Asanté™ HIV-1 *Rapid Recency®* Assay

Rapid in vitro immunoassay for determining recency of Human Immunodeficiency Virus Type 1 (HIV-1) infections

FOR RESEARCH USE ONLY
Not for use in
diagnostic procedures

Cat. No. 1130-020 (20 Tests/Pack)

Includes:

- One (1) bag containing Twenty (20) Test Strips with Desiccant
- One (1) bag containing Twenty (20) Tubes with Sample Buffer
- Specimen Collection Loops, nom. 5 µL (1 Pack of 20)

Cat. No. 1130-100 (100 Tests/Pack)

Includes:

- Five (5) bags containing Twenty (20) Test Strips with Desiccant
- Five (5) bags containing Twenty (20) Tubes with Sample Buffer
- Specimen Collection Loops, nom. 5 µL (5 Packs of 20)

Store at 2-30°C

Manufactured :
Sedia Biosciences Corporation
Beaverton, Oregon USA
☎ +1 (503) 459-4159

NAME AND INTENDED USE

The *Asanté*™ HIV-1 *Rapid Recency*® Assay is a single-use rapid in vitro immunoassay that distinguishes HIV-1 infections on the basis of recency of infection. The assay is intended for use with blood (both venous and finger-stick), serum or plasma specimens as either a laboratory or point-of-collection test to detect HIV antibodies and recency of HIV-1 infection at the same time. Rapid tests for recent infection (RTRI), like this test, are used to detect recent infection in routine HIV testing services. This allows for a rapid description of person, place, and time for all new HIV infections which forms the basis for real-time surveillance to monitor the epidemic and respond appropriately with public health response measures. Recent infection surveillance enables the identification of demographic areas and/or geographic areas (clusters) that may benefit from intensified prevention/testing activities and public health responses, with the ultimate goal of disrupting HIV transmission. **The test is intended for Research Use Only. Not for use in diagnostic procedures.**

The *Asanté* test is not intended to be used for HIV diagnosis or clinical management of HIV-positive persons because recency status does not alter ARV treatment eligibility or clinical decision making. Although the test detects recently infected individuals with mean duration of recent infection (MDRI) of about 6 months, due to the visual interpretation of the test it is not recommended that the test be used for estimation of incidence. For customers interested in a more precisely quantitative estimation of HIV incidence, please use our laboratory-based assay, LAg Avidity EIA, which can be used in combination with viral-load and ARV screening for this purpose [37,38].

BACKGROUND OF HIV-1 INCIDENCE TESTING

The public health community needs to know where and among whom HIV is spreading and where intervention can be most effective at reducing the spread of the HIV epidemic. Accurate assays for the accurate

identification of recent versus long-term infections of HIV-1 have been sought. “Recent” and “long-term” are defined by the mean duration of infection and currently inferred to be 6 months (mean duration) for “recent” infections, or longer than 12 months for “long-term” infections, for this assay (see “Interpretation of Results” below). The MDRI takes into account distribution of the recency window around the mean which can vary from <3 months to almost a year depending on individual antibody maturation kinetics.

Such information can therefore be a useful tool in surveillance, program planning, effectiveness of intervention programs and planning for vaccine or other prevention trials.

A variety of laboratory-based assays have been evaluated as an alternative to longitudinal cohort studies to determine HIV-1 incidence. These earliest methods included desensitized or “less sensitive” commercial HIV immunoassays [1-8] where the lower titers of anti-HIV antibodies typical of recent infections were used as a basis for identifying those individuals likely to be recently infected. However, since desensitized commercial HIV immunoassays were developed by modifying commercial assays that employ HIV-1 subtype B antigen(s), these tests were sometimes found to be less accurate in populations containing primarily non-subtype B infections [7,8]. To overcome this subtype bias liability and address the extreme sample dilution and assay variability problems also observed with desensitized assays, scientists at the US CDC developed the BED capture-EIA (BED-cEIA), which employed a synthetic antigen containing sequences from multiple subtypes and a simple capture format to allow the measurement of the proportion of HIV-1 antibodies which increase over time after seroconversion [9]. The BED-cEIA has been used in several studies [10-15]. However, high False Recency Rates (the frequency of false recent results from long-term infections) which give overestimations of HIV-1 incidence, have been reported at varying levels with the BED-cEIA depending on population

[16-19]. Consequently post-test adjustments have been proposed to improve the accuracy of those incidence estimates [20, 21]. Assays to determine recent HIV-1 infection based on antibody maturation as measured by antibody avidity have been studied [22-31] and have resulted in low false recency rates in those studies conducted in US populations [29,30]. However, avidity assays based on commercial assays or based on a single antigenic subtype may demonstrate the same subtype bias described above for desensitized assays. As a result, US CDC developed newer avidity assays incorporating a new recombinant protein ("rIDR-M") containing the major variants of gp41 immunodominant regions among the HIV-1 group M viruses, including a one-well avidity assay using limiting amounts of antigen [32]. Testing performed on a number of well characterized samples indicated that subtype bias is minimized by the use of the multi-subtype antigen [32-34]. Furthermore, studies evaluating one of these assays, the Sedia® HIV-1 LAg-Avidity EIA (Sedia Cat. No. 1002) have estimated proportion false recent (PFR) excluding treated patients and elite controllers, to be about 1% [33,34], suggesting improved accuracy over previous technologies. The *Asanté*™ HIV-1 *Rapid Recency*® Assay expands upon the technology in the Sedia® HIV-1 LAg-Avidity EIA by incorporating the same rIDR-M antigen to identify recent vs. long-term infections, but in a rapid lateral flow type of format to be used at point-of-care, instead of a laboratory based EIA. Like the BED-cEIA and the Sedia® HIV-1 LAg-Avidity EIA, the *Asanté*™ HIV-1 *Rapid Recency*® Assay is intended for use on specimens that have already been diagnosed as HIV-1 positive. It is not itself intended for diagnostic use or for use in making decisions about clinical care.

