INSTI®

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)

90-1008 - One INSTI® HIV-1/HIV-2 Antibody Test with support materials

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		Use by

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 in clinical laboratories and at the point-of-care (POC) including medical facilities, emergency care situations and physician's offices as a diagnostic test capable of providing results in less than one minute. Although suitable for near-patient point-of-care (POC) testing, the INSTI HIV-1/HIV-2 Antibody Test is not suitable 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.

SUMMARY

HIV stands for Human Immunodeficiency Virus. HIV is the virus that causes AIDS (Acquired Immunodeficiency Syndrome) if left untreated. AIDS is caused by at least two related retroviruses, HIV-1 and HIV-2[1]. HIV is transmitted mainly by sexual contact, exposure to blood or blood products, or from an infected mother to her unborn child. People with increased risk of HIV infection include intravenous drug-users, men having sex with men (MSM), transgendered and other key populations. Antibodies specific for HIV envelope proteins become prevalent in blood from persons infected with HIV however the presence of antibodies does not necessarily constitute a diagnosis of AIDS[5-7]. Absence of antibodies to HIV does not indicate that an individual is absolutely free of HIV-1 or HIV-2; HIV has been isolated from seronegative individuals prior to seroconversion. The INSTI HIV-1/HIV-2 Antibody Test can be used as an aid in the diagnosis of HIV-1 and/or HIV-2 infection in point of care settings. Using a rapid HIV test provides an opportunity to identify more individuals who are unaware they are living with HIV. The US Centers for Disease Control and Prevention estimates that up to 25% of persons living with HIV in the US are unaware of their infection and therefore cannot benefit from effective antiretroviral therapy[15]. Rapid HIV testing provides results during the initial visit allowing for immediate counselling and follow-up opportunities.

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 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, 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), 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 Clarifying Solution is then added to the membrane to decrease background color in order to make the control and test spots 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.[8-13]

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 (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 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.

SPECIMEN COLLECTION AND STORAGE

1. For EDTA-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. 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

The assay is packaged as a kit containing INSTI Membrane Unit, Sample Diluent (Solution 1), Color Developer (Solution 2), and Clarifying Solution (Solution 3) with support materials (lancet, pipette and alcohol swab).

2°C–30°C INSTI components should be stored at 2-30°C.
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** ⚠ 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.
3. **Color Developer** ⚠ 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. Ready to use, invert 2-3X immediately before use.
4. **Clarifying Solution** ⚠ 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.

⚠ All solutions contain 0.1% Sodium Azide as a preservative and are harmful if swallowed. All solutions are for single use only and are stable to date and under storage conditions indicated on labels.

SUPPORT MATERIALS ②

The following materials are required when testing fingerstick whole blood:
1. Single-use Alcohol Swab
2. Single-use Lancet STERILE R
3. Single-use Pipette, capable of dispensing 50µl

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.

For venipuncture blood collection:
- Venipuncture apparatus if collecting blood samples.
- Appropriate blood collection tubes.
- Precision pipette capable of delivering 50µl of sample.
- Appropriate shipping containers.
- 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-fibrinated human plasma control samples product no. 90-1034 and 90-1035 are available from bioLytical Laboratories in user-defined amounts, 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 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.
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 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.
11. Patients with elevated haemoglobin levels may test false negative with INSTI.[14]
12. Patients who are elite controllers (i.e. individuals with low or undetectable viral loads) may give a false negative INSTI HIV-1/HIV-2 Antibody Test result.

PRECAUTIONS

1. ⚠ All specimens should be handled as if capable of transmitting infectious diseases.
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 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.

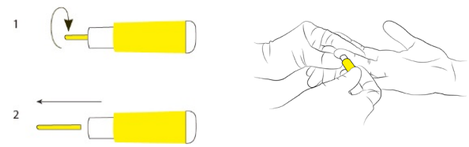
ASSAY PROCEDURE

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

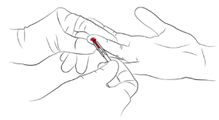
Sampling Fingerstick Blood:

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

1. Open the Sample Diluent vial cap (Solution 1) prior to proceeding with next steps of fingerstick blood collection.
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 off the protective cap from the lancet, and then pull it straight out. 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 press the lancet body firmly against the puncture site to activate the device. 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. If very little blood trickles out of the puncture, gently apply intermittent pressure near 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, Color 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 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 labelled 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 have 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.)

