

WHO Prequalification of Diagnostics Programme PUBLIC REPORT

Product: INSTI HIV-1/HIV-2 Antibody Test Number: PQDx 0002-002-00

INSTI HIV-1/HIV-2 Antibody Test with product codes, 90-1013, 90-1010, 90-1021, 90-1022, 90-1038 and 90-1064 ¹, manufactured by **bioLytical Laboratories, Rest-of-World** regulatory version was accepted for the WHO list of prequalified diagnostics and was listed on 29 August 2013.

Summary of prequalification status for INSTI HIV-1/HIV-2 Antibody Test

	Date	Outcome
Status on PQ list	29-Aug-2013	listed
Dossier assessment	15-Aug-2013	MR
Inspection status	21 – 23-Nov-2016	MR
Laboratory evaluation	13-Jun-2013	MR

MR: Meets Requirements

Report amendments and/or product changes

This public report has since been amended. Amendments may have arisen because of changes to the prequalified product for which WHO has been notified and has undertaken a review. Amendments to the report are summarized in the following table, and details of each amendment are provided below.

Public report amendment	Summary of amendment	Date of report amendment
2.0	Delisting of the product code 90-1012 INSTI HIV-1/HIV-2 Antibody Test (singles test configuration) due to general foreseeable risk of individually packaged tests being misused as self-tests	22-Mar-2018
3.0	Fulfilment of dossier related commitments; and Update to reflect latest inspection information.	20-Jul-2018
4.0	Addition of the justification for delisting of product code 90-1012; and to reflect latest information.	31-Aug-2018

¹ Other regulatory versions of the product exist and are not prequalified.

5.0	Addition of two new product codes: 90- 1038, INSTI HIV-1/HIV-2 Antibody Test, 48 tests (with pipettes but no lancet, no alcohol swabs), English language labelling, And 90-1064, INSTI HIV-1/HIV-2 Antibody Test, 48 tests, no support materials (no pipettes, no lancet, no alcohol swabs), English language labelling	06-Nov-2019
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Intended use:²

According to the claim of bioLytical Laboratories, “*INSTI HIV-1/HIV-2 Antibody Test is a single use, rapid, flow through (immunofiltration) in vitro qualitative immunoassay for the detection of antibodies to Human Immunodeficiency Virus Type 1 and Type 2 in human EDTA-venous whole blood, fingerstick whole blood, serum or EDTA-plasma. The test is intended for use by trained personnel in medical facilities, clinical laboratories, emergency care situations, physicians’ offices and field studies as a diagnostic test capable of providing results in less than one minute. Although suitable for near-patient or point-of-care (POC) testing, the INSTI HIV-1/HIV-2 Antibody Test is not suitable for self-testing. All required pre- and post-test counselling guidelines must be followed in each setting in which the INSTI HIV-1/HIV-2 Antibody Test is used. The assay is packaged as a kit containing INSTI Membrane Unit, Sample Diluent, Colour Developer, Clarifying Solution and two droppers with or without support materials (lancet, pipette and alcohol swab)*”.

Assay description:

According to the claim of bioLytical Laboratories, “*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 whole blood, serum or plasma. The test consists of a synthetic filtration membrane positioned atop an absorbent material within a plastic cartridge, referred to as the INSTI Membrane Unit. The membrane has been specifically treated with HIV-1 and HIV-2 recombinant proteins which react with HIV-1/HIV-2 antibodies in the specimen to produce a distinct visual signal on the membrane. The membrane also includes a procedural control. The procedural control consists of a protein-A treated spot capable of capturing IgG antibodies normally present in blood and blood components; IgG antibodies react with a proprietary chromatic agent to produce a visual signal on the membrane. Since IgG antibodies are present in blood in both HIV negative and HIV positive human specimens, the control spot provides a visual signal when the test is run, indicating that the*

² This product is one that uses Protein A to detect human IgG antibodies. Protein is also able to detect other classes of human antibody (IgA, IgD, IgE and IgM) but not as reliably as it does IgG. This product has been prequalified with respect to its ability to detect human IgG antibodies. Any claim to detect other types of antibodies on this kind of product has not been validated based on WHO prequalification requirements.

specimen has been added, and the test was performed correctly. If the control spot does not appear, the test is considered invalid. In the case of the test spot, recombinant HIV-1 and HIV-2 proteins, embedded in the membrane, capture HIV specific antibodies, if present in the specimen. Antibodies captured in the test spot react with a proprietary chromatic agent to produce a visible signal on the membrane. The membrane unit is designed to filter, absorb and retain the test specimen and all the test reagents in such a manner as to limit leakage and exposure of personnel to potentially infectious materials.

Reagents required to perform a test include Sample Diluent, Colour Developer and Clarifying Solution. The test is performed by adding the whole blood, serum or plasma specimen to the vial of Sample Diluent, which lyses the red blood cells. The specimen/diluent solution is then poured onto the well of the Membrane Unit. HIV-1/HIV-2 antibodies, if present in the specimen, are captured by proteins on the filtration membrane. Colour Developer is then added to the Membrane Unit. The Colour Developer reacts with the captured antibodies to generate a distinct blue dot at the location of the control spot and, in the case that HIV-1/HIV-2 antibodies are present in the specimen, a blue dot also appears at the location of the test spot on the membrane. In the final step, the Clarifying Solution is then added to the membrane to decrease background colour in order to make the control and test spots more distinct”.

Following a reactive rapid test result, the specimen should be referred for HIV confirmatory testing”.

Test kit contents:

	Item	Test/kit	Product code
1.	INSTI HIV-1/HIV-2 Antibody Test	24 Tests/kit with no support materials	90-1010
	<ul style="list-style-type: none"> • 24 Membrane Unit individually packaged • 24 Sample Diluent vials (1.5 ml) • 24 Colour Developer bottles (1.5 ml) • 24 Clarifying Solution bottles (1.5 ml) 		
2.	INSTI HIV-1/HIV-2 Antibody Test	24 Tests/kit with support materials	90-1013
	<ul style="list-style-type: none"> • 24 Membrane Units individually packaged • 24 Sample Diluent vials (1.5 ml) • 24 Colour Developer bottles (1.5 ml) • 24 Clarifying Solution bottles (1.5 ml) • 24 single-use alcohol swabs • 24 single-use sterile lancets • 24 single-use pipettes (50 µl) 		

	Item	Test/kit	Product code
3.	INSTI HIV-1/HIV-2 Antibody Test	48 Tests/kit with no support materials	90-1021
	<ul style="list-style-type: none"> • 48 Membrane Units individually packaged • 48 Sample Diluent vials (1.5 ml) • 1 Colour Developer bottle (85 ml) • 1 Clarifying Solution bottle (85 ml) 		
4.	INSTI HIV-1/HIV-2 Antibody Test	48 Tests/kit with support materials	90-1022
	<ul style="list-style-type: none"> • 48 Membrane Units individually packaged • 48 Sample Diluent vials (1.5 ml) • 1 Colour Developer bottle (85 ml) • 1 Clarifying Solution bottle (85 ml) • 48 single-use alcohol swabs • 48 single-use sterile lancets • 48 single-use pipettes (50 µl) 		
5.	INSTI HIV-1/HIV-2 Antibody Test	48 Test/kit with pipettes but no lancet, no alcohol swabs)	90- 1038
	<ul style="list-style-type: none"> • 48 Membrane Units individually packaged • 48 Sample Diluent vials (1.5 ml) • 1 Colour Developer bottle (85 ml) • 1 Clarifying Solution bottle (85 ml) • 48 single-use pipettes (50 µl) • English language labelling. 		
6.	INSTI HIV-1/HIV-2 Antibody Test	48 Test/kit with no support materials.	90-1064
	<ul style="list-style-type: none"> • 48 Membrane Units individually packaged • 48 Sample Diluent vials (1.5 ml) • 1 Colour Developer bottle (85 ml) • 1 Clarifying Solution bottle (85 ml) • English language labelling 		

Storage:

The test kit should be stored at 2 °C - 30 °C.

