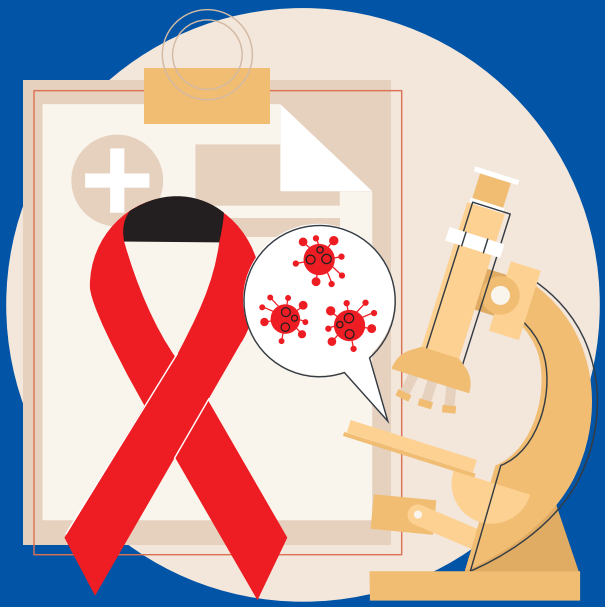
National Standard Operating Procedures for Using Rapid Test to Detect Recent HIV Infection

2023



Government of Nepal
Ministry of Health and Population
National Center for AIDS and STD Control
Teku, Kathmandu

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Date: .....10th April, 2023.....

Preface

I am pleased to present the National Standard Operating Procedures (SOP) for using rapid tests to detect recent HIV infection, which is the result of the hard work of our dedicated team. It is with great pleasure that I acknowledge and thank all members of the team for their tireless efforts in developing this document.

I would like to particularly express my appreciation to United States President Emergency Plan for AIDS Relief (PEPFAR) and the United States Agency for International Development (USAID)-supported Meeting Targets and Maintaining Epidemic Control (EpiC) Nepal project for providing invaluable technical support throughout the process.

Developing this SOP was an important task for us, as it will guide healthcare providers in using rapid tests to detect recent HIV infection, which is critical for early diagnosis and treatment and knowing the epidemic. This will ultimately contribute to our national efforts in achieving the 95-95-95 targets and ending the AIDS epidemic by 2030.

I want to congratulate the team for their outstanding work in developing this SOP, and I am confident that its implementation will have a positive impact on the lives of many people in our country.

Thanking you,

Dr. Sudha Devkota
Director
National Center for AIDS and STD Control

Abbreviations

ACD-A	Anticoagulant citrate dextrose anticoagulants
AHI	Acute HIV infection
ARVs	Antiretrovirals
ART	Antiretroviral therapy
EDTA	Ethylenediamine tetraacetic acid
EIA	Enzyme immunoassay
EpiC	Meeting Targets and Maintaining Epidemic Control
FRR	False Recent Rate
HCWM	Health care waste management
HIV	Human immunodeficiency virus
HMIS	Health Management Information System
HTS	HIV testing services
LAg	Limiting antigen
LT/R	Long term/Recent line
NCASC	National Centre for AIDS and STD Control
NPHL	National Public Health Laboratory
ONHIS	One National HIV Information System
PLHIV	People living with HIV
PQ	Prequalification
PrEP	Pre-exposure prophylaxis
RITA	Recent infection testing algorithm
RNA	Ribonucleic acid
RTRI	Rapid test for recent infection
SOP	Standard operating procedures
STI	Sexually Transmitted Infections
TRI	Test for recent infection
UNAIDS	United Nations Program on HIV/AIDS
USAID	United States Agency for International Development
V	Verification
VL	Viral load
WHO	World Health Organization

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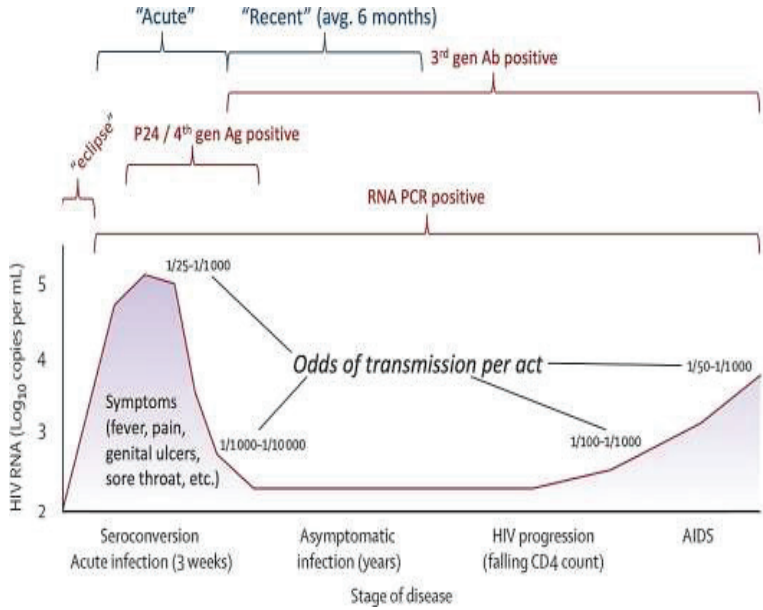
1. Introduction

