

Geenius[™] HIV 1/2 Confirmatory Assay

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A QUALITATIVE ASSAY FOR THE CONFIRMATION AND DIFFERENTIATION OF INDIVIDUAL ANTIBODIES TO HIV-1 AND HIV-2 IN WHOLE BLOOD, SERUM, OR PLASMA SPECIMENS



883601 - 2013/01



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1. INTENDED USE

The Bio-Rad Geenius™ HIV 1/2 Confirmatory Assay is a single-use immunochromatographic test for the confirmation and differentiation of individual antibodies to Human Immunodeficiency Virus Types 1 and 2 (HIV-1 and HIV-2) in fingerstick whole blood, venous whole blood, serum or plasma samples.

The Geenius™ HIV 1/2 Confirmatory Assay is intended for use as an additional test to confirm the presence of antibodies to HIV-1 and HIV-2 for specimens found to be repeatedly reactive by screening procedures.

2. SUMMARY AND EXPLANATION OF THE TEST

Discovered in 1983, the Human Immunodeficiency Virus (HIV) is a retrovirus identified as the etiologic agent for Acquired Immunodeficiency Syndrome (AIDS). AIDS is characterized by changes in the population of T-cell lymphocytes that play a key role in the immune defence system. In the infected individual the virus causes a depletion of a subpopulation of T-cells, called T-helper cells, which leaves these patients susceptible to opportunistic infections and certain malignancies. The major routes of transmission are sexual contact, contamination by blood or blood products and mother-to-newborn transmission.

At the end of 2010 there were approximately 34 million people living with HIV/AIDS worldwide, up 17% from 2001.

There were 2.7 million [2.4 -2.9] new HIV infection in 2010 including an estimated 390 000 [340 000-440 000] among children. This was 15% less than 2001 and 21% below the number of new infections at the peak of the epidemic in 1997.

While the HIV virus consists of a genomic RNA molecule protected by a capsid and an envelope, the HIV envelope is the major target for humoral antibody response. The presence of the virus in patients causes the immune system to elicit the production of antibodies to HIV. The detection of these antibodies can be used as a diagnostic tool.

The Geenius™ HIV 1/2 Confirmatory Assay is a rapid immunochromatographic test, which is simple and easy to use. The Geenius™ HIV 1/2 Confirmatory Assay utilizes immobilized antigens for the detection of antibodies to HIV-1 and HIV-2 in serum, plasma or whole blood specimens.

3. PRINCIPLE OF THE PROCEDURE

The Geenius™ HIV 1/2 Confirmatory Assay employs antibody binding protein A, which is conjugated to colloidal gold dye particles as conjugate and HIV-1 (p31, gp160, p24, gp41) and HIV-2 antigens (gp36, gp140), which are bound to the membrane solid phase. The sample is applied to the SAMPLE + BUFFER well. After the sample and buffer have migrated onto the test strip, additional buffer is added to the BUFFER well. The buffer facilitates the lateral flow of the released products and promotes the binding of antibodies to the antigens.

In a reactive sample, the anti-HIV antibodies are captured by the antigens immobilized in the TEST area (bands 1 to 6): the colloidal gold protein A binds to the captured antibodies, producing pink/purple lines.

In the absence of HIV antibodies, there are no pink/purple lines in the TEST area.

In both cases the sample continues to migrate along the membrane and produces a pink/purple line in the CONTROL (C) where protein A is immobilized.

Immunoglobulin G from sample bound to protein A is immobilized in (C) zone of the membrane solid phase to produce a pink/purple line.

This Control line serves to demonstrate that sample and reagents have been properly applied and have migrated through the device.



The Geenius™ HIV 1/2 Confirmatory Assay cassette contains a Control band (C) and six (6) test lines which are numbered on the cassette corresponding to the following:

Band 1:	gp36 (HIV-2, envelop peptide)	HIV-2 ENV
Band 2:	gp140 (HIV-2, envelop peptides)	HIV-2 ENV
Band 3:	p31 (HIV-1, polymerase peptide)	HIV-1 POL
Band 4:	gp160 (HIV-1, envelop recombinant protein)	HIV-1 ENV
Band 5:	p24 (HIV-1, core recombinant protein)	HIV-1 GAG
Band 6:	gp41 (Group M and O) (HIV-1, envelop peptides)	HIV-1 ENV
CTRL band:	Protein A	

4. REAGENTS

4.1 Description

Identification on label	Description	Presentation
Device	Nitrocellulose membrane containing HIV-1 and HIV-2 antigens in TEST area, protein A in CONTROL area and colloidal gold protein A in BUFFER well area	20 x 1 Ready for use
Buffer	Buffer dropper with preservative (sodium azide < 0.1%, gentamicin sulphate 0.125%, streptomycin sulphate 0.125%)	1 x 5 ml Ready to use
Microtubes 15 µl	15 µl Microtubes capillarity plastic pipettes (no anti-coagulant, for fingerstick protocol)	1 x 20 Ready to Use

4.2 Storage and handling requirements

The Geenius™ HIV 1/2 Confirmatory Assay (Device and Buffer) should be stored at 2°C to 30°C, until the expiration date stated on the kit.

Do not freeze. Do not open the pouch until performing a test.

The Buffer is stable until expiration date after the first use in routine.

5. WARNING AND PRECAUTIONS

For *in vitro* diagnostic use. For healthcare professional use.

