HIV-1/HIV-2Antibody Test Kit

Single-use rapid assay for the detection of antibodies to
Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1/HIV-2)

Store at 15°-30°C, 59°-86°F. For *in vitro* diagnostic use only.

Read the entire Package Insert prior to beginning the test procedure. Conformance with the test procedure is necessary to ensure accurate results. Before performing the test, all operators must become familiar with Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and other Blood-borne Pathogens in Health Care Settings[15].

COMPLEXITY: WAIVED
For Fingerstick Whole Blood.

Any modification by the laboratory to the INSTI test or the FDA approved INSTI test instructions will result in the test no longer meeting the requirements for waived category.

A CLIA Certificate of Waiver is required to perform the test in a waived setting. Additional CLIA waiver information is available at the Centers for Medicare and Medicaid website at www.cms.hhs.gov/CLIA or from your state health department.

COMPLEXITY: MODERATE
For Venous Whole Blood and Plasma Samples

NAME AND INTENDED USE: The **INSTI™ HIV-1/HIV-2Antibody Test** is a single use, rapid, *in vitro* qualitative immunoassay for the detection of antibodies to Human Immunodeficiency Virus Type 1 and/or Type 2 (HIV-1/HIV-2) in human venipuncture whole blood, fingerstick blood, or plasma specimens. The test is intended for use by trained personnel in point of care and laboratory situations to aid in the diagnosis of HIV infections. If multiple rapid HIV tests are available, this test is suitable for use in appropriate multi-test algorithms.

RESTRICTIONS

- **Sale of the INSTI™ HIV-1/HIV-2 Antibody Test is restricted to clinical laboratories**
  - **That have an adequate quality assurance program, including planned activities to provide adequate confidence that requirements for quality will be met; and**
  - **Where there is assurance that operators will receive and use instructional materials.**
- **The INSTI™ HIV-1/HIV-2Antibody Test is approved for use only by an agent of a clinical laboratory.**
- **Test subjects must receive the "Subject Information" brochure prior to specimen collection and appropriate counselling when test results are provided.**
- **The INSTI™ HIV-1/HIV-2Antibody Test is not approved for use to screen donors of blood, plasma, cells or tissues.**

SUMMARY AND EXPLANATION OF THE TEST
Acquired Immunodeficiency Syndrome (AIDS) is thought to be caused by at least two retroviruses, Human Immunodeficiency Virus Type 1 (HIV-1) and Human Immunodeficiency Virus Type 2 (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 (including sharing contaminated needles and syringes), or from an infected mother to her fetus. People with 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 asymptomatic HIV infected persons.[2-5] Antibodies specific for HIV envelope proteins are prevalent in blood or blood products from persons at high risk of contracting AIDS as well as in people with AIDS, or ARC.[5-7] The presence of antibodies to HIV indicates previous exposure to the virus, but does not necessarily constitute a diagnosis of AIDS. The prevalence of antibodies to HIV in people not known to be at risk of acquiring HIV infection is unknown, but significantly less.[5] **Absence of antibodies to HIV does not indicate that an individual is absolutely 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.

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[17]. Rapid HIV testing provides results during the initial visit allowing for immediate counselling and follow-up opportunities.

BIOLOGICAL 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 obtained from fingerstick or venipuncture, and plasma in as little as 60 seconds. The test consists of a nitrocellulose filtration membrane positioned atop an absorbent material within a plastic cartridge, referred to as the INSTI™ Membrane Unit. The membrane has been spotted with HIV-1 and HIV-2 recombinant proteins, which react with HIV antibodies in the specimen. HIV-1/HIV-2
The membrane also includes a procedure control. The procedure control consists of a protein-A spot capable of capturing human immunoglobulin G (IgG) antibodies normally present in blood and blood components. The IgG antibodies then react with a chromatic agent contained in the INSTI™ Color Developer to produce a visual blue spot 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 and the correct type and volume of specimen was added. 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 bound to the membrane capture HIV-specific antibodies, if present in the specimen. Antibodies captured in the test spot react with a chromatic agent contained in the INSTI™ Color Developer to produce a blue spot 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 fingerstick blood, venipuncture whole blood, or plasma specimen to the vial of Sample Diluent, which lyses the blood cells. This specimen/diluent solution is then poured into the well of the Membrane Unit. HIV antibodies, if present in the specimen, are captured by the HIV 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 spot at the location of the control spot and, in the case that HIV antibodies are present in the specimen, a blue spot also appears at the location of the test spot on the membrane. In the final step, the Clarifying Solution is added to the membrane to decrease background color in order to make the control and test spots more distinct.

MATERIALS PROVIDED
The INSTI™ HIV-1/HIV-2 Antibody Test kits are available in the following packaging formats:

Component	90-1018, 24 tests with support materials	90-1019, single test with support materials	90-1020, 24 tests without support materials
Outer box (24 test) or foil pouch (single test)	1	1	1
Individually Pouched Membrane Units prepared with one control and one test reaction spot	24	1	24
Solution 1 Vial, Sample Diluent (each vial contains 1.5 ml of a Tris-Glycine buffered solution containing cell lysis ingredients and an antimicrobial agent.)	24	1	24
Solution 2 Vial, Color Developer (each vial contains 1.5 ml of a borate buffered protein solution containing a blue dye color indicator and an antimicrobial agent.).	24	1	24
Solution 3 Vial, Clarifying Solution (each vial contains 1.5 ml of a Tris-Glycine buffered solution containing a detergent and an antimicrobial agent.)	24	1	24
Single Use Safety Lancet	24	1	0
Specimen Collection Capillary Pipette with 50µl fill line	24	1	0
Single Use 70%Isoproply Alcohol Swab	24	1	0
Subject Information Brochures	24	1	24
Customer Letter	1	1	1
Package Insert	1	1	1

MATERIALS REQUIRED AND AVAILABLE AS AN ACCESSORY TO THE KIT

INSTI™ HIV-1/HIV-2 Antibody Test Kit Controls (product 80-1071):

Each package of INSTI™ controls contains separateHIV-1 (12 vials, red caps), HIV-2 (12 vials, orange caps) Positive Controls, and HIV Negative Controls (12 vials, green caps, 0.4 ml per vial), and a Package Insert.