The US Centers for Disease Control and Prevention (US CDC) has conducted studies estimating the MDRI of the *Asanté*™ HIV-1 *Rapid Recency*® Assay which is approximately 170-180 days [41]. PFR is expected to be similar to the Sedia® HIV-1 LAg-Avidity EIA [40]. ARV treatment resulting in viral suppression, specifically over a long period of time, can

result in decay of HIV antibodies and additional misclassification. Therefore, as part of recent infection testing algorithm (RITA), use of viral load (and ARV, if possible) to mitigate or reduce PFR is recommended.

BIOLOGICAL PRINCIPLES OF THE TEST

The *Asanté*™ HIV-1 *Rapid Recency*® Assay is a single-use point-of-collection immunoassay for distinguishing recent HIV-1 infections from those which are long-term. Results are obtained in 20 minutes. The *Asanté*™ HIV-1 *Rapid Recency*® Assay is comprised of a Specimen Collection Loop, a capped tube containing 0.5 mL of Sample Buffer and a Test Strip. The Test Strip itself is composed of several materials which in combination are capable of detecting HIV antibodies when a blood, serum or plasma sample containing HIV antibodies is added to the Sample Buffer Tube.

The specimen may be collected as blood, serum or plasma by conventional clinical means (e.g. venipuncture, lancet finger-stick, serum or plasma separation) and a sample transferred by dipping the Specimen Collection Loop into the blood, serum, or plasma to completely fill the Loop. The loopful of sample is then transferred to the Sample Buffer Tube, and mixed with agitation to release the blood/serum/plasma into the buffer. The test is initiated by simply placing the Test Strip into the Sample Buffer Tube containing the sample, with the arrows on the Test Strip pointing down, and a timer set for 20 minutes is started. When the Test Strip is placed into the Sample Buffer mixture, the Sample Buffer mixture is absorbed into the absorbent pad at the end of the Test Strip. This absorbent pad contains additional reagents to condition the sample and prepare it for optimal reactivity in the remainder of the Test Strip. The sample mixture continues to migrate up the Test Strip by a wicking action, until it encounters a dehydrated reagent composed of Protein A conjugated to a colloidal gold reagent (”conjugate”), which is rehydrated by the liquid. This conjugate confers a reddish-purple coloration to the liquid which is used later in the Test Strip to visualize the results. Protein

A will bind to both HIV-positive (if present) and HIV-negative antibodies in the liquid containing the sample.

The reddish-purple liquid, containing the Protein A Gold, Sample Buffer and sample, continues to migrate up the Test Strip onto a nitrocellulose membrane which contains three invisible reagent lines (in order of sample contact: a Long-Term/Recent Line (referred to as simply the ”LT/R Line”), a Sample Verification Line (referred to as simply the “Verification Line”) and a Functional Control Line (referred to as simply the “Control Line”). The test results are read using this region of the membrane. The reddish-purple liquid will continue to be drawn up to the top of the Test Strip until the reddish-purple cloud that initially appeared on the membrane has cleared 20 minutes after the start of the test.

As the reddish-purple sample liquid containing antibodies bound to the conjugate crosses the membrane, it first encounters the LT/R Line, which contains the HIV-1 rIDR-M recombinant antigen bound to the membrane at a concentration that will also bind HIV-1 antibodies likely to be of higher avidity (particularly since movement of the sample is initially most rapid here) and/or present at a higher concentration in the sample, typical of a long-term infection. Since these HIV-1 antibodies will also be bound to the conjugate, the reddish-purplish colored reagent will accumulate on the LT/R Line by means of the capture of the HIV antibodies to the rIDR-M antigen. If a reddish-purple line forms on the LT/R Line, it is indicative of a long-term infection. The terms “recent” and “long-term” refer to the Mean Duration of Recent Infection (“MDRI”) as defined in the “Interpretation of Results” section below.

