INSTI HIV-1/HIV-2 Antibody Test - 48 pack

Interest in any of the products, request or order them at Bio-Connect Diagnostics.
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 qualitative immunosassay 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 near-patient or point-of-care (POC) testing. 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 near-patient or point-of-care (POC) testing.

SUMMARY

Acquired Immunodeficiency Syndrome (AIDS) is caused by at least two retroviruses, HIV-1 and HIV-2. HIV-1 and HIV-2 are similar in genomic structure, morphology and ability to cause AIDS. HIV is transmitted mainly by sexual contact, exposure to blood or body fluids, or from an infected mother to her fetus. People with increased risk of HIV infection include haemophiliacs, intravenous drug users and men having sex with men (MSM). HIV has been isolated from patients with AIDS, AIDS-related complex (ARC), and from persons at high risk of contracting AIDS. Antibodies specific for HIV envelope proteins are prevalent in sera from persons at high risk of contracting AIDS as well as in people with AIDS, or ARC. The presence of antibodies to HIV indicates previous exposure to the virus, but does not necessarily constitute a diagnosis of AIDS. The prevalence of antibodies to HIV in people not known to be at risk of acquiring HIV infection is unknown, but significantly less. Absence of antibodies to HIV does not indicate that an individual is absolutely free of HIV-1 or HIV-2, nor that the individual has been isolated from seronegative individuals prior to seroconversion. Test specificity and sensitivity depend, amongst other factors, on: a) the selection of HIV antigens used for antibody detection, b) the classes of antibodies recognized by the detection conjugate, and c) the complexity of the protocol used to perform the test. Non-specific reactions may be observed in some specimens. A reactive INSTI test result should be considered a preliminary result, and appropriate counselling provided in POC settings. Following a reactive rapid test result, a venous blood sample must be drawn in an EDTA collection tube (for whole blood or plasma) or a tube with no anticoagulant (for serum), and forwarded to a laboratory for HIV confirmatory testing.

PRINCIPLES OF THE TEST

The INSTI HIV-1/HIV-2 Antibody Test is a manual, visually read, flow through immunosassay for the qualitative detection of HIV-1/HIV-2 antibodies in human blood, serum or plasma. The test consists of a synthetic filtration membrane positioned atop an absorbent material within a plastic cartridge, referred to as the INSTI Membrane Unit. The membrane has been specifically treated with HIV-1 and HIV-2 recombinant proteins, which react with HIV-1/HIV-2 antibodies in the specimen to produce a distinct visual signal on the membrane. The membrane also includes a procedural control. The procedural control consists of a protein-A treated test spot capable of capturing IgG antibodies normally present in blood and blood components. IgG antibodies react with a proprietary chromatic agent to produce a visual signal on the membrane. Since IgG antibodies are present in blood from normal or HIV positive human specimens, the control spot provides a visual signal when the test is run, indicating that the test was performed correctly. If the control spot does not appear, the test is considered invalid. In the case of the test spot, recombinant HIV-1 and HIV-2 proteins, embedded in the membrane, capture HIV specific antibodies from the test specimen. Antibody binding results in the test spot react with a proprietary chromatic agent to produce a visible signal on the membrane. The membrane unit is designed to filter, absorb, and retain the test specimen and all the test reagents in such a manner as to limit leakage and exposure of personnel to potentially infectious materials. Reagents required to conduct a test include Sample Diluent (Solution 1), Color Developer (Solution 2) and Clarifying Solution (Solution 3). The test is performed by adding the blood, serum, or plasma specimen to the vial of Sample Diluent, which lyses the red blood cells. This specimen/diluent solution is then poured onto the well of the Membrane Unit. HIV-1/HIV-2 antibodies, if present in the specimen, are captured by proteins on the filtration membrane. Color Developer is then added to the Membrane Unit. The Color Developer reacts with the captured antibodies to generate a distinct blue dot at the location of the control spot and, in the case that HIV-1/HIV-2 antibodies are present in the specimen, a blue dot also appears at the location of the test spot on the membrane. In the final step, the Color Developer solution is then added to the membrane to breakdown background color in order to make the control and test spots more distinct.

