MULTIPLEX

HIV-1/2 Syphilis* Ab Test

***CE mark for Syphilis by self-declaration**

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

90-1028 - One INSTI® Multiplex HIV-1/2 Syphilis Ab Test with support materials (for POC use)

15°C – 30°C	Store at 15°C to 30°C	STERILE R	Sterilization using irradiation
	Caution Harmful if swallowed	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		

15°C – 30°C **Store at 15°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 MULTIPLEX HIV-1/2 Syphilis Ab Test** is a single use, rapid, flow-through *in vitro* qualitative immunoassay for the detection of antibodies to Human Immunodeficiency Virus Type 1/ Type 2 and Treponema pallidum 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 an in vitro diagnostic device capable of providing results in less than one minute. It is suitable for near-patient or point-of-care (POC) testing, and is not currently approved for self-testing. All required pre and post-test counseling guidelines must be followed in each setting in which the INSTI Multiplex antibody test is used.

The **INSTI MULTIPLEX HIV-1/2 Syphilis Ab Test** will be referred to as INSTI Multiplex Test in the remainder of this Instructions for Use.

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 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.[2,3] 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.[3,7] The presence of antibodies to HIV indicated 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.[4] **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 counseling provided in POC settings. Following a reactive HIV rapid test result, a venous blood sample must be drawn in an EDTA collection tube (for whole blood or plasma), and forwarded to a laboratory for HIV confirmatory test.

Treponema pallidum is the causative agent of syphilis. Some of the proteins of this organism are highly immunoreactive and infected persons develop antibodies soon after infection. These antibodies are unaffected by treatment and once induced they remain detectable for years. It is possible for a person to be antibody positive for *T. pallidum*, but have been cured of the infection. Following a reactive result for *T. pallidum* antibodies, a venous blood sample must be drawn in an EDTA collection tube (for whole blood or plasma) or red-top tube (for serum), and forwarded to a laboratory for syphilis confirmatory testing. A confirmatory test is required to determine active syphilis or past infection in the patient.

PRINCIPLES OF THE TEST

The **INSTI Multiplex Test** is a manual, visually read, flow through immunoassay for the qualitative detection of HIV-/HIV-2 and syphilis IgG and/or IgM[17] 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, and syphilis antigens which react with HIV-1/HIV-2 and syphilis IgG and/or IgM antibodies in the specimen to produce distinct visual signals on the membrane. The membrane also includes a procedural control. The procedural control consists of a protein-A treated spot capable of capturing IgG or IgM antibodies normally present in blood and blood components. IgG or IgM antibodies react with a proprietary chromatic agent to produce a visual signal on the membrane.

Since IgG and/or IgM antibodies can be present in blood from normal or HIV or syphilis 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 dots, recombinant HIV-1, HIV-2 and syphilis proteins, embedded in the membrane, capture specific antibodies, if present in the specimen. Antibodies captured in the test dots react with a proprietary chromatic agent to produce visible signals 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, Colour Developer and a Clarifying Solution. The test is performed by adding the blood, serum or plasma specimen to the vial of Sample Diluent, which lyses the red blood cells and dilutes the specimens. This specimen/diluent solution is then poured onto the well of the membrane unit. HIV-1/HIV-2 and syphilis 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 and test dots. In the case that HIV-1/HIV-2 and/or syphilis antibodies are present in the specimen, a blue dot also appears at the location of one or both of the test spots 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 portion 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] The syphilis antigens bound to the membrane consist of a recombinant fusion protein derived from p17 and p47 domains of *Treponema pallidum*.

Antibody Detection: The INSTI Multiplex assay uses a unique reagent to detect antibodies to HIV-1/HIV-2 and syphilis. Although primarily designed to detect the IgG class of specific antibodies, the INSTI HIV-1/HIV-2 assay portion has been shown to detect IgM antibodies in samples obtained early in HIV infection during seroconversion, and low titer anti-HIV-1 samples obtained later in infection[17]

Test Complexity: The INSTI Multiplex Test was designed to reduce protocol complexity. The INSTI Multiplex 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 test sensitivity and specificity. Total test time may vary slightly depending on specimen type but results of valid tests are usually 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

