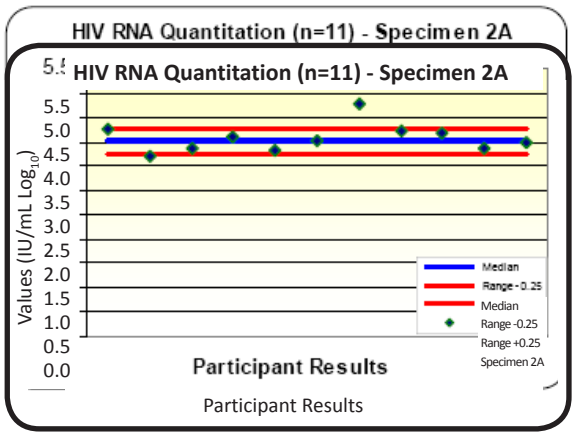
Late results will delay report preparation. If a completed questionnaire arrives **after** all data has been entered and collation of data has commenced, the received questionnaire will be marked **LATE** and the final report to NARI from RCPA will not be amended to include these late results.

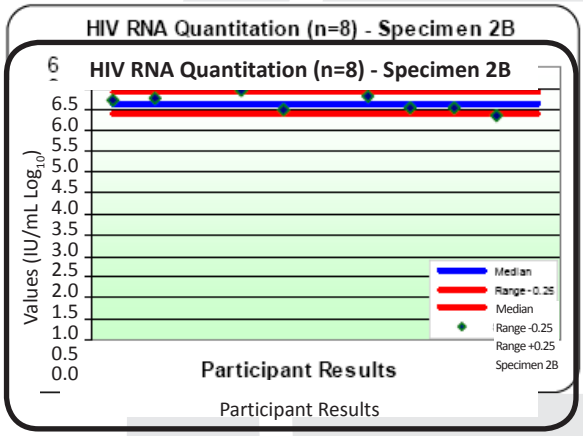
Survey Reports

Survey reports are presented in the following format:-

• **Section One** will contain a Participant Performance Table. This table identifies participants returning results inconsistent with consensus of ≥80% of participating laboratories, omission of kit details, use of expired kits, occurrence of transcription errors, inconsistent or incomplete data, inappropriate interpretative comment selection and non identification of clerical errors.

• **Section Two** contains the collated report, which is a summary of participant results, and includes discussion/comment from a scientist/pathologist with HIV expertise.





ANNEXURE IX- SECOND LINE REPORTING

1 SL + AL: SACEP REGISTER FOR ALTERNATIVE FIRST LINE & SECOND LINE ART (ADULTS AND CHILDREN)

(SACEP coordinator at CoE/pCoE and data manager at ART plus centre should maintain this register)

These columns should be on left half of register

Month: **Year:**

1	2	3	4	5	6	7		8	9	10	11	12	13	14	15	16	17	18	19	20
Sl. No (SACEP registration No) #.	ART Registration Number	Patient Name	Age	Gender	Address	Contact Number		Name of referring ART Centre	Contact no. of referring ART Centre	Name & Contact Number of NGOs linked to referring ART Centre (CCC/DLN/TI/other local NGOs)	Date of Referral (First electronic referral from ART centre seeking appointment)	Appointment Given (If Yes-Dates/If No – Reason)	Date of actual assessment by SACEP	Reason For Referral *	Latest CD4 (with date) at the time of SACEP assessment (not more than one month old))	Clinical Staging at time of SACEP assessment	Functional Status at time of SACEP assessment (WAB)	Whether VL testing recommended or not by SACEP (Y/N)	If Viral Load recommended, results (with date)/Grade of toxicity	Recommendations of SACEP (feedback to be sent to the referring ART centre& SACS) **
						Patient	Care giver													
		* 1. Suspected treatment failure 2. Side effects of drugs 3. On Second line ART from Private 4. Others																		
		** 1. Recommended for Second line ART 2. Substitution with PI containing regimen 3. Substitution of NRTI backbone 4. Substitution with other regimen – (Specify regimen code) 5. Management of complicated cases/others 6. Referred back to ART centre for review after 1/3/6 months																		
		*** 1. If viral load < 400 copies/ml, no OI, no side effects- transfer out the patient back to the referring centre 2. if viral load> 400 copies/ml at 6 month after Second line ART initiation and no OI/side effects, still transfer the patient to the referring centre and call the patient back for viral load after another six months (12th month from the time of initiation of Second line ART)																		