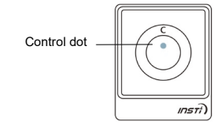
INSTI HIV-1/HIV-2 Test Controls are available separately for use only with the INSTI HIV-1/HIV-2 Antibody Test. The controls are used to verify test performance and interpretation of results. Kit controls should be run under the following circumstances:
- for new INSTI operator verification prior to 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 2°-30°C
- when the temperature of the test area falls outside of 2°-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.

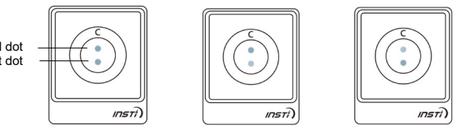
INTERPRETATION OF RESULTS

- **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.**

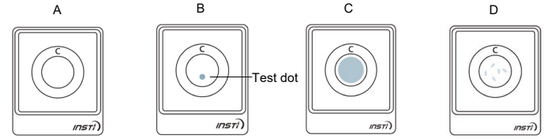
NON-REACTIVE ► One blue dot that is clearly discernable above any background tint should appear on the membrane. This is the procedural Control Spot and shows that the test has been performed correctly. The Control spot location is indicated by the letter C. No reaction should be visible at the test 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.

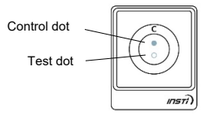


INVALID ► The test is invalid if any of the following occurs:
A. There is no dot on the membrane
B. The test dot appeared without the control dot
C. Uniform tint across the membrane
D. Only blue specks appear on the membrane



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 color than the procedural control, or vice versa.
3. Only 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 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. 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 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 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 matching 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 performance panel, (Table 3) which represent a wide range of antibody titers or classes.
2. Canadian HIV seroconversion patient samples (Table 2)
3. Prospective samples from HIV-positive patients enrolled in the Canadian Clinical Trial (Table 4)
4. Prospective negative samples from patients enrolled in the Canadian Clinical Trial (Table 5)

bioLytical 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 sensitivity 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% for fingerstick blood, EDTA blood, plasma and serum (range 99.0-99.6%) (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 substances 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.
6. The sensitivity of the INSTI HIV-1/HIV-2 test in HIV-2 positive specimens is 98.3% (Table 7).

Table 1
Anti-HIV-1 Seroconversion Panel PRB-900 Series* Boston Biomedica Inc.

INSTI HIV-1/HIV-2	Number of Panels
Detected earliest bleed of panel	14
Within 1 bleed of earliest EIA positive	8
Within 2 bleeds of earliest EIA positive	1
Unknown**	2

*PRB910, PRB904, PRB924, PRB912, PRB914, PRB916, PRB919, PRB922, PRB925, PRB926, PRB927, PRB928, PRB929, PRB934, PRB935, PRB944, PRB937, PRB938, PRB940, PRB941, PRB945, PRB947, PRB950, PRB952, PRB943
**The last bleed in the panel was positive by at least 1 EIA, negative by INSTI ion Panels PRB937 and PRB938

Table 2
Independent Study on the Performance of the INSTI HIV-1/HIV-2 Antibody Test on Canadian HIV Seroconversion Patients, n=34 patients from British Columbia and 20 from Alberta. A total of 85 serum or plasma samples collected after the initial HIV negative sample were tested in three laboratory centres:

INSTI HIV1/HIV2	Licensed EIA		Western Blot			
	POS	NEG	POS	NEG	IND	Not done
POS	69	1	35	5	24	6
NEG	14[1]	0	0	10	4	0
IND	1[2]	0	0	1	0	0

1. 13/14 had low s/co ratios (<9.0) with licensed EIA
2. s/co ratio with licensed EIA was low (5.64)
IND. indeterminate

Table 3
INSTI HIV-1/HIV-2 Antibody Test Results for Anti-HIV-1 Low Titer Performance Panel #PRB-105* Boston Biomedica Inc.