Human Immunodeficiency Virus (HIV) infection occurring within the past year, and on average within the past six months is considered a "recent" infection. Recent infection of HIV comprises of high viral load, together with immature or weak immune response.[1]

Figure 1. The natural history of HIV disease progression and options for detection of infection



1 Pettifor A, MacPhail C, Corneli A, Sibeko J, Kamanga G, Rosenberg N, et al. Continued high risk sexual behavior following diagnosis with acute HIV infection in South Africa and Malawi: implications for prevention. AIDS Behav. 2011;15(6):1243–50

HIV transmission is higher in people who have early HIV infection as viral loads during acute HIV infection (AHI) are higher and simplify high-risk behavior before diagnosis, which are major determinants of transmission. Rapid HIV recency assays, help identify individuals who have become HIV infected within the past year—on average in the past 6 months—to help estimate HIV incidence and improve the focus of programming in different key population settings.[2] See Figure 1 for details.

Rapid recency point-of-care antibody-based assays differentiate between recent HIV infection—when the antibody response is immature, as reflected by low "avidity" or binding strength of the antibody—and long-term infections in which a mature antibody response is measured by strong antibody avidity[3].The assays can yield "false-recent" results among individuals who naturally control HIV well (low virus=low antibody) or are receiving antiretroviral therapy (ART), so a recent infection result is usually confirmed using a recent infection testing algorithm in which a viral load test is conducted with results of ≥ 1,000 copies/mL confirming recent infection.[4]

Rapid recency assays only measure antibody avidity after HIV seroconversion; they do not detect HIV RNA or p24 antigen and therefore are unable to detect AHI. In typical use, they are only offered to individuals who have been confirmed to have HIV infection with a standard antibody-based national HIV testing algorithm.

The recency test provides an estimate of incidence when correctly used in representative surveys, surveillance, and outbreak investigation to understand prevention failure and helps identify sub-populations that have high levels of

2 World Health Organization. (2018). WHO working group on HIV incidence measurement and data use: 3-4 March 2018, Boston, MA, USA: meeting report. World Health Organization. https://apps.who.int/iris/handle/10665/272940.

3 Duong YT, Dobbs T, Mavengere Y, et al. Field validation of limiting-antigen avidity enzyme immunoassay to estimate HIV-1 incidence in a cross-sectional survey in Swaziland. AIDS Res Hum Retroviruses. 2019; 35(10):896–905. doi:10.1089/aid.2018.0284. pmid:31204867

4 Rice B, de Wit M, Willis R, et al. The Feasibility and Utility of HIV Recent Infection Testing in a Range of Routine Service-Provision Contexts. MeSH Consortium; 2019. Accessed October 9, 2020

new infections in routine surveillance.[5] The World Health Organization (WHO) has endorsed the use of recency assays for surveillance purposes, while program or individual-level benefits are on process of being ascertained.[6] This has shown also to be effective where surveillance data from this system can be used to rapidly detect and characterize recent HIV infection among newly diagnosed people living with HIV (PLHIV) to inform a timely and targeted public health response.[7]

The SOP intends to support the HIV testing sites to conduct recency testing and support the national HIV program for interventions related to HIV prevention and care, providing details about who carries out these tests with detailed step-by-step process, the interpretation of the results, and recording and reporting.

Recency testing in Nepal

In Nepal, a rapid test for recent infection (RTRI) was introduced by LINKAGES Nepal Project in August 2019 using The Asanté™ Rapid HIV-1 Recency Assay kit.[8] Meeting Targets and Maintaining Epidemic Control (EpiC) Nepal Project, supported by United States Agency for International Development (USAID), and funded by The U.S. President's Emergency Plan for AIDS Relief (PEPFAR), has been conducting Recency testing. Recency testing has also been a part of national Integrated Biological and Behavioral Surveillance (IBBS) Survey in Nepal among People Who Inject Drugs (PWID) in Nepal-2020.

5 Technical Guidance on the Use of Recency Assays for HIV Surveillance, PEPFAR Scientific Advisory Board, 7 September 2022

6 World Health Organization (WHO). WHO Working Group on HIV Incidence Measurement and Data Use: 3–4 March 2018, Boston, MA, USA: Meeting Report. WHO; 2018. Accessed October 9, 2020.

7 Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, et al. Prevention of HIV-1 infection with early antiretroviral therapy. N Engl J Med. 2011;365(6):493–505

8 This kit is a portable, rapid lateral flow type of format using antibody binding protein A, which is conjugated to colloidal gold dye particles as a conjugate, and HIV antigens rIDR-M, an antigen that contains the major variants of gp41 immunodominant regions among the HIV-1 group M viruses; HIV-1 (p24, gp41); HIV-2 (gp36)), which are bound to the membrane solid phase. Long term (LT) area in the kit contains HIV-1 rIDR-M recombinant antigen bound to the membrane and binds to the antibodies of HIV-1 to detect long-term infection of HIV whereas the verification (V) area, which contains p24, gp41, and gp36 recombinant antigens which bind any HIV-1 and HIV-2 antibodies present in the specimen. The Control (C) line contains goat antibodies reactive to human antibodies ("goat anti-human antibodies") which will bind human antibodies in the liquid regardless of whether those antibodies are HIV positive or negative.

