MULTIPLEX
HIV-1/2 Syphilis Ab Test

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

90-1110 - One INSTI® Multiplex HIV-1/2 Syphilis Ab Test with support materials (for POC use)

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 fingerstick blood and serum. The INSTI Multiplex Test is intended as an aid in the diagnosis of HIV1/2 and Syphilis infection in patients with signs and symptoms of HIV and Syphilis. 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. Although suitable for near-patient point-of-care (POC) testing, the INSTI Multiplex Test is not suitable 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 the 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 an increased risk of HIV infection include hemophiliacs, 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.[2,3] 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.[3] **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 the c) the 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-1/HIV-2 and syphilis IgG and/or IgM[17] antibodies in human blood and serum. 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 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 fingerstick whole blood or serum 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 spot and, 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 washing and reagent additions. These measurements 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 usually clearly readable within one minute.

SPECIMEN COLLECTION AND STORAGE

1. For serum specimens, follow venipuncture blood collection procedures using red-top (no anticoagulant) tubes.
2. If serum is to be used, separate the blood cells by centrifugation.
3. Serum 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. Do not dilute prior to testing.

KIT COMPONENTS AND STORAGE

30°C
15°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 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 MEDLANCE HTL Strefa S.A. ul. Adamowek 7, 95-035 Ozorkow, Poland.
3. Single-use Pipette 50 µL

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 sample
- Appropriate blood collection tube
- Appropriate shipping container
- Precision pipette capable of delivering 50 µL 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. 90-1124 is 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. 90-1035 is 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 Multiplex Test have not been established for body fluids other than fingerstick blood and serum.
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 validated INSTI Syphilis Controls and INSTI HIV Controls to ensure proper kit performance.
9. ⚠ Patients that are on long term antiretroviral drug therapy may give a false negative HIV test result.

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 received, follow these instructions carefully:

2. Massage the finger to allow the blood to move to the surface (fingertip will become pink). Use a 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.

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.

5. Transfer the blood held in the pipette to the Sample Diluent vial (Solution 1) immediately without any delay. 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 (See Figure below). Recap the vial and mix by inversion. Follow General Procedure after Sampling, below.

Sampling serum, 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 50 µL of serum 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 50 µL 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 test 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 (T. pallidum) 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.

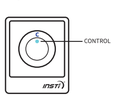
Refer to the INSTI Syphilis Test Controls Instructions for Use and the INSTI HIV-1/HIV-2 Test Controls Instructions for Use for additional information on the use of these reagents. It is the responsibility of each user of the INSTI *T. pallidum* Test Controls to establish an adequate quality assurance 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

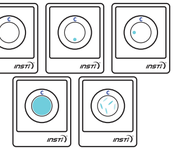
- **Do not read the results if more than 5 minutes have elapsed following the addition of Clarifying Solution.**
- **If using the syphilis control samples provided by bioLytical Laboratories:**
  - **All syphilis Positive Controls must be reactive with INSTI.**
  - **All Negative Controls must be non-reactive 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.



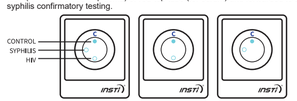
REACTIVE ► Two or three blue dots that are discernable above any background tint indicate that the specimen contains HIV-1 and/or HIV-2 and/or syphilis antibodies, depending on the position of the dots. One dot may be darker than the other. A sample giving these patterns is considered a preliminary reactive. 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 fingerstick whole blood, 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 60 µL 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

- The performance of the device has not been established in individuals under the age of 17.
- The performance of the device has only been assessed in high-risk populations.
- **Flow Times:** In some instances, samples may exhibit longer than normal flow times (from the time the Sample Diluent specimen mixture is poured into the membrane well to the time the Clarifying Solution has fully flown through the membrane). This is due to various 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 or fingerstick whole blood.
- Insufficient data are available to interpret tests performed on other body fluids, pooled blood or pooled serum 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.[8] 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.
- Patient samples with Myeloma may experience an Invalid test result with INSTI Multiplex.
- The performance of the device has not been assessed in individuals who have participated in a HIV vaccine study
- Patients who are elite controllers (i.e. individuals with low or undetectable viral loads) may give a false negative HIV antibody test result with INSTI Multiplex.
- Samples from patients with severe hypogammaglobulinemia conditions such as multiple myeloma may result in false negative HIV results or invalid results with INSTI Multiplex.
- Patients with elevated hemoglobin levels may test false negative for HIV with INSTI Multiplex.[15]
- Samples from patients with Rubella, HSV-1 and HSV-2 conditions may result in possible false positive results for HIV.