****For patients with viral load more than 400 copies/ml at Second viral load testing (at 6th month after Second line ART initiation)

These columns to be on the right side of the register

21	22	Follow up of patients on Second line								31					
		23	24	25	26	27	28	29	30						
Date of initiation of Second line/Alternative First line ART	Recommended regimen (indicate regimen number as per NACO ART Guidelines)	Any Major OI observed while on Second line ART	Date of viral load testing at 6 month after initiation of Second line ART	Result of viral load testing at 6 months	Recommendations of the SACEP after Second viral load ***	After initiation of Second line, whether Patient referred back to the referring ART centre as per guidelines at sixth month (yes/no)	Date of referral back to the nodal ART centre	Viral load testing at 12 month(if recommended by SACEP)****	Recommendations of SACEP at12th month viral load, if applicable	Repeat Viral loads (with date) for patients not eligible for Second line drugs at First SACEP review (As per guidelines, those whose VL is between 400- 5000 copies/ml)					
										1	2	3	4	5	6

2 AL: ALTERNATIVE FIRST LINE FORMAT FOR SACEP MEETING (ADULT & CHILDREN)

(To be prepared before SACEP meeting and given to all members during the meeting and to be kept at CoE/pCoE/ART plus centre)

Meeting date :

Alternative First line ART																
1	2	3	4	5	6	7	8	9	10	11	12				13	
Sl. No (SACEP registration No).	ART Registration Number	Name of patient	Age	Gender	Address	Name and address of referring ART centre	ART start date (within the program)	ART start date (outside the program)	% Adherence to First line drugs	Clinical stage at the time of assessment	CD4 counts (with date) cells/Cmm (expandable)				Type of toxicity /side effects observed	

Summary -2AL Alternative First line ART					Members Present during the SACEP
Sl. No		Name of the regimen	No of patients		1
1	Total number of patients reviewed by SACEP for Alternative First line treatment	TDF containing regimen (Adult)			2
		ATV/r based regimen (Adult)			3
		LPV/r based regimen (adult)			4
		ABC/3TC+NVP			5
		ABC/3TC+EFV			
		ABC/3TC+LPV/r			
		ZDV/3TC+LPV/r			
		d4T/3TC+LPV/r			
		TDF+3TC+ATV/r (HIV-2/dual toxicity)			
2	Total number of patients actually recommended on Alternative First line ART	TDF containing regimen (Adult)			
		ATV/r based regimen (Adult)			
		LPV/r based regimen (Adult)			
		ABC/3TC+NVP			
		ABC/3TC+EFV			
		ABC/3TC+LPV/r			
		ZDV/3TC+LPV/r			
		d4T/3TC+LPV/r			

14	15	16	17	18	19	20								21
Grade of toxicity	Photo documentation (Yes/noO	Any OI's reported	Name of drug (d4t/AZT/ NVP or EFV or any other ARV) which (probably) caused toxicity – specify	Whether recommended for Alt First line? (Yes/ No)		If recommended for Alt First line?								Remarks
					Adult Regimen			Paediatric Regimen						
					TDF based Regimen	ATV/r based Regimen	Other regi-men	ABC/3TC+ NVP	ABC/ 3TC+ EFV	ABC/ 3TC+ LPV/r	ZDV/ 3TC+ LPV/r	d4T/3TC+ LPV/r		

2 SL : SECOND LINE FORMAT FOR SACEP MEETING (ADULT & CHILDREN)

(To be prepared before every SACEP and given to members during the meeting &be kept at CoE/pCoE/ART plus Centres)