2. Requirements and process for recency testing

The National Centre for AIDS and STD Control (NCASC) provides guidance for the overall program implementation and recency testing. HIV related reports should be provided to the NCASC by reporting in One National HIV Information System (ONHIS) and to Health Management Information System (HMIS).

Guiding principles of providing recency testing services

This SOP manual is a live document and will be revised based on national and international recommendations and the decision of the NCASC. Following basic service delivery, related principles should be followed while providing recency testing services from the service delivery sites.

Consent: Clients must provide informed consent for receiving recency testing services. They should be provided with detailed information of the process and of their right to decline for the service. Also, they must sign a consent form (included in Annex 3).

Confidentiality: All the information collected should be kept confidential and should not be shared with anyone without the consent of the client.

Counseling: Recency testing service must be accompanied by appropriate and good quality information and counseling. Every individual should have the opportunity to ask questions in a private setting, if they request it.

Correct: Quality assurance, supportive supervision and monitoring should ensure that people receive a correct quality service as per SOP and national guidelines

Connections and use of result: It should be made clear to the clients that the use of data is for surveillance purpose and that no separate result would be provided to the clients with regards to the recency testing. It should be made clear that there will not be any individual level management difference based on the result.

Recommended staff structure and facility needed for recency testing services

Recommended staff structure for providing recency testing services is presented in below.

Staff: Laboratory technician or assistant

Training requirements: Laboratory technician or assistant trained for HIV and Sexually Transmitted Infections (STI) diagnosis following national training package provided by the National Public Health Laboratory (NPHL).

Role: Conduct HIV confirmation test before conducting recency testing. Conduct rapid recency testing. Coordinate with viral load testing laboratories for testing and receiving results.

Recency testing can be provided through the sites having laboratory setups and with the above-mentioned staff structure. For government-run facility, for example, the ART centers, the existing staff in the lab can provide recency testing services.

Further the sites should also have the following:

- Provision of conducting baseline and confirmatory testing for HIV.
- Facilities with no confirmatory testing for HIV can use the service from the government recognized laboratory for HIV confirmation before carrying out recency testing.
- Forms and formats and other supporting materials for recording and reporting.
- A confidential room for counseling and consent taking (existing lab room with privacy can be used for the purpose).
- Recency testing kits available and cabinet to store the kits.
- Requirements for safe health care waste management (HCWM) of the waste generated in the facility.

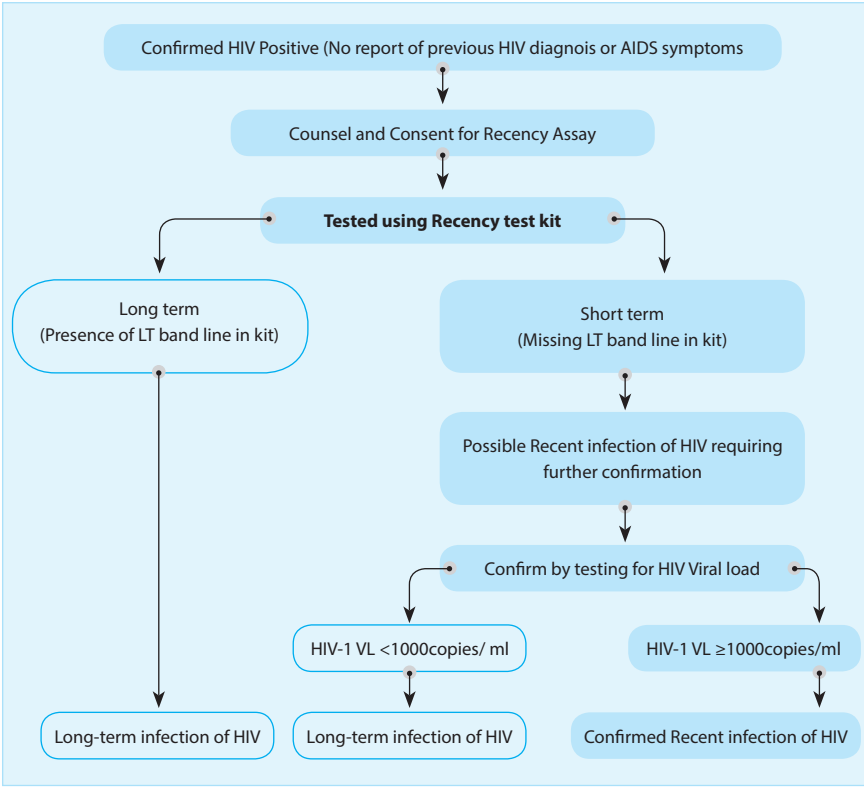
Recency service delivery package and eligibility

The recency results are for surveillance purposes only and not for programmatic or individual management. The above message should be made clear to the client and informed that no separate report will be provided regarding its results to the client.

Eligibility: All clients who have recently been confirmed as having HIV, are based on the confirmatory laboratory testing as per the national testing algorithm.[9] The confirmation can be done either during the laboratory testing of the clients or based on the recent reports of such testing by the client.

