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)

90-1015 - One INSTI® HIV-1/HIV-2 Antibody Test with support materials (for POC use)

2°C–30°C	Store at 2°C to 30°C	STERILE R	Sterilization using irradiation
⚠	Caution	LOT	Lot number
IVD	*In Vitro* diagnostic medical device	REF	Catalogue Number
	Consult instructions for use		Manufacturer
2	Do not reuse	CE	CE Mark
	Use by	X_n	R22 – Harmful if swallowed

2°C–30°C **Store at 2°C – 30°C. For *in vitro* diagnostic use only.** IVD

It is recommended that the entire Instructions for Use 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.

INTENDED USE - Not for donor screening

The **INSTI HIV-1/HIV-2 Antibody Test** is a single use, rapid, flow-through *in vitro* qualitative 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 intended for home testing. All required pre, and post-test counselling guidelines must be followed in each setting in which the INSTI HIV-1/HIV-2 Antibody Test is used. The assay is packaged as a kit containing INSTI Membrane Unit, Sample Diluent (Solution 1), Colour Developer (Solution 2), and Clarifying Solution (Solution 3) with support materials (lancet, pipette and alcohol swab).

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.[1] HIV is transmitted mainly by sexual contact, exposure to blood or blood products, or from an infected mother to her foetus. 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.[2-5] 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.[5-7] 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.[5] **Absence of antibodies to HIV does not indicate that an individual is free of HIV-1 or HIV-2; HIV 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) complexity of the protocol used to perform the test.[8] Non-specific reactions may be observed in some specimens. A reactive INSTI test result should be considered a preliminary result, with 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 immunoassay 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 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 dot provides a visual signal when the test is run, indicating that the test was performed correctly. If the control dot 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, if present in the specimen. Antibodies captured 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), Colour 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. Colour Developer is then added to the Membrane Unit. The Colour 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 Clarifying Solution is then added to the membrane to decrease background colour in order to make the control and test dots more distinct.

Antigen Selection: 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 sensitivity and specificity problems associated with tests based on viral lysates or a combination of core antigen and other viral proteins.[9-13]

Antibody Detection: The INSTI HIV-1/HIV-2 assay uses 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 low titre anti-HIV-1 samples obtained later in infection.

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 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 minute.

SPECIMEN COLLECTION AND STORAGE

1. For EDTA-whole blood, EDTA-plasma or serum specimens, follow 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. Serum or EDTA-plasma 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 one year.
4. 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.**
5. Do not dilute prior to testing.

KIT COMPONENTS AND STORAGE

2°C–30°C INSTI components should be stored at 2-30°C.
For **90-1015** all kit components are individually packaged for single use only. 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_n R22, Solution 1 vial, containing 1.5 ml of tris-glycine buffered solution containing cell lysis reagents, with adequate space for addition of blood, serum or plasma samples being 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.
3. **Colour Developer**, X_n R22, Solution 2 vial, containing 1.5 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. For single use only in the INSTI procedure. Ready to use, invert 2-3X immediately before use. Contains 0.1% sodium azide as a preservative. Stable to date and under storage conditions indicated on label.
4. **Clarifying Solution**, X_n R22, Solution 3 vial, containing 1.5 ml of a proprietary tris-glycine buffered clarifying solution designed to remove background staining from the membrane unit prior to reading the INSTI test results. Ready to use, no mixing or preparation required. For single use only in the INSTI procedure. Contains 0.1% sodium azide as a preservative. Stable to date and under storage conditions indicated on label.

SUPPORT MATERIALS

The following materials are required when testing fingerstick whole blood and included with each kit:

1. Single-use Alcohol Swab
2. Single-use Lancet STERILE R CE 0344
3. Single-use Pipette, 50µl

MATERIALS REQUIRED BUT NOT PROVIDED

- Personal protective equipment.
- Appropriate biohazard waste containers and disinfectants.
- Absorbent cotton balls for fingerstick or venipuncture wound closure.