Antigen Detection: The INSTI HIV-1/HIV-2 assay utilizes a combination of recombinant transmembrane proteins from HIV-1 (gp41) and HIV-2 (gp36). Use of these proteins overcomes species-specific and specificity problems associated with tests based on viral lysates or a combination of core antigen and other viral proteins.

Antibody Detection: The INSTI HIV-1/HIV-2 assay utilizes a unique reagent to detect antibodies to HIV-1/HIV-2. Although primarily designed to detect the IgG class of specific antibodies, the INSTI HIV-1/HIV-2 assay has been shown to detect antibodies in samples obtained early in infection, during seroconversion, and in samples from highly reactive to HIV-1 samples obtained later in infection (see tables 1-2 and 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 washes and reagents. These requirements increase the complexity of an assay and increase the number of tests which might affect the test's complexity. Total test time may vary slightly depending on specimen type; but results of valid tests are always clearly readable within one to two minutes.

SPECIMEN COLLECTION AND STORAGE

1. For EDTA-whole blood, EDTA-plasma or serum specimens, normal venipuncture blood collection procedures using lavender-top EDTA anticoagulant tubes (for whole blood and plasma) or red-top (no anticoagulant) tubes for serum. EDTA-plasma specimens may be stored at 2-8°C for up to 5 days, stored frozen at ≤-20°C for 3 months, or stored frozen at ≤-70°C for at least 1 year. Whole blood specimens collected in EDTA anticoagulant may be stored at 2-8°C and should be tested within 48 hours. Do not heat or freeze whole blood specimens.

2. Do not dilute prior to testing.

KIT COMPONENTS AND STORAGE

INSTI components should be stored at 15-30°C. For 90-1023 and 90-1026, components are provided for 48 tests. Each test requires the following materials:

- 1. Membrane Unit, individually packaged, prepared with control (IgG capture) and test (gp41 and gp36 antigen) reaction spots. For single use only in the INSTI procedure.
- 2. Sample Diluent, X, R22, Solution 1 vial, containing 1.5 ml of tris-glycine buffered solution containing cell lysates, reagents, with adequate space for addition of blood, serum or plasma samples to be tested with INSTI. Ready to use, no mixing or preparation required. Contains 0.1% sodium azide as a preservative, for single use only in the INSTI procedure. Stable to date and under storage conditions indicated on label. A blue dropper graduated to dispense 1.5 ml is included.
- 3. Color Developer, X, R22, Solution 2 bottle, containing 85 ml of a blue-coloured Borate buffered proprietary indicator solution designed to detect IgG in the control spot and specific HIV antibodies in the test spot. Ready to use, no mixing or preparation required. Contains 0.1% sodium azide as a preservative. Stable to date and under storage conditions indicated on label. A white dropper graduated to dispense 1.5 ml is included.

SUPPORT MATERIALS

- 1. Single-use Alcohol Swab
- 2. Single-use Lancet
- 3. Single-use Pipette, capable of dispensing 50μl

MATERIALS REQUIRED BUT NOT PROVIDED

- Personal protective equipment such as gloves, lab coat or gown.
- Absorbent cotton balls for fingertip or venipuncture wound closure.

For venipuncture blood collection:

- Venipuncture apparatus if collecting blood samples.
- Appropriate blood collection tubes.
- Precision pipette capable of delivering 50μl of sample.
- Appropriate shipping container.
- Personal protective equipment.
- Appropriate biohazard waste containers and disinfectants.

MATERIALS AVAILABLE AS AN ACCESSORY TO THE KIT

INSTI HIV-1/HIV-2 Test Controls: Separate HIV-negative human serum substitute and HIV-1/HIV-2 positive de-fibribrated human plasma control samples product no. 90-1037 are available from biologic Laboratories in user-defined amounts, for use in quality control procedures. Please refer to the section on Quality Controls, following the Assay Procedure, and the INSTI HIV-1/HIV-2 Test Controls instructions for use.

WARNINGS

For in vitro diagnostic use only

It is recommended that the entire Package Insert be read prior to beginning the test procedure. Although the assay is designed to be simple to use, conformance with the test procedure is necessary to ensure accurate results.

