**HIV-1/HIV-2 Antibody Test**

Single-use rapid assay for the detection of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2)

**INTENDED USE - Not for donor screening**

The INSTI HIV-1/HIV-2 Antibody Test is a single use, rapid, flow-through in vitro immunoassay for the detection of antibodies to Human Immunodeficiency Virus Type 1 and Type 2 in human EDTA whole blood, fingerstick blood, serum or EDTA-plasma. The test is intended for use by trained personnel in medical facilities, clinical laboratories, emergency care situations, and physicians' offices as a diagnostic test capable of providing results in less than one minute. Although suitable for near-patient or point-of-care (POC) testing, the INSTI HIV-1/HIV-2 Antibody Test is not suitable for home testing. All requiring care and post-test counseling guidelines must be followed in each setting in which the INSTI HIV-1/HIV-2 Antibody Test is used.

**SPECIMEN COLLECTION AND STORAGE**

1. For EDTA or whole blood, EDTA-plasma or serum specimens, follow normal venipuncture blood collection procedures using lavender top EDTA anticoagulant tubes (for whole blood and plasma) or red-top (no anticoagulant) tubes for serum.
2. If plasma or serum is to be used, separate from the blood cells by centrifugation.
3. Specimens: EDTA-plasma may be stored at 4°C for up to 5 days, stored frozen at ≤ -20°C for 3 months, or stored frozen at ≤ -70°C for one year.
4. Whole blood specimens collected in EDTA anticoagulant may be stored at 2-8°C and should be used within 48 hours. Do not heat or freeze whole blood specimens.

**KIT COMPONENTS AND STORAGE**

1. INSTI HIV-1/HIV-2 Antibody Test
2. Single-use Alcohol Swab
3. Single-use Lancing Device
4. Color Developer
5. Clarifying Solution

**STORAGE TEMPERATURES**

- INSTI HIV-1/HIV-2 Antibody Test: 15–30°C
- Single-use Alcohol Swab: -40°C to 15°C
- Lancing Device: 15–30°C
- INSTI HIV-1/HIV-2 Antibody Test: 15–30°C
- Color Developer: 15–30°C
- Clarifying Solution: 3°C to 30°C

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Personal protective equipment such as gloves, lab coat or gown.
- Appropriate biohazard waste containers.
- Absorbent cotton balls for fingerstick or venipuncture wound closure.

**SAMPLE COLLECTION**

1. EDTA or whole blood
2. EDTA-plasma
3. Serum

**ANTIGEN SELECTION**

1. The INSTI HIV-1/HIV-2 assay utilizes a combination of recombinant membrane proteins from HIV-1 (gp120) and HIV-2 (gp36). Use of these proteins overcomes sensitivity and specificity problems associated with tests based on viral lysates or a combination of core antigens and other viral proteins.

2. Antibody Detection: The INSTI HIV-1/HIV-2 assay uses a unique reagent to detect antibodies to HIV-1 and HIV-2. Although primarily designed to detect the IgG class of specific antibodies, the INSTI HIV-1/HIV-2 assay may be used as a diagnostic tool in cases of HIV infection, during seroconversion, and low titre anti-HIV-1 samples obtained late in infection (see tables 1, 2, and 3).

3. Test Complexity: The INSTI HIV-1/HIV-2 assay was designed to reduce protocol complexity. The INSTI HIV-1/HIV-2 assay does not require sample preparation, accurate timing, or several steps, which include multiple wash requirements. The requirements increase the complexity of an assay and lead to procedural errors which may adversely affect sensitivity and specificity. Total test time may vary slightly depending on specimen type; but results of valid tests are always clearly readable within one to two minutes.
7. Failure to use the recommended reagent and specimen volumes may result in leakage and/or overflow of liquids from the membrane unit.

8. If the test kit is exposed to temperatures outside of 15°-30°C, ensure it is brought to this temperature range before performing testing. Use the INSTI Controls to ensure proper kit performance.

9. Patients that have been on long term antiretroviral drug therapy may give a false negative INSTI HIV-1/HIV-2 Test result.