For venipuncture blood collection and testing:

- Venipuncture apparatus if collecting blood samples.
- Appropriate blood collection tubes.
- Appropriate shipping containers.
- Precision pipette capable of delivering 50µl of sample.

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-fibrinated human plasma control samples product no. 90-1036 are available from bioLytical Laboratories, for use in quality control procedures. Please refer to the section on Quality Control, following the Assay Procedure, and the INSTI HIV-1/HIV-2 Test Controls Instructions for Use.

WARNINGS

For *in vitro* diagnostic use only IVD

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 foil 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.
5. ⚠ 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 build-up. Check with local regulatory agencies to determine at what concentration sodium azide may cause a product to be regulated as hazardous waste.
6. The performance characteristics of the INSTI HIV-1/HIV-2 assay have not been established for body fluids other than EDTA whole blood, fingerstick blood, serum, and EDTA-plasma. The use of blood collected in anticoagulants other than EDTA has not been validated. Insufficient data are available to interpret tests performed on other body fluids, pooled blood or pooled serum and EDTA-plasma, or products made from such pools.
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 kit is refrigerated, ensure it is brought to room temperature before performing the test. Use the INSTI HIV-1/HIV-2 Test Controls to ensure proper kit performance.
9. ⚠ Patients that are 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.
11. Patients with elevated haemoglobin 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 sterilization by autoclaving 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

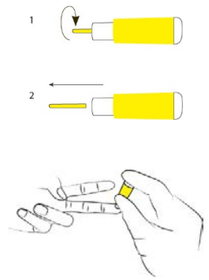
NOTE: All Membrane Units must be used immediately once opened. All reagents should be dispensed evenly in the center of the well.

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, Colour Developer, and Clarifying Solution for each test to be performed.

   ⚠ **CAUTION! The amount of sample (fingerstick blood) is critical**. To ensure that the proper amount of blood is achieved, follow these instructions carefully:

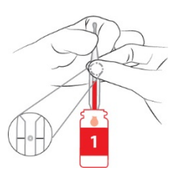
2. Massage the finger to allow the blood to move to the surface (fingertip will become pink). Use heating pad if available to warm the hand. Hand must be positioned at waist level or lower.
3. Wipe the fingertip with the alcohol swab.
4. As soon as the finger is dry, twist and remove the protective insert from the lancet. Press the finger firmly at the point just below where the lancet will be applied. With the other hand, place the lancet on the side of the fingertip and press hard until it clicks. Immediately dispose the used lancet into a proper sharps container.



5. As the blood droplet forms, 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. If very little blood trickles out of the puncture, gently apply intermittent pressure below the puncture site to obtain the required blood volume. If blood is inadequate, perform a second skin puncture using a new lancet.

⚠ **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. **NOTE:** If the sample will not expel, hold the pipette vertically and slide a finger over (without pressing) the vent hole, then squeeze the bulb. 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, Colour Developer, and Clarifying Solution for each test to be performed.
3. Using a pipette, add 50µl of whole blood, serum, plasma, or kit controls (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 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. Mix the Sample Diluent-specimen mixture by inverting several times 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 Colour Developer by slowly inverting to mix the solution thoroughly until the reagent is evenly suspended and add the entire contents to the center of the Membrane Unit well. The coloured 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 colour 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 colour on the control dot indicates that the proper specimen was added and that the assay procedure was performed correctly. The control dot 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 2°-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.

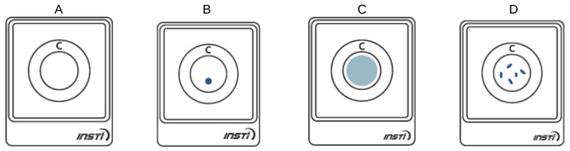
INTERPRETATION OF RESULTS

- **Do not read the results if more than 5 minutes have 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 Dot and shows that the test has been performed correctly. The control dot location is indicated by the letter C printed 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



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.

INDETERMINATE ► The test is indeterminate if a faint background ring appeared on the test area. Following an 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 a laboratory for HIV confirmatory testing.