Recent infection testing algorithm (RITA)

Figure 2. RITA used for diagnosis for recent infection[10, 11, 12]



9 National HIV Testing and Treatment Guidelines 2022 [Internet]. Kathmandu: National Centre for AIDS and STD Control, MoHP Nepal; 2022 Aug [cited 2023 Apr 13]. 158 p. Available from: http://ncasc.gov.np/uploads/frontend/publication/630f2b309ac47.pdf

10 Sedia Biosciences, Asanté™ HIV-1 Recency Assay Product Insert. August 2017

11 Technical Guidance on the Use of Recency Assays for HIV Surveillance, PEPFAR Scientific Advisory Board, 7 September 2022

12 World Health Organization (WHO). WHO Working Group on HIV Incidence Measurement and Data Use: 3–4 March 2018, Boston, MA, USA: Meeting Report. WHO; 2018. Accessed October 9, 2020.

3. Recommended flow of recency testing services

Table 1. Recommended flow of activites for recency testing

Recency testing services	Activities
Confirm for HIV infection	Confirm HIV infection using national HIV testing algorithm
Determine eligibility for recency testing	Establish eligibility based on the following criteria: • Verify that the client is HIV positive and 15 years or older. • Confirm that the client was diagnosed with HIV within 6 months of HIV recency testing. • Obtain client's consent for HIV recency testing after informing them about the test's implications and usage.

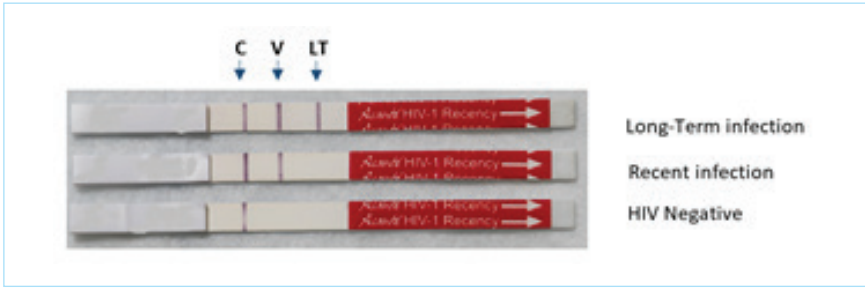
Counseling	• Provide one to one counseling during recency testing in the laboratory.
	• Communicate the purpose of recency testing to the client and include the following key messages:
	  • Recency testing identifies recent infections within the population, within the last year with a mean time of 6 months.
	  • Recency testing is for surveillance only, not for individual management.
	  • Test results will not be disclosed to the client.
	  • Confidentiality of individual information will be maintained.
	• Reinforce these messages and address any questions or concerns the client may have.
	Ensure the client fully understands the purpose of recency testing and how their privacy will be protected.

Test for recency testing	• Obtain consent from the client if agreed upon. • Conduct recency testing as per laboratory procedures outlined in chapter 4.
Recording / reporting	Record the results in the recency forms (Annex 1, Annex 2) and as detailed in chapter 8.

4. Kits used for recency testing in Nepal

A single **Asanté™ Rapid HIV-1 Recency Assay kit** pouch contains 1) Test Strip: with nitrocellulose membrane containing HIV-1 and HIV-2 recombinant antigens in TEST area, protein A in CONTROL area, and protein A-gold colloid conjugate in BUFFER well area, 2) Buffer in tubes, and 3) Collection loops for 5µl for blood/serum/plasma specimens.

Figure 3. The Asanté™ Rapid Recency Test Strip contains the Long-Term (LT), Verification (V) and Control line (C).



In addition to the kit, other materials required to conduct recency testing include a laboratory timer, centrifuge, vortex (optional), together with lancet/phlebotomy supplies for blood collection, tube stands or rack for 13 mm tubes (optional disposable foam rack is provided), absorbent pads or paper towels, biohazard bags, personal protective equipment (lab coat/apron, disposable gloves, eye protection), 10% bleach solution or 70% ethanol, and pen or permanent marker.

Sample collection and transportation, mechanism and storage of the test kit

The test can only be used on whole blood, serum, or plasma samples. A collection loop is used to collect the sample, either a fingerstick blood sample or collected blood, serum, or plasma in the sample tube. After mixing the sample with the buffer, a test strip is inserted into the liquid in the sample buffer tube and the result is read after 20 minutes.

Sample requirements

For sample of rapid receny whole blood, fingerstick or venous whole blood are acceptable. Venous blood should be collected by conventional phlebotomy methods. Ethylenediamine tetraacetic acid (EDTA) or anticoagulant citrate dextrose anticoagulants (ACD-A) should be used in blood collection. Heparin anticoagulant should not be used. For serum or plasma, it should be separated from whole blood cells using a centrifuge (3000 rpm for 10 minutes) within 24 hours of collection. Blood specimens that are to be stored for longer than 24 hours should be converted to serum or plasma and stored frozen (-20°C or colder). Serum/plasma specimens may be stored at 2–8°C for seven days. Frozen specimens may be thawed and refrozen up to 5 times.

Moreover, specimens arriving in the laboratory must have an original identifier affixed to the specimen. Specimen identity (ID)/ unique ID should be assigned and affixed to each specimen by the laboratory staff. Specimens should be shipped in compliance with proper regulations covering the transportation of etiologic agents. For shipments that are in transit for up to seven days, maintain at 2–8°C by using cold packs. For shipments that are in transit for greater than seven days, maintain the temperature at -20°C or colder by using dry ice.