Shelf-life:

15 months.

Prioritization for prequalification

bioLytical Laboratories submitted an application for prequalification of INSTI HIV-1/HIV-2 Antibody Test. Based on the eligibility criteria, INSTI HIV-1/HIV-2 Antibody Test was given priority for prequalification assessment.

Product dossier assessment

bioLytical Laboratories submitted a product dossier for INSTI HIV-1/HIV-2 Antibody Test as per the Instructions for Compilation of a Product Dossier (PQDx_018 v1). The information submitted in the product dossier was reviewed by WHO staff and external experts (assessors) appointed by WHO in accordance with the internal report on the screening and assessment of a product dossier (PQDx_009 v2). Based on the product dossier screening and assessment findings, a recommendation was made to accept the product dossier for INSTI HIV-1/HIV-2 Antibody Test for prequalification.

Manufacturing site inspection

An inspection was performed for the INSTI HIV-1/HIV-2 Antibody Test (PQDx 002-002-00) at the site of the legal manufacturer, bioLytical Laboratories, located at 1108-13351 Commerce Parkway, V6V 2X7 Richmond, British Columbia, Canada, between 21 and 23 November 2016.

The inspection was performed as per *'Information for manufacturers on WHO prequalification inspection procedures for the sites of manufacture of diagnostics'* (PQDx_014).

The inspection was based on 'ISO 13485:2003 Medical devices - Quality management systems - Requirements for regulatory purposes' and other internationally recognized standards relevant to the manufacture of in vitro diagnostics. In addition, the claims made in the submitted product dossier were verified and the adequacy of mechanisms for lot release of the product to customers was inspected. With consideration that the product should be suitable for use in resource limited settings, particular attention was paid to suitability of product labeling currently in use (including instructions for use and labeling for storage requirements), stability testing (in-use, transportation and storage stability), and effective mechanisms for customer training, service and feedback.

The manufacturer's final responses to the nonconformities found at the time of the inspection were accepted 27 March 2017. The inspection and the subsequent review of the manufacturer's action plan in response to the nonconformities found, concluded that the legal manufacturer had an acceptable quality management system and manufacturing practices in place that would ensure the consistent manufacture of a product of good quality.

Product performance evaluation

INSTI HIV-1/HIV-2 Antibody Test (bioLytical Laboratories) was evaluated by WHO at the Institute of Tropical Medicine, Antwerp, Belgium – a WHO Collaborating Centre for HIV/AIDS Diagnostics and Laboratory Support. The laboratory evaluation was conducted according to the “WHO protocol for the laboratory evaluation of HIV serology assays” (PQDx_030 v1.0), and drew the following conclusions:

INSTI HIV-1/HIV-2 Antibody Test (bioLytical Laboratories) is a flow through (immunofiltration) rapid diagnostic test for the detection of HIV-1/2 antibodies in human serum/plasma and whole blood. A volume of 50 µL of specimen is needed to perform the assay. This type of assay requires no sophisticated equipment and can therefore be performed in laboratories with limited facilities and non-laboratory settings. Reading of the results can be done visually i.e. subjectively read.

In this limited evaluation on a panel of 1079 clinically-derived specimens, we found an initial sensitivity (95% CI) of 100% (99.1% - 100%) and an initial specificity (95% CI) of 99.7% (98.9% - 100%) compared to the reference assays. The final sensitivity (95% CI) was 100% (99.1% - 100%) and the final specificity (95% CI) was 99.7% (98.9% - 100%) compared to the reference assays. In this study, 0% of the results were recorded as indeterminate. Results were interpreted independently by three technicians; the inter-reader variability was 0%. The invalid rate was 0%. Lot to lot variation was acceptable for all but one dilution series.

For eight seroconversion panels, INSTI HIV-1/HIV-2 Antibody Test detected on average 0.25 specimens (95% CI -0.64 – 0.14) later than the benchmark assay; Enzygnost Anti-HIV 1/2 Plus (Siemens Healthcare Diagnostics). For the mixed titer panel, INSTI HIV-1/HIV-2 Antibody Test correctly classified all but two specimens compared to a state-of-the-art 3rd generation antibody detection assay. For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], INSTI HIV-1/HIV-2 Antibody Test correctly classified all but 2 specimens, specifically HIV-1 subtype C and HIV-1 type O were not detected.

Labelling

- 1. Labels**
- 2. Instructions for use**

1. Labels

**1.1 Product code 90-1010 (24 Tests/kit with no support materials) and
Product code 90-1013 (24 Tests/kit with support materials)**



24 INSTI™ HIV-1/HIV-2 Antibody Tests

Intended for single use determination of HIV-1/HIV-2 antibodies in whole blood, serum or plasma.

- For *in vitro* diagnostic use only.
- No refrigeration required. Optimum storage conditions 2-30°C.
- Consult instructions for use.
- Do not reuse.
- R22 - Harmful if swallowed.

bioLytical™ Laboratories Inc.
 1108 - 13351 Commerce Parkway, Richmond, B.C., Canada, V6V 2X7 Toll Free: 1-866-674-6784
 Phone: 604-204-6784 Fax: 604-244-8399 E-mail: info@biolytical.com Website: www.biolytical.com

50-1088F

Catalogue # 90-1007
 Catalogue # 90-1013

Catalogue # 90-1009
 Catalogue # 90-1010



24 INSTI™ HIV-1/HIV-2 Antibody Tests

Intended for single use determination of HIV-1/HIV-2 antibodies in whole blood, serum or plasma.

- For *in vitro* diagnostic use only.
- No refrigeration required. Optimum storage conditions 2-30°C.
- Consult instructions for use.
- Do not reuse.
- R22 - Harmful if swallowed.

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 Phone: 604-204-6784 Fax: 604-244-8399 E-mail: info@biolytical.com Website: www.biolytical.com

50-1087



24 INSTI™ HIV-1/HIV-2 Antibody Tests

Intended for single use determination of HIV-1/HIV-2 antibodies in whole blood, serum or plasma.