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 a laboratory for HIV confirmatory testing.
2. Depending on the antibody titer, a reactive specimen may be less intense in colour than the procedural control, or vice versa.
3. Only a blue spot of colour discernibly darker than the background colour should be interpreted as reactive or positive. In rare instances, a faint background ring may appear around the 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. Colour intensity may be variable within or between the dots.
4. An invalid result indicates that the test was performed incorrectly or 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 must be repeated.
5. A test resulting in a uniform blue tint across the entire membrane, thus obscuring the control and test spots, can occur when more than 60µL 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 months.
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 mixture is poured in the membrane well to the time the Clarifying Solution has fully flowed through the membrane). This is due to variable factors such as cellular components, especially with whole blood. **In instances of long 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 as an indeterminate result.** In these instances, a venous blood sample should be drawn in a lavender-top EDTA collection tube, and forwarded to a laboratory for HIV confirmatory testing.
- The INSTI HIV-1/HIV-2 Antibody Test procedure and the interpretation of result must be followed closely when testing for the presence of antibodies to HIV in serum, plasma or whole blood.
- Insufficient data are available to interpret tests performed on other body fluids, pooled blood or pooled serum and plasma, or products made from such pools; therefore, testing of these specimens is not recommended.
- The INSTI HIV-1/HIV-2 Antibody Test has not been validated for detection of antibodies to HIV-1 Group N subtypes.
- 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 EDTA blood sample drawn for laboratory-based confirmatory testing. A person who has antibodies to HIV is presumed to be infected with the virus and appropriate counseling 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 risk of an asymptomatic person with repeated reactive serum developing AIDS is not known. The prevalence of HIV infection in various groups, as well as clinical and public health guidelines, are available in the CDC Morbidity and Mortality Report.[8]

PERFORMANCE CHARACTERISTICS

SENSITIVITY

DETECTION OF ANTIBODIES TO HIV-1 IN SPECIMENS FROM INDIVIDUALS INFECTED WITH HIV-1

A multi-center prospective study was conducted to evaluate the clinical performance of the INSTI HIV Antibody Test. There were 483 subjects known to be HIV-1 positive, and 905 subjects with unknown HIV status. The subjects with unknown HIV status were tested with INSTI and by a composite reference method (comparator method) which consisted of an licensed/approved EIA with supplemental Western blot and PCR assays as required. The result of INSTI was compared to the known or determined HIV status of the subject.

In this study, all 517/517 true HIV antibody positive subjects were identified as reactive by the INSTI HIV-1/HIV-2 Antibody Test, resulting in a relative sensitivity of 100.0% (95 % CI = 99.3% - 100.0%). There were no invalid results (0/1388) observed in this study.

Detection of HIV-1 Antibody in Fingerstick Whole Blood Specimens from HIV-1 Seropositive Individuals

Study Population	Number of Subjects	INSTI Reactive	Approved Test Reactive	True Positive
HIV status unknown	905	34	34	34
Known HIV-1 Positive	483	483	483	483
TOTAL	1,388	517	517	517

Reactivity with HIV-1: Seroconversion Panels

Thirty (30) HIV-1 seroconversion panels (Boston Biomedica Inc.) were tested with INSTI. Each panel consisted of sequential serum/plasma specimens obtained from a single individual during seroconversion. The results of this study are presented in the table below and summarizes the INSTI HIV-1/HIV-2 assay data compared to US licensed and European approved HIV antibody enzyme immunoassays (EIA). Overall the INSTI HIV-1/HIV-2 Antibody Test has similar performance to commercially available anti-HIV EIA in the detection of HIV antibodies in seroconversion samples.

INSTI HIV-1/HIV-2 TEST:	Number of Panels
Detected the earliest bleed that was detected by an EIA	15
Detected within 1 bleed of earliest EIA positive	10
Detected within 2 bleeds of earliest EIA positive	3
Unknown**	2

**The last bleed in the panel was reactive by at least 1 EIA, non-reactive by INSTI

Reactivity with HIV-1: Low Titer Panel

A single low titer HIV-1 antibody panel (#PRB-108; Boston Biomedica) was tested with the INSTI HIV-1/HIV-2 Antibody Test. This low titer panel consisted of 15 serum/plasma specimens. Results of this study are summarized in the table below. This study demonstrated that the INSTI HIV-1/HIV-2 Antibody Test has the capability of detecting antibodies to HIV-1 similar to currently available FDA licensed EIAs.

