HIV-1 and HIV-2 are similar in genomic structure, morphology and ability to cause AIDS. HIV is Human Immunodeficiency Virus Type 1 (HIV-1) and Human Immunodeficiency Virus Type 2 (HIV-2). Acquired Immunodeficiency Syndrome (AIDS) is thought to be caused by at least two retroviruses, HIV-1 infections. If multiple rapid HIV tests are available, this test is suitable for use in appropriate qualitative immunoassay for the detection of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1). US Centers for Disease Control and Prevention estimates that up to 25% of persons living with HIV in the United States have undiagnosed HIV. HIV-infected persons. Antibodies specific for HIV envelope proteins are prevalent in blood or blood plasma of HIV-infected persons.2-5 Antibodies recognized by the detection conjugate, and c) complexity of the protocol used to perform the test. The INSTI™ HIV-1 Antibody Test result is a manual, visually read, flow-through immunoassay for the detection of antibodies to HIV-1 and HIV-2 in human serum or plasma. The test is intended for use by trained personnel in point of care and laboratory situations to aid in the diagnosis of HIV-1 infections. Multiple rapid HIV tests are available, this test is suitable for use in appropriate qualitative immunoassay for the detection of antibodies to HIV-1. The membrane 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 or 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 read 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 Solutions (Solution 3). The test is performed by adding the fingertip 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 Antibody Test kits are available in the following packaging formats: 

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**NAME AND INTENDED USE:** The INSTI™ HIV-1 Antibody Test is a single use, rapid, in vitro immunoassay for the detection of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) in human serum, whole 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-1 infections. Multiple rapid HIV tests are available, this test is suitable for use in appropriate qualitative immunoassay for the detection of antibodies to HIV-1. Material required but not provided: Appropriate blood collection tubes. Absorbent cotton for fingertip or venipuncture wound closure. Appropriate biohazard waste containers. Absorbent cotton for fingertip or venipuncture wound closure. **WARNINGS** For in vitro diagnostic use only: 1. Read the entire Package Insert prior to beginning the test procedure. 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. 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 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 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 give a false Negative INSTI™ HIV-1 Antibody Test result.
8. Specimens from patients with multiple myeloma, may result in false Non-Reactive or invalid results with the INSTI™ HIV-1 Antibody Test.

9. Patients with elevated hemoglobin levels may test false Non-Reactive with the INSTI™ HIV-1 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.

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 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.
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.

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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 Antibody Test are ready to use as supplied. All Membrane Units must be used immediately once opened. All reagents should be dispersed 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-s 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 vial 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.

Quality Control

Procedure Control
The INSTI™ HIV-1 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 Antibody Test Kit Controls are available separately for use only with the INSTI™ HIV-1 Antibody Test. The controls are used to verify test performance and interpretation of results. The Positive Control and the Negative Control are to be run on separate Membrane Units. The HIV-1 Positive Control has 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 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 Antibody Test.

**INSTI™ HIV-1 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 Antibody Test Controls package insert for additional information on the use of these reagents. It is the responsibility of each user of the INSTI™ HIV-1 Antibody 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 Controls 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-1 were 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-1 antibodies. As illustrated, one spot may be darker than the other.

**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:** Following a reactive rapid test result, a venous blood specimen must be drawn in an appropriate anticoagulant collection tube for HIV confirmatory testing.

**LIMITATIONS OF THE TEST**

1. The INSTI™ HIV-1 Antibody 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 into the 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 control 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 at the 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 Antibody Test detects antibodies to HIV-1 and is useful as an aid in the diagnosis of infection with HIV-1. Because a variety of factors may cause non-specific reactions, a patient found to be Reactive using the INSTI™ HIV-1 Antibody 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 antibody to HIV-1 and give a false Non-Reactive INSTI™ HIV-1 Antibody Test result.

9. Specimens from patients with multiple myeloma, may result in false Non-Reactive or invalid results with the INSTI™ HIV-1 Antibody Test.

10. Patients with elevated hemoglobin levels may test false Non-Reactive with the INSTI™ HIV-1 Antibody Test.

11. A person who has antibodies to HIV-1 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 plasma, 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**

<table>
<thead>
<tr>
<th>Test Group</th>
<th>Total Specimens</th>
<th>Reactive</th>
<th>Non-Reactive</th>
<th>True Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known HIV-1 Positive</td>
<td>1075</td>
<td>1074</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>High Risk</td>
<td>702</td>
<td>22</td>
<td>1</td>
<td>510</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1777</td>
<td>1097</td>
<td>1896</td>
<td>760</td>
</tr>
</tbody>
</table>

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).

**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 for HIV-1 by an FDA licensed test and of those 22 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 Antibody 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 1095/1099=99.8% (95% CI = 99.3% - 99.9%). The rate of invalid tests was 4/1857 (0.2%).