5.1 Health and Safety precautions

- This test kit should be handled only by adequately qualified personnel trained in laboratory procedures and familiar with their potential hazards. Wear appropriate protective clothing, gloves and eye/face protection and handle appropriately with the requisite Good Laboratory Practices.
- The test kit contains human blood components. No known test method can offer complete assurance that infectious agents are absent. Therefore, all human blood derivatives, reagents and human specimens should be handled as if capable of transmitting infectious disease, following recommended Precautions for as defined by local, regional and national regulations.
- Biological spills: Human source material spills should be treated as potentially infectious.

Spills not containing acid should be immediately decontaminated, including the spill area, materials and any contaminated surfaces or equipment, with an appropriate chemical disinfectant that is effective for the potential biohazards relative to the samples involved (commonly a 1:10 dilution of household bleach, 70-80% Ethanol or Isopropanol, an iodophor [such as 0.5% Wescodyne™ Plus, etc.), and wiped dry.

NOTE: Do not place solutions containing bleach into the autoclave

- Dispose of all specimens and material used to perform the test as though they contain an infectious agent. Laboratory, chemical or biohazardous wastes must be handled and discarded in accordance with all local, regional and national regulations.
- For hazard and precaution recommendations related to some chemical components in this test kit, please refer to the pictogram(s) mentioned on the labels and the information supplied at the end of instruction for use. The Safety Data Sheet is available on www.bio-rad.com.

5.2 Precautions related to the procedure

5.2.1 Preparing

- Read the Product Insert completely before using this assay. Follow the instructions carefully as not doing so may result in inaccurate test results.
- Use of this test kit with sample types other than those specifically approved for use with this device may result in inaccurate test results.
- This test should be performed at 18°C to 30°C. If stored refrigerated, before use wait at least 30 min for the reagents to stabilize at room temperature.
- DO NOT USE the test device if there is no desiccant packet in the device pouch. Discard the test device and use a new device from a pouch that contains a desiccant.
- DO NOT USE the test device if the device pouch is damaged.
- Each test device is for single use only.
- Do not use the test device or kit reagent beyond their expiration dates. Always check expiration dates prior to testing.
- Do not mix reagents from different lot numbers of kits.
- Adequate lighting is required to read the test results.

- If the test kit is stored at temperatures outside the storage temperature 2°C to 30°C, or used outside the operating temperature 18°C to 30°C, use the Geenius™ HIV 1/2 Confirmatory Controls, Ref: 72329, to ensure proper performance of the test.

5.2.2 Processing

- After the closed bag has been opened, the device must be used within 60 min.
- Do not change the assay procedure.

6. SPECIMENS

The Geenius™ HIV 1/2 Confirmatory Assay can be performed on venous or fingerstick whole blood, serum or plasma samples.

6.1 Specimen types

Venous Whole Blood

Draw blood following laboratory procedure for obtaining venous blood. Collect sample in a tube containing citrate, heparin or EDTA. Be sure the tube of blood is well mixed before sampling. Use a laboratory pipette to withdraw 15 µl of the blood. Test immediately, following Test Procedure instructions.

Fingerstick Whole Blood

Clean the finger of the person being tested with an antiseptic wipe. Allow the finger to dry thoroughly or wipe dry with a sterile gauze pad. Using a sterile lancet, puncture the skin just off the center of the finger and wipe away the first drop with sterile gauze and avoid squeezing the fingertip to accelerate bleeding as this may dilute the blood with excess tissue fluid. Collect 15 µl of the sample from the second drop touching the disposable Microtube pipette provided to the drop of blood until the pipette is full, following the procedure below.

Test immediately, following Test Procedure Instructions.

Serum or Plasma

Draw blood following laboratory procedure for obtaining serum or plasma samples. Collect serum samples in clotting agent-containing tubes that do not contain any anticoagulant (serum). Collect plasma samples in tubes containing citrate, heparin, or EDTA anticoagulants. Collect sample in a clean container following standard laboratory procedures. Be sure that the tube of serum or plasma is well mixed before sampling. Use a laboratory pipette to withdraw 5 µl of the sample. Test immediately following Test Procedure instructions.

6.2 Specimen Handling

Fingerstick whole blood should be tested immediately after collection.

Venous whole blood, specimens may be tested immediately or stored at 2°C to 8°C for up to 3 days following collection before being tested.

DO NOT FREEZE WHOLE BLOOD.

Serum and plasma specimens may be tested immediately or stored at 2°C to 8°C for up to 7 days following collection before being tested.

For long-term storage, the serum and plasma specimens should be frozen (at -20°C or colder).

Samples should not be used if they have incurred more than 5 freeze-thaw cycles. Mix samples thoroughly and gently after thawing, and bring to room temperature.

No interference has been shown in samples containing up to 200 mg/l of bilirubin, or in lipemic samples containing up to 33 g/l of triolein, or in hemolyzed samples containing up to 2 g/l of hemoglobin. Abnormally high albuminemia or proteinemia (120 g/l) did not show either any interference.

6.3 Specimen Shipping

If specimens are to be shipped, they should be packed in compliance with regulations covering the transportation of etiologic agents.

Venous whole blood, specimens should be shipped refrigerated with cold packs or wet ice.

Serum and plasma specimens should be shipped frozen in dry ice.

7. PROCEDURE

7.1 Materials required

Materials provided

- Device (20 units), Buffer Dropper (1 x 5 ml) and Microtubes 15 µl (1 x 20) per kit.
- See § 4.1 Description.