	Specimen Number														
Test	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
INSTI HIV-1/HIV-2	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**N**	**P**	**P**	**P**
Abbott EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Abbott HIVAB HIV-1/HIV2 (rDNA)EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Cambridge Biotech Recombigen HIV-1 EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Syva EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Organon Teknika Vironostika Anti-HIV Uni-Form II	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Murex HIV 1/2 EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Ortho HIV-1/HIV-2 EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Sorin ETI-Ab-HIV 1/2K EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Syva Microtrak II EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Behringwerke ENZ PLUS Anti HIV 1/2 EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Biotest Anti-HIV-1/HIV-2 Recombinant EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Boehringer Mannheim Anti HIV-1/HIV-2 EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
IAF Biochem Detect-HIV-EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Diagnostic Pasteur Genelavia EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
bioMerieux VIDAS anti-HIV-1/2 EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Murex Wellcozyme HIV-1/HIV-2 EIA	P	P	P	P	P	P	P	P	P	P	N	N	P	P	P
Behringwerke Enzygnost Anti HIV 1+2 EIA	N	P	N	P	P	P	P	P	P	P	P	N	P	P	P
Cellular Products HIV-1 EIA	N	P	P	P	P	P	P	P	N	P	P	N	P	P	P
Genetic Systems LAV EIA	N	P	P	P	P	P	P	P	N	P	P	N	P	P	P
Genetic Systems HIV-1/HIV-2 EIA	N	P	N	P	P	P	P	P	P	P	P	N	P	P	P

*These samples were confirmed positive (P) by EIA and Western Blotting
(Data obtained from Boston Biomedica package insert, May 1995 p.2)

Interfering Substances and Unrelated Medical Conditions

To assess the impact of unrelated medical conditions or interfering substances on the sensitivity of the INSTI HIV-1/HIV-2 Antibody Test, 195 serum/plasma specimens from a variety of medical conditions unrelated to HIV-1 infection and 217 specimens with interfering substances were spiked with an HIV-1 positive specimen; see table in the Specificity section for list of medical conditions and substances tested. All spiked specimens gave reactive results.

DETECTION OF ANTIBODIES TO HIV-2 IN SPECIMENS FROM INDIVIDUALS INFECTED WITH HIV-2

A total of 137 individual HIV-2 positive samples were obtained from European sources. 49 sera from individuals with chronic HIV-2 infection were reactive on the INSTI HIV-1/HIV-2 Antibody Test. An additional 88 HIV-2 positive serum and plasma samples were prepared as contrived whole blood; all 88 contrived samples were reactive on the INSTI HIV-1/HIV-2 Antibody test. Combining the results of the two studies, the relative sensitivity of the INSTI HIV-1/HIV-2 Antibody test for the detection of HIV-2 antibodies in these studies was calculated to be 100% (137/137).

HIV-1 SUBTYPE TESTING

To assess the sensitivity of the INSTI HIV-1/HIV-2 Antibody test for HIV-1 variants from various geographic regions, a total of 118 individual confirmed HIV-1 antibody-positive non-B subtype serum/plasma specimens were tested; of these 118 samples, 109 were non-B subtypes including 23 sub-type O samples. All 118 of these specimens were reactive using INSTI, generating an overall sensitivity of the INSTI HIV-1/HIV-2 Antibody Test for HIV-1 non-B subtypes of 100%.