The liquid sample continues to migrate up the Test Strip, next encountering the Verification Line, which contains p24, HIV-1 gp41, and HIV-2 gp36 recombinant viral antigens bound to the membrane which bind HIV-1 or HIV-2 antibodies present in the specimen. (It is important in areas where HIV-2 is likely to be encountered, that HIV-2 specimens be subsequently identified and excluded from test results as they will likely

be reported as recent infections regardless of true recency of infection.) Since these HIV antibodies will also be bound to the conjugate, the reddish-purplish liquid will accumulate on the Verification Line by means of the capture of the HIV antibodies to the viral proteins. In a valid test, a reddish-purple Verification Line forms in the presence of HIV antibodies. This line must be present to have a valid LT/R Line result. The appearance of a Verification Line serves to verify that only specimens that have been determined to be HIV-positive are being tested. If no Verification Line appears from a previously diagnosed HIV specimen, test should be rerun with a freshly prepared sample and assay. In addition, the specimen's diagnostic status should also be confirmed based on prior documentation of an approved antibody-based diagnostic test algorithm result, or if not available, evaluated by an approved diagnostic test algorithm. The Verification Line is not intended to determine the diagnostic status of the individual but only to verify that the test is suitable for use on the sample tested for recency information.

Finally, the liquid sample will continue to migrate up the strip, encountering the Control Line. The Control 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. If the correct sample type has been used and the test is run correctly, based on the procedures outlined below, the Control Line should be present: a clearly visible reddish-purple line. The appearance or non-appearance of the Control Line is determined by the amount of antibodies detected. Any test result of the other two reaction lines when the Test Strip yields a missing Control Line should be considered an invalid and the test should be rerun with a fresh sample and assay.

The results of the test are interpreted at 20 minutes after adding the Test Strip to the Sample Buffer containing the sample. After 20 minutes, the sample containing antibodies will have had adequate time to migrate up

the entire Test Strip encountering both the colored Protein A-gold colloid conjugate and the three reaction lines to give a test result. Refer to the "Interpretation of Results" section below.

MATERIALS PROVIDED

The *Asanté*™ HIV-1 *Rapid Recency*® Assays are available in 20 Test Packs (Cat. No. 1130-020) and 100 Test Packs (Cat. No. 1130-100).

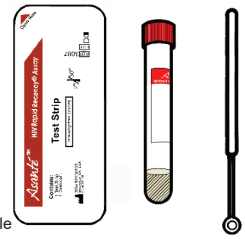
Each Test Pack contains the following single-use components (components must not be reused):

Cat. No. 1130-020

- 1 Bag of 20 Test Strips with Desiccant in sealed foil pouches
- 1 Bag of 20 Sample Buffer Tubes
- 1 Pack of 20 Specimen Collection Loops
- 1 Disposable Foam Sample Buffer Tube Rack

Cat. No. 1130-100

- 5 Bags of 20 Test Strips with Desiccant in sealed foil pouches
- 5 Bags of 20 Sample Buffer Tubes
- 5 Packs of 20 Specimen Collection Loops
- 1 Disposable Foam Sample Buffer Tube Rack



Note: Each bag of Specimen Collection Loops contained minimum 20 loops.

MATERIALS REQUIRED BUT NOT PROVIDED

- Lancet for finger-stick blood collection.
- Phlebotomy supplies for blood collection, optional. (Samples may be collected using the provided Specimen Collection Loops).
- Alcohol or antiseptic wipes.
- Timer or watch.

- Mechanical pipette with disposable tip(s) capable of transferring 5 µL, optional. (May be used to transfer a blood, serum or plasma sample from a specimen tube into the Sample Buffer Tube instead of using the Specimen Collection Loop).
- *Asanté*™ Rapid Test Strip Reader (Research Use Only), optional, if read visually. Recommended for maximum accuracy and to avoid subjective reads. Contact Sedia at customerservice@sediabio.com for availability, pricing, and other product use information.
- 10% bleach solution (0.5% sodium hypochlorite) for disinfection.
- Personal protection equipment. (PPE) (disposable gloves, lab coat, safety glasses or goggles, as appropriate).
- Biohazardous waste container.

WARNINGS

1. The *Asanté*™ HIV-1 *Rapid Recency*® Assay is for Research Use Only and is not intended for use in diagnostic procedures.
2. The *Asanté*™ HIV-1 *Rapid Recency*® Assay is a secondary assay that is intended to be performed only on specimens that have been previously diagnosed as HIV-1 positive. It is not intended as a test to identify HIV-1 infected individuals or as a confirmatory diagnostic test.
3. HIV-2 specimens will react with the Verification Line but are unlikely to react with the LT/R Line and are therefore likely to give a “recent” result regardless of 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 to confirm that they are not HIV-2-reactive specimens.

4. Be sure to read this product insert completely before performing the test. It is important to follow the instructions carefully to avoid obtaining inaccurate results.
5. The *Asanté*™ HIV-1 *Rapid Recency*® Assay is intended only for use with venous or finger-stick blood, serum or plasma specimens. Testing with any other specimen type may not give accurate results. Do not use heparin anticoagulant.
6. Lipemic, hemolyzed or microbially contaminated blood, serum or plasma, or old or partially or fully coagulated blood may cause the assay to run improperly, or not at all or may give erroneous results. Samples tested as whole or finger-stick blood should be tested immediately after collection before coagulation occurs if no anticoagulant is present. Otherwise, if an anticoagulant is present, test as soon as possible but in no case more than 24 hours after collection. Specimens to be stored for longer than 24 hours should be converted to serum or plasma and stored frozen.
7. The desiccant provided will change color if the desiccated state is compromised. Check the color of the indicator dye on the desiccant. Stable (dry) desiccant, indicated acceptable humidity while stored, will be either blue or orange. Compromised (wet) desiccant, indicating unacceptable humidity while stored, will be either pink or green. Pouches with compromised desiccant (pink or green dyed) indicate that the strip within that pouch cannot be used, so the test strip must be discarded.
8. Check the expiration date of the kit and each dated component (Pouched Test Strip and Sample Buffer Tube) prior to use. Do not use any materials after the expiration date printed on the component's package labeling.