MATERIALS REQUIRED BUT NOT PROVIDED
- Personal protective equipment such as gloves, lab coat or gown.
- Appropriate biohazard waste containers.
- Absorbent cotton for fingerstick or venipuncture wound closure.

For venipuncture blood collection and plasma specimens:
- Venipuncture apparatus if collecting blood specimens.
- Appropriate blood collection tubes.
- Precision pipette capable of delivering 50µl of specimen.
- Appropriate shipping containers.
- Personal protective equipment.
- Appropriate biohazard waste containers and disinfectants.
- Centrifuge to process a plasma specimen.
- Timer

WARNINGS
For *in vitro* diagnostic use only

1. **Read the entire Package Insert prior to beginning the test procedure. Complete conformance with the test procedure is necessary to ensure accurate results.**
2. **Before performing testing, operators must read and become familiar with Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus and other Blood-borne Pathogens in Health-Care Settings[15].**
3. **Do not use the Membrane Unit if the foil pouch has been previously opened or if the packaging integrity of any component has been compromised. Once the Membrane Unit has been opened, it must be used immediately.**
4. **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.**
5. **The performance characteristics of the INSTI™ HIV-1/HIV-2 Antibody Test have not been established for body fluids other than venipuncture whole blood, fingerstick blood, and plasma. Insufficient data are available to interpret tests performed on other body fluids, pooled blood plasma, or products made from such pools.**
6. **If the test kit is exposed to temperatures outside of 15° – 30°C, (59° – 86°F), ensure it is brought to this temperature range before performing testing. Use the INSTI™ Controls to ensure proper kit performance.**
7. **Patients that are receiving highly active antiretroviral therapy (HAART) may have undetectable levels of antibody to HIV-1 and/or HIV-2 and give a false Non-Reactive INSTI™ HIV-1/HIV-2 Antibody Test result.**
8. **Specimens from patients with multiple myeloma, may result in false Non-Reactive or invalid results with the INSTI™ HIV-1/HIV-2 Antibody Test.**
9. **Patients with elevated hemoglobin levels may test false Non-Reactive with the INSTI™ HIV-1/HIV-2 Antibody Test**.

PRECAUTIONS

Safety Precautions

1. All specimens should be handled as if capable of transmitting infectious agents.
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 in a biohazard waste container. The preferred method of disposal is sterilization by autoclaving for a minimum of one hour at 121°C. Disposable materials may be incinerated. Liquid waste may be mixed with sodium hypochlorite (bleach) in volumes such that the final mixture contains 1.0% sodium hypochlorite (using a freshly prepared solution containing 10% household bleach). Allow at least 60 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.
9. For additional information on bio-safety refer to "Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and other Blood-borne Pathogens in Health Care Settings"[15] and "Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations for Postexposure Prophylaxis"[16].

Handling Precautions

1. Use all alcohol swabs, safety lancets, capillary pipette, INSTI™ solution vials and membrane units only once and dispose of properly (see Safety Precautions). **Do not reuse any of these test components**.
2. Do not mix reagent vials and membrane units from different lots.
3. Do not use the test beyond the expiration dates printed on the outer packaging, reagent vials and membrane unit pouch.
4. Avoid microbial contamination and exercise care in handling the kit components.
5. All Membrane Units must be used immediately in the test procedure once the membrane unit pouch is opened.
6. When collecting fingerstick blood with the capillary pipette, ensure blood flows to the black fill line. **Do not squeeze the capillary pipette bulb while collecting the specimen**. (See Test Procedure).

STORAGE INSTRUCTIONS

Store unused INSTI™ kits unopened at 15°-30°C, 59°-86°F. Do not open the Membrane Unit pouch until ready to use.

INSTRUCTIONS FOR USE

Workspace Preparations

- Ensure the workspace is clean and uncluttered. Preferably, cover the workspace with a clean, disposable absorbent workspace cover.
- Gather support materials (swab, lancet, pipette), 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.
- Gather the required materials you will need.
- Refer to the External Quality Control section of this package insert to determine when INSTI™ Controls should be run.
- Put on the gloves and any other personal protective equipment as required in accordance with the Safety Precautions section of this package insert.

Prior to testing provide the "Subject Information" brochure to the individual being tested.

SPECIMEN COLLECTION AND TESTING PROCEDURE

The **INSTI™ HIV-1/HIV-2 Antibody Test** can be used for testing fingerstick whole blood, venipuncture whole blood and plasma specimens.

Fingerstick Whole Blood:

Caution: The amount of specimen (fingerstick blood) is critical. To ensure that the proper amount of blood is achieved, follow these instructions carefully:

1. 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 should be positioned at waist level or lower for optimal blood flow.
2. Wipe the fingertip with the alcohol swab.
3. As soon as the finger is dry, twist and remove the protective tab from the lancet (Figure A). Grasp 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 lightly press the tip of the lancet on the finger and then push down to release the needle (Figure B). Immediately dispose the used lancet into a proper sharps container.