- For *in vitro* diagnostic use only.
- No refrigeration required. Optimum storage conditions 2-30°C.
- Consult instructions for use.
- Do not reuse.
- R22 - Harmful if swallowed.

bioLytical™ Laboratories Inc.
 1108 - 13351 Commerce Parkway, Richmond, B.C., Canada, V6V 2X7 Toll Free: 1-866-674-6784
 Phone: 604-204-6784 Fax: 604-244-8399 E-mail: info@biolytical.com Website: www.biolytical.com

50-1087

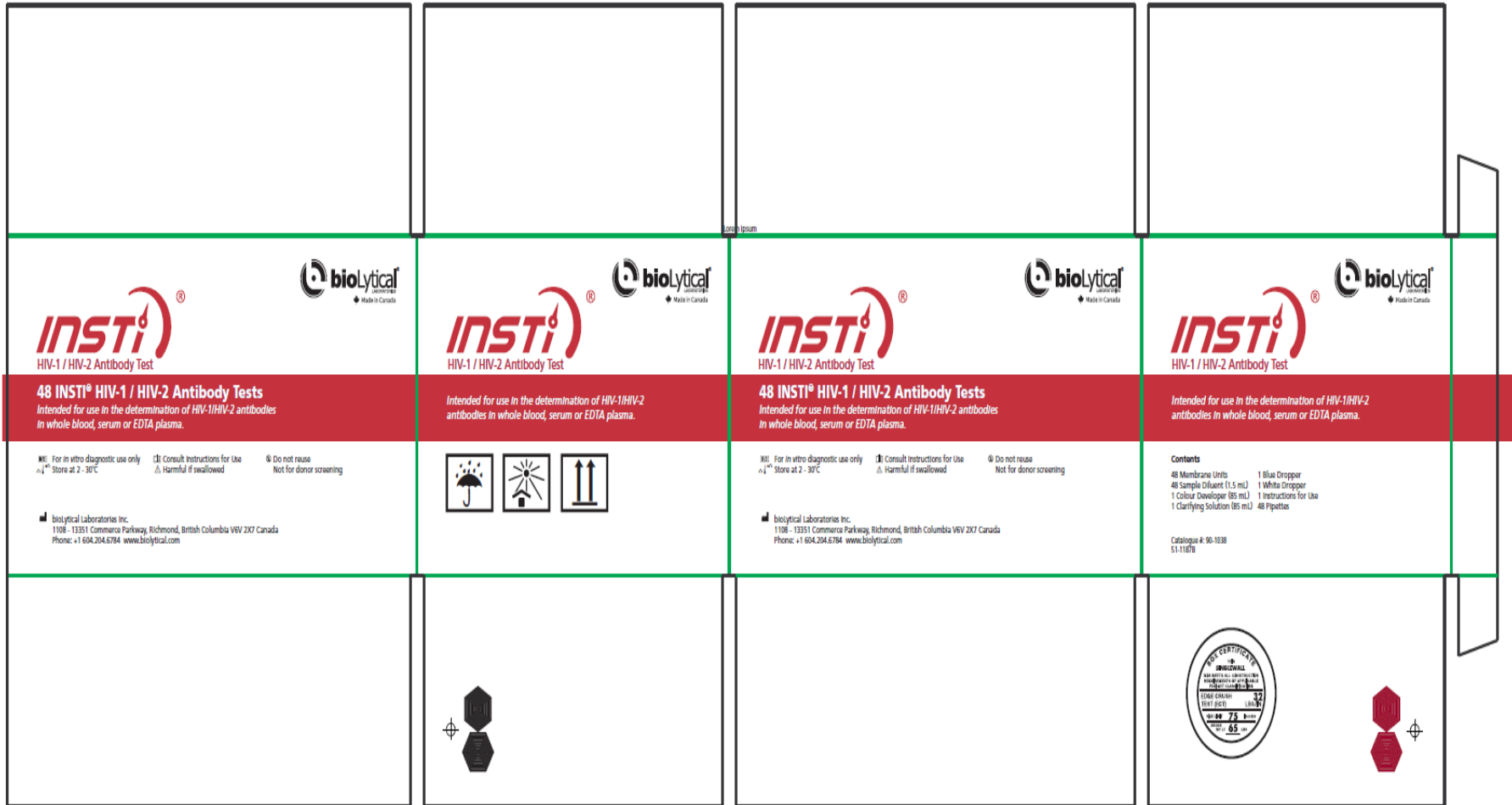
90-1007 / 90-1013 Contents:

- 24 Membrane Units
- 24 Sample Diluents
- 24 Colour Developers
- 24 Clarifying Solutions
- 24 Lancets
- 24 Pipettes
- 24 Alcohol Swabs
- 1 Package Insert

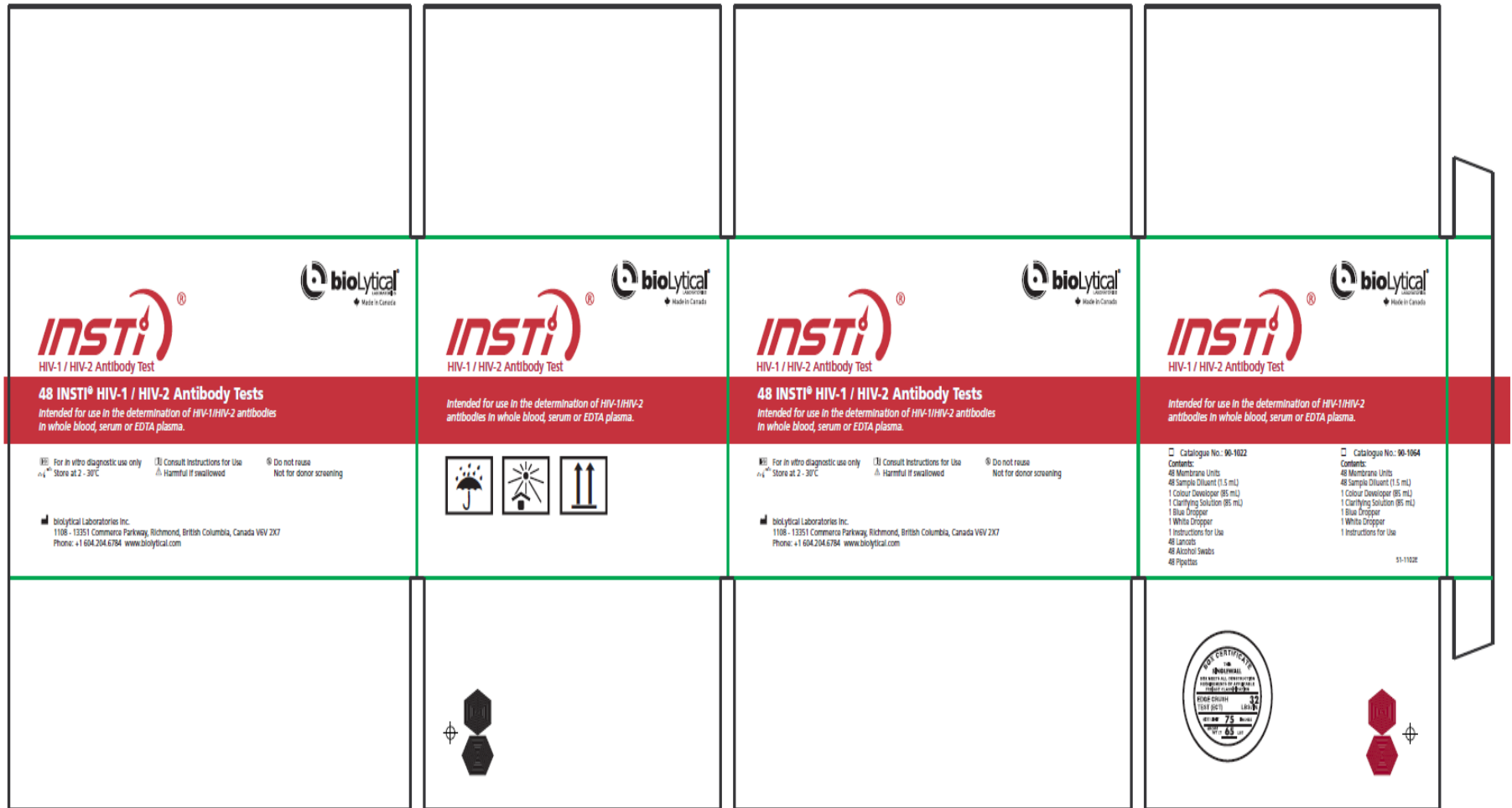
90-1009 / 90-1010 Contents:

- 24 Membrane Units
- 24 Sample Diluents
- 24 Colour Developers
- 24 Clarifying Solutions
- 1 Package Insert

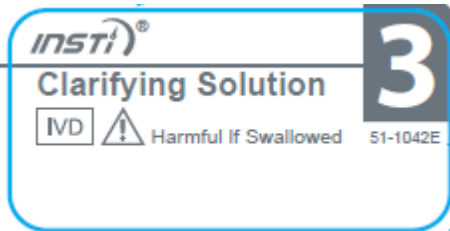
1.2 INSTI HIV-1/HIV-2 Antibody Test, 48s (90-1038) Box Artwork



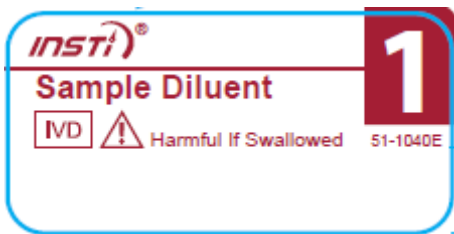
1.3 INSTI HIV-1/HIV-2 Antibody Test, 48s, without support (90-1064) Box Artwork



1.4 Clarifying solution



1.5 Sample diluent



1.6 Colour developer



1.7 Membrane unit label



2. Instructions for use³

³English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.



HIV-1/HIV-2 Antibody Test

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

- 90-1022** - 48 INSTI® HIV-1/HIV-2 Antibody Tests with support materials (for POC use)
- 90-1038** - 48 INSTI® HIV-1/HIV-2 Antibody Tests without support materials (for laboratory use only)
- 90-1064** - 48 INSTI® HIV-1/HIV-2 Antibody Tests without support materials (for laboratory use only)

30°C
 2°C
 Store at 2°C to 30°C

STERILE R Sterilization using irradiation

Caution
 Harmful if swallowed

LOT Lot number

REF Catalogue Number

IVD In Vitro diagnostic medical device

MANUFACTURER Manufacturer

Do not reuse

This side up

Use by

Keep dry

30°C
 2°C
 Store at 2°C – 30°C. For *in vitro* diagnostic use only. **IVD**

It is recommended that the entire Instructions for Use be read prior to beginning the test procedure. Although the assay is designed to be simple to use, conformance with the test procedure is necessary to ensure accurate results.

INTENDED USE - Not for donor screening

The INSTI HIV-1/HIV-2 Antibody Test is a single use, rapid, *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 EDTA whole blood, fingerstick blood, serum or EDTA-plasma. The test is intended for use by trained personnel in medical facilities, clinical laboratories, emergency care situations, and physicians' offices as a diagnostic test capable of providing results in less than one minute. Although suitable for near-patient or point-of-care (POC) testing, the INSTI HIV-1/HIV-2 Antibody Test is not suitable for home testing. All required pre and post-test counselling guidelines must be followed in each setting in which the INSTI HIV-1/HIV-2 Antibody Test is used. The assay is packaged as a kit containing INSTI Membrane Unit, Sample Diluent (Solution 1), Color Developer (Solution 2), Clarifying Solution (Solution 3) and two droppers with or without support materials (lancet, pipette and alcohol swab).

SUMMARY

Acquired Immunodeficiency Syndrome (AIDS) is caused by at least two retroviruses, HIV-1 and HIV-2. HIV-1 and HIV-2 are similar in genomic structure, morphology and ability to cause AIDS.¹ HIV is transmitted mainly by sexual contact, exposure to blood or blood products, or from an infected mother to her foetus. People with increased risk of HIV infection include haemophiliacs, intravenous drug-users and men having sex with men (MSM). HIV has been isolated from patients with AIDS, AIDS-related complex (ARC), and from persons at high risk of contracting AIDS.^{2,5} Antibodies specific for HIV envelope proteins are prevalent in sera from persons at high risk of contracting AIDS as well as in people with AIDS, or ARC.^{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. A reactive INSTI test result should be considered a preliminary result, with appropriate counselling provided in POC settings. Following a reactive rapid test result, a venous blood sample must be drawn in an EDTA collection tube (for whole blood or plasma) or a tube with no anticoagulant (for serum), and forwarded to a laboratory for HIV confirmatory testing.

PRINCIPLES OF THE TEST

The INSTI HIV-1/HIV-2 Antibody Test is a manual, visually read, flow through immunoassay for the qualitative detection of HIV-1/HIV-2 antibodies in human blood, serum or plasma. The test consists of a synthetic filtration membrane positioned atop an absorbent material within a plastic cartridge, referred to as the INSTI Membrane Unit. The membrane has been specifically treated with HIV-1 and HIV-2 recombinant proteins, which react with HIV-1/HIV-2 antibodies in the specimen to produce a distinct visual signal on the membrane. The membrane also includes a procedural control. The procedural control consists of a protein-A treated spot capable of capturing IgG antibodies normally present in blood and blood components. IgG antibodies react with a proprietary chromatic agent to produce a visual signal on the membrane. Since IgG antibodies are present in blood from normal or HIV positive human specimens, the control spot provides a visual signal when the test is run, indicating that the test was performed correctly. If the control spot does not appear, the test is considered invalid. In the case of the test spot, recombinant HIV-1 and HIV-2 proteins, embedded in the membrane, capture HIV specific antibodies, if present in the specimen. Antibodies captured in the test spot react with a proprietary chromatic agent to produce a visible signal on the membrane. The membrane unit is designed to filter, absorb, and retain the test specimen and all the test reagents in such a manner as to limit leakage and exposure of personnel to potentially infectious materials.

Reagents required to conduct a test include Sample Diluent (Solution 1), Color Developer (Solution 2) and Clarifying Solution (Solution 3). The test is performed by adding the blood, serum, or plasma specimen to the vial of Sample Diluent, which lyses the red blood cells. This specimen/diluent solution is then poured onto the well of the Membrane Unit. HIV-1/HIV-2 antibodies, if present in the specimen, are captured by proteins on the filtration membrane. Color Developer is then added to the Membrane Unit. The Color Developer reacts with the captured antibodies to generate a distinct blue dot at the

location of the control spot and, in the case that HIV-1/HIV-2 antibodies are present in the specimen, a blue dot also appears at the location of the test spot on the membrane. In the final step, the Clarifying Solution is then added to the membrane to decrease background color in order to make the control and test spots more distinct.