15°C – 30°C INSTI components should be stored at 15-30°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 and/or IgM capture), HIV test (gp41 and gp36 antigen) and *T. pallidum* (p17-p47 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, invert 2-3X immediately before use.
3. **Colour Developer**, ⚠ Solution 2 vial, containing 1.5 mL of a blue-coloured borate buffered proprietary indicator solution designed to detect IgG and IgM in the control spot and specific HIV and *T. pallidum* antibodies in the test spots.. 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 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 *T. pallidum* Antibody Positive Control: Separate vials of anti-*T. pallidum* positive de-fibrinated human plasma control sample, product no. 90-1032 are available from bioLytical Laboratories.
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, the INSTI Multiplex Test Controls Instructions for Use 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 test kit is exposed to temperatures outside of 15°–30°C, ensure it is brought to this temperature range before performing testing. Use the Syphilis INSTI Controls and validated HIV Controls to ensure proper kit performance
9. ⚠ Patients that are on long term antiretroviral drug therapy may give a false negative 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 for HIV with INSTI Multiplex.
11. Patients with elevated haemoglobin levels may test false negative for HIV with INSTI Multiplex.[15]
12. Because the INSTI Multiplex Test has a lower affinity to IgM antibody class compared to IgG, patients in the early primary stage of syphilis infection may test negative for *T. pallidum* antibodies with INSTI Multiplex.

PRECAUTIONS

1. ⚠ All specimens should be handled as if capable of transmitting infectious diseases. It is recommended that Directive 2000/54/EC, 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 INSTI 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 pipette bulb while sampling.

6. Transfer the blood held in the pipette to the Sample Diluent vial (Solution 1). Align the tip of the pipette with the Sample Diluent vial and squeeze the bulb to dispense the sample (See Figure A).
NOTE: If the sample will not expel, hold the pipette vertically and slide a finger over (without pressing) the vent hole, then squeeze the bulb (See Figure B). Recap the vial and mix by inversion. Follow General Procedure after Sampling, below.

Sampling Whole Blood, serum, 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 2-3 times.
⚠ 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 INSTI Membrane Unit without touching the center well. Place the unit on a level surface. For sample identification purposes the bottom 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. Re-suspend the Colour Developer by slowly inverting to mix the solution thoroughly until the reagent is evenly suspended. Open the Colour Developer 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 have elapsed following the addition of Clarifying Solution.**

NOTE: INSTI tests should be read and interpreted under adequate lighting.

QUALITY CONTROL

Kit Controls:
The INSTI Multiplex Test has a built-in IgG and IgM 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.)

Separate Syphilis Controls and HIV Controls are available for use with the INSTI Multiplex Test. The controls are used to verify Syphilis and HIV 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 INSTI kits is received
- when temperature during storage of the kit falls outside of 15°–30°C
- when the temperature of the test area falls outside of 15°-30°C
- at regular intervals as determined by the user facility.

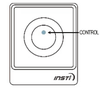
Refer to the INSTI *T. pallidum* Test Controls Instructions for Use and the INSTI HIV-1/HIV-2 Test Controls Instructions for Use for additional information on the use of these reagents. It is the responsibility of each user of the INSTI *T. pallidum* Test Controls to establish an adequate quality assurance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

⚠ **CAUTION! It is not recommended to use external controls that have not been validated for the INSTI Multiplex Test as these may not produce the expected results.**

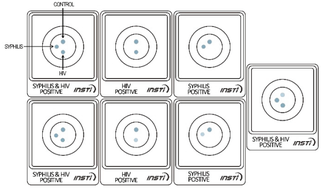
INTERPRETATION OF RESULTS

- **Do not read the results if more than 5 minutes have elapsed following the addition of Clarifying Solution.**
- **If using the syphilis control samples provided by bioLytical Laboratories, all syphilis 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.**

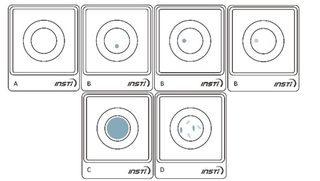
NON-REACTIVE ► One blue dot that is clearly discernable above any background tint should appear on the membrane. This is the procedural Control Dot and shows that the test has been performed correctly. The control dot is located towards the top of the read frame furthest from the plastic tab on the Membrane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



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

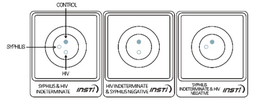


INVALID ► The test is invalid if any of the following occurs:
A. There is no dot on the membrane
B. The test dots appeared without the control dot
C. Uniform tint across the membrane
D. Only scattered blue specks appear on the membrane



NOTE: Invalid tests with fingerstick blood should be repeated with a fresh sample using a new membrane unit, kit components and support materials. Invalid tests with whole blood, plasma or serum samples 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 areas. Following an indeterminate INSTI test result, a venous blood sample must be drawn in an EDTA collection tube (for whole blood or plasma) or red-top tube (for serum), and forwarded to a laboratory for HIV and/or syphilis confirmatory testing.