A sample can be rejected if it is labeled or unlabeled improperly; it has insufficient specimen volume; it is without documentation or with discrepant documentation; it is improperly collected or preserved; it has leaked in transit or otherwise shows evidence of contamination; or it contains lipemic, hemolyzed or microbially contaminated blood, serum, or plasma.

Store the unopened kit at 2-30°C until the product expiration date. This test should be performed at 15°C to 37°C. If the kits are stored refrigerated, it needs to be kept at room temperature for a while to allow the test sets to come to ambient temperature before performing the test.

Step wise process at laboratory for testing

- Read the instructions provided with the kit thoroughly first. Each test component (test strip, buffer, and collection loop) is intended for a single use. If a test must be repeated, use all new components for the retest.

- Bring all reagents and specimens to room temperature (15–37°C) before beginning the testing. Allow for at least 30 minutes for warm-up. All test components and specimens must be at room temperature before use.
- Tap the lower side of the required number of capped sample buffer tubes sharply against the edge of a benchtop or similar hard surface 2–3 times, to dislodge sample buffer that may be trapped inside the cap.
- Label the tubes with the client ID facing toward the laboratory professional and place the buffer tubes in a test tube rack. Do not test more than five (for beginners) or 10 (for experts) samples in a batch.
- Remove the cap from the sample buffer tube and place the tube in a test tube rack. Discard the cap. Inspect the specimen collection loop to make sure the round end of the Loop is intact. Use a specimen collection loop to transfer the sample to the sample buffer tube.

For a Fingerstick blood sample: Wipe and clean the finger where the blood is to be collected with an antiseptic or alcohol wipe. Allow the finger to dry completely before collecting the sample. With a sterile lancet, puncture the skin off the center of the finger pad. Gently squeeze the finger to yield a drop of blood. Touch the round end of the loop to the drop of blood, allowing the blood on the finger to wick up into the loop.

For Venous blood, serum, or plasma samples: Using the collection loop, touch the round end of the loop to the blood, serum, or plasma in the sample tube to draw a liquid specimen into the loop, filling the loop.

- Visually inspect the loop to make sure that it is filled with specimens without bubbles. The loop in the tube to thoroughly mix the sample with the sample buffer. Transfer the loopful of specimens directly into the open sample buffer tube and then discard the loop. Open the foil pouch containing the test strip and remove the test strip.
- Insert the test strip into the liquid in the sample buffer tube with an arrow pointing down toward the liquid. Set a timer to 20 minutes. Wait for 20 minutes and remove the test strip from the sample buffer tube.
- Read the results on the Test Strip. After reading the results, dispose of the used test strip and sample buffer tube following universal precautions.

5. Test validation criteria and result interpretation

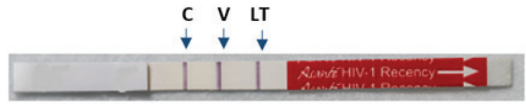
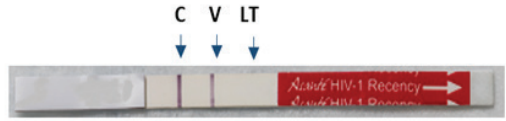
Band reactivity—All visible bands. Even a faint band must be considered reactive.

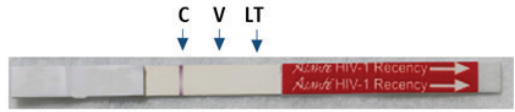
Valid—A test is valid only if a reddish-purple line appears in the control (C) area, whether the V or LT line gives a reactive or non-reactive result.

Invalid—If a reddish-purple line does not appear in the control (C) area, the test is invalid. An invalid test cannot be interpreted. It is necessary to repeat sample testing with a new device.

Upon validation, the interpretation of the test results is detailed in the Table below.

Table 2. Interpretation of the test results

Test Result	Asanté™ Rapid HIV-1 Recency Assay Kit
Long-Term infection: A sample is considered long-term when all three reactive lines appear as reddish-purple, i.e., the Control line (C), the Verification line (V), and the Long-Term/Recent line (LT/R).	
Recent infection: A sample is considered a recent infection when the C line and V lines are both visible as reddish-purple lines, but the LT line is not.	

Negative Result: A sample is an unconfirmed negative when only the C line appears as reddish-purple lines and the V and LT lines are not visible.	
Invalid Test: A test result is considered invalid: If the top, control Line, does not appear as a reddish-purple line, regardless of the presence or absence of any other lines (strips A–D at right). Or If the LT line is present, but the verification line is absent, the results are also invalid (strip E, at right).	

6. Post-test considerations

A result with only the C line, i.e., HIV-negative, is very rare. If it does occur, the test should be repeated, and the results recorded. Additionally, a sample giving a negative result should be confirmed with an approved HIV diagnostic test. Specimens that give invalid results should be retested with a new sample aliquot dispensed into a fresh sample buffer and tested with a new test strip.

