

	TITLE: Policy and Procedure for Point Of Care Testing		POLICY #: PHU 002-2019	
	UNIT: Public Health Unit		PROGRAM: HIV Program	
	SOURCE: Communicable Disease Control			
	APPROVED BY: Medical Health Officer	APPROVAL DATE:	May 6, 2019	
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INSTI HIV-1/HIV-2 Antibody Test Kit – BioLytical Laboratories

1.0 Purpose:

Acquired Immunodeficiency Syndrome (AIDS) is caused by two retroviruses, HIV-1 and HIV-2. HIV-1 and HIV-2 are similar in genomic structure, morphology and ability to cause AIDS.¹ HIV is transmitted mainly by sexual contact, exposure to blood or blood products, or from an infected mother to her fetus. People with increased risk of HIV infection include hemophiliacs, intravenous drug-users and men having sex with men (MSM). HIV has been isolated from patients with AIDS, AIDS-related complex (ARC), and from persons at high risk of contracting AIDS.²⁻⁵ Antibodies specific for HIV envelope proteins are prevalent in sera from persons at high risk of contracting HIV as well as in people with AIDS, or ARC.⁵⁻⁷

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
- c) Complexity of the protocol used to perform the test.

⁸ Non-specific reactions may be observed in some specimens. A reactive INSTI test result should be considered a preliminary result, with appropriate counseling provided in point-of-care test (POCT) 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 the Roy Romanow Provincial Laboratory (Formerly known as Saskatchewan Disease Control Laboratory) for HIV confirmatory testing.

2.0 Principle:

The INSTI™ HIV-1/HIV-2 Rapid Antibody Test is a single use, rapid, flow-through *in vitro* qualitative immunoassay test for the detection of antibodies to Human Immunodeficiency Virus Type 1 and Type 2 in human whole blood, fingerstick blood, serum or EDTA-plasma. The test is intended for use by trained and licensed personnel in medical facilities, clinical laboratories, emergency care situations, and physicians' offices as a screening test capable of providing results in less than one minute. Its rapid result makes it easy to be included in a variety of workflow models and it works with most testing algorithms. Although suitable for near-patient or 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), and Clarifying Solution (Solution 3) with support materials (lancet, pipette and alcohol swab).

3.0 Kit Components and Storage:

INSTI Reagents should be stored at room temperature 2°C -30°C. For kit 90-1008, each package contains the following components:

- 1 lancet
- 1 pipette
- 1 alcohol swab
- **Membrane Unit**, individually packaged, prepared with control (IgG capture) and test (gp41 and gp36 antigen) reaction spots. It is 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 and an antimicrobial agent, with adequate space for addition of blood and serum or plasma samples being tested with INSTI. It is ready for use, no mixing or preparation required. It contains 0.1% sodium azide as a preservative, for single use only in the INSTI procedure. Stable to date and under storage conditions indicated on label.
- **Color Developer**, Solution 2 vial, contains 1.5ml of a blue-coloured Borate buffered proprietary indicator solution designed to detect IgG in the control spot and an antimicrobial agent (specific HIV antibodies in the test spot). It is for single use only in the INSTI procedure. Ready to use, invert 2 to 3 times immediately before use. It contains 0.1% sodium azide as a preservative. Stable to date and under storage conditions indicated on label.
- **Clarifying Solution**, Solution 3 vial, contains 1.5 ml of a proprietary tris-glycine buffered clarifying solution containing detergent which is designed to remove background staining from the membrane unit prior to reading the INSTI test results and it also contains an antimicrobial

agent. It is ready to use, no mixing or preparation required. For single use only in the INSTI procedure. Contains 0.1% sodium azide as a preservative. Stable to date and under storage conditions indicated on label.

4.0 Support Materials/Equipment:

- INSTI HIV-1/HIV-2 Test Controls: Separate HIV-negative human serum substitute, HIV-1 positive and HIV-2 positive defibrinated human plasma control samples product no. 80-1037 (BioLytical Laboratories) are available from Roy Romanow Provincial 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 package insert.
- Personal protective equipment such as gloves, lab coat or gown.
- Precision pipette capable of delivering 50µl of sample.
- Appropriate biohazard waste containers.
- Absorbent cotton balls for fingerstick or venipuncture wound closure.
- Disinfectants

4.1 For venipuncture blood collection:

- Venipuncture apparatus if collecting blood samples.
- Appropriate blood collection tubes.
- Appropriate shipping containers.
- Personal protective equipment such as gloves, lab coat or gown
- Appropriate biohazard waste containers and disinfectants.

5.0 Specimen Collection and Storage:

- 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.
- 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 anticoagulant may be stored at 4°C and should be tested within 48 hours. Do not heat or freeze whole blood specimens.
- Do not dilute prior to testing.