Material required provided separately

- Geenius™ HIV 1/2 Confirmatory Controls, Ref: 72329.

Materials required but not provided

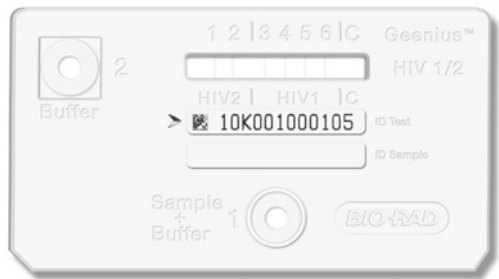
- Clock, watch or other timing device.
- Pipettor capable of delivering 5 µl (serum/plasma) and 15 µl (venous blood) of sample.
- Disposable gloves.
- Biohazard disposal containers.

7.2 Reagent preparation

All components for the Geenius™ HIV 1/2 Confirmatory Assay are ready-to-use as supplied.

7.3 Assay Procedure

Whole Blood PROCEDURE

<p>1. Remove the Geenius™ HIV 1/2 Confirmatory Assay device from its pouch and place it on a flat surface (it is not necessary to remove the desiccant from the pouch). NOTE: If desiccant packet is missing from the pouch, DO NOT USE. Discard test device and use a new test device.</p> <p>Label the test device with patient ID or identification number (see Figure 1 below). Note that the Geenius™ HIV 1/2 Confirmatory Assay device has six (6) blue coloured lines in the Test area (no blue line in Control area); If any of the 6 coloured lines are absent, DO NOT USE. Discard the test device and use a new test device.</p>	<p style="text-align: center;">Figure 1</p> 
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Venous Whole Blood

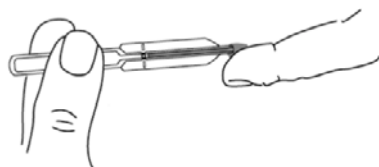
See specimen preparation on § 6.1 Specimen types.

Fingerstick Whole Blood

See specimen preparation on § 6.1 Specimen types.

Step 1:

Hold the 15µL Microtube horizontally and touch the blood drop with the tip. Capillary action will automatically draw the sample to the fill line and stop.



Step 2:

To expel the sample, align the tip of the tube with the sample target and squeeze the bulb. If a sample won't expel, hold the tube vertically and slide a finger over the vent hole. Then align the tip with the sample target and squeeze the bulb.



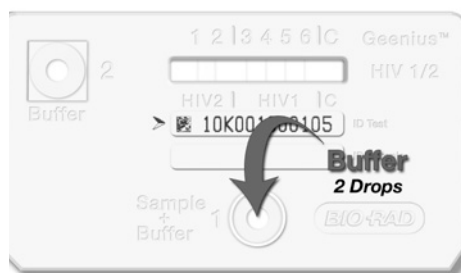
2. Dispense 15 µl of whole blood to the centre of the SAMPLE + BUFFER Well 1 of the device (see Figure 2 below).
For venous whole blood use a laboratory pipette.
For Fingerstick whole blood, follow the protocol using the Microtube 15 µl of the kit (see step 1 and 2 above).

Figure 2



3. **Immediately** following the addition of the sample, use the Buffer dropper to **add 2 drops** (60 µl) of Buffer, into the SAMPLE + BUFFER Well 1 (see Figure 3 below).

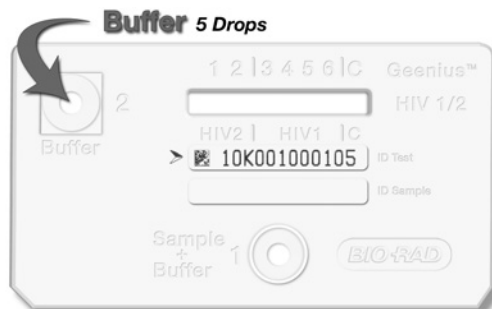
Figure 3



4. **Wait 5-7 minutes** the 6 blue coloured TEST lines should have disappeared from the rectangular TEST area. If not, discard the test device and repeat the procedure with a new test device. **NOTE:** A slight bluish-greenish colour may remain on the membrane, but none of the actual coloured lines should be seen at this point.

Use the Buffer dropper to **add 5 drops** (150 µl) of Buffer into BUFFER Well 2 (see Figure 4 below).

Figure 4



5. Read the test results between 20 to 30 minutes after the addition of the Buffer to BUFFER Well 2

Do not read results after 30 minutes

Read results in a well-lit area.

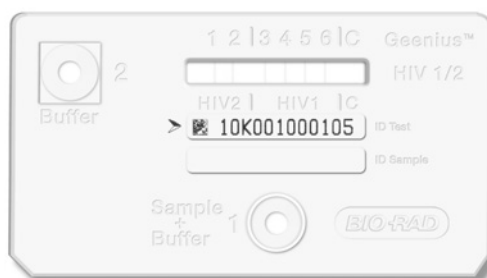
NOTE: Discard the used pipette tips, test device and any other test materials into a biohazard container.

Serum or Plasma PROCEDURE

See specimen preparation on § 6.1 Specimen types.