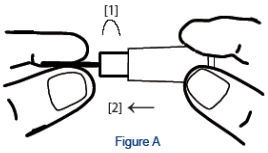


Figure A

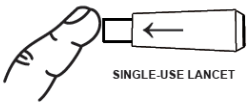
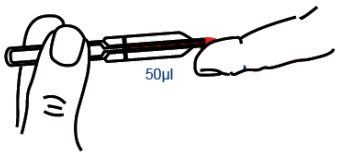


Figure B

4. As the blood bubbles up, hold the capillary pipette horizontally and touch the tip of the pipette to the blood specimen. Capillary action automatically draws the specimen to the black 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 the volume of blood is inadequate, perform a second finger puncture using a new lancet and capillary pipette.



CAUTION! Filling is automatic: Never squeeze the pipette while sampling.

5. 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 specimen. Note: If the specimen will not expel, hold the pipette vertically and slide a finger over (without pressing) the vent hole, then squeeze the bulb (see illustrations below). Recap the vial and mix by inversion.

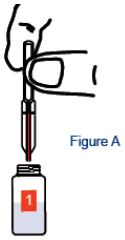


Figure A

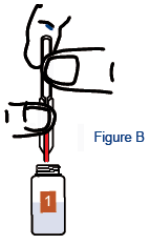


Figure B

Follow the Test Procedure, below.

Venipuncture Whole Blood

1. Using standard venous phlebotomy procedures, collect a whole blood specimen in a tube containing any of the following anticoagulants: EDTA (lavender top), sodium heparin (green top), sodium citrate (light blue top). **Other anticoagulants have not been validated and may give an incorrect result.**
2. If not testing at the time of specimen collection, whole blood specimens may be stored for up to 5 days at 2°-24°C. Prior to testing, mix the blood by gentle inversion several times. **Do not heat or freeze whole blood specimens.**
3. Using a calibrated 50µl precision pipette and clean unused tip, collect 50µl of whole blood from the collection tube.
4. Transfer the blood held in the pipette to the Sample Diluent vial (Solution 1). Recap the vial and mix by inversion. Follow the Test Procedure, below.

Plasma

1. Using standard venous phlebotomy procedures, collect a whole blood specimen in a tube containing any of the following anticoagulants: EDTA (lavender top), sodium heparin (green top), sodium citrate (light blue top). **Other anticoagulants have not been validated and may give an incorrect result.**
2. Centrifuge the tube of blood at 1000-1200 x g for approximately 5 minutes to separate the blood cells from the plasma. Plasma may be stored at 2°-24°C for up to 5 days prior to testing. Carefully uncap the tube so as not to produce any aerosols.
3. Using a calibrated 50µl precision pipette and clean unused tip, collect 50µl of the separated plasma from the collection tube.
4. Transfer the plasma held in the pipette to the Sample Diluent vial (Solution 1). Recap the vial and mix by inversion. Follow the Test Procedure, below.

TEST PROCEDURE

Note: All components for the **INSTI™ HIV-1/HIV-2 Antibody Test** are ready to use as supplied. **All Membrane Units must be used immediately once opened.** All reagents should be dispensed evenly in the center of the well.

For Testing Fingerstick Whole Blood, Venipuncture Whole Blood, plasma and INSTI™ Controls:

1. Tear open the pouch and carefully remove the Membrane Unit without touching the center well. Place the unit on a level surface. For specimen 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. 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 Diluent-specimen mixture should be absorbed through the membrane in less than 30 seconds; however, absorption times will vary slightly depending upon specimen type. (see Note, below)

3. Re-suspend the Color Developer by slowly inverting to mix the solution thoroughly, until 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 reduce the background color and facilitate reading. Immediately read the result while the membrane is still wet. **Do not read the results if more than 5 minutes has elapsed following the addition of Clarifying Solution.**

Note: If at the any period during Test Procedure steps 2, 3, or 4 the solutions completely stop flowing into the Membrane Unit, the procedure must be halted, a new specimen collected, and the procedure re-started from the beginning with fresh INSTI™ components.

QUALITY CONTROL

Procedure Control

The INSTI™ HIV-1/HIV-2 Antibody Test has a built-in procedure control that demonstrates assay validity and adequate specimen addition. A blue color in the control spot indicates that the proper specimen was added and that the test procedure was performed correctly. The control spot must appear on all valid INSTI™ tests. (Refer to Interpretation of Results in this package insert.)

External Quality Control

INSTI™ HIV-1/HIV-2Antibody Test Kit Controls are available separately for use only with the INSTI™ HIV-1/HIV-2Antibody Test. The controls are used to verify test performance and interpretation of results. The Positive Controls and the Negative Control are to be run on separate Membrane Units. The HIV-1 and HIV-2Positive Controls have been manufactured to produce a faint blue color in the test spot. The

Negative Control will produce a blue color in the control spot, but no color in the test spot, for a Non-Reactive test result. Use of non-validated kit control material manufactured by other sources may not produce the required results and therefore would be inadequate for quality assurance programs for the INSTI™ HIV-1/HIV-2 Antibody Test.

INSTI™ HIV-1/HIV-2 Positive and Negative Controls should be run under the following circumstances:

- **for new INSTI™ operator verification prior to performing testing on patient specimens**
- **when switching to a new lot number of INSTI™ test kits**
- **whenever a new shipment of kits is received**
- **when temperature during storage of the kit falls outside of 15˚-30˚C (59˚-86˚F)**
- **when the temperature of the test area falls outside of 15˚-30˚C (59˚-86˚F)**
- **At regular intervals as determined by the user facility.**

Refer to the INSTI™ HIV-1/HIV-2Antibody Test Kit Controls package insert for additional information on the use of these reagents. It is the responsibility of each user of the INSTI™ HIV-1/HIV-2Antibody Test to establish an adequate quality assurance program to ensure proper performance under their specific locations and conditions of use. Contact bioLytical Laboratories' Technical Support if the INSTI™ HIV-1/HIV-2Controls do not produce the expected results.