Please note the following:

1. Following a reactive or indeterminate INSTI test result, a venous blood sample must be drawn in an EDTA collection tube (for whole blood or plasma) or red-top tube (for serum), and forwarded to a laboratory for HIV and/or syphilis 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 solid 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.
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 of the assay membrane is obstructed.
6. An individual who has a non-reactive result but was involved in HIV-risk activity is recommended to obtain additional testing over the next months.
7. To significantly reduce the risk of HIV or syphilis 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 flown 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 an appropriate collection tube, and forwarded to a laboratory for HIV and/or syphilis confirmatory testing.
- The INSTI Multiplex Test procedure and the interpretation of result must be followed closely when testing for the presence of antibodies to HIV and/or syphilis 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 Multiplex Test has not been validated for detection of antibodies to HIV-1 Group N subtypes.
- The INSTI Multiplex Test detects antibodies to HIV-1/HIV-2 and *T. pallidum*, and is useful in establishing infection with HIV and/or syphilis. Because a variety of factors may cause non-specific reactions, a patient found to be positive for HIV or syphilis using the INSTI Multiplex assay should have a 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 results 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.[5] The presence of antibodies to *T. pallidum* may indicate current or past syphilis infection, and a blood sample should be collected and sent to a laboratory for confirmation of infection status. Antibodies to the syphilis antigens used in this test may persist for decades, even in spite of successful therapy. A positive syphilis test may not be an indication of an ongoing infection.

HIV PERFORMANCE CHARACTERISTICS (Note: The HIV-1/HIV-2 portion of the Multiplex assay is identical to CE Marked INSTI HIV-1/HIV-2 Antibody Test product 90-1015. All data presented in this section is based on the data presented in the INSTI HIV-1/HIV-2 Antibody Test Instructions for Use, document 51-1037)

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/HIV-2 (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[1]	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 assess 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	19	0	19
Lipemic	20	0	20
Visual Hemolysis	5	0	5
Elevated Triglyceride	19	0	19
Elevated Hemoglobin	20	0	20
Elevated Albumin	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.

HIV REPRODUCIBILITY
The reproducibility of the INSTI HIV-1/HIV-2 portion of the Multiplex Test was tested at 3 laboratory sites using 3 lots of the INSTI HIV-1/HIV-2 Antibody Test 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 positive while 41% (33/81) were negative.

SYPHILIS (*T. pallidum*) PERFORMANCE CHARACTERISTICS

In-house Studies
Data from parallel INSTI Multiplex and *T. pallidum* Particle Agglutination (TP-PA) in-house testing of frozen, archived serum and plasma samples obtained from commercial sources is provided below. INSTI Multiplex *T. pallidum* antibody test results were compared to a CE marked *T. pallidum* Particle Agglutination (TP-PA) assay, n=524 serum/plasma specimens known to be TP-PA negative or positive.

INSTI Multiplex *T. pallidum* Antibody Results	TP-PA Final Interpretation		
	Positive	Negative	Total
Reactive	138	5	143
Non-Reactive	7	374	381
Total	145	379	524

NOTE: In an independent study conducted at the Centre National de la Reference de la Syphilis, Paris, France[16], the sensitivity of INSTI Multiplex on samples from patients with known secondary syphilis was 100% (41/41).

Field Study
In an independent prospective field study conducted in Bangalore India in 2012-2013 on a population at risk for HIV and STI (n=1010 plasma), the performance of the INSTI Multiplex Test was assessed against the syphilis and HIV status for each patient as determined by an algorithm of syphilis serology methods (RPR, TPHA) and HIV antibody tests (two rapid lateral flow HIV-1/HIV-2 assays plus one rapid HIV-1/HIV-2 ELISA test. The results are presented below.

Syphilis Performance of the INSTI Multiplex Test against the Rapid Plasma Reagin (RPR) test for the prospective field study population, n=1010.

Rapid Plasma Reagin (RPR)Test	INSTI Multiplex *T. pallidum* Antibody Positive	INSTI Multiplex *T. pallidum* Antibody Negative
Positive	12[1]	5[2]
Negative	2[3]	991
Total	14	996

1. 11/12 were confirmed positive by *T. pallidum* Haemagglutination Assay (TPHA) and 1/12 is considered false positive by INSTI and RPR.
2. 3/5 were confirmed positive by TPHA, ie INSTI false negative and 2/5 were negative by TPHA, ie RPR biological false positive.
3. 2/2 were confirmed positive by TPHA, ie RPR false negative

The number of true *T. pallidum* antibody positive patients (n=16) in this prospective study, as determined by TPHA is too low for calculation of relative sensitivity of the INSTI Multiplex test for detection of *T. pallidum* antibodies. Of the total 994 samples that were considered negative for active syphilis (by RPR and/or TPHA), INSTI Multiplex was negative for 993, for a negative percent agreement of **99.9%.** (It is important to note that a negative RPR result does not rule out past syphilis infection, therefore this is not a measure of true *T. pallidum* antibody specificity.)