9. Ensure that the specimen is introduced into the Sample Buffer in the Sample Buffer Tube. Failure to add the sample properly may result in an invalid test result.
10. The Control Line only indicates addition of specimen to the test and does not guarantee sufficiency of specimen volume having been added.

PRECAUTIONS

1. The *Asanté*™ HIV-1 *Rapid Recency*® Assay should be performed at ambient temperature (i.e. 15-37°C).
2. The *Asanté*™ HIV-1 *Rapid Recency*® Assay should be performed in a well-lit area.
3. Do not drink, eat, smoke, or apply cosmetics while handling specimens.
4. Practice Universal Precautions [35] when handling whole blood, serum or plasma specimens, and used assay components.
5. Wear gloves, a lab coat, and eye protection when handling specimens or materials exposed to blood or blood components. Wash hands thoroughly after handling specimens and tests. Use of disposable gloves is recommended.
6. Used gloves and used test supplies should be discarded as biohazardous waste after use. Lancets, syringes and other sharps should be disposed of in a puncture-resistant container prior to disposal as biohazardous waste.
7. Liquid wastes should be first mixed with appropriate chemical disinfectants such as 10% household bleach (0.5% sodium hypochlorite) before disposal. (CAUTION: Do not autoclave solutions containing bleach).

8. Wipe all work areas before and after testing with an appropriate chemical disinfectant such as 10% household bleach. Wipe all spills thoroughly with disinfectant.
9. Each test component (Pouched Test Strip, Sample Buffer Tube and Specimen Collection Loop) is intended for a single use. Do not use more than once. If a test must be repeated, use all new components for the retest with a freshly collected sample aliquot.
10. Pouched Test Strips and Sample Buffer Tubes are matched to work with each other in each kit. Don't interchange or use Sample Buffer Tubes and Pouched Test Strips from different kit lots.
11. Avoid handling kit components to minimize contamination. In particular, avoid handling the Results Region (i.e. membrane) of the Test Strip. Refer to the section, "Results Region of the Test Strip", below.
12. After performing the test, results may be read either visually or using an *Asanté*™ Rapid Test Strip Reader. Visual reads are subjective and may be less accurate than those obtained with the *Asanté*™ Rapid Test Strip Reader, depending on the skill level, lighting conditions, and experience of the operator performing the visual reads. If read visually, read the results using adequate lighting to maximize accuracy. For optimal results, read the Test Strips using the *Asanté*™ Rapid Test Strip Reader, especially if the test is used in incidence surveillance. Do not use other readers as they may not give accurate results, may not be designed to target the 3 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 (*Asanté*™ Rapid Test Strip Reader)", section below. The *Asanté*™ Rapid Test Strip Reader is for Research Use Only.

STORAGE CONDITIONS

Unused *Asanté*™ HIV-1 *Rapid Recency*® Assays may be stored unopened at 2-30°C until the product expiration date. Do not open the Pouched Test Strip or Sample Buffer Tube until ready to perform a test. If the test is stored refrigerated, take the test out of the refrigerator and bring to ambient temperature (15-37°C) before opening the component packaging.

TEST PREPARATION

1. Be sure to receive training on how to perform and interpret the results of this test. Training materials can be obtained by emailing Sedia customer support at customerservice@sediabio.com
2. Specimens suitable for testing with the *Asanté*™ HIV-1 *Rapid Recency*® Assay include whole blood (either venous or finger-stick), serum or plasma.
3. Ensure you have all specimen collection and test materials needed before starting.
4. Allow the *Asanté*™ HIV-1 *Rapid Recency*® Assay to come to ambient temperature (15-37°C) before running the test.

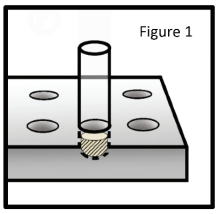


Figure 1

5. Remove the cap from the Sample Buffer Tube and place the tube in a test tube rack. Discard the cap. See Figure 1 at right. Proceed to Step 6A or 6B depending on the sample to be tested.

6A. Finger-stick 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 side of the finger-tip. Gently squeeze the finger to yield a drop of blood. Do not squeeze excessively or "milk" the finger. See Figure 2 at right.

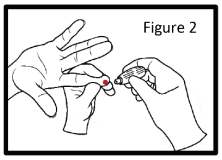


Figure 2

Remove a Specimen Collection Loop from the pouch of Loops in the Pack. Touch the round end of the Loop to the drop of blood, allowing the blood on the finger to fill the Loop. See Figure 3 at right.