INTERPRETATION OF RESULTS

- **Do not read the results if more than 5 minutes has elapsed following the addition of Clarifying Solution.**
- **If using the controls provided by bioLytical, the Positive Control must be Reactive with INSTI™ and the Negative Control 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.**
- **Follow CDC guidelines to inform the test subject of the Test Result and its interpretation [additional reference (see Bibliography)].**

NON-REACTIVE ► Only the control spot shows blue color development. The visible control spot indicates that the test has been performed correctly and a proper specimen was added. As illustrated, the control spot is located towards the top of the read frame furthest from the plastic tab on the Membrane Unit. No blue spot should be visible at the test spot, located below the control. A Non-Reactive result indicates that antibodies to HIV-1and/or HIV-2were not detected in the specimen.

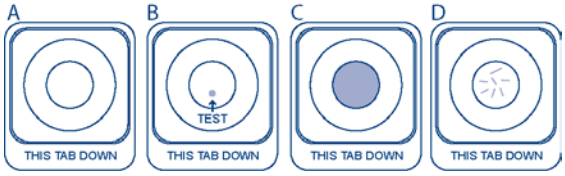
REACTIVE ► Both the control spot and the test spot show blue color development. This Reactive result indicates that the specimen is **preliminary positive** for HIV antibodies. As illustrated, one spot may be darker than the other.

Note:

1. **Following a reactive rapid test result, a venous blood specimen must be drawn in an appropriate anticoagulant collection tube for HIV confirmatory testing.**
2. A reactive test spot may be less intense in color than the control spot, or vice versa.
3. In rare instances, a faint ring may appear at the test spot; this should be interpreted as a Reactive result.

INVALID ► The test is invalid if any of the following occurs:

A. There is no blue color on the control spot or the test spot
B. There is blue color on the test spot but not on the control spot
C. Uniform tint across the membrane
D. Only blue specks appear on the membrane



Note: An **invalid** test result means that the test was run incorrectly or insufficient specimen was added. Invalid test results cannot be interpreted. **Repeat the test with a fresh specimen using a new Membrane Unit, kit components and support materials. Contact bioLytical Laboratories' Technical Support if you are unable to produce a valid result upon repeat testing.**

LIMITATIONS OF THE TEST

1. The INSTI™ HIV-1/HIV-2Antibody Test must be used in accordance with the instructions in this package insert to obtain accurate results.
2. In some instances, specimens 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. As long as the contents from all three INSTI™ solution bottles completely flow through the membrane, regardless of flow time, the test can be properly interpreted according to the Interpretation of Results section of this package insert. **In occasional instances of long flow times, a faint result in the form of a ring may appear atthe test spot location. This should be considered as a Reactive result.** In these instances, a venous blood specimen should be drawn in an anticoagulant blood collection tube, and forwarded to a laboratory for HIV confirmatory testing. **If any of the solutions completely stop flowing into the Membrane Unit, the procedure must be halted, a new specimen collected, and the procedure re-started from the beginning with fresh INSTI™ components.**
3. For a Reactive result, the intensity of the test spot does not necessarily correlate with the titer of antibody in the specimen.
4. The test is approved by FDA for use with fingerstick whole blood, venipuncture whole blood, and plasma specimens only. Other specimen types have not been evaluated and may give an incorrect result.
5. Use of other anticoagulants not listed in the Specimen Collection and Testing Procedure for Venipuncture Whole Blood and Plasma section of this package insert has not been evaluated and may give incorrect results.
6. Reading the test results after more than 5 minutes has elapsed following the addition of Clarifying Solution might produce erroneous results.
7. The INSTI™ HIV-1/HIV-2Antibody Test detects antibodies to HIV-1 and/or HIV-2 and is useful as an aid in the diagnosis of infection with HIV-1and/or HIV-2. Because a variety of factors may cause non-specific reactions, a patient found to be Reactive using the INSTI™ HIV-1/HIV-2Antibody Test should have a blood specimen 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.
8. Patients that are receiving highly active antiretroviral therapy (HAART) may have undetectable levels of HIV antibodies and give a false Non-Reactive INSTI™ HIV-1/HIV-2Antibody Test result.
9. Specimens from patients with multiple myeloma, may result in false Non-Reactive or invalid results with the INSTI™ HIV-1/HIV-2Antibody Test.
10. Patients with elevated hemoglobin levels may test false Non-Reactive with the INSTI™ HIV-1/HIV-2Antibody Test.
11. A person who has antibodies to HIV is presumed to be infected with the virus, except a person who has participated in an HIV vaccine study may develop antibodies to the vaccine and may or may not be infected with HIV. Clinical correlation is indicated with appropriate counseling, medical evaluation, and possibly additional testing to decide whether a diagnosis of HIV infection is accurate.

PERFORMANCE CHARACTERISTICS

SENSITIVITY:

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

A sensitivity study was performed in 14 clinical trial sites using freshly obtained matching fingerstick whole blood, EDTA whole blood, and EDTA plasma specimens collected from 1076 individuals known to be infected with HIV-1. Additionally, matching fingerstick whole blood, EDTA whole blood, and EDTA plasma specimens were collected from 782 previously unscreened individuals from populations at high risk for HIV-1 from which 22 were confirmed seropositive by an FDA licensed test. For the 1098 total HIV-1 positives, results for fingerstick whole blood, venipuncture whole blood and plasma are shown in **Tables 1, 2, 3 and 4**.