**Table 2**

<table>
<thead>
<tr>
<th>Test Group</th>
<th>Total Specimens</th>
<th>Reactive</th>
<th>Non-Reactive</th>
<th>True Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known HIV-1 Positive</td>
<td>1075</td>
<td>1074</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>High Risk</td>
<td>702</td>
<td>22</td>
<td>1</td>
<td>510</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1777</td>
<td>1097</td>
<td>1896</td>
<td>760</td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>Test Group</th>
<th>Total Specimens</th>
<th>Reactive</th>
<th>Non-Reactive</th>
<th>True Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known HIV-1 Positive</td>
<td>1075</td>
<td>1075</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>High Risk</td>
<td>702</td>
<td>22</td>
<td>2</td>
<td>700</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1777</td>
<td>1097</td>
<td>1896</td>
<td>760</td>
</tr>
</tbody>
</table>

1. Confirmed by licensed HIV-1 Western Blot.
Comparison of the INSTI™ HIV-1 Antibody Test and Licensed or Approved Anti-HIV EIA Tests Using a Low Titer HIV-1 Antibody Panel

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

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

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

Table 7: Unrelated Medical Conditions and Potentially Interfering Substances

Table 8: INSTI™ HIV-1 Antibody 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).

Reactivity With HIV-1 Seroconversion Panels

INSTI™ HIV-1

INSTI™ HIV-1 Antibody Test was capable of detecting levels of antibodies to HIV-1 similar to FDA licensed or approved EIA's.

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

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 1386 individuals identified as HIV negative using an approved comparator assay, 2 did not provide a fingerstick specimen. Of the remaining 1384 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 or of those, 21 were Reactive with INSTI™; an additional high risk specimen (1/782) was INSTI™ false Reactive. Of the 1388 matching

Reactivity With HIV-1 Low Titer Panel

One HIV-1 low titer panel was tested with 2 production lots of INSTI™ and results were compared to three FDA licensed or approved HIV EIA’s. The results of this study are shown in Table 7. In this study, the INSTI™ HIV-1 Antibody Test was capable of detecting levels of antibodies to HIV-1 similar to FDA licensed or approved EIA's.

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

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 1386 individuals identified as HIV negative using an approved comparator assay, 2 did not provide a fingerstick specimen. Of the remaining 1384 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 9, 10, 11, 12, and 13.

Table 9

<table>
<thead>
<tr>
<th>Test Group</th>
<th>Total Specimens</th>
<th>INSTI Non-Reactive</th>
<th>INSTI Reactive</th>
<th>INSTI™ Inval®</th>
<th>Approved Test Non-Reactive</th>
<th>Approved Test Reactive</th>
<th>Test Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low or Unknown Risk</td>
<td>628</td>
<td>0</td>
<td>628</td>
<td>0</td>
<td>628</td>
<td>0</td>
<td>628</td>
</tr>
<tr>
<td>High Risk</td>
<td>782</td>
<td>22</td>
<td>760</td>
<td>22</td>
<td>780</td>
<td>22</td>
<td>780</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1410</td>
<td>22</td>
<td>1388</td>
<td>22</td>
<td>1386</td>
<td>22</td>
<td>1386</td>
</tr>
</tbody>
</table>

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 10 and 11 below). From Table 9 above, the overall specificity of the INSTI™ HIV-1 Antibody Test in fingerstick whole blood specimens from the combined high risk and low or unknown risk populations, minus the invalid results, was calculated to be 1375/1382 = 99.5% (95% CI = 99.0% - 99.8%). From Table 10, 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 11, the specificity of the INSTI™ HIV-1 Antibody Test from low or unknown risk populations was calculated to be 620/626 = 99.0% (95% CI = 97.9% - 99.6%).

Table 10

<table>
<thead>
<tr>
<th>INSTI™ Test Result</th>
<th>Reactive</th>
<th>Non- Reactive</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approved Test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>22</td>
<td>780</td>
<td>782</td>
</tr>
<tr>
<td>Non- Reactive</td>
<td>0</td>
<td>620</td>
<td>620</td>
</tr>
<tr>
<td>TOTAL</td>
<td>22</td>
<td>620</td>
<td>642</td>
</tr>
</tbody>
</table>

The overall specificity of the INSTI™ HIV-1 Antibody 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 11

<table>
<thead>
<tr>
<th>INSTI™ Test Result</th>
<th>Reactive</th>
<th>Non- Reactive</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approved Test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>22</td>
<td>780</td>
<td>782</td>
</tr>
<tr>
<td>Non- Reactive</td>
<td>0</td>
<td>620</td>
<td>620</td>
</tr>
<tr>
<td>TOTAL</td>
<td>22</td>
<td>620</td>
<td>642</td>
</tr>
</tbody>
</table>

The overall specificity of the INSTI™ HIV-1 Antibody 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 Antibody Test, 195 serum/plasma specimens from a cross section of medical conditions unrelated to HIV-1 infection and 217 specimens with potentially interfering substances were tested with the INSTI™ HIV-1 Antibody Test. The results are presented in Table 14.