5.1 Special Safety 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.

6.0 Quality Control:

6.1 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 user verification**
- **When switching to new lot number of INSTI test kits**
- **If a site conducts >24 point-of-care tests per day, the controls should be run everyday**
- **If a site conducts <24 point-of-care tests per day, the controls should be run approximately once per 24 specimens, but no less than once per week**
- **If a site does no point-of-care tests in a given week, controls do not have to be run**

in that week. However, controls must be run prior to conducting a client test, if it has been a week since the last controls were run

Refer to the INSTI HIV-1/HIV-2 Test Controls package insert for additional information on the use of these reagents. New lot numbers are tested at the Roy Romanow Provincial Laboratory (formerly known as Saskatchewan Disease Control Laboratory) prior to distribution to POC (Point of Care) sites. 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. This can be achieved through participation in the external quality control program provided by the College of Physicians and Surgeons.

7.0 Procedure:

Note: All Test Units must be used immediately once opened. All reagents should be dispensed evenly in the center of the well of the membrane unit.

7.1 Sampling Fingerstick Blood:

- Take one sealed pouch that contains support materials (swab, lancet, pipette), INSTI Membrane Unit, and one vial each of Sample Diluent, Color Developer, and Clarifying Solution for each test to be performed.

Caution: The amount of sample (fingerstick blood) is critical.

- To ensure that the proper amount of blood is achieved, follow these instructions carefully:
 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 must be positioned at waist level or lower.
 2. Wipe the fingertip with the alcohol swab.
 3. As soon as the finger is dry, twist off the green protective cap from the lancet, and then pull it straight out. 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. Immediately dispose the used lancet into a proper sharps container.
 4. As the blood bubbles up, hold the pipette horizontally and touch the tip of the pipette to the blood sample. Capillary action automatically draws the sample to the fill line and stops. If very little blood trickles out of the puncture, gently apply intermittent

pressure near the puncture site to obtain the required blood volume. If blood is inadequate, perform a second skin puncture using a new lancet.

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

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

7.2 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.
- Gather 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.
- 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 (Point of Care) settings, for INSTI kit controls, it is important to use a 50µl pipette device to add the control material to the Sample Diluent vial. Do not use the disposable single-use pipette provided for finger stick blood collection.

7.3 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.

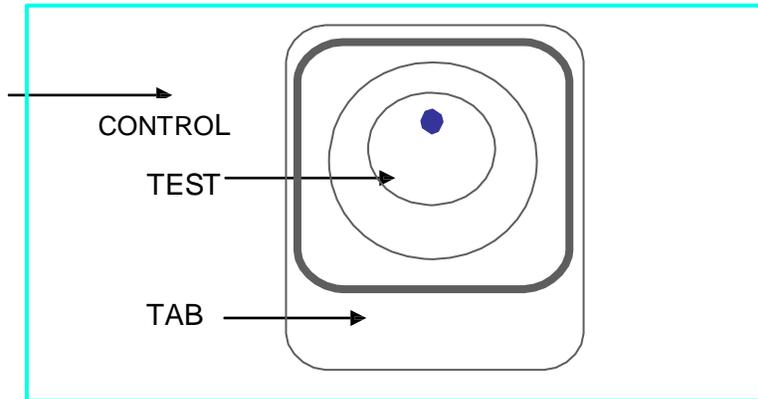
NOTE: At this point, it is important that the following steps be performed immediately and in sequence.

- Remix the Sample Diluent-specimen 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.
- Resuspend the Color Developer by slowly inverting to mix the solution thoroughly. Continue this process until careful visual observation confirms that the reagent is evenly suspended. Open the Color Developer and add the entire contents to the center of the Membrane Unit well. The colored solution should flow through completely in about 20 seconds.
- Open the Clarifying Solution and add the entire contents 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 has elapsed following the addition of Clarifying Solution.**

8.0 Interpretation/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 the Roy Romanow Provincial Laboratory (RRPL), 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 RRPL immediately.

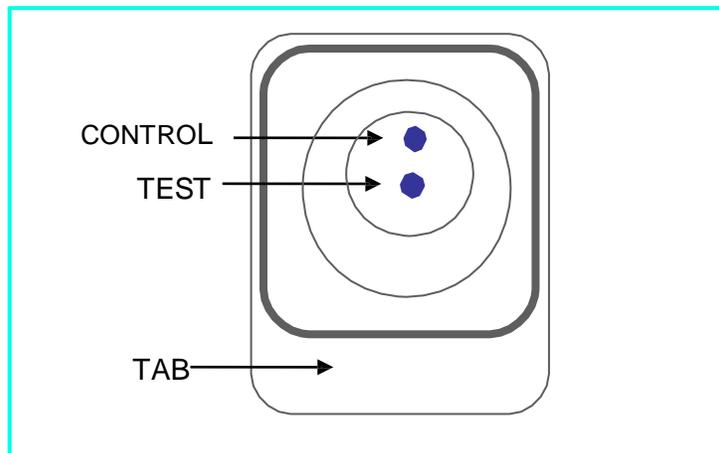
8.1. Non-reactive INSTI Test Result:



In this scenario, no reaction is visible below the control (only one blue dot appears; which is the control spot). This indicates that antibodies to HIV were not detected. A small number of HIV infected individuals may have a non-reactive result if they are in the period of HIV seroconversion. For such individuals consider repeating the test in two to three weeks, or submit a venous sample.

