INSTI® HIV-1/HIV-2 Antibody Test

Guidelines

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INTRODUCTION

The INSTI® HIV-1/HIV-2 Antibody test is a single-use, rapid, in vitro qualitative device for the detection of antibodies to Human Immunodeficiency Virus (HIV). The test is intended for use by trained personnel in point of care and laboratory settings to aid in the diagnosis of HIV infections. Results are available in as little as *60 seconds* and should be read and interpreted within five minutes.

Based on US clinical trials, INSTI® has a proven accuracy of *99.9% sensitivity* and *100% specificity in venous blood and plasma (≥ 99.8% sensitivity and ≥ 99.5% specificity in finger stick blood)*[1]. The test consists of a filtration membrane positioned atop an absorbent material within a plastic cartridge, referred to as the INSTI® Membrane Unit. The membrane has been spotted with HIV-1 and HIV-2 recombinant proteins, which react with HIV antibodies in the specimen. If present, the HIV antibodies are captured visually after pouring the three solutions provided in the kit.

Although a variety of factors may cause non-specific reactions[2], when a patient is found to be reactive using the INSTI® HIV-1/HIV-2 Antibody Test (preliminary positive), a second "confirmatory" test should be run according to your algorithm. A non-reactive test result is considered conclusive and does not require confirmatory testing. A person with HIV antibodies 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.

Please refer to the INSTI® HIV-1/HIV-2 Antibody Test Kit package insert for any additional information on the test procedure beyond what is described in this protocol. Conformance with the test procedure is necessary to ensure accurate results. All customer questions can be answered by phone on the technical support line at: 1-866-674-6784 or by email to customercare@biolytical.com.

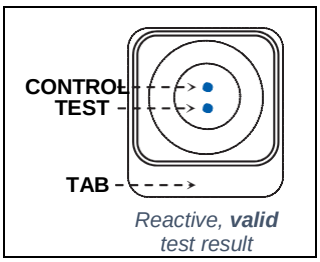
[1] INSTI® HIV-1/HIV-2 Antibody Test, 50-1080 (E) INSTI HIV1_HIV2 IFU (US), Richmond, BC: bioLytical Laboratories Inc; 2015
[2] Specimens from patients with multiple myeloma or receiving HAART may result in false non-reactive or invalid results.

HOW IT WORKS

The INSTI® test kit includes one of each of the following:

- Instructions for Use / Package Insert
- Membrane Unit
- Sample Diluent (“**Solution 1**”)
- Color Developer (“**Solution 2**”)
- Clarifying Solution (“**Solution 3**”)
- Lancet
- Pipette
- Alcohol Swab

The membrane unit displays information on two separate spots: the procedural “control dot” and the “test dot”. The control dot captures human immunoglobulin G (IgG) antibodies normally present in blood and blood components (irrespective of HIV status). The IgG antibodies react with Solution 2 to produce a visible blue dot on the membrane. The control dot provides a visible signal when the test is run indicating that the test was performed correctly, the correct sample type and volume was added, and the results are considered valid. If there is no control dot, the test is considered invalid. Invalid test results cannot be interpreted. Repeat the test with a fresh specimen using a new Membrane Unit, kit components and support materials. Contact bioLytical Laboratories’ Technical Support if you are unable to produce a valid result upon repeat testing. The test dot captures recombinant HIV-1 and HIV-2 proteins if they are present in the specimen. These will also react with Solution 2 to produce a visual signal if positive (a blue test dot), or no signal if negative (absence of a blue test dot). Within the membrane unit there is an absorbent pad that absorbs all the fluid used in the reagents to avoid spills and display dot in as little as 60 seconds. Over time, the solutions in the absorbent pad spread uniformly and can stain the membrane from below. For this reason, it is essential to read and interpret the test result within **5 minutes** of completing the test.



STORAGE AND HANDLING

The INSTI® test kit has a 15 month shelf life and must be stored unopened at room temperature, **15° - 30°C (59° - 86°F)**. If the storage conditions deviate from the specified temperature range, external controls must be run before administering any further tests[3]. It is the responsibility of each INSTI® user to establish an adequate quality assurance program to ensure the proper performance under their facilities' locations and conditions of use.