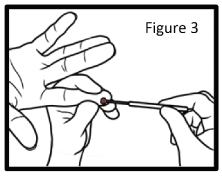


Figure 3

Be sure there are no bubbles and the Loop is completely filled with blood. See Figure 4 at right.

Proceed to Testing Procedure below.

Figure 4

6B. Venous blood, serum or plasma samples

Venous blood should be collected by standard phlebotomy methods and serum/plasma separated from blood cells. Plasma specimens should be collected with specimen collection tubes containing EDTA or ACD (acid/citrate/dextrose) anticoagulants. Do not use heparin anticoagulant.

Samples may be transferred from the blood, serum or plasma specimen collection tube using either a Specimen Collection Loop, or using a pipette with a disposable tip capable of accurately measuring 5 mL.

Transfer using the Specimen Collection Loop.
Dip the round end of the Loop to the blood, serum or plasma in the collection tube sufficiently to draw liquid specimen up into the Loop, completely filling the Loop. See Figure 5 at right. Visually inspect the Loop to make sure that it is completely filled with specimen and does not contain a bubble. **Inspect carefully to confirm that the droplet of specimen is not just a large bubble.**

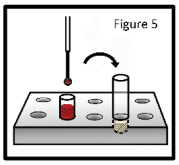


Figure 5

Transfer the loopful of sample directly into the open Sample Buffer Tube. Agitate the Loop in the tube to thoroughly mix the sample with the Sample Buffer.
See Figure 6 at right.

Proceed to Testing Procedure below.

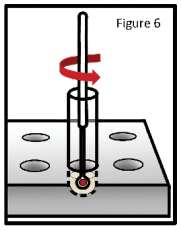


Figure 6

Transfer using a Pipette. Using a pipette with a clean disposable pipette tip, transfer 5 µL of specimen to the Sample Buffer Tube and into the Sample Buffer. Agitate the Sample Buffer Tube to mix the specimen. See Figure 7 at right.

Proceed to Testing Procedure below.

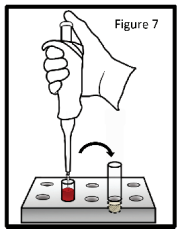


Figure 7

Testing Procedure

Once the blood, serum or plasma has been collected and mixed with the Sample Buffer, the test can be performed on the diluted sample.

1. Open the foil pouch containing the Test Strip and remove the Test Strip. See Figure 8 at right. Do not touch the middle of the Test Strip which displays the test results. Inspect the desiccant packet inside the foil pouch and make sure that one is present and that the indicator dye is blue or orange in color. If no desiccant packet is present, discard the Test Strip and obtain another Test Strip. If the desiccant is pink or green, the seal may have been compromised and the Test should be discarded, and this step should be repeated with another Pouched Test Strip before proceeding.

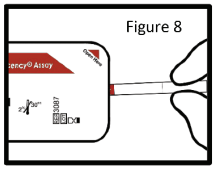


Figure 8

2. Insert the Test Strip into the liquid in the Sample Buffer Tube with the arrows pointing down toward the liquid. See Figure 9 at right. Set a timer to 20 minutes, or note the time on a watch.

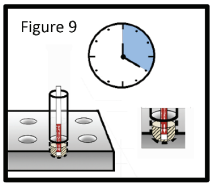


Figure 9

3. Wait for 20 minutes and remove the Test Strip from the Sample Buffer Tube. Dab the lower end of the strip onto a filter paper to remove excess buffer, then lay flat on the bench.

4. Immediately after removing the Test Strip from the Sample Buffer Tube, read the test results on the Test Strip visually or with the *Asanté*™ Rapid Test Strip Reader (available separately). See Figure 10 at right. Be sure to use the appropriate size cartridge to hold the strip. (Newer strips are 5 mm, prior versions of strips are 4.5 mm). For maximum accuracy, the results should be read with the *Asanté*™ Rapid Test Strip Reader. Refer to Interpretation of Results section below.

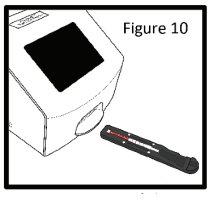


Figure 10

IMPORTANT: Do not read the results earlier than 20 minutes after placing the Test Strip into the Sample Buffer Tube containing the specimen. Results should be interpreted within 1 minute of removing the strip. After reading the results, dispose of the used Test Strip and Sample Buffer Tube in accordance with Universal Precautions.

QUALITY CONTROL

The *Asanté*™ HIV-1 *Rapid Recency*® Assay has a built-in procedural control that establishes assay validity. A reddish-purple line in the Control Line region of the Test Strip membrane indicates that a proper specimen was collected and run in the test, and that the Test Strip functioned properly. This Control Line will appear on all valid tests whether or not the LT/R or Verification Lines give a reactive or non-reactive result. If the Control Line does not appear on a given test, the test is invalid and the results of the other reaction lines of the Test Strip should be ignored, regardless of their presence or absence. An invalid test will then need to be repeated with a new specimen, Specimen Collection Loop, Sample Buffer Tube, and Test Strip.