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 14.

Table 14

Unrelated Medical Condition (n=195) | No. of Specimens | INSTI™ Reactive | INSTI™ Nonreactive |
-------------------------------------|------------------|-----------------|-------------------|
Toxoplasmosis                        | 20               | 20              | 0                 |
Rheumatoid Factor                    | 20               | 20              | 0                 |
Multiple Myeloma                     | 10               | 0               | 10                |
Syphilis                             | 30               | 30              | 0                 |
SLE                                  | 5                | 5               | 0                 |
Rubella                              | 20               | 20              | 0                 |
Cytomegalovirus                      | 20               | 20              | 0                 |
Epstein Barr Virus                    | 20               | 20              | 0                 |
HTLV-III 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 (>5.0mg/dL)       | 19               | 19              | 0                 |
Lipemic                              | 20               | 20              | 0                 |
Visual Hemolysis                     | 5                | 5               | 0                 |
Elevated Triglyceride (>250mg/dL)    | 15               | 15              | 0                 |
Elevated Hemoglobin (>12g/100ml)     | 20               | 20              | 0                 |
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                 |

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 Antibody Test was tested at 3 laboratory sites using 3 lots of the INSTI™ HIV-1 Antibody 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 Antibody Test was 405/405 = 100%.

CLIA WAIVER Study

The performance of the INSTI™ HIV-1 Antibody Test was evaluated in a prospective study conducted over 4 months at 3 geographically diverse sites located in Arizona, Pennsylvania and Florida. At each site, INSTI HIV-1 testing was conducted by operators who had no laboratory experience and were representative of users at CLIA waived testing sites. The subjects known to be HIV positive were tested with INSTI HIV-1 and their HIV status was not known to the operators. Fingerstick blood from each subject with unknown HIV status was tested with INSTI HIV-1 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 the INSTI HIV-1 Antibody Test was compared to the HIV status of the subject. The positive percent agreement and negative percent agreement between INSTI HIV-1 and the HIV status for the study specimens is presented in Table 15 below. There were no INSTI HIV-1 invalid results reported.

Table 15

<table>
<thead>
<tr>
<th>Study Population</th>
<th>Number of Subjects</th>
<th>Positive Percent Agreement</th>
<th>% Confidence Interval</th>
<th>Negative Percent Agreement</th>
<th>% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV status unknown</td>
<td>905</td>
<td>95%</td>
<td>89.9% - 99.9%</td>
<td>99.6%</td>
<td>(869/871)</td>
</tr>
<tr>
<td>Known HIV-1 Positive</td>
<td>483</td>
<td>100%</td>
<td>99.2% - 100%</td>
<td>99.9%</td>
<td>(483/483)</td>
</tr>
<tr>
<td>Total</td>
<td>1388</td>
<td>99.3%</td>
<td>99.2% - 99.9%</td>
<td>99.8%</td>
<td>(988/997)</td>
</tr>
</tbody>
</table>

Percent of invalid results was 0.0% (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 HIV-1 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 HIV-1 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 16 Performance of the INSTI™ HIV-1 Antibody Test Run by Intended Users with Weakly Reactive Samples

<table>
<thead>
<tr>
<th>Intended Users</th>
<th>Dilution</th>
<th>Percent Reactive</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weakly Reactive</td>
<td>1:600</td>
<td>88.3% (53/60)</td>
<td>77.8% - 94.2%</td>
</tr>
<tr>
<td>Weakly Reactive</td>
<td>1:800</td>
<td>80.0% (48/60)</td>
<td>68.2% - 88.2%</td>
</tr>
<tr>
<td>Weakly Reactive</td>
<td>1:1200</td>
<td>66.1% (39/60)</td>
<td>53.4% - 76.9%</td>
</tr>
<tr>
<td>Weakly Reactive</td>
<td>1:1600</td>
<td>34.5% (20/58)</td>
<td>23.6% - 47.3%</td>
</tr>
</tbody>
</table>

- **Expected results:** There should be a greater number of INSTI HIV-1 Antibody Test reactive results than non-reactive results.
- **Expected results:** There should be an equal distribution of reactive and non-reactive INSTI HIV-1 Antibody Test results.
- **Expected results:** There should be a greater number of INSTI HIV-1 Antibody Test non-reactive results than reactive results.
- A total of 3 INSTI HIV-1 Antibody Test invalid results were obtained: 1 invalid for Weakly Reactive 3 sample and 2 invalids for Weakly Reactive 4 sample.
- 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.

### Bibliography

11. World Health Organization/Global Programme on AIDS. Operational characteristics of commercially available assays to detect antibodies to HIV-1 and/or HIV-2 in human sera. Geneva, Switzerland: WHO documents GPA/BRM/89.4, GPA/BRM/90.1, GPA/RES/A09.1, GPA/RES/A09.6, GPA/RES/A/90.6 and GPA/RES/A/93.4.