As 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.

ANALYTICAL END-POINT SENSITIVITY:

WHO International Standard for Syphilis (1st IS for human syphilitic plasma IgG; NIBSC code:05/122; 0.3 IU/ml) were diluted at 1:4 and 1:8 in negative matrix respectively and tested on 3 lots of INSTI HIV/Syphilis rapid test kits at n=20 per dilution level per kit lot. The 1:4 dilution of WHO international standards for syphilis showed 100% sensitivity for syphilis, which was defined as analytical end-point sensitivity of INSTI HIV/Syphilis rapid test.

CLINICAL EVALUATION

Syphilis

The clinical evaluation conducted in Alberta compared the performance of the test from fingerstick whole blood specimens with standard laboratory tests. Of the 1500 participants with eligible HIV POCT results, 55.9% were men with the majority (42.2%) between 26-35 years, 12 were pregnant and the majority (85.1%) of tests were conducted in a correctional facility. Of the 1508 participants with eligible syphilis POCT results, 55.9% were men with the majority (42.2%) between 26-35 years, 12 were pregnant and the majority (85.0%) were conducted in a correctional facility. In summary, the test performance on completed POCT reveals a PPA of 100.0%, and NPA of 99.7% for HIV (1498 completed POCT) and a PPA of 74.3% and NPA of 99.8% for syphilis (with any RPR value) when compared to serology (1494 completed POCT). The INSTI Multiplex has the greatest PPA to those who have RPR dilutions greater than or equal to 8 dilutions; a RPR titre of > 8 dilutions is generally considered to be a marker for an infectious case. The PPA, NPA, PPV, and NPV of the tests are summarized in below table 1 for primary analysis.

Table 1 Primary INSTI Multiplex Test Performance Calculations

INSTI Multiplex Test	PPA (%) (95% CI)	NPA (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
PPA and NPA relative to the overall interpretation of the serology results:				
HIV	100.0% (85.1%-100.0%) N=22 22/(22+0)	99.7% (99.2%-99.9%) N=1476 1471/(1471+5)	81.5% (63.5%-91.8%) N=27 22/(22+5)	100.0% (99.7%-100.0%) N=1471 1471/(0+1471)
Syphilis	74.3% (70.4%-77.8%) N=529 393/(393+136)	99.8% (99.3%-99.9%) N=965 963/(963+2)	99.5% (98.2%-99.9%) N=395 393/(393+2)	87.6% (88.6%-89.4%) N=1099 963/(136+963)
Syphilis sub-analysis for different RPR dilution values:				
Syphilis (RPR Non-Reactive)	25.8% (18.9%-34.2%) N=124 32/(32+92)	99.8% (99.3%-99.9%) N=965 963/(963+2)	94.1% (80.9%-98.4%) N=34 32/(32+2)	91.3% (89.4%-92.8%) N=1055 963/(963+92)
Syphilis (RPR 1-8 dilutions)	75.6% (68.3%-81.7%) N=156 118/(118+38)	99.8% (99.3%-99.9%) N=965 963/(963+2)	98.3% (94.1%-99.5%) N=120 118/(118+2)	96.2% (94.8%-97.2%) N=1001 963/(963+38)
Syphilis (RPR ≥ 8 dilutions)	97.6% (94.8%-98.9%) N=249 243/(243+6)	99.8% (99.3%-99.9%) N=965 963/(963+2)	99.2% (97.1%-99.8%) N=245 243/(243+2)	99.4% (98.7%-99.7%) N=969 963/(963+6)

A total of 1508 participants had a completed syphilis serology and POCT and were included in the below table. Syphilis stages for the 39 negative POCT results that had RPR dilutions <8 were: previously positive (n=29), primary (n=3), early latent (n=3), late latent (n=3), and other (n=1). Syphilis staging for the 104 negative POCT results that had a non-reactive RPR were: previously positive (n=85), false positive (n=9), late latent (n=5), early latent (n=3), primary (n=1), and other (n=1). Below table 2 presents the Syphilis results by EIA, RPR and RPR Dilutions:

Table 2 Syphilis Results by EIA, RPR and RPR Dilutions

INSTI Multiplex (Syphilis) Test Results (N=1508)	EIA reactive			EIA Non-Reactive
	RPR Reactive (>=8 dils)	RPR Reactive (<8dils)	RPR Non-Reactive (Null)	RPR not done (Null)
Positive	243	118	33	2
Negative	6	39	104	963
Indeterminate	0	0	0	0
Total:	249	157	137	965

Table 3 demonstrates the INSTI Multiplex performance with respect to the syphilis staging for EIA-positive results. Concordance between the INSTI Multiplex with syphilis diagnosis ranged from 90.3%-100% for early infectious syphilis (primary, secondary, early latent, and early neurosyphilis), and 65.2% to 100% for late syphilis (late latent and late neurosyphilis) were observed.

Table 3 INSTI Multiplex test performance based on the Syphilis Staging for EIA Positive Results:

Syphilis Staging for EIA Positive Results (N=543)				
		INTSI Multiplex Result		Total
		Positive (N) (%)	Negative (N) (%)	
Newly Diagnosed	Primary	56 (90.3%)	6 (9.7%)	62
	Secondary	19 (100%)	0	19
	Early Latent	124 (93.2%)	9 (6.8%)	133
	Early Neurosyphilis	2 (100%)	0	2
	Late Latent	15 (65.2%)	8 (34.8%)	23
	Late Neurosyphilis	1 (100%)	0	1
	Subtotal	**217 (90.4%)**	**23 (9.6%)**	**240**
Other	Previously Positive	176 (60.5%)	115 (39.5%)	291
	Biological False Positive	1 (10.0%)	9 (90.0%)	10
	Other	0	2 (100%)	2
	Subtotal	**177 (58.4%)**	**126 (41.6%)**	**303**
Grand Total		**394 (72.6%)**	**149 (27.4%)**	**543**

SYPHILIS PERFORMANCE WITH PREGNANT SAMPLES:

A total of 80 pregnant samples were tested to evaluate the performance of Syphilis test dot in the INSTI Multiplex Test, 31/31 of the syphilis positive pregnancy samples were reactive and 49/49 of the syphilis negative pregnancy samples were non reactive. Below is the tabular data of this study:

Comparator Method (Abbott Architect)		INSTI Multiplex Result		Total
Pregnancy sample Staging[1]		Positive	Negative	
1st Trimester	Positive	6[a]	0	6
	Negative	0	16	16
2nd Trimester	Positive	13[b]	0	13
	Negative	0	16	16
3rd Trimester	Positive	12[c]	0	12
	Negative	0	17	17
Total		31	49	80
Sensitivity		100 % (95% CI: 88.78 to 100)		
Specificity		100% (95% CI: 92.75 to 100)		

[1]Syphilis staging: [a]1x secondary and 5x primary; [b]3x secondary; 9x primary; 1x unknown; [c]4 x secondary and 8x primary.

In addition, 12 pregnant samples were identified in the clinical study conducted in Alberta, out of which 8 were EIA reactive and 4 were EIA non-reactive. Out of 8 EIA reactive, 5 (two in first trimester and three in second trimester) were detected as positive on INSTI Multiplex with fingerstick blood specimens.

HIV

Out of 1500 participants who had a completed HIV serology and a completed, valid HIV POCT (note that 15 participants were excluded for an incomplete HIV serology, seven for an incomplete POCT, and four with an invalid POCT). Of the 24 serological positive results, 4 were newly diagnosed HIV cases and 20 were previously reported cases. Nine of the 20 previously reported cases were on antiretroviral therapy, including the 1 indeterminate POCT.