Table 1
Detection of Antibody to HIV-1 in Fingerstick Whole Blood Specimens from Seropositive Individuals and Individuals at High Risk of HIV Infection

Test Group	Total Specimens	INSTI™ Reactive	INSTI™ Non-Reactive	INSTI™ Invalid[1]	Approved Test Repeatedly Reactive	Approved Test Non-Reactive	True Positive[2]
Known HIV-1 Positive	1075	1074	1	0	1075	0	1075
High Risk	782	22[2]	756[3]	4	22	760	22
TOTAL	1857	**1096**	757	4	**1097**	760	1097

[1] Invalid results were not included in the calculation of sensitivity. The 4 specimens which gave invalid results on INSTI™ were Non-Reactive on the approved test.
[2] Confirmed by licensed HIV-1 Western Blot
[3] Of the 22 true positive specimens, 1 was Non-Reactive on INSTI™ (false Non-Reactive). One other specimen was false Reactive on INSTI™.

Fingerstick Whole Blood Specimens

Of the 1076 known HIV-1 positive individuals, one did not provide a fingerstick specimen. Of the 1075 fingerstick specimens collected from the known HIV-1 positive patients that were repeatedly Reactive by an FDA approved test, 1074 gave a Reactive result with INSTI™. Within the high risk group, 22 specimens were confirmed seropositive by an FDA licensed test and of those, 21 were Reactive with INSTI™. One specimen was false Non-Reactive on INSTI™. One additional fingerstick specimen from the high risk population was INSTI™ false Reactive (See **Table 2** below). The overall sensitivity of the INSTI™ HIV-1/HIV-2Antibody Test in **fingerstick whole blood** specimens for the confirmed HIV-1 positives from the combined high risk and known HIV-1 positive populations was calculated to be 1095/1097= 99.8% (95% CI = 99.3% - 99.9%). The rate of invalid tests was 4/1857 (0.2%).

Table 2
Comparison of Results for Fingerstick Whole Blood Specimens from Seropositive Individuals and Individuals at High Risk of HIV Infection

		Approved Test Result		
		Reactive	Non-Reactive	TOTAL
INSTI™ Test Result	Reactive	1095	1[1]	1096
	Non-Reactive	2[2]	755	757
	Invalid	0	4	4
	TOTAL	1097	760	1857

[1] The one specimen that gave a false Reactive result on INSTI™ was from an individual at high risk for HIV infection.
[2] Of the two false Non-Reactive specimens on INSTI™, one was from an individual known to be infected with HIV-1 and one was from an individual at high risk for HIV infection

Table 3
Detection of Antibody to HIV-1/HIV-2 in Venipuncture Whole Blood Specimens from Seropositive Individuals and Individuals at High Risk of HIV Infection

Test Group	Total Specimens	INSTI™ Reactive	INSTI™ Non-Reactive	INSTI™ Invalid	Approved Test Repeatedly Reactive	Approved Test Non-Reactive	True Positive[1]
Known HIV-1 Positive	1076	1075	1	0	1076	0	1076
High Risk	782	22	760	0	22	760	22
TOTAL	1858	**1097**	761	0	**1098**	760	1098

Of the 1076 known HIV-1 positive EDTA whole blood specimens, 1075 gave Reactive results with INSTI™. Within the high risk group, 22 EDTA whole blood specimens were confirmed seropositive by an FDA licensed test and these same 22 were Reactive with INSTI™. The overall sensitivity of the INSTI™ HIV-1/HIV-2Antibody Test in **venipuncture whole blood** specimens for the confirmed HIV-1

positives from the combined high risk and known HIV-1 positive populations was calculated to be 1097/1098 = 99.9% (95% CI = 99.5% - 100%).

Table 4
Detection of HIV-1 Antibody in Plasma Specimens from Seropositive Individuals and Individuals at High Risk of HIV Infection

Test Group	Total Specimens	INSTI™ Reactive	INSTI™ Non-Reactive	INSTI™ Invalid	Approved Test Repeatedly Reactive	Approved Test Non-Reactive	True Positive[1]
Known HIV-1 Positive	1076	1075	1	0	1076	0	1076
High Risk	782	22	760	0	22	760	22
TOTAL	1858	**1097**	761	0	**1098**	760	1098

[1]Confirmed by licensed HIV-1 Western Blot

Plasma Specimens

Of the 1076 known HIV-1 positive EDTA plasma specimens, 1075 gave Reactive results with INSTI™. Within the high risk group, 22 plasma specimens were confirmed seropositive by an FDA licensed test and these same 22 were Reactive with INSTI™. The overall sensitivity of the INSTI™ HIV-1/HIV-2 Antibody Test in **plasma** specimens for the confirmed HIV-1 positives from the combined high risk and known HIV-1 positive populations was calculated to be 1097/1098 = 99.9% (95% CI = 99.5% - 100%).

Detection of Anti-HIV-1 non-B Subtypes
To assess the sensitivity of the INSTI™ HIV-1/HIV-2Antibody Test for detection of antibodies to non-B subtypes of HIV-1, a total of 207 serum/plasma specimens collected from individuals from various geographic regions who were infected with non-B subtypes of HIV-1 were tested. Of these 207 specimens, a total of 206 were Reactive with the INSTI™ HIV-1/HIV-2Antibody Test, for an overall sensitivity of 99.5% (95% CI = 97.3-99.9%). One subtype A specimen tested false Non-Reactive. The INSTI™ results and HIV-1 non-B subtype listings are presented in **Table 5** below.

Table 5:
Sensitivity of the INSTI™ HIV-1/HIV-2Antibody Test for Detection of Antibodies to HIV-1 Non-B Subtypes

HIV Subtype	Number of Specimens	INSTI™ Reactive
A	28	27
C	57	57
D	22	22
E	7	7
F	9	9
G	10	10
H	2	2
J	4	4
K	1	1
O	23	23
AE	11	11
AG	31	31
CRF06	1	1
CRF11	1	1
TOTAL	207	206

Reactivity With HIV-1Seroconversion Panels

Twenty three commercial seroconversion panels were tested and the INSTI™ results were compared with FDA licensed or approved anti-HIV EIA's. The results of this study are shown in **Table 6**. In this study, the INSTI™ HIV-1/HIV-2 Antibody Test demonstrated the ability to detect HIV-1 antibodies during seroconversion similar to FDA licensed or approved anti-HIV EIAs.