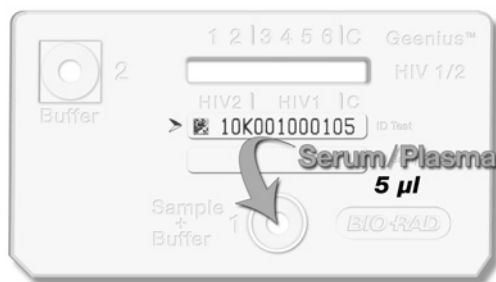
1. Remove the Geenius™ HIV 1/2 Confirmatory Assay device from its pouch and place it on a flat surface (it is not necessary to remove the desiccant from the pouch). **NOTE:** If desiccant packet is missing from the pouch, DO NOT USE. Discard test device and use a new test device. Label the test device with patient ID or identification number (see Figure 1 below). Note that the Geenius™ HIV 1/2 Confirmatory Assay device has six (6) blue coloured lines in the Test area (no blue line in Control area); If any of the 6 coloured lines are absent, DO NOT USE. Discard the test device and use a new test device.

Figure 1



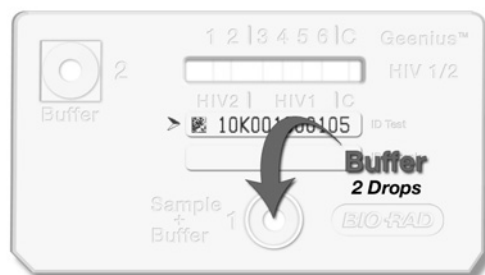
2. Using a laboratory pipette, dispense 5 µl of serum/plasma to the centre of the SAMPLE + BUFFER Well 1 of the device (see Figure 2 below).

Figure 2



3. Immediately following the addition of the sample, use the diluent dropper bottle to **add 2 drops** (60 µl) of Buffer into the SAMPLE + BUFFER Well 1 (see Figure 3 below).

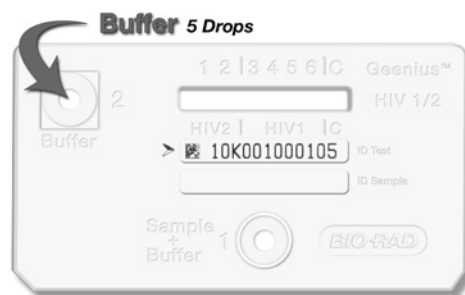
Figure 3



4. **Wait 5-7 minutes.** All the 6 blue coloured TEST lines should have disappeared from the rectangular TEST area. If not, discard the test device and repeat the procedure with a new test device. NOTE: A slight bluish-greenish colour may remain on the membrane, but none of the actual coloured lines should be seen at this point.

Use the diluent dropper bottle to **add 5 drops** (150 µl) of Buffer into BUFFER Well 2 (see Figure 4 below).

Figure 4



5. Read the test results between 20 to 30 minutes after the addition of the Buffer to BUFFER Well 2

Do not read results after 30 minutes

Read results in a well-lit area.

NOTE: Discard the used pipette tips, test devices and any other test materials into a biohazard container.

7.4 Quality Control

7.4.1 Built-in Control Feature

The control line serves as a built-in internal control and gives confirmation of sample addition and proper test performance. A pink/purple line will appear in the CONTROL (C) area if the test has been performed correctly and the device is working properly (Please see: Interpretation of Test Results).

7.4.2 External Quality Control

Geenius™ HIV 1/2 Confirmatory Controls, Ref: 72329 is available separately for use with the Geenius™ HIV 1/2 Confirmatory Assay.

It is recommended to perform the Geenius™ HIV 1/2 Confirmatory Controls under the following circumstances:

- When opening a new test kit lot.
- Whenever a new shipment of test kits is received.
- If the temperature of the test storage area falls outside of 2°C to 30°C.
- If the temperature of the testing area falls outside of 18°C to 30°C.
- At periodic intervals as indicated by the user facility.

7.5 Test Validation criteria

BAND reactivity

All visible bands. Even a faint band must be considered as reactive.

Validation criteria

VALID:

A test is valid only if a pink/purple line appears in the CONTROL (C) area, whether or not a line appears in the TEST line area.

(The Control Band must be strong: a faint band is not acceptable for the Control Band)

INVALID:

If there is no distinct pink/purple line visible (including a faint band) in the CONTROL (C) area, then the test is INVALID.

An INVALID test cannot be interpreted. It is necessary to repeat sample testing with a new device.

7.6 Interpretation of the Results

The following definitions describe the criteria employed by the Geenius™ HIV 1/2 Confirmatory Assay to determine the presence or absence of antibodies to HIV-1 and/or HIV-2.

The user subsequently analyzes the combined type specific band profiles for each assay according to the criteria listed in the Interpretation of Results Table below.

7.6.1 Interpretation criteria

HIV-1 Interpretation criteria

Interpretation	Bio-Rad criteria
POSITIVE	Any 2 bands of the 4 HIV-1 test lines with at least 1 ENV - gp160 (Band 4) or gp41 (Band 6)
NEGATIVE	No Band
INDETERMINATE	1ENV (Band 4 or 6) 1GAG (Band 5) 1POL (Band 3) 1GAG and 1POL (Bands 5 and 3)

HIV-2 Interpretation criteria

Interpretation	Bio-Rad criteria
POSITIVE	2 HIV-2 bands must be present: gp36 and gp140 (Band 1 and 2)
NEGATIVE	No Band

INDETERMINATE	1 ENV: gp36 (Band 1) or gp140 (Band 2) gp36 (Band 1) alone gp140 (Band 2) alone
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GLOBAL HIV-1/HIV-2 Interpretation criteria

The following Interpretation of Results table describes the criteria employed by the Geenius™ HIV 1/2 Confirmatory Assay to interpret the combined type specific band patterns observed for each assay.