	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)

Table 4
INSTI HIV-1/HIV-2 Sensitivity in matching fingerstick blood, EDTA whole blood, plasma and serum samples collected from patients (n=3507) enrolled in the Canadian Clinical Trial of INSTI.

	Fingerstick Blood	EDTA Whole Blood	Plasma	Serum
Number of Confirmed Positive Samples[1]	820	836	838	396[2]
Number of Positive Samples by INSTI	817	831	834	392
Calculated Sensitivity (95% C.I.)	**99.6%** (98.9-99.9%)	**99.4%** (98.6-99.7%)	**99.5%** (98.8-99.8%)	**99.0%** (97.4-99.6%)
Positive Predictive Value of INSTI	97.84%	98.90%	99.90%	100%

1. Samples were confirmed HIV positive by the approved laboratory-based screen test of record, and by Western Blot
2. Serum samples were collected from a portion (n=1346) of the study patients (n=3507)
Note: INSTI invalid results were not included in the table and calculations

Table 5
INSTI HIV-1/HIV-2 Specificity in matching fingerstick blood, EDTA whole blood, plasma and serum samples collected from patients (n=3507) enrolled in the Canadian Clinical Trial of INSTI.

	Fingerstick Blood	EDTA Whole Blood	Plasma	Serum
Number of HIV Negative Samples[1]	2506	2630	2638	949
Number of Negative Samples by INSTI	2488	2621	2637	949
Calculated Specificity (95% C.I.)	**99.3%** (98.9-99.5%)	**99.7%** (99.4-99.8%)	**99.96%** (99.8-100%)	**100%** (99.6-100%)
Negative Predictive Value of INSTI	99.90%	99.80%	99.80%	99.58%

1. Samples were negative by the approved laboratory-based screen test of record.
Note: INSTI invalid results were not included in the table and calculations.

Table 6
INSTI HIV-1/HIV-2 Antibody Test Reactivity with Specimens from Individuals with potentially Interfering Medical Conditions and Specimens with Interfering Substances, n=388.

Specimen Type	INSTI Positive*	INSTI Negative **	Invalid
Anaemia	1	2	-
Carcinoma/Cancer	24	5	-
Chlamydia	0	2	-
Cytomegalovirus (CMV)	0	5	-
Diabetes	17	6	-
Epstein Barr Virus (EBV)	0	5	-
Haemolysed	0	12	-
Haemophilia	0	1	-
Hepatitis A Virus (HAV)	4	1	-
Hepatitis B Virus (HBV)	7	4	-
Hepatitis C Virus (HCV)	46	7	-
Herpes	63	12	-
HTLV I	0	7	-
HTLV II	0	7	-
Lipid Abnormalities	58	4	-
Lymphoma	1	1	-
MAC/TB	2	0	-
Malaria	0	1	-
Myeloma	0	7	3[1]
Multiple Transfusion Recipients	2	6[2]	-
Neuropathies	28	0	-
PCP	2	0	-
Rheumatoid Factor	0	5	-
Rubella	0	5	-
Lupus	0	5	-
Syphilis	0	10	-
Toxoplasmosis	1	10	-
Yeast/Candida	0	2	-

1. Weak or no IgG control spot visible
2. One sample was weak EIA positive but not confirmed
*All INSTI Positive Samples were confirmed HIV Positive
**All INSTI Negative Samples were confirmed HIV Negative

Table 7
INSTI HIV-1/HIV-2 Antibody Test's Sensitivity in HIV-2 Positive Specimens

Sample Source	France[1]	France[2]	Nigeria[3]	U.K.[4]	Total
Positive Samples	49	88	24	76	237
INSTI Positives	49	88	24	72*	233
Sensitivity	100%	100%	100%	94.7%	98.3%

* One positive, one negative and two indeterminate on repeat testing
1. Tests performed in France using serum samples
2. Tests performed in-house using whole blood spiked with plasma (13 samples) and serum (75 samples)
3. Tests performed in-house using plasma samples
4. Tests performed in the U.K. using 33 plasma samples and 43 unknown (serum or plasma samples)

REPRODUCIBILITY

Two studies were conducted to evaluate the reproducibility of INSTI HIV-1/HIV-2 Antibody Test. For each study, 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 in triplicate at 3 sites on 3 separate days using 3 lots of INSTI.
The overall reproducibility of INSTI for HIV-1 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 positive while 41% (33/81) were negative.
The overall reproducibility of INSTI for HIV-2 was 99.8% (566/567, one antibody negative sample was read as indeterminate at 1 site). At the 2 lowest level antibody samples, 85.2% of the results were positive with Panel Member 3 (69/81), while 63% of the results were negative with Panel Member 4 (51/81).