10. Samples from patients with severe hypogammaglobulinemia conditions such as multiple myeloma may result in false negative or invalid results with INSTI.15

11. Patients with elevated hemoglobin levels may test false negative with INSTI.15

PRECAUTIONS

1. All specimens should be handled as if capable of transmitting infectious diseases. It is recommended that BioSafety Level 2 practices, or equivalent regulations, be observed.14

2. Thoroughly wash hands after handling or performing this test.

3. Do not smoke, eat, or drink in areas where specimens or kit reagents are being handled.

4. Wear a lab coat and disposable gloves while handling kit reagents or specimens. Do not pipette by mouth.

5. Avoid contact with skin and eyes. If contact occurs, wash affected areas with water.

6. Avoid forming aerosols.

7. Dispose of all specimens and materials used to perform the test as if they contained infectious agents. The preferred method of disposal is incineration for a minimum of one hour at 121°C followed by incineration. Liquid waste not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 0.5% sodium hypochlorite (a solution containing 10% household bleach). Allow at least 30 minutes for decontamination to be completed. Do not autoclave solutions that contain bleach.

8. Spills should be cleaned up and decontaminated in accordance with the user facility’s established procedures for handling biohazardous spills.

ASSAY PROCEDURE

NOTE: All Membrane Units must be used immediately once opened. All reagents should be dispensed evenly in the center of the well.

Sampling Fingerstick Blood:

1. Gather support materials (swab, lancet, pipette), one sealed test pouch containing INSTI Membrane Unit, and one vial each of the Sample Diluent, Color Developer, and Clarifying Solution for each test to be performed.

2. Massage the finger to allow the blood to move to the surface (fingertip will become pink). Use heating pad if available to warm the hand. Hand must be positioned at waist level or lower.

3. Wipe the fingertip with the alcohol swab.

4. As soon as the finger is dry, twist and remove the protective insert from the lancet. Press the finger firmly at the point just below where the lancet will be applied. With the other hand, hold the lancet by the body and lightly press the tip of the lancet on the finger and then push down to release the needle (see diagram below). Immediately dispose the used lancet into a proper sharps container.

5. As the blood bubbles up, hold the pipette horizontally and touch the tip of the pipette to the blood sample. Capillary action automatically draws the sample to the fill line and stops. All visible trickles out of the puncture. Gently apply intermittent pressure near the puncture site to obtain the equilibrated blood volume. If blood is inadequate, perform a second skin puncture using a new lancet.

Y CAUTION! Filling is automatic: Never squeeze the tube while sampling.

6. Transfer the blood held in the pipette to the Sample Diluent vial (Solution 1). Align the tip of the pipette with the Sample Diluent vial and squeeze the bulb to dispense the sample (See Figure A). NOTE: If the sample will not expel, hold the pipette vertically and apply gentle pressure from below until the vent hole, then squeeze the bulb (See Figure B). Recap the vial and mix by inversion. Follow General Procedure after Sampling, below.

Sampling EDTA Whole Blood, serum, EDTA-plasma and Test Controls:

1. Bring specimens to room temperature and mix each specimen thoroughly prior to use. Do not heat or repeatedly freeze/thaw specimens.

2. Gather one sealed test pouch containing INSTI Membrane Unit, and one vial each of the Sample Diluent, Color Developer, and Clarifying Solution for each kit (See Note) to the Sample Diluent vial. Recap the vial and mix by inversion. Adding an excessive amount of specimen may cause the device to overflow or leak.

NOTE: In POC settings, for INSTI kit controls, it is important to use a 50μl pipette device to add the control material to the Sample Diluent vial. Do not use the disposable single-use pipette provided for finger stick blood collection.

General Procedure after Sampling:

1. Tear open the pouch and carefully remove the Membrane Unit without touching the center well. Place the unit on a level surface. For sample identification purposes the tab of the Membrane Unit may be labeled with the patient’s name or number. NOTE: At this point, it is important that the following steps be performed immediately and in sequence.