Table 6:
HIV-1 Seroconversion Panel PRB-900 Series[1] Boston Biomedica Inc.

INSTI™ HIV-1	Number of Panels (n=23)
Detected the earliest bleed that was detected by an EIA	18
Within 1 bleed of the earliest bleed that was detected by an EIA	4
Unknown[2]	1

[1] PRB904, PRB910, PRB914, PRB916, PRB919, PRB924, PRB925, PRB926, PRB927,PRB928, PRB929, PRB934, PRB935, BRB937, PRB938, PRB940, PRB941 PRB943, PRB944, PRB945,PRB947, PRB950, PRB952.

[2] The last bleed in the panel PRB937 was positive by at least 1 EIA, negative by INSTI™.

Reactivity With HIV-1 Low Titer Panel
One HIV-1 low titer panel was tested with 3 production lots of INSTI™ and results were compared to three FDA licensed or approved HIV EIAs. The results of this study are shown in **Table 7**. In this study, the INSTI™ HIV-1/HIV-2Antibody Test was capable of detecting levels of antibodies to HIV-1 HIV-1/HIV-2similar to FDA licensed or approved EIAs.

Table 7:
Comparison of the INSTI™ HIV-1/HIV-2Antibody Test and Licensed or Approved Anti-HIV EIA Tests Using a Low Titer HIV-1 Antibody Panel.

PANEL	MEMBER	INSTI™[1]	EIA #1	EIA #2	EIA #3
PRB 108	1	**R**	**RR**	**RR**	**RR**
	2	NR	NR	NR	NR
	3	**R**	**RR**	**RR**	**RR**
	4	**R**	**RR**	**RR**	**RR**
	5	**R**	**RR**	**RR**	**RR**
	6	**R**	**RR**	**RR**	**RR**
	7	**R**	**RR**	**RR**	**RR**
	8	**R**	**RR**	**RR**	**RR**
	9	**R**	**RR**	**RR**	NR
	10	**R**	**RR**	NR	NR
	11	**R**	**RR**	**RR**	**RR**
	12	**R**	**RR**	NR	NR
	13	**R**	**RR**	NR	NR
	14	**R**	**RR**	NR	NR
	15	**R**	**RR**	**RR**	**RR**

NR = Non Reactive; R = Reactive; RR = Repeatedly Reactive
[1]Identical INSTI™ results were obtained across the three production lots tested.

Unrelated Medical Conditions and Potentially Interfering Substances
To assess the impact of unrelated medical conditions or potentially interfering substances on the sensitivity of the INSTI™ HIV-1/HIV-2Antibody Test, 195 serum/plasma specimens from a cross section of medical conditions unrelated to HIV infection and 217 specimens with potentially interfering substances were spiked with an HIV-1 positive specimen to give a low level of reactivity in the INSTI™ HIV-1/HIV-2Antibody Test. The results are presented in **Table 8**.

Table 8
INSTI™ HIV-1/HIV-2Antibody Test Reactivity with HIV-1 Spiked Specimens from Individuals with Unrelated Medical Conditions (n=195) and with HIV-1 Spiked Specimens containing Potentially Interfering Substances (n=217).

Unrelated Medical Condition (n=195)	No. of Specimens	INSTI™ Reactive	INSTI™ Nonreactive
Toxoplasmosis	20	20	0
Rheumatoid Factor	20	20	0
Multiple Myeloma	10	5[1]	0
Syphilis	30	30	0
SLE	5	5	0
Rubella	20	20	0
Cytomegalovirus	20	20	0
Epstein Barr Virus	20	20	0
HTLV-I/II panel	15	15	0
Hepatitis B Virus	20	20	0
Hepatitis A Virus	15	15	0
Potentially Interfering Substance (n=217)			
Icteric	20	20	0
Elevated Bilirubin (≥8.0mg/dL)	19	19	0
Lipemic	20	20	0
Visual Hemolysis	5	5	0
Elevated Triglyceride (≥292mg/dL)	19	19	0
Elevated Hemoglobin (>12g/100mL)	20	19[2]	1
Elevated Albumin (11.5-13.0g/dL)	15	15	0
EDTA	13	13	0
Sodium Heparin	13	13	0
Sodium Citrate	13	13	0
Bacterially Contaminated	60	60	0

[1]Up to 5 specimens from individuals with multiple myeloma produced invalid INSTI™ results depending on the INSTI™ kit lot tested.
[2]Of the 20 specimens from individuals with elevated hemoglobin, one tested false Non-Reactive in 2 out of 3 INSTI™ kit lots.

Related statements are listed in the **Warnings** and **Limitations** sections of this Package Insert.

In addition, a study was performed to assess the potential effect of common blood tube anticoagulants on assay sensitivity. Venipuncture blood was collected from 13 volunteer subjects in each of 3 tubes containing one of three anticoagulants (EDTA, sodium heparin, and sodium citrate). A total of13 specimens for each anticoagulant type were spiked with an HIV-1 positive specimen to give a low level of reactivity in the INSTI™ HIV-1/HIV-2Antibody Test. Aliquots of the spiked specimens were stored refrigerated (2° – 8°C) and at ambient temperature (20° – 24°C) and tested at day 3 and day 7 over a 7 day period. There was no effect of the anticoagulants on sensitivity with specimens held up to 7 days at 2° - 24°C. Results are shown in **Table 8**.