Recent studies including United Nations Program on HIV/AIDS (UNAIDS) and WHO now recommend that where possible, viral load (VL) testing should be incorporated into a multi-test algorithm incorporating serological assays to measure HIV incidence to reduce the False Recent Rate (FRR) in HIV incidence estimates. If VL testing is performed on specimens tested with the Asanté™ HIV-1 Rapid Recency™ Assay, those specimens classified as "Recent" by this assay, but which have a VL < 1000 copies/mL, should be reclassified as "Long Term" infections. True recent cases should meet both criteria so that they are "Recent" by the Asanté™ HIV-1 Rapid Recency™ Assay and have VL > 1000 copies/ml (i.e., not suppressed) which will exclude persons likely to be on ART and elite controllers.

HIV-2 specimens will react with the V Line but are unlikely to react with the LT/R line and are therefore likely to give a "recent" result regardless of the duration of infection. If this assay is used in a region where HIV-2 specimens are likely to be encountered, specimens that are reported as "recent" (i.e., no LT/R line) should be retested with serology test(s) to confirm that they are HIV-1, not HIV-2 specimens. HIV-2 specimens should not be used in the estimation of HIV-1 incidence rates using this assay.

7. Quality assurance

It is necessary to perform quality assurance of the test kits for getting the best results. For internal quality assurance, there is built-in "Control" line in test kits and previously tested plasma samples can be used as external controls. The laboratory technician/assistants can see the inbuilt control results in the kit itself during test procedure whereas the laboratory professionals can use external plasma controls (known samples) also as external quality assurance. It is recommended to perform the controls with each lot of assay run, when opening a new test kit lot and whenever a new shipment of test kits is received. Any external plasma controls has to be stored at -20°C, to be brought to room temperature (15–37°C) before use and it has to be returned to -20°C after use. The plasma used as controls can be frozen and thawed up to five times.

Regarding the built-in control, "C" line serves as a built-in internal control and gives confirmation of the sample addition and proper test performance. A reddish-purple line will appear in the C position, regardless of whether the LT/R or V line gives a reactive or non-reactive result if the test has been performed correctly and the device is working properly (Please see Table 2 for Interpretation of the test results). The C line serves as a built-in internal control and gives confirmation of sample addition and proper test performance. If a reddish-purple C line does not appear, the test is "invalid".

Warning and Precautions

- This test kit should be handled only by adequately qualified personnel trained in laboratory procedures and familiar with their potential hazards. Use of this test kit with sample types other than those specifically approved for use with this device may result in inaccurate test results.
- The test device should not be used if there is no desiccant packet in the device pouch, if the device pouch is damaged, or if the pouch contains any kit component beyond its stated expiration date.

- The Test Strip should not be opened from its packaging until ready to be used. After performing the test, the result should be read in the presence of adequate lighting. For optimal results, the test strip should be read using the Asanté™ Rapid Test Strip Reader, especially if the test is used in incidence surveillance. Other readers should not be used as they may not give accurate results, may not be designed to target the three reaction lines on the test, and may measure line intensity in a different manner used to determine cutoff values used in the "Interpretation of Results."
- Components from any other type of test kit should not be used as a substitute for the components included in the test kits.
- When removing the specimen Collection Loop in a pouch, it should not be touched. Additionally, the reading area (i.e., membrane) of the test strip should not be touched to avoid contamination.
- It is important to read results within the allotted time specified, at 20 minutes after placing the Test Strip into the Sample Buffer Tube containing the specimen.
- It is recommended not to drink, eat, smoke, or apply cosmetics while handling the specimens.
- Universal precautions should be followed such as the use of gloves, a lab coat, and eye protection while handling whole blood, serum, or plasma specimens and unused assay components. Hands should be washed thoroughly after handling specimens and tests. The use of disposable gloves is recommended.
- Used gloves and test supplies should be discarded as hazardous waste after use. Syringes and other sharps should be disposed of in a puncture-resistant container before disposal as hazardous waste.
- All work areas or spills, if any, should be wiped before and after testing with an appropriate chemical disinfectant such as 10% household bleach or 70% Ethanol. Liquid wastes should be first mixed with appropriate chemical disinfectants such as 10% household bleach (0.5% sodium hypochlorite) before disposal.
- Assay quality controls:
  - Store controls at -20°C.
  - Bring controls to room temperature before use. Return controls to -20°C after use.
  - In-house controls
  - Negative—one vial (100µl)
  - Recent Infection—one vial (100µl)
  - Long-Term Infection—one vial (100µl)

8. Recording and reporting

Once the recency testing is done, and results are obtained in the lab, the results are recorded by the case manager in the recency testing form (Annex 1, Annex 2).

Indicator	Definition	Numerator	Denominator
HTS_RECENT	Number of newly diagnosed HIV-positive persons who received testing for recent infection with a documented result	Number of newly diagnosed HIV-positive persons who received a test for recent infection with a documented result during the reporting period	N/A
HTS_RECENT (RTRI Recent)	Number of person with RTRI result of recent (HTS_RECENT) in rapid test kits		
HTS_RECENT (RITA RECENT)	Percentage of person with RTRI result of recent and VL result ≥ 1000 copies / ml among number of individuals who tested for HIV and received a positive result	Number of person with RTRI result of recent and VL result ≥ 1000 copies / ml	Number of individuals who tested for HIV and received a positive result (HTS_TST_POS)

9. Related documents

- Sedia Biosciences, Asanté™ HIV-1 Recency Assay Product Insert. August 2017
- Technical Guidance on the Use of Recency Assays for HIV Surveillance, PEPFAR Scientific Advisory Board, 7 September 2022
- World Health Organization (WHO). WHO Working Group on HIV Incidence Measurement and Data Use: 3–4 March 2018, Boston, MA, USA: Meeting Report. WHO; 2018. Accessed October 9, 2020.