2. Remix the Sample Diluent-specimen mixture and pour the entire contents to the center of the Membrane Unit well. (NOTE: Do this within 5 minutes after the specimen has been added to the Sample Diluent vial). The sample should be absorbed through the membrane in less than 30 seconds; however, absorption times will vary slightly depending upon sample type.

3. Resuspend the Color Developer by slowly inverting to mix the solution thoroughly. Continue this process until careful visual observation confirms that the reagent is evenly suspended. Open the Color Developer and add the entire contents to the center of the Membrane Unit well. The colored solution should flow through completely in about 20 seconds.

4. Open the Clarifying Solution and add the entire contents to the center of the Membrane Unit well. This will lighten the background color and facilitate reading. Immediately read the result while the membrane is still wet. Do not read the results if more than 5 minutes has elapsed following the addition of Clarifying Solution.

QUALITY CONTROL

Kit Controls:

The INSTI HIV-1/HIV-2 Antibody Test has a built-in IgG capture procedural control that demonstrates assay validity and adequate sample addition. A blue color in the control spot indicates that the proper specimen was added and that the assay procedure was performed correctly. The control spot will appear on all valid INSTI tests. (Refer to Interpretation of Results, below.)

INSTI HIV-1/HIV-2 Test Controls are available separately for use only with the INSTI HIV-1/HIV-2 Antibody Test. The controls are used to verify test performance and interpretation of results. Kit controls should be run under the following circumstances:

- for new INSTI operator verification prior to performing testing on patient specimens
- when switching to a new lot number of INSTI test kits
- whenever a new shipment of kits is received
- when temperature during storage of the kit falls outside of 15°-30°C
- when the temperature of the test area falls outside of 15°-30°C
- at regular intervals as determined by the user facility.

Refer to the INSTI HIV-1/HIV-2 Test Controls instructions for use for additional information on the use of these reagents. It is the responsibility of each user of the INSTI HIV-1/HIV-2 Antibody Test to establish an adequate quality assurance program to ensure proper performance under their specific locations and conditions of use.

INTERPRETATION OF RESULTS

- Do not read the results if more than 5 minutes has elapsed following the addition of Clarifying Solution.
- If using the control samples provided by bioLytical, all Positive Controls must be reactive with INSTI and all Negative Controls must be non-reactive with INSTI. Controls that produce incorrect or invalid results must be re-tested with INSTI. If results are still incorrect or invalid, inform bioLytical Laboratories immediately.

NON-REACTIVE ► One blue dot that is clearly discernable above any background tint should appear on the membrane. This is the procedural Control Spot and shows that the test has been performed correctly. The control is located towards the top of the read frame furthest from the plastic tab on the Membrane Unit. No reaction should be visible at the test spot, located below the control. A non-reactive result indicates that antibodies to HIV-1/HIV-2 were not detected in the specimen.

REACTIVE ► Two blue dots that are discernable above any background tint indicate that the specimen contains HIV-1/HIV-2 antibodies. One dot may be darker than the other. A sample giving this pattern is considered a preliminary reactive. Following a reactive rapid test result, a venous blood sample should be drawn in a lavender-top EDTA collection tube (for whole blood or plasma) or red-top tube (for serum), and forwarded to a laboratory for HIV confirmatory testing.

NOTE: Invalid tests with fingerstick blood samples in POC settings should be repeated with a fresh sample using a new membrane unit, kit components and support materials. Invalid tests with EDTA whole blood, EDTA plasma or serum samples in laboratory settings should be repeated using a new membrane unit and kit components.
Please note the following:
1. Following a reactive or indeterminate INSTI test result, a venous blood sample must be drawn in a lavender-top EDTA collection tube (for whole blood or plasma) or red-top tube (for serum), and forwarded to laboratory for HIV confirmatory testing.
2. To perform a second antibody titer, a reactive specimen may be less intense in color than the procedural control, or vice versa.
3. A blue spot of color discernibly darker than the background color should be interpreted as reactive or positive. In rare instances, a faint background ring may appear around the control or test spot; this should not be interpreted as a reactive result. Only tests exhibiting distinct fully formed blue test dot combined with a distinct fully formed blue control dot should be interpreted as reactive. Color intensity may be variable within or between the dots.
4. In an invalid test that indicates the test was performed incorrectly, or if there is a problem with the sample or device. The absence of a distinct control dot usually indicates that the sample volume was insufficient. An invalid test result must be repeated.
5. A test result in a uniform blue tint across the entire membrane, thus obscuring the control and test spots, can occur when more than 60 μL of whole blood is used and the flow through the assay membrane is obstructed.
6. An individual who has a non-reactive result but was involved in HIV-risk activity is likewise recommended to obtain additional testing over the next month.
7. To significantly reduce the risk of HIV transmission, it is advisable to refrain from high risk activities such as unprotected sex and needle sharing at all times.