Antigen Selection: The INSTI HIV-1/HIV-2 assay utilizes a combination of recombinant transmembrane proteins from HIV-1 (gp41) and HIV-2 (gp36). Use of these proteins overcomes sensitivity and specificity problems associated with tests based on viral lysates or a combination of core antigen and other viral proteins.⁹⁻¹³

Antibody Detection: The INSTI HIV-1/HIV-2 assay uses a unique reagent to detect antibodies to HIV-1/HIV-2. Although primarily designed to detect the IgG class of specific antibodies, the INSTI HIV-1/HIV-2 assay has been shown to detect antibodies in samples obtained early in infection, during seroconversion, and low titre anti-HIV-1 samples obtained later in infection (see tables # 1, 2 and 3).

Test Complexity: The INSTI HIV-1/HIV-2 assay was designed to reduce protocol complexity. The INSTI HIV-1/HIV-2 assay does not require sample preparation, accurate timing, or several steps, which include multiple washes and reagents. These requirements increase the complexity of an assay and lead to procedural errors which may adversely affect sensitivity and specificity. Total test time may vary slightly depending on specimen type; but results of valid tests are always clearly readable within as little as one minute.

SPECIMEN COLLECTION AND STORAGE

- For fingerstick blood collection procedures, follow the Assay Procedure instructions below.
- For EDTA-whole blood, EDTA-plasma or serum specimens, follow normal venipuncture blood collection procedures using lavender-top EDTA anticoagulant tubes (for whole blood and plasma) or red-top (no anticoagulant) tubes for serum collection. **Other anticoagulants have not been validated and may give an incorrect result.**
- If plasma or serum is to be used, separate from the blood cells by centrifugation.
- Serum or EDTA-plasma may be stored at 2-8°C for up to 5 days, stored frozen at ≤ -20°C for 3 months, or stored frozen at ≤ -70°C for one year.
- Whole blood specimens collected in EDTA, anticoagulants may be stored at 2-8°C and should be tested within 48 hours. **Do not heat or freeze whole blood specimens.**
- Do not dilute prior to testing.

KIT COMPONENTS AND STORAGE

INSTI components should be stored at 2-30°C. For **90-1022, 90-1038 and 90-1064**, components are provided for 48 tests. Each test requires the following materials:

- Membrane Unit**, individually packaged, prepared with control (IgG capture) and test (gp41 and gp36 antigen) reaction spots. For single use only in the INSTI procedure.
- Sample Diluent**, Solution 1 vial, containing 1.5 ml of tris-glycine buffered solution containing cell lysis reagents, with adequate space for addition of blood, serum or plasma samples being tested with INSTI. Ready to use, no mixing or preparation required.
- Color Developer**, Solution 2 bottle, containing 85 ml of a blue-coloured Borate buffered proprietary indicator solution designed to detect IgG in the control spot and specific HIV antibodies in the test spot. Ready to use, no mixing or preparation required. A blue dropper individually wrapped with 1.5mL mark is included.
- Clarifying Solution**, Solution 3 bottle, containing 85 ml of a proprietary tris-glycine buffered clarifying solution designed to remove background staining from the membrane unit prior to reading the INSTI test results. Ready to use, no mixing or preparation required. A white dropper individually wrapped with 1.5mL mark is included.

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

SUPPORT MATERIALS

The following materials are required when testing fingerstick whole blood:

- Single-use Alcohol Swab (included in **90-1022**)
- Single-use Lancet (included in **90-1022**)
- Single-use Pipette, capable of dispensing 50µl (included in **90-1022** and **90-1038**, not included in **90-1064**)

MATERIALS REQUIRED BUT NOT PROVIDED

- Personal protective equipment such as gloves, lab coat or gown.
- Appropriate biohazard waste containers.
- Absorbent cotton balls for fingerstick or venipuncture wound closure.

For venipuncture blood collection:

- Venipuncture apparatus if collecting blood samples.
- Appropriate blood collection tubes.
- Precision pipette capable of delivering 50µl of sample.
- Appropriate shipping containers.
- Personal protective equipment.

MATERIALS AVAILABLE AS AN ACCESSORY TO THE KIT

INSTI HIV-1/HIV-2 Test Controls: Separate HIV-negative human serum substitute and HIV-1/HIV-2 positive de-fibrinated human plasma control samples product no. 90-1034 and 90-1035 are available from bioLytical Laboratories in user-defined amounts, for use in quality control procedures. Please refer to the section on Quality Control, following the Assay Procedure, and the INSTI HIV-1/HIV-2 Test Controls Instructions for Use.

WARNINGS

For *in vitro* diagnostic use only **IVD**

It is recommended that the entire Instructions for Use be read prior to beginning the test procedure. Although the assay is designed to be simple to use, conformance with the test procedure is necessary to ensure accurate results.

- Do not mix reagents from different lots.**
- Do not use reagents or kits beyond the stated expiration date.
- Do not use the Membrane Unit if the foil pouch has been previously opened or if the packaging integrity has been compromised. Once the Membrane Unit has been opened, it must be used immediately.
- Avoid microbial contamination of reagents.
- 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.
- The performance characteristics of the INSTI HIV-1/HIV-2 assay have not been established for body fluids other than EDTA whole blood, fingerstick blood, serum, and EDTA-plasma. The use of blood collected in anticoagulants other than EDTA has not been validated. Insufficient data are available

to interpret tests performed on other body fluids, pooled blood or pooled serum and EDTA-plasma, or products made from such pools.

- Failure to use the recommended reagent and specimen volumes may result in leakage and/or overflow of liquids from the membrane unit.
- If the test kit is stored refrigerated, ensure it is brought to ambient temperature before performing the test. Use the INSTI Controls to ensure proper kit performance.
- Patients that have been on long term antiretroviral drug therapy may give a false negative INSTI HIV-1/HIV-2 Test result.
- Samples from patients with severe hypogammaglobulinemia conditions such as multiple myeloma may result in false negative or invalid results with INSTI.
- Patients with elevated haemoglobin levels may test false negative with INSTI.¹⁵

PRECAUTIONS

- All specimens should be handled as if capable of transmitting infectious diseases. It is recommended that BioSafety Level 2 practices, or equivalent regulations, be observed.¹⁴
- Thoroughly wash hands after handling or performing this test.
- Do not smoke, eat, or drink in areas where specimens or kit reagents are being handled.
- Wear a lab coat and disposable gloves while handling kit reagents or specimens. Do not pipette by mouth.
- Avoid contact with skin and eyes. If contact occurs, wash affected areas with water.
- Avoid forming aerosols.
- Dispose of all specimens and materials used to perform the test as if they contained infectious agents. The preferred method of disposal is sterilization by autoclaving for a minimum of one hour at 121°C followed by incineration. Liquid waste not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 0.5% sodium hypochlorite (a solution containing 10% household bleach). Allow at least 30 minutes for decontamination to be completed. **Do not autoclave solutions that contain bleach.**
- Spills should be cleaned up and decontaminated in accordance with the user facility's established procedures for handling biohazardous spills.