10. Frequently asked questions

What is a rapid test for HIV-1 recent infection (RTRI)?

A test for recent infection measures maturation of HIV antibody avidity (i.e., antibody binding strength). Avidity increases after seroconversion, as part of the immune response, and the timing of this maturation varies among individuals. A RTRI can help classify HIV-1 infections as recent or long-term using a simple lateral flow format.

What is a Recent Infection Testing Algorithm (RITA)?

RITA is a combination of laboratory tests used to classify an HIV infection as recent or long-term. RITA helps to reduce false recent classification when individuals are on ART and virally suppressed or are elite controllers. If a client tests recent on the TRI, VL testing should be conducted to improve the accuracy of classification. Those who test recent on the RTRI and have a viral load ≥1,000 copies/mL should be classified as RITA-recent.

What is a recent HIV infection?

The mean duration of recent infection may differ by manufacturers, however, there is a distribution around the mean, which can vary from ≤ 3 months to >9 months across individuals due to variations in the immune response. Therefore, at the individual level, it is appropriate to interpret recent HIV infection as having been acquired approximately within the past 12 months.

What proportion of persons newly diagnosed with HIV is expected to be recent?

The expected proportion will vary by country based on local epidemic factors such as HIV incidence and program contexts such as testing strategy, frequency, and coverage among those at risk. The observed range based on RITA (RTRI and additional VL testing among those RTRI-recent) classification from October 2020-September 2021 was from 3% to 19% across PEPFAR supported countries. This proportion may also be affected by the fraction of individuals testing HIV-positive who are re-testers and may not be “newly diagnosed”. Current data from countries implementing routine recent infection surveillance suggest that 30%–50% of HTS clients may be re-testers. People who re-test (and go unrecognized at the time of testing) can increase the denominator of the recent

infection calculation and reduce the proportion classified as recent. Appropriate pre-test counseling, and if available, unique ID and case surveillance systems can help recognize people who are retesting and should not be tested with RTRI and thereby improve accuracy of recent infection surveillance. VL testing as a part of RITA is also strongly recommended to better recognize and reclassify re-testers.

Who can perform the RTRI?

RTRIs should be performed by well-trained and certified lab staff and can be conducted at HIV testing location, in a health facility, or in a laboratory as an additional test for surveillance among persons who are newly diagnosed with HIV using the national diagnostic testing algorithm.

Can clinical history be used to improve recency classification?

Yes. In addition to VL testing as a part of RITA, recency classification can also be improved by using clinical history to identify and exclude from TRI testing persons presenting to the HTS with advanced HIV disease, low CD4+ T cell count, prior HIV diagnosis, and prior/long-term ARV use. However, HIV diagnosis and subsequent ARV initiation with or without suppressed VL within the last four weeks of RTRI testing, should not affect the TRI result.

Are there situations when a person tests recent on the assay but was infected over a year ago?

Yes. The mean duration of recent infection is about six months, but there is a distribution around the mean, which can vary from ≤3 months to >9 months across individuals due to the variation in the immune response. Based on this distribution, although we expect most persons who test RTRI- recent to have acquired HIV approximately within the last 12 months, there may be some who have had HIV for more than 12 months. Prior ART (particularly for longer duration) or weak antibody development among elite controllers can increase the likelihood of testing false-RTRI-recent.

Is it possible that a person is classified as long-term on the assay but was infected recently in the last 6-12 months (i.e. after a recent negative test result)?

Yes. Individuals transition from recent to long-term status at varying time periods after infection (≤3 to >9 months). This means that individuals may reach the antibody avidity threshold, but are still within 12 months of infection and will test as RTRI-LT.

Does the RTRI detect acute HIV infection?

No. The RTRI does not detect acute HIV infection. Persons who have acute HIV infection do not have HIV antibodies and will test HIV-negative by a national HIV testing algorithm that uses HIV antibody tests. Thus, in Nepal where the national HIV testing algorithm is based on antibody detections, persons with acute infection will not be detected and will not be offered recency testing or included in recent infection surveillance.

What should be done for persons who have an HIV diagnosis and an RTRI result with only the control line (i.e., RTRI inconclusive or negative)?

In general, RTRI should be repeated, and the results recorded. Irrespective of RTRI results, the national HIV testing algorithm is always the final HIV result. It is important to review inconclusive results in the context of the total number of HIV-positives tested.

How should an RTRI result with a faint long-term line be interpreted?

Any visible line, even if weak, should be interpreted as present. The long-term line measures the evolving antibody avidity, and a person could truly be at the transition point between recent and long-term infection. In this case, the test may show a faint long-term line which may be detected differently by different testers.

Should persons using pre-exposure prophylaxis (PrEP) receive testing for recent infection?