When testing is performed in an outreach setting, operators should check and record the ambient temperature throughout the duration of testing. If the temperature falls outside 15° - 30°C (59° - 86°F), controls should be run.

Please follow the bio-safety guidelines outlined in the Centers for Disease Control and Prevention (CDC) Universal Precautions[4], and other applicable safety guidelines for the management of occupational exposures. Important safety precautions:

- Thoroughly wash hands before and after handling or performing a test.
- Do not smoke, eat, or drink in areas where specimens or kit reagents are being handled.
- Wear disposable gloves while handling kit reagents and specimens.
- Avoid contact with skin and eyes. If contact occurs, wash affected areas with water.
- Spills should be cleaned up and decontaminated according to facility protocol.

Important handling guidelines:

- Do not open the membrane unit pouch until ready to use to completion.
- Once membrane unit pouch is open, perform test immediately.
- Do not mix and match membrane units and solutions from different lots.
- Do not use the test beyond the expiration dates printed on the outer packaging.
- *All components are single-use only*. All used materials exposed to blood should be disposed of in a designated biohazard waste container. All other components, such as Solution 2 and 3 bottles, plus the outer pouches may be disposed of in regular waste.

[3] Please see Quality Control section for more information and Appendix for example templates of control and temperature logs.
[4] CDC. Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens in health care settings. MMWR 1988; 37(24):377-388

GUIDELINE FOR OBTAINING FINGERSTICK BLOOD SAMPLE FOR AN INSTI® TEST

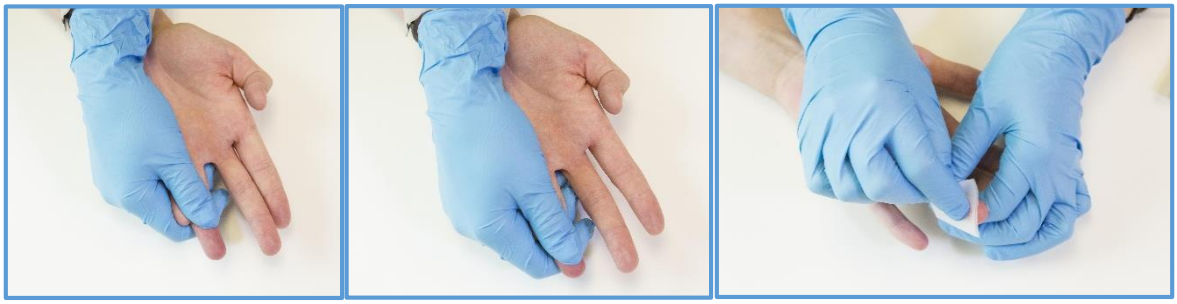
WORKSPACE PREPARATION:

1. Gather all support materials (alcohol swab, pipette, and lancet) included with the test kit.
2. Preferably, cover the workspace with a clean, disposable absorbent workplace cover.

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

OBTAINING FINGERSTICK BLOOD SAMPLE

1. With gloves on, squeeze fingers toward the fingertip while observing which finger is optimal (look for one that has the deepest color for the best blood flow). Wipe finger with alcohol swab and allow to dry. Hand should be positioned at waist level or below for optimal blood flow.

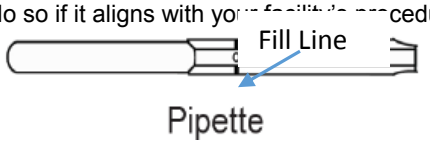


2. Twist and pull to remove protective tip from the lancet. Hold the finger and position the lancet off center, press down to trigger the lancet and puncture the skin. Avoid firing the lancet too close to the pad of the finger or nail bed. Discard lancet into a sharps container.

3. With the pipette ready in hand, pulse the finger to generate a contained bead of blood[5]. Angle the capillary tube horizontally or slightly downwards (at a 45° angle) when collecting the blood sample.