Second line ART																
1	2	3	4	5	6	7	8	9	10	11				12	13	14	15	16
Sl. No (SACEP registration No).	ART Registration no.	Name of patient	Age	Gender	Address	Name , address of referring ART centre	ART start date (within the programme)	ART start date (outside the programme)	Adherence to First line drugs	CD4 counts (with date) Cells/cmm all values to be given				Recommended for Viral Load Test by SACEP (Y/N)	Viral Load result with date	Clinical Status (OI, Clinical staging)	Whether recommended for Second line ART ? (Yes/No)	Remarks
New cases																		
Follow up cases																		

New Cases: Those PLHIV who are being referred to SACEP for the First time and re-referred cases who were previously not given appointment or were not previously eligible (<400 copies/ml).

Follow up casesincludes:

- Those PLHIV who have come for review with their First VL report or
- Repeat VL results after initiation of Second line (6th/12th month) , or
- Those PLHIVs who were not eligible earlier and have come for review of repeat viral loads results by SACEP (400- 5000 copies/ml)

2SL Summary -Second Line ART		
Sl. No		**No of patients**
1	Total no. of patients referred to SACEP for suspected treatment failure	
2	Total no. of patients supposed to attend SACEP for suspected treatment failure	
3	Total Number of patients actually reviewed by SACEP for suspected treatment failure	
4	Total number of patients recommended for Viral Load test	
5	Total number of patients recommended for Second Line ART (After VL)	
6	Total number of patients actually initiated on Second Line ART (After minimum 2 counselling sessions)	

Members Present during the SACEP	
1	
2	
3	
4	
5	
6	

3AL+SL COMBINED MONTHLY REPORTING FORMAT FOR SECOND & ALTERNATIVE FIRST LINE ART

(to be sent to NACO by 4th of every month to Secondline2008@gmail.com)

(Provided separately in Excel form)

artdrugs@gmail.com, Concerned SACS (JD, CST), RC & linked CoE/ART plus/pCoE)

1. Name of ART Centre							
2. CMIS Code of ART centre (same as code for First line)							
3. Name of the District							
4. Name of the State							
5. Name of Nodal Officer							
6. Report for the period	Month		Year				

Second line ART						
7. Details of PLHIV referred to SACEP for suspected treatment failure	**Adult**			**Children**		**Total**
	Male	**Female**	**TS/TG**	**Male**	**Female**	
7.1 Cumulative number of PLHIV referred to SACEP for assessment at the beginning of this month						
7.2 Number of new PLHIV referred to SACEP for assessment during this month						
7.3 Cumulative number of PLHIV referred to SACEP for assessment at the end of this month = 7.1 + 7.2						
7.4a Cumulative number of patients referred from this CoE/ART plus centre (The reporting centre) (out of 7.3)						
7.4b Cumulative number of patients referred from referring ART centres linked to this CoE /ART plus (out of 7.3)						
7.4c Cumulative number of patients given appointment for SACEP review at end of this month (out of 7.3)						
7.4d Cumulative number of patients actually reviewed by SACEP at end of this month (out of 7.4c)						
7.4e Cumulative number of PLHIV found eligible for Viral Load (out of 7.4d)						

7.4f Cumulative number of PLHIV actually underwent Viral Load (out of 7.4e)						
8.1 Cumulative number of PLHIV found eligible for Second line at beginning of this month						
8.2 Number of PLHIV found eligible for Second line during this month						
8.3 Cumulative number of PLHA found eligible for Second line at the end of this month = 8.1 + 8.2						
8.4 Cumulative number of patients ever started on Second line ART (Number at the beginning of this month) (8.7 of previous month)						
8.5 Number of new patients started on Second line ART during this month						
8.6 Number of patients "restarted "on Second line ART during this month						
8.7 Cumulative number of patients ever started on Second line ART (Number at the end of this month) = 8.4+8.5+8.6						
9.1 Cumulative number of patients on Second line who died since the beginning of the programme						
9.2a Cumulative number of patients on Second line who are "transferred out" to other COE/ART plus						
9.2b Cumulative number of patients on Second line who are "transferred out" to the referring centres (ART centres)						
9.2c Cumulative number of patients on Second line who are "transferred in" (from other COE/ART plus)						
9.3 Number of all patients on Second line treatment whose treatment status in this month is "stopped treatment"						
9.4 Cumulative Number of patients on Second line who are lost to follow-up (LFU)						
9.5 Cumulative Number of patients on Second line treatment who did not return to the ART centre (Defaulter)/whose treatment status is "MIS" in this month						
9.6 Total number of patients alive and on Second line ART (OT) at the end of this month = 8.7+ 9.2c - (9.1+9.2a+9.3+9.4+9.5)						
9.7a Out of 9.6, the number of patients on Second line ART initiated on DOTS this month						