HIV-2 RESULT	HIV-1 RESULT	GLOBAL ASSAY INTERPRETATION
Negative	Negative	HIV NEGATIVE
Indeterminate	Negative	HIV-2 INDETERMINATE
Negative	Indeterminate	HIV-1 INDETERMINATE
Indeterminate	Indeterminate	HIV INDETERMINATE
Negative	Positive	HIV-1 POSITIVE
Indeterminate	Positive	HIV-1 POSITIVE
Positive	Negative	HIV-2 POSITIVE
Positive	Indeterminate	HIV-2 POSITIVE
Positive	Positive case 1 = 1 ENV HIV-1 (gp 160 or gp41) + GAG or POL case 2 = 2 ENV HIV-1 (gp 160 and gp41) +/- GAG and/or +/-POL	HIV-2 POSITIVE (with HIV-1 cross-reactivity) HIV POSITIVE UNTYPABLE

8. TEST LIMITATION

8.1 General Limitations

1. Visual reading can introduce some variability in the final conclusion between two different technicians or two different tests: this difference may be linked to the subjectivity of the visual interpretation.
2. For a reactive result, the intensity of the test lines does not necessarily correlate with the titer of antibody in the sample.
3. A person who is confirmed HIV-1 Positive or HIV-2 Positive is presumed to be infected with the virus, except that a person who has participated in an HIV vaccine study may develop antibodies to the vaccine and may or may not be infected with HIV.
4. Individuals infected with HIV-1 and/or HIV-2 who are receiving highly active antiretroviral therapy (HAART) may produce false negative results.
5. The variability of HIV-1 (group M and group O) and HIV-2 viruses does not exclude the possibility of false negative reactions. No known test method can offer complete assurance that the HIV virus is absent.
6. A nonreactive result does not preclude the possibility of exposure to HIV or infection with HIV. An antibody response to a recent exposure may take several months to reach detectable levels. A positive screening test associated with a negative confirmatory test may occur during the first stage of infection; hence, a negative result indicates that the tested sample does not contain anti-HIV antibodies detectable with Geenius™ HIV 1/2 Confirmatory Assay. Such a result does not, however, exclude the possibility of a recent HIV-1/HIV-2 infection. A new sample should be tested later.
7. An indeterminate result does not preclude the possibility of exposure to HIV or infection with HIV. An antibody response to a recent exposure may take several months to reach detectable levels. A positive screening test associated with an indeterminate confirmatory test may occur during the first stage of infection; hence, an indeterminate result indicates that the tested sample may contain anti-HIV antibodies detectable with Geenius™ HIV 1/2 Confirmatory Assay. Such a result does not, however, exclude the possibility of a recent HIV-1/HIV-2 infection. A new sample should be tested later.
8. The Geenius™ HIV 1/2 Confirmatory Assay is intended as an aid in the diagnosis of infection with HIV-1 and or HIV-2. HIV and AIDS related conditions are clinical syndromes and their diagnosis can only be established clinically.
9. The Geenius™ HIV 1/2 Confirmatory Assay must ONLY be used with capillary blood, whole venous blood, serum or plasma. Using other types of samples or testing of venipuncture whole blood samples collected using a tube containing an anticoagulant other than citrate, heparin or EDTA may not yield accurate results.
10. The Geenius™ HIV 1/2 Confirmatory Assay must be used in accordance with the instructions in this product insert to obtain accurate results.
11. Reading test results earlier than 20 minutes or later than 30 minutes since the addition of Running Buffer to BUFFER Well 2 may yield erroneous results.

8.2 Assay Interpretation limitations

An “indeterminate” profile does not exclude one of the following situations: seroconversion, or a cross-reaction with other retroviruses. The homology between HIV-1 and HIV-2 viruses can lead to cross reactivity between both anti-HIV-1 and anti-HIV-2 antibodies against HIV-2 and HIV-1 viruses.

Samples which meet HIV-1 positive criteria show in very rare cases some cross reactivity on one of the HIV-2 Envelop bands. Nevertheless, such rare profile of single HIV-1 infection does not also exclude in very rare cases the possibility of a secondary HIV-2 seroconversion (surinfection).

Samples which meet HIV-2 positive criteria can show cross reactivity on one or more HIV-1 bands. In most of the cases, an HIV-1 indeterminate profile associated to an HIV-2 positive

profile confirms a single HIV-2 infection. However it does not exclude the possibility of a secondary HIV-1 seroconversion (surinfection).

Samples that meet both HIV-1 and HIV-2 positive criteria are generally HIV-2 positive samples which show HIV-1 cross reactivity when they have only one detected envelop band (gp160 or gp41). Such profiles do not exclude the rare possibility of HIV-1-HIV-2 coinfection.

HIV Untypable samples with all 4 envelop bands detected (all of the HIV-1 env and HIV-2 env) are in most of the cases HIV-2 positive samples with HIV2 reactivity that cannot be visually differentiated from HIV-1 reactivity. Such profiles do not exclude the possibility of HIV 1/2 coinfection.

Samples which meet both HIV-1 and HIV-2 positive criteria are in very rare cases HIV-1 positive samples which show HIV-2 cross-reactivity.