SPECIFICITY

A specificity study was performed using 1386 freshly obtained specimens collected from low or unknown risk and high risk individuals as part of a multicenter prospective clinical trial. Of the 1386 samples, 1376 gave a Non-Reactive result with INSTI and 4 were invalid. INSTI HIV-1/HIV-2 Antibody Test results were compared to results from a composite reference method (comparator method) which consisted of an FDA approved EIA with supplemental Western blot and PCR as required. A total of 7 INSTI false Reactive results (1 from the high risk group, 6 from the low or unknown risk group) were obtained from the 1382 specimens from HIV-negative individuals that produced valid INSTI results. From this data, the overall specificity of the INSTI HIV-1/HIV-2 Antibody Test in fingerstick whole blood specimens from the combined high risk and low or unknown risk populations, minus the invalid results, was calculated to be 1375/1382 = 99.5% (95% CI = 99.0% - 99.8%).

Performance of the INSTI HIV-1/HIV-2 Antibody Test on Fingerstick Whole Blood Specimens from Individuals Presumed to be Negative for HIV Infection

Test Group	Total Specimens	INSTI Non-Reactive[3]	Approved Test Non-Reactive[2]	True Negative[2]
Low Risk	626	620	626	626
High Risk	782	756[1]	760[2]	760
TOTAL	1408	1376	1386	1386

[1] 4 invalid results were not included in the calculation of specificity. The 4 specimens which gave invalid results on INSTI were Non-Reactive on the approved test.
[2] 22 Reactives were confirmed by licensed HIV-1 Western Blot and excluded from the calculation of specificity.
[3] Of the 22 INSTI Reactive specimens, one was Non-Reactive by the approved test, i.e. INSTI false Reactive.

Interfering Substances and Unrelated Medical Conditions

To asses the impact of unrelated medical conditions or interfering substances on the specificity of the INSTI HIV-1/HIV-2 Antibody Test, 195 serum/plasma specimens from a variety of medical conditions unrelated to HIV-1 infection and 217 specimens with interfering substances were analyzed. Five specimens from individuals with multiple myeloma gave invalid results. No false reactive results were obtained.

Medical Condition (n=195)	No. of Specimens	INSTI Reactive	INSTI Nonreactive
Toxoplasmosis	20	0	20
Rheumatoid Factor	20	0	20
Multiple Myeloma	10	0	5
Syphilis	30	0	30
SLE	5	0	5
Rubella	20	0	20
Cytomegalovirus	20	0	20
Epstein Barr Virus	20	0	20
HTLV-I/II panel	15	0	15
Hepatitis B Virus	20	0	20
Hepatitis A Virus	15	0	15
Interfering Substances (n=217)			
Icteric	20	0	20
Elevated Bilirubin (≥8.0mg/dL)	19	0	19
Lipemic	20	0	20
Visual Hemolysis	5	0	5
Elevated Triglyceride (≥292mg/dL)	19	0	19
Elevated Hemoglobin (>12g/100mL)	20	0	20
Elevated Albumin (11.5-13.0g/dL)	15	0	15
EDTA	13	0	13
Sodium Heparin	13	0	13
Sodium Citrate	13	0	13
Bacterially Contaminated	60	0	60

In addition, a total of 208 specimens from pregnant women in various trimesters of pregnancy confirmed to be HIV-1 negative by a 3rd Generation HIV EIA were tested. One sample (1/208) produced invalid result, all other INSTI results were non-reactive.

EQUIVALENCE STUDIES

The INSTI HIV-1/HIV-2 Antibody Test was evaluated using matched serum and plasma specimens, Testing was performed with 50 anti-HIV-1 negative specimens (25 serum and 25 plasma) and 50 anti-HIV-1-spiked positive specimens. All samples produced acceptable assay performance. These results indicate 100% relative sensitivity and 100% relative specificity with the matched serum and plasma panel provided, and that serum and plasma sample types are equivalent.

REPRODUCIBILITY

The reproducibility of the INSTI HIV-1/HIV-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, 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 samples were read as weak positive at 1 site). For the 1 very low level antibody sample, 59% (48/81) of the results were reactive while 41% (33/81) were non-reactive.

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TECHNICAL INFORMATION

For further information or assistance, contact the Technical Services at 1-604-644-4677.

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