Detection of Antibodies to HIV-2 in Specimens From Individual Subjects Infected with HIV-2
A total of 199 repository serum/plasma samples that were indicated as being HIV-2 antibody positive were obtained from commercial sources. A total of 9 of these specimens were also positive for HIV-1 antibodies on an FDA-approved HIV-1/HIV-2 antibody differentiation assay and were excluded from the analysis. Of the remaining 190 specimens that were reactive for HIV-2 only, INSTI was reactive on 189, for a calculated relative sensitivity of 99.7% (189/190). The results are presented in Table 9

Table 9
Detection of Antibody to HIV-2 in Specimens from HIV-2 Seropositive Individuals and Individuals from an HIV-2 Endemic Region

Specimen Group	Total Specimens	True HIV-2 Positive[1]	INSTI HIV-1/HIV-2 Reactive	Licensed HIV-1/HIV-2 Differentiation Assay Repeatedly Reactive for HIV-2 only
Known HIV-2 Positive	199[2]	190	189	190
Endemic subjects	500[3]	12	12	12
Total	699	202	201	202

[1]Determined by an approved HIV-1/HIV-2 differentiation assay or HIV-2 RNA testing
[2]9/199 specimens were HIV-1 positive or undifferentiated by an approved HIV-1/HIV-2 differentiation assay and were excluded from the sensitivity analysis
[3]34/500 specimens were HIV-1 positive or undifferentiated by an approved HIV-1/HIV-2 differentiation assay and were excluded from the sensitivity analysis

In a separate study, a total of 500 plasma specimens collected from HIV-2 endemic regions (Ivory Coast) were tested by INSTI HIV-1/HIV-2 and the FDA-approved HIV-1/HIV-2 differentiation assay. Of these specimens, a total of 34 tested either positive for HIV-1 or were undifferentiated on the differentiation assay and were excluded from the analysis. Of the remaining 466 specimens, a total of 12 were confirmed as HIV-2 positive by the differentiation assay or an HIV-2 RNA quantitative assay[18]. INSTI was reactive in all 12 of these specimens. The results are presented in **Table 9**.

In the combined specimen groups, a total of 202 specimens were confirmed reactive for HIV-2 only, by the FDA-approved differentiation assay or by an HIV-2 RNA quantitative assay[18]. INSTI was reactive on 201/202 of these specimens for a calculated **sensitivity of 99.5% (95% CI= 97.2%-99.9%)**.

SPECIFICITY:

A specificity study was performed in the same 14 clinical trial sites using freshly obtained matching fingerstick whole blood, EDTA whole blood, and EDTA plasma specimens collected from 1410 low or unknown risk and high risk individuals. Of the 1388 individuals identified as HIV negative using an approved comparator assay, 2 did not provide a fingerstick specimen. Of the remaining 1386 fingerstick specimens, 1376 gave a Non-Reactive result with INSTI™, and 4 were invalid. Within the high risk group, 22 specimens were confirmed seropositive by Western Blot and of those, 21 were Reactive with INSTI™; an additional high risk specimen (1/782) was INSTI™ false Reactive. Of the 1388 matching EDTA whole blood and plasma specimens, 1388 gave Non-Reactive results with INSTI™. Results are shown in **Tables, 10, 11, 12, 13, and 14**.

Table 10
Performance of the INSTI™ HIV-1/HIV-2Antibody Test on Fingerstick Whole Blood Specimens from Individuals Presumed to be HIV Negative and Individuals at High Risk of HIV Infection

Test Group	Total Specimens	INSTI™ Non-Reactive	INSTI™ Reactive	INSTI™ Invalid[1]	Approved Test Non-Reactive	Approved Test Reactive	True Negative[2]
Low or Unknown Risk	626	620	6	0	626	0	626
High Risk	782	756	22[3]	4	760	22	760
TOTAL	1408	1376	28	4	1386	22	1386

[1] 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] 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.

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 (see **Tables 11 and 12** below). From **Table 10** above, the overall specificity of the INSTI™ HIV-1/HIV-2Antibody 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%). From **Table 11**, the specificity in the high risk populations, minus the invalid results, was calculated to be 755/756 = 99.9% (95% CI = 99.3% - 100%). From **Table 12**, the specificity of the INSTI™ HIV-1/HIV-2Antibody Test from low or unknown risk populations was calculated to be 620/626 = 99.0% (95% CI = 97.9% - 99.6%).

Table 11
Comparison of Results for Fingerstick Whole Blood Specimens from Individuals at High Risk of HIV Infection

		Approved Test Result		
		Reactive	Non-Reactive	TOTAL
INSTI™ Test Result	Reactive	21	1	22
	Non-Reactive	1	755	756
	Invalid	0	4	4
	TOTAL	22	760	782

Table 12
Comparison of Results for Fingerstick Whole Blood Specimens from Low and Unknown Risk Individuals Presumed to be Negative for HIV Infection

		Approved Test Result		
		Reactive	Non-Reactive	TOTAL
INSTI™ Test Result	Reactive	0	6	6
	Non-Reactive	0	620	620
	TOTAL	0	626	626

Table 13
Performance of the INSTI™ HIV-1/HIV-2Antibody Test on Venipuncture Whole Blood Specimens from Individuals Presumed to be HIV Negative and Individuals at High Risk of HIV Infection

Test Group	Total Specimens	INSTI™ Non-Reactive	INSTI™ Reactive	INSTI™ Invalid	Approved Test Non-Reactive	Approved Test Reactive	True Negative[1]
Low or Unknown Risk	628	628	0	0	628	0	628
High Risk	782	760	22	0	760	22	760
TOTAL	1410	1388	22	0	1388	22	1388

[1] Reactives were confirmed by licensed HIV-1 Western Blot and excluded from the calculation of specificity.