9. PERFORMANCES CHARACTERISTICS

9.1 Precision Study

A precision panel (N=6) made of 3 serum and 3 whole blood samples of different HIV status (HIV negative, HIV-1 positive, HIV-2 positive) was tested. For each precision study and panel member, an agreement percentage was determined as the number of responses correctly identified compared to the sample status.

9.1.1 Repeatability

Precision panel was tested in 10 replicates during the same run. Repeatability measurement was found with agreements of 100% for HIV negative, HIV-1 positive and HIV-2 positive.

Panel member	Repeatability results for Serum					Repeatability results for Whole blood				
	N	NEG	IND	POS	agreement	N	NEG	IND	POS	agreement
HIV NEG	10	10	0	0	100%	10	10	0	0	100%
HIV-1 POS	10	0	0	10	100%	10	0	0	10	100%
HIV-2 POS	10	0	0	10	100%	10	0	0	10	100%

9.1.2 Intermediate precision

Run and Day precision

Serum precision panel was tested in duplicate per run, with 2 runs per day during 10 days and whole blood precision panel in triplicate per run, with 2 runs per day during 3 days. A run-to-run and day-to-day precision was found with agreements of 100% for HIV negative, HIV-1 positive and HIV-2 positive samples.

Panel member	Run and day precision results for Serum					Run and day precision results for Whole blood				
	N	NEG	IND	POS	agreement	N	NEG	IND	POS	agreement
HIV NEG	40	40	0	0	100%	18	18	0	0	100%
HIV-1 POS	40	0	0	40	100%	18	0	0	18	100%
HIV-2 POS	40	0	0	40	100%	18	0	0	18	100%

Lot and Operator precision

Precision panel was tested in duplicate on 2 lots of reagent and by 3 operators with 1 run per day during 3 days. An inter-operator and inter-batch precision was found with agreements of 100% for HIV negative, HIV-1 positive and HIV-2 positive samples.

Panel member	Lot and Operator precision results for Serum					Lot and Operator precision results for Whole blood				
	N	NEG	IND	POS	Agreement	N	NEG	IND	POS	Agreement
HIV NEG	36	36	0	0	100%	36	36	0	0	100%
HIV-1 POS	36	0	0	36	100%	36	0	0	36	100%
HIV-2 POS	36	0	0	36*	100%	36	0	0	36	100%

* 2 replicates gave HIV-1 cross reactivity

9.2 Clinical performance

9.2.1 Diagnostic Specificity

Blood donors

A total of 400 specimens (serum, plasma and venous blood) drawn from 300 non selected known and first time donors, were tested on the Geenius™ HIV 1/2 Confirmatory Assay in a blood bank site. 398 specimens tested negative and 2 tested indeterminate. Indeterminate results representing 0.5% (2/400) of total specimens have not been considered as false positive. Overall specificity (true negative/ true negative + false positive) on the 398 specimens was 100.0% (398/398) with a confidence interval at 95% of [99.1; 100.0].

Specificity on blood Donors	Total number specimens	Negative	Indeterminate	Positive	Specificity (%)	95 CI (%)
Serum (SSTII Gel sep)	100	98	2 (**)	0	100.0 (98/98)	[96.3 - 100.0]
Plasma (*) (EDTA-K2)	100	100	0	0	100.0 (100/100)	[96.4 - 100.0]
Whole venous blood (EDTA-K2)	200	100	0	0	100.0 (200/200)	[98.2 - 100.0]
TOTAL 300 donors	400	398	2 (**)	0	100.0 (398/398)	[99.1 - 100.0]

(*) specimens of plasma paired to whole venous blood samples obtained from the same 100 donors

(**) Indeterminate results have not been considered as false positive / further investigation is needed

Hospitalized patients and pregnant women

A total of 508 specimens from 326 hospitalized patients were tested on the Geenius™ HIV 1/2 Confirmatory Assay at 2 different sites. Among these patients, 99 had serum sampling alone, 100 had whole blood sampling alone, 72 had both serum and whole blood sampling, 30 patients had both serum, plasma and whole blood sampling, and 25 had serum, plasma and capillary blood sampling. 30 serum from pregnant women from 2 sites were also tested.

Whole venous blood and plasma samples were collected on EDTA-K2 tubes and serum on SSTII with gel separator tubes. No anticoagulant was used for capillary blood collection.

529 specimens tested negative and 9 tested indeterminate. Indeterminate results representing 1.7% (9/538) of total specimens have not been considered as false positive. Overall specificity (true negative/ true negative + false positive) on the 529 specimens was 100.0% (529/529) with a confidence interval at 95% of [99.3; 100.0].

Site	Patients	Fresh Serum /SSTII Gel	Fresh Plasma /EDTA-K2	Fresh venous blood /EDTA-K2	Fresh Capillary blood	Total specimens	Pregnant women (frozen serum)	GrandTotal specimens
Site 1	99	99	/	/	/	99	10	109
Site 2	227	/	/	100	/	100	/	100
		72	/	72	/	144	/	144
		30	30	30	/	90	/	90
		25	25	/	25	75	/	75
Site 5		/	/	/	/		20	20
Total	326	226	55	202	25	508	30	538
Negative		221	54	201	25	501	28	529
Indeterminate	/	5(*) (***)	1(*) (***)	1(*) (***)	0	7 (***)	2 (***)	9 (***)
Positive		0	0	0	0	0	0	0
Specificity (%)	/	100.0 (221/221)	100.0 (54/54)	100.0 (201/201)	100.0 (25/25)	100.0 (501/501)	100.0 (28/28)	100.0 (529/529)
95 CI (%)	/	[98.3 - 100.0]	[93.4 - 100.0]	[98.2 - 100.0]	N/A(**)	[99.3 - 100.0]	N/A(**)	[99.3 - 100.0]