9.7b Out of 9.6, the number of patients on Second line ART initiated on non-DOTS anti-tuberculosis treatment this month						
9.7c Out of 9.6, the total number of pregnant women on Second line ART this month						
Alternative First line ART						
10.1 Cumulative number of PLHIV referred to SACEP for assessment at the beginning of this month						
10.2 Number of new PLHIV referred to SACEP for assessment during this month						
10.3 Cumulative number of PLHIV referred to SACEP for assessment at the end of this month(10.1+10.2)						
10.3a Number referred from CoE/ART plus (The reporting centre) (out of 10.3)						
10.3b Number referred from other ART centres (out of 10.3)						
11.1 Cumulative number of PLHIV found eligible for Alternative First line ART at beginning of this month (11.3 of previous month)						
11.2 Number of PLHIV found eligible for Alternative First line ART during this month						
11.3 Total number of PLHIV found eligible for Alternative First line ART till the end of this month(11.1+11.2)						
12.1 Cumulative number of patients ever started on Alternative First line ART (Number at the beginning of this month)						
12.2 Number of new patients started on alternative First line ART during this month						
12.3 Cumulative number of patients ever started on Alternative First line ART (Number at the end of this month) =12.1+12.2						
13.1 Cumulative number of patients on alternate First line who died since the beginning of the programme						
13.2a Cumulative number of patients on Alternative First line who are “transferred out” to other CoE/ART Plus Centres						
13.2b Cumulative number of patients on Alternative First line who are “transferred out” to the referring ART centre						
13.2c Cumulative number of patients on Alternative First line who are “transferred in” from other centres (CoE/ART plus)						

13.3 The number of all patients on Alternative First line treatment whose treatment status in this month is "stopped treatment"						
13.4 Cumulative Number of patients receiving Alternative First line who are lost to follow-up (LFU)						
13.5 The number of patients on Alternative First line treatment who did not return to the ART centre (Defaulter)/, whose treatment status is "MIS" in this month						
13.6 Total number of patients alive and on Alternative First line ART= 12.3+13.2c-(13.1+13.2a+13.3+13.4+13.5)						
13.7 Out of 13.6, the number of patients on Alternative First line ART initiated on DOTS this month						
13.8 the number of patients on Alt First line ART initiated on non-DOTS anti-tuberculosis treatment this month						
13.9 the total number of pregnant women on Alternative First line ART this month						
14. Treatment Adherence				**Number**		
14.1a Of all patients who are on Second line ART this month (9.6), the number who have NOT been assessed for adherence (refer guideline)						
14.1b Of all patients on Second line ART (9.6) this month and who have been assessed for adherence, how many had 95% adherence or better (refer guideline)						
14.2a Of all patients who are on alternative First line ART this month (13.6) the number who have NOT been assessed for adherence (refer guideline)						
14.2b Of all patients on alternative First line ART (13.6) this month and who have been assessed for adherence, how many had 95% adherence or better (refer guideline)						
15 a. Primary Regimen of PLHIV started on Second line/Alternative First line ART at the end of the month						