[5] Although it is not necessary to wipe the first drop of blood you may do so if it aligns with yo[illegible] cedure.

4. Do not squeeze the bulb. The capillary action, assisted by gravity, will automatically draw the blood to the pipette's fill line.



5. For best results, hover the pipette on the bead of blood and do not make contact with the finger.

6. Depending on how the patient bleeds, you may need to pulse or milk the finger to generate new beads until you have the required 50 µl (up to the black line on the pipette). If the bead of blood breaks, wipe the finger and pulse again to generate a fresh bead of blood.

7. Transfer the blood to the open **Solution 1** bottle by squeezing the bulb to dispense the specimen. Note: if the specimen does not expel, cover the vent hole with a gloved finger and squeeze the bulb again. Recap the vial and immediately follow the INSTI test procedure below.

VENIPUNCTURE AND PLASMA:

If you are using standard venous phlebotomy procedures instead of fingerstick blood, please refer to page two of the Package Insert for the whole blood procedure.

HOW TO PERFORM AN INSTI® TEST ON FINGERSTICK BLOOD

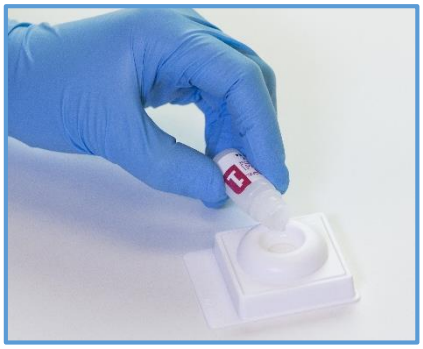
1. Open sealed test pouch and place the membrane unit on a level surface so the tab faces towards you. Do not touch the center well. Uncap **Solution 1** (red cap) and reserve the cap.

2. Add 50µL of fingerstick blood, with the capillary pipette provided, to the Sample Diluent (**Solution 1**). Cap the solution bottle and invert several times to mix well[6].

   NOTE: For EDTA whole blood, serum, and plasma, or as an alternative when running INSTI® Test Controls use a calibrated 50µL laboratory pipette to draw up sample.

NOTE: ***Only mix Solution 1 once you are ready to pour into the membrane unit. At this point, it is important that the following steps be performed immediately and in sequence*.**

3. Pour the diluted, mixed sample from Solution 1 into the membrane unit. (Note: do this within 5 minutes of adding the blood to Solution 1.) Allow the solution to be fully absorbed through the membrane (time will vary slightly, less than 30 seconds).



4. Re-suspend the Color Developer by inverting several times to mix well. Pour the Color Developer (**Solution 2**) to the membrane unit (within less than 20 seconds).

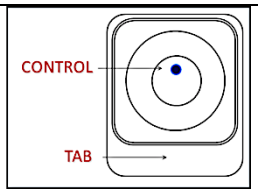
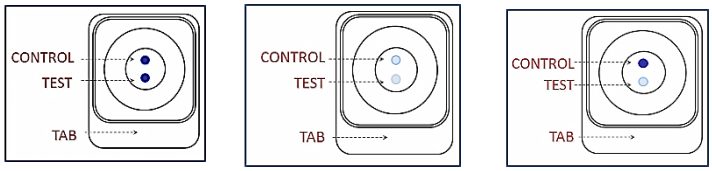
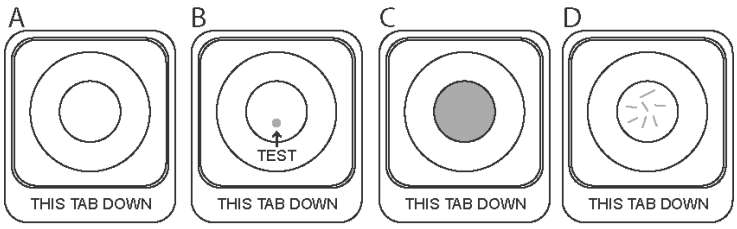
[1] Although the test is intended to be performed immediately, the sample is stable **in Solution 1** for up to five minutes.