(*) 1 patient had 1 indeterminate result for both serum, venous blood and plasma

(**) not applicable with N<30 population

(***) Indeterminate results have not been considered as false positive / further investigation is needed

Blood donors giving false positive results at screening

A total of 275 serum specimens drawn from blood donors giving false positive results with HIV ELISA screening assays, were tested on the Geenius™ HIV 1/2 Confirmatory Assay at two clinical sites. 258 specimens tested negative and 17 tested indeterminate. Indeterminate results representing 6.2% (17/275) of total specimens have not been considered as false positive. Overall specificity (true negative/ true negative + false positive) on the 258 specimens was 100.0% (258/258) with a confidence interval at 95% of [98.6; 100.0].

Specificity on blood Donors	Total number specimens	Negative	Indeterminate	Positive	Specificity (%)	95 CI (%)
TOTAL 275 donors	275	258	17 (*)	0	100.0 (258/258)	[98.6 - 100.0]

(*) Indeterminate results have not been considered as false positive / further investigation is needed

9.2.2 Diagnostic Sensitivity

HIV-1 infected patients

A total of 599 specimens from 263 patients confirmed as HIV-1 infected from 2 sites (155 patients at site 1 and 108 patients at site 2) were tested on the Geenius™ HIV 1/2 Confirmatory Assay

On 1 site,, 108 fresh serum and paired plasma , 5 fresh serum and 50 genotyped HIV-1 strains (2 CRF01, 5 CRF02, 1 CRF05, 1 CRF06, 2 CRF09, 1 CRF11, 1 CRF12, 1 CRF13, 1 CRF14, 1 CRF15, 1 CRF18, 1 CRF19, 1 CRF22, 1 CRF27, 1 CRF30, 1 CRF36, 1 CRF42, 4 subtype A, 5 subtype B, 2 subtype C, 2 subtype D, 2 subtype F, 2 subtype G, 2 subtype H, 2 subtype J, 1 subtype K, 5 group O) samples were tested.

On the second site, among the 108 patients, 82 had whole blood, serum and plasma samplings, 20 had both whole blood, capillary whole blood, serum and plasma samplings, and 6 had capillary whole blood, serum and plasma sampling.

Whole venous blood and plasma samples were collected on EDTA-K2 tubes and serum samples on SSTII with gel separator tubes.

All the 599 specimens tested HIV-1 positive, leading to an overall sensitivity of 100.0% (599/599) with a confidence interval at 95% of [99.4 - 100.0].

HIV-1 sensitivity on patients was 100% (263/263).

On the total of 599 specimens, 3 specimens were found HIV untypable instead of HIV-1 positive, therefore HIV-1 differentiation capacity of the Geenius™ HIV 1/2 Confirmatory Assay was 99.5% (596/599) with a confidence interval at 95% of [98.5 - 99.9].

Site	Patients	Fresh Serum (SSTII Gel)	Genotyped serum	Fresh Plasma (EDTA-K2)	Fresh venous blood (EDTA-K2)	Fresh Capillary blood	Total specimens
Site 1 N= 155	100 5 50	/ 5 /	/ / 50	100 / /	100 / /	/ / /	200 5 50
Site 2 N= 108	82 20 6	82 20 6	/ / /	82 20 6	82 20 /	/ 20 6	246 80 18
Total	263	113	50	208	202	26	599
HIV-1 Positive		113	49	207	201	26	
HIV untypable		0	1	1(*)	1(*)	0	
Sensitivity (%)		100.0 (113/113)	100.0 (50/50)	100.0 (208/208)	100.0 (202/202)	100.0 (26/26)	100.0 (599/599)
95 CI (%)		[97.8 - 100.0]		[98.2 - 100.0]	[98.2 -100.0]	N/A(**)	[99.4 - 100.0]

(*) specimens of plasma paired to venous blood sample obtained from the same HIV-1 infected patient

(**) not applicable with N<30 population

HIV-2 infected patients

A total of 283 specimens from 172 patients confirmed as HIV-2 infected (serum, plasma, venous blood and capillary blood with some paired samples drawn from the same patients) were tested on the Geenius™ HIV 1/2 Confirmatory Assay at three clinical sites. 66 serum specimens were obtained from two clinical sites samples collections. All others specimens were freshly obtained from patients. Whole venous blood and plasma samples were collected on EDTA-K2 or EDTA-K3 tubes and serum collected samples on SSTII with gel separator or dry tubes.

281 specimens tested HIV positive and 2 tested HIV-2 indeterminate. The two HIV-2 indeterminate results (gp140 not detected) were obtained on serum and whole blood drawn from the same patient found gp105 negative with a CE-marked HIV I/II confirmation assay. Indeterminate results representing 0.7% (2/283) of total specimens have not been considered as false negative. Overall sensitivity (true positive/ true positive + false negative) on the 281 specimens was 100.0% (281/281) with a confidence interval at 95% of [98.7; 100.0].

172 specimens over 283 were correctly found HIV-2 positive (with or without cross HIV-1 reactivity) or HIV-2 indeterminate and 111 HIV untypable, therefore HIV-2 differentiation capacity of the Geenius™ HIV 1/2 Confirmatory Assay was 60.8% (172/283) with a confidence interval at 95% of [54.8 - 66.5].