Regimen	Adult	Pediatrics	Total
Tenofovir+ Lamivudine + Nevirapine	1	1	**2**
Tenofovir + Lamivudine + Efavirenz	1	1	**2**
Zidovudine + Lamivudine + Atazanavir/Ritonavir	1	1	**2**
Zidovudine + Lamivudine + Lopinavir/Ritonavir	1	1	**2**
Tenofovir + Lamivudine+ Atazanavir/Ritonavir	1	1	**2**

Tenofovir + Lamivudine +Lopinavir/Ritonavir	1	1	**2**
Stavudine + Lamivudine + Lopinavir/Ritonavir	1	1	**2**
Stavudine + Lamivudine + Atazanavir/Ritonavir	1	1	**2**
Abacavir + Lamivudine + Nevirapine	1	1	**2**
Abacavir + Lamivudine + Efavirenz	1	1	**2**
Abacavir + Lamivudine + Lopinavir/Ritonavir	1	1	**2**
Zidovudine + Lamivudine + Lopinavir/Ritnovir	1	1	**2**
Stavudine + Lamivudine + Lopinavir/Ritonavir	1	1	**2**
Abacavir + Lamivudine + Didanosine + Lopinavir/Ritonavir	1	1	**2**
Others	1	1	**2**
Total	15	15	30

15.b. Total Number of Patients on CPT Prophylaxis		
Regimen	Adults	Pediatrics
Cotrimoxazole (DS)		
Cotrimoxazole (SS)		
Cotrimoxazole Suspension		
Total number of patients		

15.c. Opportunistic Infections					
OI's	**Number of episodes of OIs this month**		**OI's**	**Number of episodes of OIs this month**	
	Adults	Pediatrics		Adults	Pediatrics
1(a) Tuberculosis (Pulmonary)			8 Toxoplasmosis		
1(b)Tuberculosis (Extra-Pulmonary)			9 CMV Retinitis		
2 Candidiasis			10 Bacterial Infections(skin)		
3 Diarrhoea			11 Herpes Simplex		
4 PCP			12 Malignancy		
5 Herpes Zoster			13 Others		
6 Bacterial Infections (Respiratory)			14 Others		
7 Cryptococcal Meningitis					

15.d Were there any specific side effects noted for Second line/Alternative First line ART during the month			
	Male		Female
Particulars	Adult	Pediatrics	Adult
1. AZT Induced Anaemia			
2. Peripheral Neuropathy			
3. Hepatitis			
4. Lipodystrophy			
5.Pancreatitis			
6. Skin Reaction			
7. CNS Side Effects			
8. IRIS			

4 AL : LIST OF PATIENTS ON ALTERNATIVE FIRST LINE ART																
1	2	3	4	5	6	7	8	9	10		11	12	13	14	15	16	17
Sl. No (SACEP registration No).	ART Reg. No.	Name	Age	Sex	Address	Telephone	Date of ART start (1st line)	Current CD4 count	Type of Toxicity		Probable drug causing toxicity	SACEP recom-mendations	Date of ART start (alternative First line)	Alternative First line Regimen*	Status of patient (OT/ MIS/LFU/TO/Death)	To be filled by CoE/PCoE/ART plus centre only	
									Clinical Symptoms	Laboratory tests						Patient referred back to the referring ART centre (yes/no?)	Date of refer-ral back to the referring ART centre

4 SL : LIST OF PATIENTS ON SECOND LINE ART

1	2	3	4	5	6	7	8	9	10		11			12			13	14	15	16	17
Sr. No.((SACEP Regn. No)	ART Reg. No.	Name	Age	Sex	Address	Telephone	Date of ART start (1st line)	Current regimen (at the time of SACEP Referral)	CD4 count		Viral load at the time of SACEP assessment with date			SACEP recommendations			Date of ART start (Second line)	Second line ART regimen initiated	Status of patient (OT/MIS/LFU/TO/ Death)	To be filled by CoE/PCoE/ART plus centre only	
									Baseline	At the time of At the time of referral to SACEP	Baseline	After 6 months on 2nd Line	After 12 months on 2nd Line	After Baseline VL	After 6 months on 2nd Line	After 12 months on 2nd Line				Patient re-ferred back to the refer-ring ART centre (yes/ no?)	Date of referral back to the refer-ring ART centre
1																					
2																					
3																					
4																					
5																					
6																					
7																					
8																					
9																					
10																					
	Total																				