5. After Solution 2 has flowed through the membrane, add the Clarifying Solution (**Solution 3**) to the membrane unit. This will reduce the background color and produce more distinct control and/or test dots.

6. **Read and interpret results immediately, or in less than <u>five minutes</u> of adding the final solution**.

INTERPRETING RESULTS

Results must be read within five minutes of pouring the Clarifying Solution (**Solution 3**). All interpretations should be made with the tab facing towards the operator.

NON-REACTIVE	 Only the control dot appears blue.	HIV antibodies were not detected in the sample. The visible control indicates the test was performed correctly and enough specimen was added.
REACTIVE	 Both the control and test dots appear blue. One dot may be darker than the other and may be non-homogeneous in appearance. The color intensity of the test dot does not necessarily correlate with the amount of antibody in the sample. If you see a faint background ring around the test dot (rare), this should be interpreted as a reactive result.	Sample is **preliminary positive** for HIV antibodies. All reactive test results should be followed up by confirmatory testing.
INVALID	A. There is no blue on the control spot. B. The test dot is blue but not the control spot. C. There is uniform tint across the membrane. D. Only blue specks appear on the membrane. 	The test was run incorrectly, not enough sample was added, a procedural error (e.g. added sample directly to the membrane unit, didn't mix bottles 1 & 2, etc.) or there was a problem with the device. **Do not interpret invalid test results.** Repeat the test with a fresh blood sample using a new test kit.[7]

[1] Contact bioLytical Laboratories' Technical Support if you are unable to produce a valid result upon repeat testing.

QUALITY CONTROL

Internal Controls:

Built-in sample addition control, or the “control dot”, is used to demonstrate the assay validity and adequate sample addition and the procedure was followed correctly. A blue color in the control dot is a visual indicator that the proper specimen was added and that the assay procedure was performed correctly. The presence of a control dot signifies the following:

- The test has been performed correctly.
- The correct amount and type of specimen has been added to the membrane unit.
- In combination with the test dot, is a valid reactive result.
- In the absence of a test dot, this is a valid non-reactive result.

External Controls:

The INSTI® Test Control sets are available as an accessory to the test kit. The control sets are used to verify test performance and interpretation of results. They can be stored at refrigerator, or freezer temperature which will dictate their shelf life. Please refer to the following table:

Temperature Range	Expiry
≤ - 20°C (- 4°F)	12 months
2°C to 8°C (36°F to 46°F)	12 months

Each package of INSTI® controls contains HIV-1 and/or HIV-2 Positive Controls (1 vial, 1 ml per vial), HIV Negative Controls (1 vial, 1 ml per vial), and a Package Insert. Each control vial contains enough material to conduct 20 tests. The positive source material has been heat inactivated at 60°C (140°F) for 60 minutes, but should be treated as biohazardous material.

Control set storage temperatures should always be monitored and logged. External controls should be used according to the facility’s quality system and are advisable to use in the circumstances outlined on page 11.

If there are any further questions regarding the external control sets please refer to the INSTI® HIV-1/HIV-2 Antibody Test Kit Controls package insert for additional information on the operation and control kit solutions.

Manufacturing Facility Controls:

The INSTI® test kits and accessories are manufactured by bioLytical Laboratories in British Columbia, Canada. The facility has an ISO 13485 certified quality system that meets FDA and Good Manufacturing Practices (GMP) requirements for manufacturing medical devices.

HOW TO PERFORM CONTROL TESTING

Please refer to the INSTI® HIV-1/HIV-2 Antibody Test Kit Controls package insert before conducting any Control testing.

The INSTI® HIV-1/HIV-2 Antibody Test Kit Controls have been designed for use with the INSTI® HIV-1/HIV-2 Antibody Test to validate the correct performance of the test procedure in the hands of the operator.

The HIV-1 Positive, HIV-2 Positive and Negative Controls are used to ensure that the test functions correctly. Controls are also run as part of your laboratory's standard quality control procedures. The HIV-1 and HIV-2 Positive Controls will produce a weakly reactive result in the INSTI® HIV-1/HIV-2 Antibody Test.