Table 4 HIV TEST RESULTS

INSTI Multiplex (HIV) Test Results (N=1500)	Serological HIV Result[1]			
	Newly Positive	Previously Positive	Negative	Total
Reactive	3	19	5	27
Indeterminate	1	1	0	2
Non-Reactive	0	0	1471	1471
Total:	4	20	1476	1500

[1]For HIV, a fourth generation EIA (Architect HIV Ag/Ab Combo; Abbott Laboratories, Illinois, USA) was used as the initial screening test and if positive it was repeated in duplicate. Confirmation was done using the GeeniusTM HIV - 1/2 Antibody Differentiation Assay. For samples that screened positive on the EIA but were not confirmed in the Geenius, an additional specimen was requested for HIV RNA (qualitative) testing.

HIV PERFORMANCE CHARACTERISTICS (Note: The HIV-1/HIV-2 portion of the Multiplex assay is identical to INSTI HIV-1/HIV-2 Antibody Test product 90-1008 licensed by Health Canada. All data presented in this section is based on the data presented in the INSTI HIV-1/HIV-2 Antibody Test Instructions for Use 51-1028).

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

INDEPENDENT STUDY

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 with 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

SPECIFICITY

Interfering Substances and Unrelated Medical Conditions

To assess the impact of unrelated medical conditions or interfering substances on the specificity of the INSTI Multiplex Test, samples from a variety of medical conditions unrelated to HIV and Syphilis infection and interfering substances were analyzed.

INSTI Multiplex Test Reactivity with Specimens from Individuals with potentially Interfering Medical Conditions and Specimens with Interfering Substances:

		Negative				Weak Positive			
Condition	N	INSTI HIV Reactive	INSTI HIV Non-Reactive	INSTI Syphilis Reactive	INSTI Syphilis Non-Reactive	INSTI HIV Reactive	INSTI HIV Non-Reactive	INSTI Syphilis Reactive	INSTI Syphilis Non-Reactive
Elevated Bilirubin	10	0	10	0	10	10	0	10	0
Elevated Triglyceride	10	0	10	0	10	10	0	10	0
Elevated Lipid	10	0	10	0	10	10	0	10	0
Hemolysis	5	0	5	0	5	4	0	4[1]	0
Elevated Albumin	5	5*	0	1**	4	NR[2]	NR[2]	NR[2]	NR[2]
Rheumatoid Factor	10	0	10	0	10	10	0	10	0
Pregnancy	10	0	10	0	10	10	0	10	0
Toxoplasmosis	10	0	10	2**	8	10	0	10	0
SLE	10	0	10	1**	9	10	0	10	0
HCV	10	0	10	0	10	10	0	10	0
CMV	10	0	10	0	10	10	0	10	0
EBV	10	0	10	0	10	10	0	10	0
Rubella	10	0	10	0	10	10	0	10	0
HSV-1	10	0	10	0	10	10	0	10	0
HSV-2	10	0	10	0	10	10	0	10	0
Myeloma	10	0	8[3]	0	8[3]	10	0	10	0
Dengue	10	0	10	0	10	10	0	10	0
Chikungunya	10	0	10	0	10	10	0	10	0
Pregnancy (1ST trimester)	50	0	50	0	50	n/a	n/a	n/a	n/a
Pregnancy (2ND trimester)	50	n/a	n/a	0	50	n/a	n/a	n/a	n/a
Pregnancy (3RD trimester)	50	n/a	n/a	0	50	n/a	n/a	n/a	n/a
Pregnancy (unspecified)	40	n/a	n/a	0	40	n/a	n/a	n/a	n/a
Cytomegalovirus	5	0	5	0	5	n/a	n/a	n/a	n/a
Epstein-Barr Virus	5	0	5	0	5	n/a	n/a	n/a	n/a
Influenza vaccine recipient	5	0	5	0	5	n/a	n/a	n/a	n/a
Vaccine-induced HIV seropositivity	5	0	5	0	5	n/a	n/a	n/a	n/a
Malaria	5	0	5	0	5	n/a	n/a	n/a	n/a
Visceral Leishmaniasis	5	0	5	0	5	n/a	n/a	n/a	n/a
Tuberculosis	5	0	5	0	5	n/a	n/a	n/a	n/a
Brucellosis	5	0	5	0	5	n/a	n/a	n/a	n/a
Leprosy	5	0	5	0	5	n/a	n/a	n/a	n/a
Leptospirosis	5	0	5	0	5	n/a	n/a	n/a	n/a
Chlamydia trachomatis	5	0	5	0	5	n/a	n/a	n/a	n/a
Human papillomavirus	5	0	5	0	5	n/a	n/a	n/a	n/a
Trichomoniasis	5	0	5	0	5	n/a	n/a	n/a	n/a
Anti E. Coli Human Antibodies	5	0	5	0	5	5	0	5	0
Human Anti-Mouse Antibody	5	0	5	0	5	5	0	5	0
Systemic Lupus Erythematosus	5	0	5	0	5	5	0	5	0
Anti-Nuclear Antibodies	5	0	5	0	5	5	0	5	0
Sickle-Cell Disease	5	0	5	0	5	5	0	5	0
Thyroiditis	5	0	5	0	5	5	0	5	0
Albumin	5	0	5	0	5	5	0	5	0
Hepatitis A	5	0	5	0	5	n/a	n/a	n/a	n/a
Hepatitis B	5	0	5	0	5	n/a	n/a	n/a	n/a
Lyme Disease	5	0	5	0	5	n/a	n/a	n/a	n/a