5 SL: CENTRE WISE (LINKED CENTRE INFORMATION) BREAK UP FOR PLHIV INITIATED ON SECOND LINE ART

S. No	Names of Linked ART Centres	Cumulative no of patients Referred to SACEP (7.3)	Cumulative no of patients given appointment for SACEP review (7.4c)	Cumulative no of patients actually reviewed by SACEP (7.4d)	Cumulative no of patients eligible for viral load test (7.4e)	Cumulative number of PLHIV actually underwent Viral Load (7.4f)	Cumulative number of PLHA found eligible for Second line (8.3)	Cumulative number of patients ever started on Second line ART (8.7)	Cumulative number of patients on Second line who died (9.1)	Alive and on Second line ART (9.6)		
										At CoE/ ART plus centre	At referring centre	Total
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
	Total											

ANNEX X

Dr. Ashok Kumar, M.D
Deputy Director General
Head, Central TB Division
Project Director RNTCP

Telephone- +91-11-23062980
Telefax- +91-11-23063226
Email: ddgtb@rntcp.org

भारत सरकार
Government of India
स्वास्थ्य सेवा महानिदेशालय
Directorate General of Health Services
स्वास्थ्य एवं परिवार कल्याण मंत्रालय
Ministry of Health and Family Welfare
निर्माण भवन, नई दिल्ली - ११० १०८
Nirman Bhawan, New Delhi - 110 108

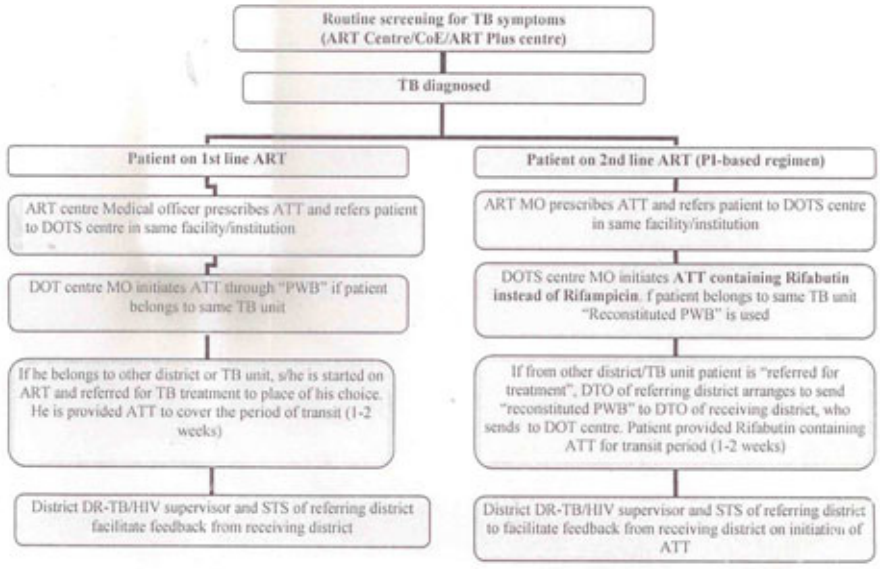
Ref No. Z.28015/53/2007-TB
Dated the 18th Feb. 2013.