1. Do not mix reagents from different lots.
2. Do not use reagents or kits beyond the stated expiration date.
3. Do not use the Membrane Unit if the pouch has been previously opened or if the packaging integrity has been compromised. Once the Membrane Unit has been opened, it must be used immediately.
4. Avoid microbial contamination of reagents.

- Sodium azide is present at 0.1% in all assay reagents. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. If products containing sodium azide are discarded into a drain, flush with large amounts of water to prevent azide buildup. Check with local regulatory agencies to determine at which concentration sodium azide may cause a product to be regulated as hazardous waste.
5. The performance characteristics of the INSTI HIV-1/HIV-2 assay have not been established for body fluids other than EDTA whole blood, fingertip blood, serum, and EDTA-plasma. The use of blood collected in anticoagulants other than EDTA has not been validated. Insufficient data are available...
Patients that have been on long term antiretroviral drug therapy may give a false negative INSTI.

All specimens should be handled as if capable of transmitting infectious diseases. It is

Adding an excessive amount of specimen

2. To prepare the Color Developer and Clarifying Solution bottles, remove the caps and seals from the

5. As the blood bubbles up, hold the pipette horizontally and touch the tip of the pipette to the blood

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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

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

2. To prepare the Color Developer and Clarifying Solution bottles, remove the caps and seals from the

NOTE: The Color Developer and Clarifying Solution may form bubbles during handling. This
does not affect the performance of the test. To ensure that the proper volume of solution is
used in the test, completely fill the dropper with the solution up to the 1.5 ml mark. If
bubbles form in the dropper, ensure that the fluid level below the bubbles is at the 1.5 ml
mark. Use within one month after opening. Recap the droppers when not in use.

3. Gather one sealed test pouch containing INSTI Membrane Unit, and one vial of Sample Diluent for
each test to be performed.

4. Using a pipette, add 50 μl of whole blood, serum, plasma, or kit controls (see Note) to the Sample

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

General Procedure after Sampling:

1. Tear open the pouch and carefully remove the Membrane Unit without touching the center well.

NOTE: At this point, it is important that the following steps be performed immediately and in

2. Remix the Sample Diluent-specimen mixture and pour the entire contents to the center of the

3. Open the Color Developer bottle and with the blue dropper, slowly draw solution up to the 1.5 ml mark. Add the solution to the center of the Membrane Unit well. The colored solution should flow through completely in about 20

4. Open the Color Developer bottle and with the blue dropper, slowly draw solution up to the 1.5 ml mark. Add the solution 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
• 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,
informe biolitical Laboratories immediately.

NON-REACTIVE
The 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 must 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.

INVALID
The test is invalid if any of the following occurs:
A. There is no dot on the membrane
B. The test dot appeared without the control dot
C. Uniform tint across the membrane
D. Only blue specks appear on the membrane
**Limitations of the Test**

1. To significantly reduce the risk of HIV transmission, it is advisable to refrain from high-risk activities.
2. An individual who has a non-reactive result but was involved in HIV-risk activity is likewise
3. **The sensitivity of the INSTI HIV-1/HIV-2 assay was**
4. **The relative sensitivity of the INSTI HIV-1/HIV-2 assay for early antibody detection was also**
5. **EDTA-plasma samples, which were also analyzed for anti-HIV-antibodies using ELISA and Western**
6. **The prevalence of HIV infection in various groups, as well as clinical and public health guidelines,**
7. To significantly reduce the risk of 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**: Instances of prolonged flow times, samples may exhibit longer than normal flow times (from the time the sample
- **Insufficient data are available to interpret tests performed on other body fluids, pooled blood or**
- **The INSTI HIV-1/HIV-2 Antibody Test has not been validated for detection of antibodies to HIV-1**
- **The INSTI HIV-1/HIV-2 Antibody Test detects antibodies to HIV-1/HIV-2 and is useful in establishing infection with HIV. Because a variety of factors may cause non-specific reactions, a patient found to be positive using the INSTI HIV-1/HIV-2 assay should have an EIA blood sample drawn for laboratory-based confirmatory testing. A person who has antibodies to HIV is presumed to be infected with the virus and therefore requires consulting and medical evaluation should be offered. The presence of HIV antibodies indicates past exposure to HIV but is not a diagnosis of AIDS, which can only be made by a physician. However, a non-reactive test does not rule out past exposure to HIV. The presence of HIV antibodies indicates past exposure to HIV but is not a diagnosis of AIDS, which can only be made by a physician. However, a non-reactive test does not rule out past exposure to HIV.**