LIMITATIONS OF THE TEST

Flow Times
- In some instances, samples may exhibit longer than normal flow times (from the time the Sample Diluent specimen is placed into the membrane well to the time the color bands are fully flowed through the membrane). This is due to variable factors such as cellular components, especially white blood cells. For a sample of normal flow times, a faint shadow in the form of a ring may appear at the test spot location, but this should not be interpreted as a reactive result. This should be considered an indeterminate result.

Performance Characteristics

Sensitivity and Specificity:
- The sensitivity of a test is the ability of a test to detect truly infected people; whereas, the specificity of a test is the ability of a test to identify all non-infected individuals. Thus, a sensitive test should not produce false negatives, and a specific test should not produce false positives. There is no single standard for detecting the sensitivity and specificity of an antibody test for HIV in various sera, plasma, or whole blood.
- However, the generally accepted method to express the sensitivity and specificity of a given test is in terms of the detection rate which is compared to results of approved supplemental assay results, such as ELISA and Western Blot.
- The sensitivity and specificity of the INSTI HIV-1/2 Antibody test were determined using matched fingerstick blood, EDTA whole blood, serum and plasma samples from patients throughout the CDC Morbidity and Mortality Report.

Table 4

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HIV-2
The sensitivity of INSTI HIV-1/HIV-2 antibody test evaluated in an independent European study with 49 sera from Western Blot confirmed HIV-2 infected patients at the chronic stage of the infection was 100%.
An additional study conducted in-house with 88 different HIV-2 positive serum and plasma samples obtained from European sources and added into individual whole blood (to simulate HIV-2 positive blood) also showed 100% sensitivity of INSTI for HIV-2 antibody detection.

HIV-1 SUBTYPE TESTING
Forty eight samples from 48 patients infected with HIV-1 non B strains were tested. All samples were genotyped by deoxyribonucleotide sequencing of the entire HIV-1 protease gene and the first 450 codons of reverse transcriptase, for subtype determination. The subtype distribution was the following: A7, C8, D8, F6, G8, J1, CRF AG: 5, CRF AE: 5.
The sensitivity of the INSTI HIV-1/HIV-2 Antibody Test on the 48-non B HIV positive samples tested was 100%.

REPRODUCIBILITY
The reproducibility of the INSTI HIV-1/HIV-2 Antibody Test was tested at 3 laboratory sites using 3 lots of reagent controls from 3 inactivated HIV+ patient plasma samples, consisting of 4 antibody positive, 1 very low antibody level sample, and 4 antibody negative samples was tested at each site. A total of 729 tests were conducted, 243 at each site. For the 4 antibody positive and 4 antibody negative samples, the overall reproducibility was 99.7% (726/729, two antibody negative samples were read as weak positive at 1 site). For the 1 very low antibody sample, 59% (48/81) of the results were positive while 41% (33/81) were negative.

BIBLIOGRAPHY
11. World Health Organization/Global Programme on AIDS. Operational characteristics of commercially available assays to detect antibodies to HIV-1 and/or HIV-2 in human sera. Geneva, Switzerland: WHO documents GPA/BMP/89.4; GPA/BMP/90.1; GPA/RES/DIA90.1; GPA/RES/DIA91.6; GPA/RES/DIA92.6 and GPA/RES/DIA93.4.