Subject: Strengthening supply chain system for prompt initiation of Rifabutin containing Anti-TB Treatment (ATT) for HIV infected TB patient receiving Protease Inhibitor (PI) based ART

Dear All (State TB Officers and Project Directors SACS in all 35 states/UTs in India)

As you are aware all HIV infected individuals are routinely screened for TB at ART centres and those diagnosed with TB are categorised as per RNTCP guidelines and prescribed anti-TB treatment (ATT) by ART medical officer. The patients are then sent to institutional DOT centre in the same health facility for dispensing drugs. If patient belongs to same TB unit he is initiated on ATT through a patient wise box (PWB) from institutional DOT centre, but if he belongs to other TB unit or district, he will be provided initial ATT to cover the period of transit and then "referred for treatment" by the RNTCP to a DOT centre nearest to her/his residence. RNTCP supervisors of the referring district co-ordinates with receiving district for treatment initiation and feedback regarding the same. This mechanism applies equally in special cases like treatment of TB among HIV infected individual on Protease Inhibitor (PI) based ART.

ATT in patients on Protease Inhibitor (PI) containing ART is different compared to those on 1st line ART. This is because of the significant drug interaction between PIs and Rifampicin. Rifampicin is a potent inducer of hepatic enzymes that metabolise PIs leading to their sub-therapeutic blood levels. These patients are prescribed Rifabutin containing ART instead of Rifampicin since Rifabutin is a less potent inducer and can be administered without compromising efficacy of ART or ATT. This calls for special arrangements to provide Rifabutin based ATT at NACO Centres of Excellence (CoE) and ART-Plus centres where PI based ART is initiated. NACP and RNTCP recommend following mechanism for provision of Rifabutin containing ATT in this special case scenario.



Contd/2

Provision of Rifabutin

The procurement and dispensing of Rifabutin containing ATT shall be done by RNTCP. The State TB Officer is to either procures or receives Rifabutin from Central TB Division and supply to districts having COE and ART-plus centres. DTO of these districts should reconstitute PWB and prolongation pouches (PP) replacing Rifampicin with Rifabutin, as per the requirement at COE and ART-plus centres and supply the reconstituted PWB/PP to DOT centre in these facilities. The DTO should also arrange to transfer the PWB to other district where the patient wants to complete his anti-TB treatment.

Recording and reporting:

Details of TB treatment should be recorded in NACP as well as RNTCP records like patients TB treatment card, ART white card, RNTCP "referral for treatment register" and the HIV/TB register at ART centres. The HIV/TB register at ART centre is a key record for follow-up and reporting hence, it should have clear mention of ATT regimen e.g. patient is "**currently on PI based ART with Rifabutin containing ATT**" in remarks column. This information should form basis of reporting on TB in the 2nd line ART report from COE/ART plus centre to NACO.

Supervision and monitoring:

RNTCP District DR-TB/HIV supervisor, district ICTC supervisor, STS of concerned TU and staff nurse at ART centre should co-ordinate flow of information between the ART centre to DOT centre and DTO. They should also ensure uninterrupted supply of reconstituted PWB/PP from DTC to DOT centre and further to neighbouring district if required. The DR-TB/HIV supervisor and District ICTC supervisor should also facilitate feedback on initiation of ATT and inform staff nurse at ART centre to update HIV/TB register.

This mechanism is critical for successful management of TB in patients on 2nd line ART. You are kindly requested to ensure smooth implementation of these activities considering their importance in treatment outcome in this patient group

With Regards,

18/2/13

Dr.Ashok Kumar
Deputy Director General (TB)

19/02/2013

Dr.R.S.Gupta
Deputy Director General (NACO)

To,

1. Project Director, SACS, All States/UTs
2. State TB Officers, All States/UTs

Cc for Information to:

1. PPS to Secretary & DG (NACO)/MOHFW/GOI
2. PPS to Addl. Secretary, NACO/MOHFW/GOI
3. Asstt. DG (CST), NACO/MOHFW/GOI
4. NPO (ART), NACO/MOHFW/GOI

(2)

ACKNOWLEDGEMENTS

"The National AIDS Control organisation wishes to acknowledge contributions made by the guidelines team at NACO, Regional Coordinators, Joint Directors of SACS, Program Directors of CoEs, and other contributors in updating these guidelines.

We also acknowledge the technical support provided by World Health Organisation country office for India in developing and updating these 2013 guidelines.

The support of International Training and Education Centre for Health (I-TECH), India for printing these guidelines is also acknowledged"

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