ASSAY PROCEDURE

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

Fingerstick Blood Sample Collection:

- Gather support materials (alcohol swab, lancet, pipette), one sealed test pouch containing INSTI Membrane Unit, and one vial of Sample Diluent for each test to be performed. Also for each test to be performed, 1.5 ml of Color Developer and 1.5 ml of Clarifying Solution will be drawn using the provided droppers.

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

- Massage the finger to allow the blood to move to the surface (fingertip will become pink). Use heating pad if available to warm the hand. Hand must be positioned at waist level or lower.
- Wipe the fingertip with the alcohol swab.
- As soon as the finger is dry, twist off the protective cap from the lancet, and then pull it straight out (see Figure A). Press the finger firmly at the point just below where the lancet will be applied. With the other hand, hold the lancet by the body and press the lancet body firmly against the puncture site to activate the device (see Figure B). Immediately dispose of the used lancet into a proper sharps container.

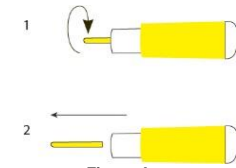


Figure A



Figure B

- As the blood bubbles up, hold the pipette horizontally and touch the tip of the pipette to the blood sample (see Figure C). Capillary action automatically draws the sample to the fill line and stops. If very little blood trickles out of the puncture, gently apply intermittent pressure near the puncture site to obtain the required blood volume. If blood is inadequate, perform a second skin puncture using a new lancet.



Figure C

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

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



Figure D

Sampling EDTA Whole Blood, Serum, EDTA-plasma and Test Controls:

- Bring specimens to room temperature and mix each specimen thoroughly prior to use. **Do not heat or repeatedly freeze/thaw specimens.**
- To prepare the Color Developer and Clarifying Solution bottles, remove the caps and seals from the bottles, and replace the caps with the droppers provided with the kit.

NOTE: The Color Developer and Clarifying Solution may form bubbles during handling. This does not affect the performance of the test. To ensure that the proper volume of solution is used in the test, completely fill the dropper with the solution up to the 1.5 ml mark. If bubbles form in the dropper, ensure that the fluid level below the bubbles is at the 1.5 ml mark. Use within one month after opening. Recap the bottles when not in use.

- Gather one sealed test pouch containing INSTI Membrane Unit, and one vial of Sample Diluent for each test to be performed.
- Using a pipette, add 50µl of whole blood, serum, plasma, or kit controls (see Note) to the Sample Diluent vial. Recap the vial and mix by inversion. Adding an excessive amount of specimen may cause the device to overflow or leak.

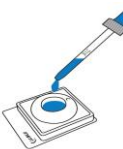
NOTE: In POC settings, for INSTI kit controls, it is important to use a 50µl pipette device to add the control material to the Sample Diluent vial. Do not use the disposable single-use pipette provided for fingerstick blood collection.

General Procedure after Sampling:

- Tear open the pouch and carefully remove the Membrane Unit without touching the center well. Place the unit on a level surface. For sample identification purposes the tab of the Membrane Unit may be labeled with the patient's name or number.

NOTE: At this point, it is important that the following steps be performed immediately and in sequence.

- Mix the Sample Diluent-specimen by inverting several times mixture and pour the entire contents to the center of the Membrane Unit well. (**Note:** Do this within 5 minutes after the specimen has been added to the Sample Diluent vial). The sample should be absorbed through the membrane in less than 30 seconds; however, absorption times will vary slightly depending upon sample type.
- Ensure that the Color Developer is resuspended prior to use. Slowly draw Color Developer solution up to the 1.5 ml mark. Add the solution to the center of the Membrane Unit well. The colored solution should flow through completely in about 20 seconds.
- Slowly draw Clarifying solution up to the 1.5 ml mark. Add the solution to the center of the Membrane Unit well. This will lighten the background color and facilitate reading. Immediately read the result while the membrane is still wet. **Do not read the results if more than 5 minutes have elapsed following the addition of Clarifying Solution.**



QUALITY CONTROL

Kit Controls:

The INSTI HIV-1/HIV-2 Antibody Test has a built-in IgG capture procedural control that demonstrates assay validity and adequate sample addition. A blue color in the control spot indicates that the proper specimen was added and that the assay procedure was performed correctly. The control spot will appear on all valid INSTI tests. (Refer to Interpretation of Results, below.)

INSTI HIV-1/HIV-2 Test Controls are available separately for use only with the INSTI HIV-1/HIV-2 Antibody Test. The controls are used to verify test performance and interpretation of results. Kit controls should be run under the following circumstances:

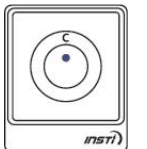
- for new INSTI operator verification prior to performing testing on patient specimens
- when switching to a new lot number of INSTI test kits
- whenever a new shipment of kits is received
- when temperature during storage of the kit falls outside of 2°-30°C
- when the temperature of the test area falls outside of 15°-30°C
- at regular intervals as determined by the user facility.

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

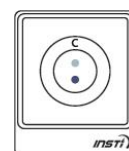
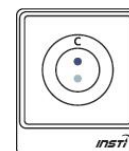
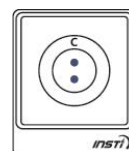
INTERPRETATION OF RESULTS

- Do not read the results if more than 5 minutes has elapsed following the addition of Clarifying Solution.**
- If using the control samples provided by bioLytical, all Positive Controls must be reactive with INSTI and all Negative Controls must be non-reactive with INSTI. Controls that produce incorrect or invalid results must be re-tested with INSTI. If results are still incorrect or invalid, inform bioLytical Laboratories immediately.**

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

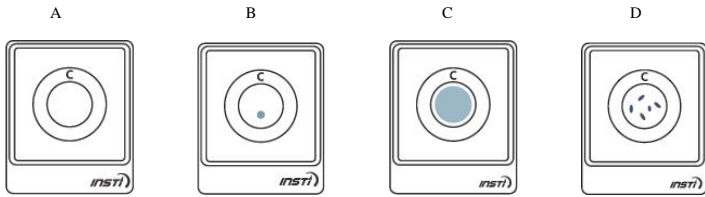


REACTIVE ▶ Two blue dots that are discernible above any background tint indicate a reactive specimen. One dot may be darker than the other. A sample giving this pattern is considered a preliminary reactive. Following a reactive rapid test result, a venous blood sample must be drawn in a lavender-top EDTA collection tube (for whole blood or plasma) or red-top tube (for serum), and forwarded to a laboratory for HIV confirmatory testing.



INVALID ▶ The test is invalid if any of the following occurs:
A. There is no dot on the membrane

- B. The test dot appeared without the control dot
- C. Uniform tint across the membrane
- D. Only blue specks appear on the membrane



NOTE: Invalid tests with fingerstick blood samples in POC settings should be repeated with a fresh sample using a new membrane unit, kit components and support materials. Invalid tests with EDTA whole blood, EDTA plasma or serum samples in laboratory settings should be repeated using a new membrane unit and kit components.