HIV Performance of the INSTI Multiplex Test against the HIV test algorithm results for the prospective field study population, n=1010

Panel	INSTI Multiplex HIV-1/HIV-2 Positive	INSTI Multiplex HIV-1/HIV-2 Negative
HIV known positive (n=136)	136	0
HIV negative (n=874)	0	874
Total	136	874

The positive and negative percent agreement for HIV antibody detection between the INSTI Multiplex Test and the patients' HIV status determined by the three-test HIV algorithm was 100%.

Mixed Titre Panel
The INSTI Multiplex Test was tested against a commercial syphilis mixed titre performance panel to compare performance to other *T. pallidum* antibody tests for detection of high and low levels of *T. pallidum* antibodies. The results are presented below.

Syphilis Mixed Titre Performance Panel 1111-272-00123 (ZeptoMetrix Corporation)

	Panel Member ID Number									
***T. pallidum* Test Method**	1	2	3	4	5	6	7	8	9	10
US FDA Licensed Test										
Pheonix Biotech Trep Sure	P	P	P	N	N	N	N	N	P	P
Trinity Captia IgG	P	P	P	N	N	N	N	N	P	P
Trinity Captia IgM	N	N	N	N	N	N	N	N	P	N
Serodia-TP.PA	P	P	P	N	N	N	N	N	P	P
INSTI Multiplex *T. pallidum* Antibody	P	P	P	N	N	N	N	N	P	P

N = negative P = positive

Whole Blood Testing
Performance of the INSTI Multiplex Test compared to TP-PA for EDTA Whole Blood Specimens, un-spiked (n=105) and spiked with T.pallidum (n=64) .

INSTI Multiplex *T. pallidum* Antibody Results	TP-PA Final Interpretation		
	Positive	Negative	Percent Agreement
Reactive	62	0	Positive percent agreement 96.9% (62/64)
Non-Reactive	2	105	Negative percent agreement 100% (105/105)
Total	64	105	169

The positive percent and negative percent agreement was 96.9% and 100% for this subset of specimens, comparing favorably to the corresponding values obtained from in-house testing (95.2% and 98.7%) indicating that there is no performance difference in the detection of *T. pallidum* antibodies in whole blood, serum or plasma samples.

INSTI Multiplex Test Result compared to TP-PA for specimens that tested positive for other diseases or medical conditions (n = 380)

Condition	No. of Specimens	INSTI Multiplex Syphilis Reactive[1]	INSTI Multiplex Syphilis Nonreactive[2]
Cytomegalovirus (CMV)	10	1	9
Epstein Barr Virus (EBV)	9	0	9
Helicobactor pylori	10	0	10
Hepatitis A Virus (HAV)	40	6[3]	34[4]
Hepatitis B Virus (HBV)	40	1[5]	39[6]
Hepatitis C Virus (HCV)	121	6[7]	115[8]
Human Immunodeficiency Virus (HIV)	25	1	24
Herpes Simplex Virus (HSV)	10	0	10
Lyme Disease	5	0	5
Myeloma	10	0	10
Pregnancy	50	0	50
Rheumatoid Factor	5	0	5
Rubella	10	0	10
Systemic Lupus Erythematosus (SLE)	5	1	4
Toxoplasmosis	20	0	20
Varicella Zoster Virus (VZV)	10	0	10

[1] all were TP-PA positive unless otherwise indicated.
[2] all were TPPA negative unless otherwise indicated
[3] 2 specimens tested negative on TP-PA, ie INSTI false positive
[4] 2 specimens tested positive on TP-PA, ie INSTI false negative
[5] 1 specimen tested negative on TP-PA test, ie INSTI false positive
[6] 1 specimen tested positive on TP-PA, ie INSTI false negative
[7] 2 specimens tested negative on TP-PA, ie INSTI false positive.
[8] 3 specimens tested positive on TP-PA test, ie INSTI false negative.

HIV and Syphilis Reproducibility
The reproducibility of the HIV-1/HIV-2 and syphilis portions of the Multiplex Test was tested using 3 distinct lots of the INSTI Multiplex Test components by 3 operators over 3 separate days. A panel of 5 blind-coded plasma samples, designed to produce HIV and syphilis results ranging from strongly reactive to weakly reactive to negative, was used for the study. Each panel member sample was tested 33 times, for a total of 165 INSTI Multiplex tests. For HIV. 165/165 results were in agreement with the expected results across all operators, component lots and days of testing for an overall reproducibility of 100%. For syphilis, 164/165 results were in agreement with the expected results across all operators, component lots and days of testing for an overall reproducibility of 99.4%.

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TECHNICAL INFORMATION
NOTE: The HIV assay portion of the INSTI Multiplex Test is CE Marked through CE0543 and the syphilis assay portion is CE Marked through bioLytical Laboratories by self-declaration.

For further information, assistance, or problem reporting, contact Customer Service at +1-604-644-4677.

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