**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 not to detect non-infected individuals. Thus, a sensitive test should not produce false negatives, and a specific test should not produce false positives. There is no standard for detecting the sensitivity or specificity of an antibody test for HIV in human sera, plasma or whole blood. However, the generally accepted method to express the sensitivity and specificity of a given test in terms of the detection rate is to compare results to approved supplemental assay results, such as ELISA and Western Blot.
  - Based on these criteria, the sensitivity and specificity of the INSTI HIV-1/HIV-2 assay was determined using fingerstick blood, EDTA whole blood, serum and EDTA-plasma samples, which were also analyzed for anti-HIV-antibodies using ELISA and Western Blot. Samples tested using the INSTI HIV-1/HIV-2 test fall into 4 categories:
  1. Twenty five commercial seroconversion panels (Table 1) and one HIV-1 low titer antibody
  2. Canadian HIV seroconversion patient samples (Table 2).
  3. Prospective samples from HIV-infected patients in the Canadian Clinical Trial (Table 4)
  4. Prospective negative samples from patients enrolled in the Canadian Clinical Trial (Table 5)

**Biological Laboratories’ Canadian Clinical Trial data show**

1. The relative sensitivity of the INSTI HIV-1/HIV-2 assay for early antibody detection was assessed using standardized seroconversion panels from Boston Biomedica Inc. Table 1 summarizes the INSTI HIV-1/HIV-2 assay data compared to a number of US licensed and European approved enzyme immunoassays (EIA) using the commercial panels.
2. The relative specificity of the INSTI HIV-1/HIV-2 assay for early antibody detection was also assessed using Canadian seroconversion patients. Table 2 summarizes the data from the Canadian seroconversion patients.
3. The sensitivity of the INSTI HIV-1/HIV-2 assay was 99.5% for fingerstick blood, EDTA blood, plasma and serum (range 99.0-99.9%) (Table 4) Indeterminate and invalid results were eliminated from evaluation.
4. The specificity of the INSTI HIV-1/HIV-2 assay was 99.3% (range 99.3-100%) for fingerstick blood, EDTA blood, plasma and serum (Table 5). Indeterminate and invalid results were eliminated from evaluation.
5. INSTI HIV-1/HIV-2 Antibody Test results were not affected by most potentially interfering conditions or substrates as illustrated in Table 6. Samples from patients with severe hypogammaglobulinemia conditions such as multiple myeloma may result in false negative or invalid results with INSTI.

**Table 1**

<table>
<thead>
<tr>
<th>Antibody Test</th>
<th>Number of Panels</th>
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<tr>
<td>Anti-HIV-1 Serocconversion Panel PRB-900 Series* Boston Biomedica Inc.</td>
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**Table 2**

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**Reproducibility**

The reproducibility of the INSTI HIV-1/HTLV-2 antibody test was tested at 3 laboratory sites using 3 lots of the INSTI device on 3 separate days. A panel of 9 blind-coded plasma samples, consisting of 4 antibody positive, 4 antibody negative, 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% (646/648, two antibody negative results were positive while 41% (33/81) were negative.

**Clinical Evaluation**

The sensitivity of the INSTI HIV-1/HTLV-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-2 Subtyping**

Forty-eight samples from 48 patients infected with HIV-1 non B strains were tested. All samples were genotyped by dideoxynucleotide 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: 7
- C8: 5
- D1: 6
- F6: 7
- G8: 1
- J1: 2
- CRF AG: 5
- CRF AE: 5

The sensitivity of the INSTI HIV-1/HTLV-2 Antibody Test on the 48 non-B HIV positive samples tested was 100%.

**BIBLIOGRAPHY**