INDETERMINATE ► The test is indeterminate if a faint background ring appeared on the test area. Following an indeterminate INSTI test result, a venous blood sample must be drawn in a lavender-top EDTA collection tube (for whole blood or plasma) or red-top tube (for serum), and forwarded to a laboratory for HIV confirmatory testing.



Please note the following:

1. Following a reactive or indeterminate INSTI test result, a venous blood sample must be drawn in a lavender-top EDTA collection tube (for whole blood or plasma) or red-top tube (for serum), and forwarded to a laboratory for HIV confirmatory testing.
2. Depending on the antibody titer, a reactive specimen may be less intense in color than the procedural control, or vice versa.
3. Only a blue spot of color discernibly darker than the background color should be interpreted as reactive or positive. In rare instances, a faint background ring may appear around the test spot; this should not be interpreted as a reactive result. Only tests exhibiting distinct fully formed blue test dot combined with a distinct fully formed blue control dot should be interpreted as reactive. Color intensity may be variable within or between the dots.
4. An invalid result indicates that the test was performed incorrectly or there is a problem with the sample or device. The absence of a distinct control dot usually indicates that the sample volume was insufficient. An invalid test must be repeated.
5. A test resulting in a uniform blue tint across the entire membrane, thus obscuring the control and test spots, can occur when more than 60µl of whole blood is used and the flow through the assay membrane is obstructed.
6. An individual who has a non-reactive result but was involved in HIV-risk activity is likewise recommended to obtain additional testing over the next months.
7. To significantly reduce the risk of HIV transmission, it is advisable to refrain from high risk activities such as unprotected sex and needle sharing at all times.

LIMITATIONS OF THE TEST

- **Flow Times**
In some instances, samples may exhibit longer than normal flow times (from the time the Sample Diluent specimen mixture is poured in the membrane well to the time the Clarifying Solution has fully flowed through the membrane). This is due to variable factors such as cellular components, especially with whole blood. **In instances of long flow times, a faint shadow in the form of a ring may appear at the test spot location, but this should not be interpreted as a reactive result. This should be considered as an indeterminate result.** In these instances, a venous blood sample should be drawn in a lavender-top EDTA collection tube, and forwarded to a laboratory for HIV confirmatory testing.
- The INSTI HIV-1/HIV-2 Antibody Test procedure and the interpretation of result must be followed closely when testing for the presence of antibodies to HIV in serum, plasma or whole blood.
- Insufficient data are available to interpret tests performed on other body fluids, pooled blood or pooled serum and plasma, or products made from such pools; therefore, testing of these specimens is not recommended.
- The INSTI HIV-1/HIV-2 Antibody Test has not been validated for detection of antibodies to HIV-1 Group N subtypes.
- The INSTI HIV-1/HIV-2 Antibody Test detects antibodies to HIV-1/HIV-2 and is useful in establishing infection with HIV. Because a variety of factors may cause non-specific reactions, a patient found to be positive using the INSTI HIV-1/HIV-2 assay should have an EDTA blood sample drawn for laboratory-based confirmatory testing. A person who has antibodies to HIV is presumed to be infected with the virus and appropriate counseling and medical evaluation should be offered. The presence of HIV antibodies indicates past exposure to HIV but is not a diagnosis of AIDS, which can only be made by a physician. However, a non-reactive test does not rule out past exposure to HIV. The risk of an asymptomatic person with repeated reactive serum developing AIDS is not known. The prevalence of HIV infection in various groups, as well as clinical and public health guidelines, are available in the CDC Morbidity and Mortality Report.⁵

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity:

The sensitivity of a test is the ability of a test to detect truly infected people; whereas, the specificity of a test is the ability of a test to identify all non-infected individuals. Thus, a sensitive test should not produce false negatives, and a specific test should not produce false positives. There is no single standard for detecting the sensitivity or specificity of an antibody test for HIV in human sera, plasma or whole blood. However, the generally accepted method to express the sensitivity and specificity of a given test in terms of the detection rate is to compare results to approved supplemental assay results, such as ELISA and Western Blot. Based on these criteria, the sensitivity and specificity of the INSTI HIV-1/HIV-2 assay was determined using matching fingerstick blood, EDTA whole blood, serum and EDTA-plasma samples, which were also analyzed for anti-HIV-antibodies using ELISA and Western Blot.

Samples tested using the INSTI HIV-1/HIV-2 test fall into 4 categories:

1. Twenty five commercial seroconversion panels (Table 1) and one HIV-1 low titer antibody performance panel, (Table 3) which represent a wide range of antibody titers or classes.
2. Canadian HIV seroconversion patient samples (Table 2)
3. Prospective samples from HIV-positive patients enrolled in the Canadian Clinical Trial (Table 4)
4. Prospective negative samples from patients enrolled in the Canadian Clinical Trial (Table 5)

bioLytical Laboratories' Canadian Clinical Trial data show:

1. The relative sensitivity of the INSTI HIV-1/HIV-2 assay for early antibody detection was assessed using standardized seroconversion panels from Boston Biomedica Inc. Table 1 summarizes the INSTI HIV-1/HIV-2 assay data compared to a number of US licensed and European approved enzyme immunoassays (EIA) using the commercial panels.

2. The relative sensitivity of the INSTI HIV-1/HIV-2 assay for early antibody detection was also assessed using Canadian seroconversion patients. Table 2 summarizes the data from the Canadian seroconversion patients.
3. The sensitivity of the INSTI HIV-1/HIV-2 assay was ≥99% for fingerstick blood, EDTA blood, plasma and serum (range 99.0-99.6%) (Table 4) Indeterminate and invalid results were eliminated from evaluation.
4. The specificity of the INSTI HIV-1/HIV-2 assay was ≥99.3% (range 99.3-100%) for fingerstick blood, EDTA blood, plasma and serum (Table 5). Indeterminate and invalid results were eliminated from evaluation.
5. INSTI HIV-1/HIV-2 Antibody Test results were not affected by most potentially interfering conditions or substances as illustrated in Table 6. Samples from patients with severe hypogammaglobulinemia conditions such as multiple myeloma may result in false negative or invalid results with INSTI.
6. The sensitivity of the INSTI HIV-1/HIV-2 test in HIV-2 positive specimens is 98.3% (Table 8).