The overall specificity of the INSTI™ HIV-1/HIV-2Antibody Test in **venipuncture whole blood** specimens from the combined HIV negative high risk and low or unknown risk populations, was calculated to be 1388/1388 = 100% (95% CI = 99.7% - 100%).

Table 14
Performance of the INSTI™ HIV-1/HIV-2Antibody Test on Plasma Specimens from Individuals Presumed to be HIV Negative and Individuals at High Risk of HIV Infection

Test Group	Total Specimens	INSTI™ Non-Reactive	INSTI™ Reactive	INSTI™ Invalid	Approved Test Non-Reactive	Approved Test Reactive	True Negative[1]
Low or Unknown Risk	628	628	0	0	628	0	628
High Risk	782	760	22	0	760	22	760
TOTAL	1410	1388	22	0	1388	22	1388

[1] Reactives were confirmed by licensed HIV-1 Western Blot and excluded from the calculation of specificity.

The overall specificity of the INSTI™ HIV-1/HIV-2Antibody Test in **plasma** specimens from the combined HIV negative high-risk and low or unknown risk populations, was calculated to be 1388/1388 = 100% (95% CI = 99.7% - 100%).

Unrelated Medical Conditions and Potentially Interfering Substances
To assess the impact of unrelated medical conditions or potentially interfering substances on the specificity of the INSTI™ HIV-1/HIV-2Antibody Test, 195 serum/plasma specimens from a cross section of medical conditions unrelated to HIV infection and 217 specimens with potentially interfering substances were tested with the INSTI™ HIV-1/HIV-2Antibody Test. The results are presented in **Table 15**.

In addition, a study was performed to assess the potential effect of common blood tube anticoagulants on assay specificity. Venipuncture blood was collected from 13 volunteer subjects in each of 3 tubes containing one of three anticoagulants (EDTA, sodium heparin, and sodium citrate). Aliquots of the spiked specimens were stored refrigerated (2° – 8°C) and at ambient temperature (20° – 24°C) and tested at day 3 and day 7 over a 7 day period. There was no effect of the anticoagulants on specificity with specimens held up to 7 days at 2° - 24°C. Results are shown in **Table 15**.

Table 15
INSTI™ HIV-1/HIV-2Antibody Test Specificity with Specimens from Individuals with Unrelated Medical Conditions (n=195) and with Specimens containing Potentially Interfering Substances (n=217).

Unrelated 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[1]
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
Potentially Interfering Substance (n=217)			
Icteric	20	0	20
Elevated Bilirubin (≥8.0mg/dL)	19	0	19
Lipemic	20	0	20
Visual Hemolysis	5	0	5
Elevated Triglyceride (≥292mg/dL)	19	0	19
Elevated Hemoglobin (>12g/100mL)	20	0	20
Elevated Albumin (11.5-13.0g/dL)	15	0	15
EDTA	13	0	13
Sodium Heparin	13	0	13
Sodium Citrate	13	0	13
Bacterially Contaminated	60	0	60

[1]Up to 5 specimens from individuals with multiple myeloma produced invalid INSTI™ results depending on the INSTI™ kit lot tested. A related statement is listed in the **Warnings** and **Limitations** sections of this Package Insert.

Reproducibility
The reproducibility of the INSTI™ HIV-1/HIV-2Antibody Test was tested at 3 laboratory sites using 3 lots of the INSTI™ HIV-1/HIV-2Antibody Test on 3 separate days with 9 operators (3 per site). A panel of 5 blind-coded contrived plasma specimens, consisting of 4 HIV-1 antibody positive (one strong

positive and three low positives) and 1 HIV-1 antibody negative specimen was tested at each site. A total of 405 tests were conducted, 135 at each site, with a total of 81 tests per panel specimen. The overall reproducibility of the INSTI™ HIV-1/HIV-2Antibody Test was 405/405 = 100%.

CLIA WAIVER Study

The performance of the INSTI HIV-1/HIV-2Antibody Test was evaluated in a prospective study conducted over 4 months at 3 geographically diverse sites located in Arizona, Pensylvania and Florida. At each site, INSTI testing was conducted by operators who had no laboratory experience and were representative of users at CLIA waived testing sites (intended use). The 11 operators (intended users) who participated in the study were not given any training on the use of the test. There were 905 subjects with unknown HIV status and 483 subjects known to be HIV positive. The subjects with unknown HIV status were tested with INSTI and the comparator method. The subjects known to be HIV positive were tested with INSTI and their HIV status was not known to the operators. Fingerstick blood from each subject with unknown HIV status was tested with INSTI by the operators at each site. HIV status for each subject with unknown HIV status was determined by a composite reference method (comparator method) which consists of an FDA approved EIA with supplemental Western blot and PCR assays as required. The result of INSTI was compared to the HIV status of the subject. The positive percent agreement and negative percent agreement between INSTI and the HIV status for the study specimens is presented in **Table 16** below. There were no INSTI HIV-1/HIV-2invalid results reported.

Table 16
Positive Percent Agreement and Negative Percent Agreement between the INSTI HIV-1/HIV-2 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 intended use 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 17 below shows performance of the test with samples near the cutoff of the assay in the hands of intended users (across all sites).

Table 17 Performance of the INSTI™ HIV-1/HIV-2Antibody 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.

Using risk analysis as a guide, analytical flex studies were conducted. The studies demonstrated that the test is insensitive to stresses of environmental conditions and potential user errors.

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ADDITIONAL REFERENCE FROM INTERPRETATION SECTION:

CDC. Revised Guidelines for HIV Counseling, Testing and Referral and Revised Recommendations for HIV Screening of Pregnant Women. MMWR 2001; 50(19);32-35.

TECHNICAL INFORMATION / CUSTOMER SERVICE

For further information or assistance, or to report problems, contact bioLytical Laboratories Technical Support / Customer Service at 1-866-674-6784.

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