Untrained User Evaluation Study
The performance of the INSTI HIV-1/HIV-2 Antibody Test by untrained users was evaluated in a prospective study conducted at 3 US sites. 11 operators with no previous laboratory experience and no training on the use of the test participated in the study. 1,388 subjects were tested in this study, 905 subjects with unknown HIV status and 483 subjects known to be HIV-1 positive; operators were blinded to the HIV status of the subjects being tested. Fingerstick blood was drawn from each subject for INSTI testing and the INSTI result compared to the HIV status of the subject. Subjects with unknown HIV status were tested with INSTI and a composite reference method (comparator method) which consisted of an FDA approved EIA with supplemental Western blot and PCR assays as required. The positive percent agreement and negative percent agreement between INSTI and the HIV status for the study specimens is presented in Table 8 below. There were no INSTI HIV-1 invalid results reported.

Table 8
Positive Percent Agreement and Negative Percent Agreement between the INSTI HIV-1 Antibody Test and the HIV Status of Individuals with Known and Unknown HIV Status.

Study Population	Number of Subjects	Positive Percent Agreement	95% Confidence Interval	Negative Percent Agreement	95% Confidence Interval
HIV status unknown	905	100% (34/34)	89.9% - 100%	99.8% (869/871)	99.2% - 99.9%
Known HIV-1 Positive	483	100% (483/483)	99.2% -100%	N.A.	N.A.
Total	1,388	100% (517/517)	99.3% - 100%	99.8% (869/871)	99.2% - 99.9%

Percent of invalid results was 0% (0/1388) with 95% CI: 0% to 0.3%

Additionally, a study was conducted to evaluate the ability of untrained operators to detect HIV antibodies in weakly reactive samples. Randomly coded panels consisting of 4 contrived weakly reactive plasma samples were tested with INSTI at 3 sites by 10 untrained operators (60 measurements in total per sample). The testing was done over 5 consecutive days with samples integrated into the daily workflow at each site. The samples were prepared from a dilution series of single HIV-1 positive plasma control material and represent INSTI results that are at, slightly above and slightly below the cutoff in this dilution series. The same panel was also tested by trained laboratory professionals to verify that the dilution series gave the expected reactivities.

Table 9 below shows performance of the test with samples near the cutoff of the assay in the hands of intended users (across all sites).

Table 9 Performance of the INSTI HIV-1/HIV-2 Antibody Test Run by Intended Users with Weakly Reactive Samples

		Intended Users	
Sample	**Dilution**	**Percent Reactive**	**95% Confidence Interval**
Weakly Reactive 1[a]	1:600	88.3%[e] (53/60)	77.8% - 94.2%
Weakly Reactive 2[a]	1:800	80.0% (48/60)	68.2% - 88.2%
Weakly Reactive 3[b]	1:1200	66.1%[e] (39/59)[d]	53.4% - 76.9%
Weakly Reactive 4[c]	1:1600	34.5% (20/58)[d]	23.6% - 47.3%

[a] Expected results: There should be a greater number of INSTI reactive results than non-reactive results.
[b] Expected results: There should be an equal distribution of reactive and non-reactive INSTI results.
[c] Expected results: There should be a greater number of INSTI non-reactive results than reactive results.
[d] A total of 3 INSTI invalid results were obtained: 1 invalid for Weakly Reactive 3 sample and 2 invalids for Weakly Reactive 4 sample.
[e] Two out of 10 intended users had a lower number of reactive results with weakly reactive samples, as compared with other intended users.

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TECHNICAL INFORMATION

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