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

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

*PRB910, PRB904, PRB924, PRB912, PRB914, PRB916, PRB919, PRB922, PRB925, PRB926, PRB927, PRB928, PRB929, PRB934, PRB935, PRB944, PRB937, PRB938, PRB940, PRB941, PRB945, PRB947, PRB950, PRB952, PRB943

**The last bleed in the panel was positive by at least 1 EIA, negative by INSTI on Panels PRB937 and PRB938

Table 2

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

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

1. 13/14 had low s/co ratios (<9.0) with licensed EIA

2. s/co ratio with licensed EIA was low (5.64)

IND: indeterminate

Table 3

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

Test	Specimen Number														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
INSTI HIV-1/HIV-2	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Abbott EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Abbott HIVAB HIV-1/HIV2 (rDNA)EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Cambridge Biotech Recombigen HIV-1 EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Syva EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Organon Teknika Vironostika Anti-HIV Uni-Form II	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Murex HIV 1/2 EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Ortho HIV-1/HIV-2 EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Sorin ETI-Ab-HIV 1/2K EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Syva Microtrak II EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Behringwerke ENZ PLUS Anti HIV 1/2 EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Biotech Anti-HIV-1/HIV-2 Recombinant EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Boehringer Mannheim Anti HIV-1/HIV-2 EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
IAF Biochem Detect-HIV-EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Diagnostic Pasteur Genelavia EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
bioMerieux VIDAS anti-HIV-1/2 EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Murex Wellcozyme HIV-1/HIV-2 EIA	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P
Behringwerke Enzygnost Anti HIV 1+2 EIA	N	P	N	P	P	P	P	P	P	P	P	N	P	P	P
Cellular Products HIV-1 EIA	N	P	P	P	P	P	P	P	N	P	P	N	P	P	P
Genetic Systems LAV EIA	N	P	P	P	P	P	P	P	N	P	P	N	P	P	P
Genetic Systems HIV-1/HIV-2 EIA	N	P	N	P	P	P	P	P	P	P	P	N	P	P	P

*These samples were confirmed positive (P) by EIA and Western Blotting (Data obtained from Boston Biomedica package insert, May 1995 p.2)

Table 4

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

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

1. Samples were confirmed HIV positive by the approved laboratory-based screen test of record, and by Western Blot

2. Serum samples were collected from a portion (n=1346) of the study patients (n=3507)

Note: INSTI invalid results were not included in the table and calculations

Table 5

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

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

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

Table 6
INSTI HIV-1/HIV-2 Antibody Test Reactivity with Specimens from Individuals with potentially Interfering Medical Conditions and Specimens with Interfering Substances, n=666.

Specimen Type	INSTI Positive*	INSTI Negative **	Invalid
Anaemia	1	2	-
Carcinoma/Cancer	24	5	-
Chlamydia	0	2	-
Cytomegalovirus (CMV)	0	10	-
Diabetes	17	6	-
Epstein Barr Virus (EBV)	0	10	-
Haemolysed	0	12	-
Haemophilia	0	1	-
Hepatitis A Virus (HAV)	4	1	-
Hepatitis B Virus (HBV)	7	4	-
Hepatitis C Virus (HCV)	46	7	-
Herpes	63	12	-
HTLV I	0	7	-
HTLV II	0	7	-
Lipid Abnormalities	58	4	-
Lymphoma	1	1	-
MAC/TB	2	0	-
Malaria	0	1	-
Myeloma	0	7	3 ¹
Multiple Transfusion Recipients	2	6 ²	-
Neuropathies	28	0	-
PCP	2	0	-
Rheumatoid Factor	0	5	-
Rubella	0	10	-
Lupus	0	5	-
Syphilis	0	10	-
Toxoplasmosis	1	10	-
Yeast/Candida	0	2	-
Pregnancy	0	200	-
Dengue	0	10	-
Chikungunya	0	10	-
Elevated Bilirubin	0	10	-
Elevated Triglyceride	0	10	-
Elevated Lipid	0	10	-
Elevated Albumin	5	0	-
Sodium heparin	0	10	-
Sodium citrate	0	10	-

Table 7
INSTI HIV-1/HIV-2 Antibody Test Reactivity with Specimens from Therapeutic Drugs. n=100

Specimen Type	INSTI Positive*	INSTI Negative **	Invalid
Acetaminophen	0	5	-
Acetylsalicylic Acid	0	5	-
Ampicillin	0	5	-
Erythromycin	0	5	-
Gentamycin	0	5	-
Ibuprofen	0	5	-
Tetracycline	0	5	-
Artesunate	0	5	-
Chloroquine	0	5	-
Doxycycline	0	5	-
Primaquine	0	5	-
Mefloquine	0	5	-
Ethambutol	0	5	-
Isoniazid	0	5	-
Pyrazinamide	0	5	-
Rifampin	0	5	-
Benznidazole	0	5	-
Diethylcarbamazine	0	5	-
Nifurtimox	0	5	-
Suramin	0	5	-

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

The following substances were evaluated for potential interference as per CLSI EP7-A2. These compounds were chosen to represent common over-the-counter therapeutics (acetaminophen, acetylsalicylic acid, ibuprofen), frequently prescribed antibiotics (ampicillin, erythromycin, tetracycline, gentamycin), and medications frequently prescribed for treatment of malaria (malaria, artesunate, chloroquine, doxycycline, primaquine, mefloquine) tuberculosis (ethambutol, isoniazid, pyrazinamide, rifampin) or parasitic diseases (benznidazole, diethylcarbamazine, nifurtimox, suramin).

Each substance was evaluated on both Negative and contrived Low Positive samples and compared to a solvent control. No interference was observed for any of the substances at the maximum concentrations tested, as reported in Table 8.

Table 8
Potential interfering substances and maximal concentration evaluated for performance.

Substance	Concentration (µg/mL)
Acetaminophen (APAP)	91
Acetylsalicylic acid (ASA)	1172
Ampicillin	53
Erythromycin	60
Ibuprofen	30
Tetracycline Hydrochloride	210
Gentamycin Sulfate	15

Malarone (Atovaquone/Proguanil)	600/240
Artesunate	180
Chloroquine	600
Doxycycline	60
Primaquine	160
Mefloquine	540
Ethambutol	720
Isoniazid	180
Pyrazinamide	1,100
Rifampin	360
Benznidazole	130
Diethylcarbamazine	120
Nifurtimox	180
Suramin	120

Table 9
INSTI HIV-1/HIV-2 Antibody Test's Sensitivity in HIV-2 Positive Specimens

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

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

HIV-1 SUBTYPE TESTING

Forty eight samples from 48 patients infected with HIV-1 non B strains were tested. All samples were genotyped by dideoxynucleotide sequencing of the entire HIV-1 protease gene and the first 450 codons of reverse transcriptase, for subtype determination. The subtype distribution was the following:
A:7,C:8,D:8,F:6,G:8,J: 1,CRF AG: 5,CRF AE: 5
The sensitivity of the INSTI HIV-1/HIV-2 Antibody Test on the 48 non-B HIV positive samples tested was 100%.

REPRODUCIBILITY

Two studies were conducted to evaluate the reproducibility of INSTI HIV-1/HIV-2 Antibody Test. For each study, a panel of 9 blind-coded plasma samples consisting of 4 antibody positive, 1 very low antibody level sample, and 4 antibody negative samples, was tested in triplicate at 3 sites on 3 separate days using 3 lots of INSTI.
The overall reproducibility of INSTI for HIV-1 was 99.7% (646/648, two antibody negative samples were read as weak positive at 1 site). For the 1 very low level antibody sample, 59% (48/81) of the results were positive while 41% (33/81) were negative.
The overall reproducibility of INSTI for HIV-2 was 99.8% (566/567, one antibody negative sample was read as indeterminate at 1 site). At the 2 lowest level antibody samples, 85.2% of the results were positive with Panel Member 3 (69/81), while 63% of the results were negative with Panel Member 4 (51/81).

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TECHNICAL INFORMATION

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