Yes. PrEP may slow the maturation of antibodies, which can delay identification as HIV-positive on routine diagnostic tests and result in infection classified as recent for a longer time than the mean duration of recent infection (about six months). These events (breakthrough infection while on PrEP) are likely to be rare. Persons using PrEP who are newly diagnosed with HIV, are eligible for recency testing and inclusion in recent infection surveillance.

What are the different types of analyses that can be done using RTRI data?

Recent surveillance data can be used to characterize new HIV diagnoses in a population by recent infection status and other characteristics of interest routinely captured such as demographics or risk information. Additionally, these data can be used to estimate the proportion recently infected among

a proxy population of persons at-risk (those who test HIV-negative within the HTS plus those who are classified as recently infected). These analyses can help monitor for trends in the overall population or among specific subpopulations. Additionally, geospatial analysis of new HIV diagnoses by recency status can help to detect potential hotspots where there is ongoing transmission and need for further investigation to identify gaps and address them through program strengthening and response. Many of these analyses can be built into national recent infection surveillance dashboards to facilitate timely and routine data use by Ministries of Health and key stakeholders.

Can we estimate HIV incidence using routine recent infection surveillance?

Many factors need to be carefully considered in use of routine recent infection surveillance to estimate population HIV incidence, including recency assay-specific performance characteristics, timing, and coverage of HIV testing among persons at-risk within the population, and coverage and quality of recency testing among those newly diagnosed with HIV. Approaches that adequately measure or adjust for these factors may make it possible in the future to use routine recent infection surveillance to estimate HIV incidence.

Are the recency tests WHO prequalified, and can it be used for recent infection surveillance?

Currently, there are no recency tests kits that have been fully WHO prequalified. TRIs are used for surveillance and not for HIV diagnosis or individual-level clinical management or care. WHO does not currently have a pathway for prequalification (PQ) assessment of any tests for recent infection surveillance or incidence estimation, including the RTRI and LAg- Avidity Enzyme immunoassay (EIA) assay.

11. Annex

Annex 1: HIV Recency Form

HIV RECENCY TESTING FORM

Service date: ..... (MM/DD/YYYY)

Name of the client: .....

Name of site: ..... **UIC (ONHIS):** .....

District: ..... **Hotspot area:** .....

TICK (√) THE APPLICABLE BOXES			
Testing Site:	ART sites ☐ Others ☐	Referred from for testing or tested by:	Community Led testing (CLT) ☐ Self-Testing ☐ Online Reach ☐ EPOA ☐ Others .....

RECENCY TESTING SECTION			
Offered Recency Testing	Yes ☐ No ☐	Consent is taken	Yes ☐ No ☐
Date of HIV positive diagnosis:	……/……./……. (MM/DD/YYYY)	Rapid test for recent infection (RTRI) done:	Yes ☐ No ☐
Result of RTRI	Recent Long Term Negative Invalid		
Viral load (VL) Count	…….. copies/ml	Viral load count date	……/……../……. (MM/DD/YYYY)
Confirmed result through VL testing	Yes ☐ No ☐	Confirmed result received by client	Confirmed Recent ☐ Confirmed Long Term ☐

Annex 2: HIV Recency Testing Register

RECENY TESTING

MONTH: ---------- YEAR: ----------

SN	Registration					Demographic Information																
						Client's Name and Caste			Age											Address				
	Date			MR No	SR No				Sex														
	DD	MM	YY			First Name (Optional)	Last Name (Compulsory)	Caste Code	F	M	S.G.M.	Marital Status*	Educational Status**	Education Level***	Risk Group	Employment Status****	Average Monthly Income (NPR)	Contact Number:	District	Municipality	Ward Number	Hotsppot	Client Code
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24

	Recency Testing Section								
Referred from for testing or tested by	Offered for Recency Testing	Consent provided	Date of HIV diagnosed	Rapid Test for recent infection (RTRI)	Result of RTRI	Viral load count date	Viral load count (copies/ml)	Confirmed result through VL testing	Confirmed result received by client
25	26	27	28	29	30	31	32	33	34
			DD/MM/YY			DD/MM/YY			
			DD/MM/YY			DD/MM/YY			

Annex 3: HIV Recency Consent Form

CONSENT FORM FOR RECENCY TESTING

Lab No: | | | | | | | | | |

Date DD MM YYYY

PATIENT INFORMATION

Name:_____ Age: _____

Gender
☐ Male
☐ Female
☐ Others

Testing site:_____

Date of HIV diagnosis:_____

Site of HIV diagnosis: _____

Lab diagnosis no:_____

CONSENT:

I, the undersigned provide my consent* to get my blood tested for recency testing for HIV. The significance, relevant information, and counseling have been provided to me.

I understand that I would not be given any separate results for this test . I also understand that my individual information will be kept confidential and that the result will be used for epidemiological analysis and program purposes. I authorize the following person/agency to collect and use the result from this test.

Name _____

Signature of Patient _____

Signature of Counselor _____

In case of minors, consent form to be signed by either of the Parents / Legal Guardian

In adoption cases, consent form to be signed by Orphanage / NGO / Adopting Parents

In case of incapacitated or hospitalized patients, consent is to be signed by next of kin or doctor

Government of Nepal
Ministry of Health and Population
National Center for AIDS and STD Control
Teku, Kathmandu