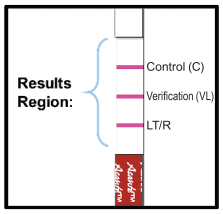
Before starting a new lot of *Asanté*™ HIV-1 *Rapid Recency*® Assays, or at the beginning of each day of testing, it is recommended that the following samples be tested as Controls to verify the performance of the test kits:

1. A known HIV-negative serum or plasma specimen.
2. A known recent HIV-1 infection serum or plasma specimen.
3. A known long-term HIV-1 infection serum or plasma specimen.
4. A known HIV-2 positive serum or plasma specimen.

External controls for the *Asanté*™ HIV-1 *Rapid Recency*® Assay are in development but not commercially available. Contact customerservice@sediabio.com for more information on availability.

RESULTS REGION OF THE TEST STRIP

Test results are interpreted in the Results Region of the Test Strip, either visually or using the *Asanté*™ Rapid Test Strip Reader. Refer to the Results Region of the Test Strip in the figure at right.

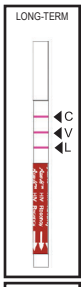


- The Control Line is a functional control line that indicates that the assay was performed as recommended. Absence of Control Line indicates either the assay is not functioning properly, or that sample collection/addition was inadequate or improper.
- The Verification Line is meant to verify HIV-positive serostatus as previously determined by an approved HIV diagnostic algorithm.
- The LT/R Line is a test line to differentiate if the specimen is either a long-term infection (when the line is present) or recent infection (when the line is absent). Recent infection is defined as an infection which has seroconverted within a mean duration of approximately the last 180 days.

INTERPRETATION OF RESULTS (VISUAL)

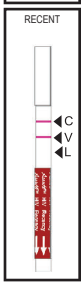
Long-Term Infection

The figure at right shows an example of a Long-Term Infection test result. A sample is considered long-term when all three reactive lines appear as reddish-purple lines, i.e., the top Control Line, the middle Verification Line, and the bottom LT/R Line should all be visible. A long-term HIV-1 infection is interpreted as an infection that was likely acquired more than 1 year ago.



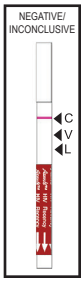
Recent Infection

The figure at right shows an example of a Recent Infection test result. A sample is considered a recent infection when the top two reactive lines appear as reddish-purple lines, i.e., the top Control Line and the middle Verification Line are both visible, but the bottom LT/R Line is not. Although the mean duration is about 6 months, recency at the individual level can vary around the mean. Therefore, in the context of an individual a recent HIV-1 infection is interpreted as an infection that was likely acquired within the past year.



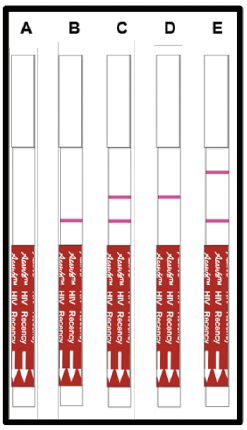
Negative/Inconclusive

The figure at right shows an example of a Negative or Inconclusive test result. Except for the negative QC specimen, all other samples should be interpreted as inconclusive when only the Control Line is present. A sample giving a Negative test result should be verified with an approved HIV diagnostic test if previously diagnosed HIV positive. (The *Asanté*™ HIV-1 Rapid Recency® Assay should only be run on diagnosed HIV-1



positive specimens). All client samples are interpreted as inconclusive (neither recent nor long-term) when only the top line (i.e. the Control Line) appears as a reddish purple line and the middle Verification Line and lower LT/R Line are not visible.

Invalid



The figure at right shows several examples of Invalid test results. 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).

If a specimen gives a positive LT/R Line, but negative Verification Line, the results are also invalid (Strip E at right).

Specimens that give invalid results should be retested with a new sample aliquot dispensed into a fresh Sample Buffer Tube and tested with a new Test Strip. If the retest still gives an invalid result, a new specimen should be collected and retested. An invalid result indicates an inadequate or improper sample was collected, the assay was not performed correctly, or the assay is not functioning properly.

INTERPRETATION OF RESULTS (USING OPTIONAL *Asanté*™ RAPID TEST STRIP READER, FOR RESEARCH USE ONLY)

To minimize subjective variability of individual visual reads of the Test Strips, the *Asanté*™ Rapid Test Strip Reader (Cat. No. 1200) is recommended to read the Test Strips. Follow the instructions provided with the

Test Strip Reader using the settings specified for the *Asanté*™ HIV-1 *Rapid Recency*® Assay.

Control Line

Test Strips that do not display an adequate Control Line (Result ≥ 3.000 on the *Asanté*™ Rapid Test Strip Reader) regardless of the results of other lines on the Test Strip, are considered INVALID and will report an INVALID result on the Test Strip Reader. An invalid result on the Control Line means that the Test Strip should be discarded, the remaining Test Strip results ignored, and the specimen should be retested with a new sample aliquot dispensed into a fresh Sample Buffer Tube and tested with a new Test Strip. If the retest still gives an invalid result, a new specimen should be collected and retested. An invalid result indicates an inadequate or improper sample was collected, the assay was not performed correctly, or the assay is not functioning properly.