INSTI® HIV-1 Positive, HIV-2 Positive and Negative controls should be used according to Good Laboratory Procedures. They 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

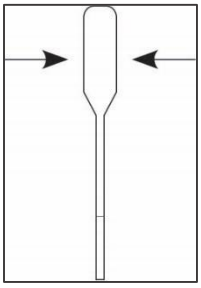
The positive control and negative control must be run on separate membrane units. The positive control is designed to produce a faint blue test dot and a control dot. The negative control will produce a blue dot in only the control dot position.

Controls that produce incorrect or invalid results must be re-tested using a new INSTI® kits. If results are still incorrect or invalid inform bioLytical Laboratories technical support immediately.

SAMPLING OF INSTI CONTROLS

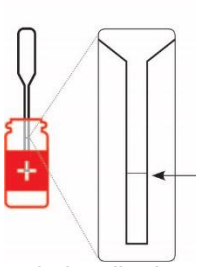
- Remove from storage and bring Controls to room temperature. Mix each sample before use. Return unused sample to storage.[8]

- Gather the membrane units, one for the HIV-1 positive control, one for the HIV-2 positive control (if required) and one for the negative control. **Use a new pipette for each Control sample collection**.



- Take the pipette and lightly depress the bulb to prepare for collection.

- With the bulb depressed, insert pipette tip into a control vial. Slowly release the bulb and watch the control solution travel up the stem of the pipette. Do not allow liquid to enter the bulb of the pipette, ensure the liquid only reaches the fill line (see diagram below).



INSTI Control Kit Bulb Pipette: solution must fill to the black line.

- Take pipette and depress the bulb to release the contents into the **Solution 1** bottle. Dispose of pipette (they are single use only “consumables”).

    - Note: alternatively, a calibrated 50µL laboratory pipette can be used to sample the controls. Please ensure that a new pipette tip is used with each sampling on the control material.

- Proceed with standard testing procedure (recap and invert Solution 1 several times to mix contents, pour **Solution 1**, invert to mix **Solution 2** and pour, pour **Solution 3**).

- Refer to page 10 for Interpretation of Results.

EXPECTED RESULTS:

- If expected results are obtained, kits are safe to use.

- If expected results are not obtained, repeat the test with a fresh specimen using a new Membrane Unit, kit components and support materials. Contact bioLytical Laboratories’ Technical Support if you are unable to produce a valid result upon repeat testing.

[8] Avoid excessive freeze/thaw cycles with the samples. Controls can be stored at ≤ -20°C (-4°F), or between 2 - 8°C (35 – 46°F) until expiry. Do not re-freeze once the vials have been opened.

AMBIENT TEMPERATURE LOG

INSTI® HIV-1/HIV-2 ANTIBODY TESTS

TESTING LOCATION:_____

DATE	TIME	TEMPERATURE (°C / °F)	CORRECTIVE ACTION/COMMENTS	STAFF INITIALS

EXTERNAL CONTROL TEST LOG

DATE	TIME	TEST KIT LOT #	TEST KIT EXPIRE DATE	NEW LOT # or SHIPMENT? Y/N	CONTROL KIT LOT #	CONTROL KIT EXPIRE DATE	DATE CONTROLS OPENED	HIV-1 POSITIVE CONTROL RESULT	HIV-2 POSITIVE CONTROL RESULT	HIV NEGATIVE CONTROL RESULT	RESULTS OK? Y/N	IF NO, CORRECTIVE ACTION TAKEN	STAFF INITIALS

INSTI® HIV-1/HIV-2 Antibody Test
CLIENT TEST RESULT LOG

Clinic Name: _____ **Date:** _____

Testing Location: _____

Client ID/Name	Counselor Code or Initials	Test Date (mm/dd/yy)	Test Performed	Lot # and Expiry Date	Test Time (AM/ PM)	Testing Location Temp (°C / °F)	Test Result (Reactive, Nonreactive, Invalid)	Date Client Received Result (mm/dd/yy)	Confirmatory Test	Confirmatory Test Result (+ / -)	Date Client Received Confirmatory Test Result