*4 of 5 INSTI HIV Positive Samples were confirmed HIV Positive by ARCHITECT; one sample had insufficient volume
** All INSTI Syphilis Positive Samples were confirmed TP-PA positive
[1] 1 of 5 test result was Invalid.
[2] Samples not tested (NR – not run) due to confirmed HIV and/or Syphilis results identified during specificity testing;
[3] 2 of 10 test results were invalid.

Drug Interferences

A drug interference study was performed with ten common therapeutic drugs representing common over-the-counter anti-inflammatory drugs, anti-bacterial, antiparasitic, anti-tuberculosis, antimalarial, and anti-viral drugs used in HIV/Syphilis treatment. Each drug was evaluated at approximately 3x the highest concentration reported followed a drug therapeutic dosage as recommended by EP07-A2 or at the recommended concentrations as per CLSI EP37. No interference was observed at the following tested concentrations in the table below:

Exogenous interference performance of INSTI Multiplex Test:

Drugs	Concentration (µg/mL)
Acetaminophen	156
Acetylsalicylic acid	1172
Ampicillin	75
Erythromycin	138
Ibuprofen	219
Tetracycline Hydrochloride	210
Gentamycin Sulfate	15
Malarone (Atovaquone/Proguanil)	600/240
Artesunate	180
Chloroquine	600
Doxycycline	60
Primaquine	160
Mefloquine	540
Ethambutol	720
Isoniazid	180
Pyrazinamide	1,100
Rifampin	360
Benznidazole	130
Diethylcarbamazine	120
Nifurtimox	180
Suramin	120

EQUIVALENCE STUDIES

The INSTI Multiplex Test Equivalence was demonstrated using matched donations of HIV and Syphilis antibody negative serum. EDTA whole blood, plasma-EDTA, plasma-sodium heparin, and plasma-sodium citrate from 25 individual donors. Each matrix specimen was tested at the negative and low positive anti-HIV and anti-Syphilis antibody concentrations. The results indicate 100% relative sensitivity and specificity with the matched whole blood, serum and plasma samples and they are equivalent.

REPEATABILITY STUDY

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%.

REPRODUCIBILITY STUDY

A study was conducted to evaluate the reproducibility of INSTI Multiplex Test with a panel of 6 blind-coded whole blood and plasma samples were tested. The panels included Negative, HIV-1/HIV-2, Syphilis weak positive (2xLoD) contrived whole blood specimens and Controls (HIV-1 positive, HIV-2 negative, Syphilis positive, Negative control) in plasma matrix.
INSTI® Multiplex reproducibility testing was completed using three lots of kits over five days with 1 run per day (alternating morning and afternoon) at each of three testing sites (three kit lots per site). Each panel member was tested at n=5 per run for a total of 225 replicates per panel member.
Overall, 1351 tests were performed to determine the reproducibility of the INSTI® Multiplex Test. 1 invalid result was observed (invalid rate of 0.07%). The overall reproducibility rate for each panel member and each of HIV and Syphilis test dots was 100% (CI of 98.3% - 100%).

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TECHNICAL INFORMATION

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

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GLOSSARY OF SYMBOLS

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		Use by
	Do not use if damaged		Keep dry
	This side up		Keep away from direct sunlight

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