Verification Line

A Verification Line result ≥ 2.800 on the *Asanté*™ Rapid Test Strip Reader indicates a “Positive” result for the Verification Line, verifying that the specimen contains HIV-reactive antibodies. Verification Line result < 2.800 indicates a “Negative” result for the Verification Line indicative of a specimen that contains no detectable HIV antibodies. Confirmation of borderline results should be performed according to the next section on Confirmatory Testing.

Results inconsistent with prior HIV diagnostic testing should be confirmed with an approved HIV diagnostic test algorithm.
The Verification Line should not be used for diagnostic purposes, but only as an alert to verify the original diagnostic results if confirmation has not already been done with an approved diagnostic test.

LT/R Line

A LT/R Line result ≥ 3.000 on the *Asanté*™ Rapid Test Strip Reader indicates the infection is a "Long-Term" one as long as the Verification Line is positive and the Control Line gives a valid result. Recency Line result < 3.000 indicates the infection is a "Recent" one as long as the Verification Line is positive and the Control Line gives a valid result. Confirmation of borderline results should be performed according to the next section on Confirmatory Testing. **A negative Verification Line with a LT/R Line result ≥ 3.000 should be considered an invalid result and retested or the original diagnostic result should be confirmed with an approved diagnostic test algorithm.**

The terms "Recent" or "Long-Term" here refer to infections shorter or longer, respectively, than the Mean Duration of Recent Infection (MDRI) for the test. The MDRI, defined as the time from HIV seroconversion to the assay's cut-off for recent infection (LT/R Line = 3.000), is approximately 180 days [41].

Confirmatory Testing of the Initial *Asanté*™ HIV-1 *Rapid Recency*® Assay Result

To minimize the effect of strip to strip variability, Confirmatory Testing is recommended for specimens giving a borderline result on either the LT/R Line, or the Verification Line. Borderline results are those where the Verification Line is > 2.400 and < 3.200, or where the LT/R Line is > 2.600 and < 3.400.

Verification Line

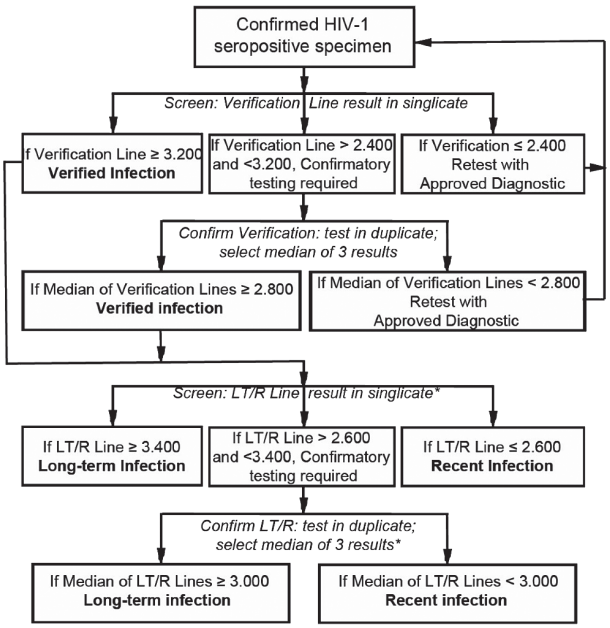
Specimens yielding a Verification Line result on a single initial test > 2.400 and < 3.200 should be repeat tested using two new Sample Buffer preparations of the specimen, with two more Test Strips and read on the *Asanté*™ Rapid Test Strip Reader. The median of all three results (i.e. the initial and two confirmatory tests) should be used as the final

determinant against a cutoff of 2.800 to determine confirmation of the positive infection status of the specimen. For example, if a specimen is initially tested and the Verification Line gives a result on the Test Strip Reader of 2.731 (normally a “Negative” result), and on confirmatory testing gives results of 2.914 and 2.952 (both “Positive” results), the final result is the median, or 2.914, and should be classified as “Positive”. Refer to the figure below for the full algorithm used for testing and interpretation.

LT/R Line

Specimens yielding LT/R Line results on a single initial test > 2.600 and < 3.400 should be repeat tested using two new Sample Buffer preparations of the specimen, with two more Test Strips and read on the *Asanté*™ Rapid Test Strip Reader. The median of all three results (i.e. the initial and two confirmatory tests) should be used as the final determinant against a cutoff of 3.000 to determine the recency status of the specimen. For example, if a specimen is initially tested and the LT/R Line gives a result on the Reader of 2.879 (normally a “Recent” result), and on confirmatory testing gives results of 3.020 and 3.054 (both “Long-Term” results), the final result is the median, or 3.020, and should be classified as “Long-Term”. Refer to the figure below for the full algorithm used for testing and interpretation.

The flow chart below outlines the algorithm for reading the *Asanté*™ HIV-1 *Rapid Recency*® Assay results on the *Asanté*™ Rapid Test Strip Reader to determine recency of infection.



*If previously tested in triplicate to confirm Verification Line, results, use median of LT/R Line Confirmatory testing.

LIMITATIONS OF THE TEST

1. The *Asanté*™ HIV-1 *Rapid Recency*® Assay must be used according to the instructions in this product insert to obtain accurate results.