It is imperative that despite a negative rapid HIV test result, all patients who have had any HIV risk activity in the previous three months should be informed of the benefits of repeat HIV testing.

8.2. Reactive INSTI Test Result:

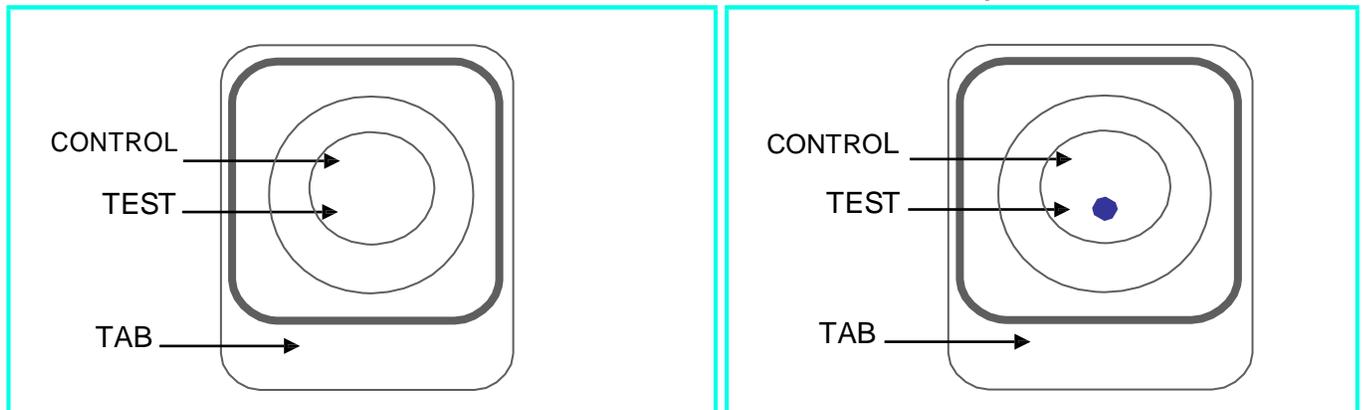


In this scenario a reaction is visible below the control. The appearance of two blue dots

indicates the presence of HIV antibodies.

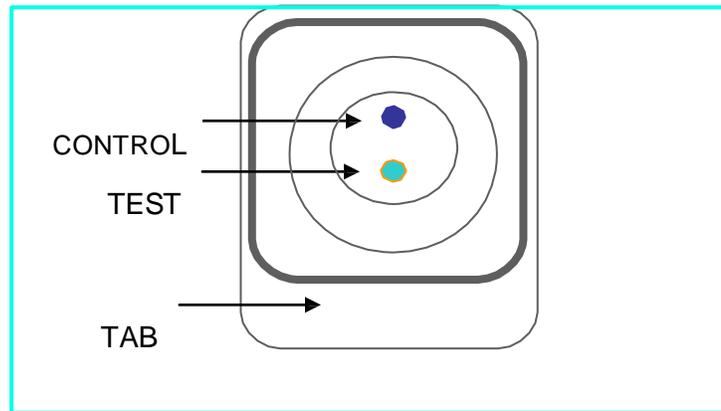
All reactive results from HIV POC (point of care) testing need to be confirmed, as false positives may occur when testing populations with low HIV prevalence. It is therefore essential that confirmatory testing at RRPL is conducted in order to rule out the possibility of false positive results.

8.3 Invalid INSTI Test Result:



In this scenario no dot appears on the membrane or the test dot appears without the control dot. It is recommended that an invalid test be repeated with another kit. If the result is still invalid, it should be explained to the patient that collection of a venous blood sample is necessary to send to RRPL for testing as there may be interfering substances which may invalidate the POCT. It is necessary to wait for the result from the RRPL for further counselling.

8.4 Indeterminate INSTI Test Result:



In this scenario, a faint shadow in the form of a ring (when cellular components in the blood cause long flow times) may appear at the test spot location, but this should not be interpreted as a reactive result. This should be considered an indeterminate result. The test should be repeated with a new kit. If the result is still indeterminate, it should be explained to the patient that collection of a venous blood sample is necessary to send to RRPL to be processed for confirmatory HIV testing.

9.0 Expected Values: - Negative.

10.0 Method Limitations:

10.1 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 RRPL for HIV confirmatory testing.

- The INSTI HIV-1/HIV-2 assay 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 assay has not been validated for detection of antibodies to HIV-1 Group O or N subtypes.
- The INSTI HIV-1/HIV-2 assay detects antibodies to HIV-1/HIV-2 and is useful in establishing infection with HIV. As a result of variety of factors which 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, even though the presence of HIV antibodies indicates past exposure to HIV but it does not a diagnosis of AIDS infection, which can only be made by a physician.
- 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.⁸
- Samples from patients with severe hypogammaglobulinemia conditions such as multiple myeloma may result in false or invalid results with INSTI.

11.0 Author:

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