2. Results must be read immediately (within 1 minute) after removing the strips from Sample Buffer Tubes but no earlier than 20 minutes after inserting the Test Strip into the Sample Buffer Tube containing the specimen. Reading results at times shorter or longer than recommended times may give inaccurate results.
3. The *Asanté*™ HIV-1 *Rapid Recency*® Assay is for Research Use Only. It is not intended for use in diagnostic procedures. The Assay is intended for use only with venous or finger-stick blood, serum or plasma collected as described.
4. The US CDC has conducted an evaluation of the performance of this assay using a well-characterized panel of cross-sectional specimens with known HIV serology status and recent or long-term status based on comparative LAg-Avidity EIA results. This US CDC evaluation has determined the accuracy of the test and estimated the window period of recent infection which is inferred to be approximately 180 days [40, 41]. Antibody development varies from individual to individual, therefore transition from recent to long-term can occur early or later, compared to the mean duration, among different individuals. Furthermore additional independent evaluation of the assay is planned to generate more data in specimens from individuals of known duration of infection. The False Recency Rate (FRR) of this assay has been estimated to be similar to the Sedia® HIV-1 LAg-Avidity EIA or about 1% excluding treated patients and elite controllers [33, 34, 40, 41]. For updates on the most current information on these parameters, check the Sedia Biosciences website at www.sediabio.com.
5. Persons with diagnosis of AIDS or low CD4+ T cell counts (below 200 cells per µL), recipients of anti-retroviral therapy and known “elite controllers” (HIV-infected individuals with known low or undetectable viral loads) should be excluded from the study populations to reduce the likelihood of misclassification of recency of infection.

Incorporation of viral load testing will reduce the risk of such individuals being misclassified as recent (see “Recommended Recent Infection Algorithm” below).

6. Recent studies [37, 38] and UNAIDS/WHO [39] now recommend that where possible, viral load (VL) testing should be incorporated into a multi-test algorithm (recent infection testing algorithm [RITA]) incorporating serological assays to improve on the accuracy of recent infection classification. If viral load 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. RITA 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.
7. The Verification Line of the *Asanté*™ HIV-1 *Rapid Recency*® Assay does not distinguish between HIV-1 and HIV-2 and will bind to both HIV-1 and HIV-2 antibodies since it contains antigens to both. The LT/R Line of the Assay contains only an HIV-1 specific antigen. HIV-2 positive persons will always classify as recent, irrespective of the duration of their infection in the Assay, yielding false recent results in long-term HIV-2 infections. If this assay is used in areas or populations where HIV-2 is endemic, or on specimens otherwise suspected of being HIV-2 positive, type-specific diagnosis should be performed before final recency classification is made, to exclude HIV-2 positive specimens from recency analysis.
8. This product is provided as a Research Use Only product to users requiring the means to detect the recency of infection in specimens and individuals that have already been diagnosed using an approved HIV diagnostic test algorithm. This assay should not be used for any diagnostic use or case management for individual

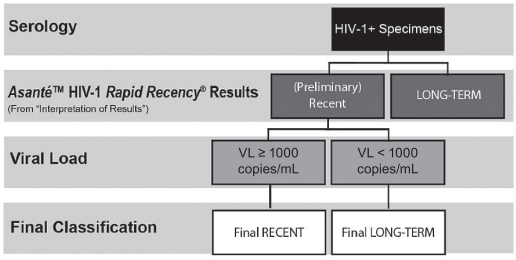
patients in the absence of a locally approved algorithm for HIV diagnostic testing. The US CDC has conducted a preliminary research study of the product's Verification Line and LT/R Line performance characteristics. Results may be found as reported [41].

RECOMMENDED RECENT INFECTION ALGORITHM

Based on recommendations by UNAIDS/WHO [39], US CDC [38] and CEPHIA [37], it is recommended that the user incorporate viral load testing into the test algorithm of population surveys analyzed by serological HIV incidence assays (also referred to as HIV recency assays), such as the *Asanté*™ HIV-1 *Rapid Recency*® Assay as shown below to reduce and minimize the impact of false recent infections, primarily attributable to elite controllers and subjects on ARV.

Viral load testing is necessary only on *Asanté*™ HIV-1 Rapid Recency® Assay samples classified as "recent infections" (usually <10% of total positives in most populations).

Recommended *Asanté*™ HIV-1 Rapid Recency® Assay and Viral Load Algorithm



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SYMBOLS AND ABBREVIATIONS

The following symbols appear in *Asanté*™ HIV-1 *Rapid Recency*® Assay labeling.

REF	Part Number		Manufacturer
LOT	Lot number (batch code)	USA	Country of manufacture
	Consult instructions for use		Do not use if package is damaged and consult instructions for use
	Do not reuse		

Asanté™ HIV-1 *Rapid Recency*® Assay

Ordering Information

REF	Cat. No. 1130-100	100 Bulk Packaged Tests
REF	Cat. No. 1130-020	20 Bulk Packaged Tests

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Related Products Available from Sedia Biosciences:

SEDIA® HIV-1 LAg-Avidity EIA (Cat. No. 1002)

Asanté™ Rapid Test Strip Reader (Cat. No. 1200)

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