Sites	Patients	Fresh Serum (SSTII Gel)	Frozen serum	Fresh Plasma (EDTA-K2)	Fresh venous blood (EDTA-K2 or K3)	Fresh Capillary blood	Total specimens
Site 1	5 16	5 /	/ 16	5 /	2 /	3 /	15 16
Site 2	50	/	50	/	/	/	50
Site 3	101	101	/	/	101	/	202
Total	172	106	66	5	103	3	283
HIV-2 Positive	/	33	15	3	28	3	82
HIV-2 positive with HIV-1 reactivity		33	19	1	35	0	88
HIV untypable		39	32	1	39	0	111
Indeterminate		1(*)(***)	0	0	1(*)(***)	0	2 (***)
Sensitivity (%) 95 CI (%)		100.0 (171/171) [97.9 - 100.0]		100.0 (5/5) N/A**	100.0 (102/102) [96.5 - 100.0]	100.0 (3/3) N/A**	100.0 (281/281) [98.7 - 100.0]

(*) specimens of serum paired to venous blood sample obtained from the same HIV-2 infected patient

(**) not applicable with N<30 population

(***) Indeterminate results have not been considered as false negative / further investigation is needed

HIV-1/HIV-2 co-infected patients

A total of 22 specimens from 15 patients confirmed as HIV-1/ HIV-2 coinfectd (13 serum, 2 plasma and 7 paired whole venous blood drawn from same 7 patients) were tested on the Geenius™ HIV 1/2 Confirmatory Assay at two clinical sites. Six serum and 2 plasma specimens were obtained from one clinical samples collection site and the seven paired serum-whole venous blood were freshly obtained from another clinical site patients.

Overall sensitivity was 100% (22/22) (serum and whole venous blood) without indeterminate results.

At the first intent, all specimens were correctly found HIV untypable (HIV-1 positive with two envelopes detection and HIV-2 positive), except one whole blood and one serum specimens. Whole blood was improperly found HIV-2 positive due to recent surinfection. After recall few weeks later, this patient was correctly found HIV untypable. Serum was improperly found HIV-2 positive with HIV-1 reactivity instead of HIV untypable but was also improperly found HIV-2 positive (without HIV-1 reactivity) on several CE-marked HIV differentiation assays. 21 over 22 specimens were correctly found HIV untypable after one patient recall. Therefore, HIV-1+2 differentiation capacity of the Geenius™ HIV 1/2 Confirmatory Assay was 95.5% (21/22).

HIV-1 seroconversion samples

Sensitivity of Geenius™ HIV1/2 Confirmatory Assay has been estimated with 32 seroconverter panels (154 samples). 41.6% (64/154) were positive with Geenius™ HIV1/2 Confirmatory Assay, meanwhile 12.3% (19/154) were positive with a CE-marked Western Blot assay. The detection of the first positive bleed point was in average earlier of 1.4 (44/32) time-points per panel with Geenius™ HIV 1/2 Confirmatory Assay.

When testing 83 early-seroconversion samples (negative or indeterminate by Western Blot), 10.8% (9/83) were positive with Geenius™ HIV1/2 Confirmatory Assay.

Based on 10 seroconversion samples tested in a clinical site and comparison to the same reference Western blot assay, Geenius™ HIV1/2 Confirmatory Assay was more sensitive for the detection of antibodies to gp41 and had a similar sensitivity for the detection of antibodies to gp160.

Geenius™ HIV1/2 Confirmatory Assay complies with the state of art in term of sensitivity estimated with HIV seroconversion panels.

9.3 Analytical Specificity

9.3.1 Cross Reactivity

251 potentially cross-reacting samples representing 29 different diseases/ states testing positive for the following markers were tested on the Geenius™ HIV 1/2 Confirmatory Assay in different clinical sites.

HTLV I/ II (20), Hepatitis C (10 HCV), Hepatitis B (10 anti-HBS) and Hepatitis A (10 HAV IgG); Cytomegalovirus (10 CMV IgG), Epstein-Barr (10 EBV IgG), Herpes Simplex (10 HSV), Rubella IgG (10), Toxoplasmosis IgG (5), Syphilis IgG (10), Candida (10), Malaria (26), Dengue (2), Leishmaniosis (2), Vaccinia (10), Influenza vaccine (5 Flu), Dialysis (10), HAMA (10), Rheumatoid factor (10), Multi-transfusion (10), Myeloma (5) Hemophiliac (10), Autoimmune as Systemic Lupus Erythemateous (12 SLE), Scleroderma (2), Sjogrens (2), Mixed connective tissue (2 MCTD), anti-nuclear antibody (3 ANA), Cancer (5), Cirrhosis (5) and Multipareous women (5).

Over the total 251 difficult samples, 245 specimens tested negative and 6 specimens tested indeterminate (they were indeterminate with HIV-1 Western-Blot and positive for Malaria). Indeterminate results representing 2.4% (6/251) of total specimens have not been considered as false positive. Overall specificity (true negative/ true negative + false positive) was 100.0% (245/245) with a confidence interval at 95% of [98.5 - 100.00].

9.4 Hook effect

Possible hook effect was studied by testing 2 HIV-1 and 2 HIV-2 high titer specimens, neat and diluted. Neither negative or lower intensity results were observed with the neat high titer HIV-1 and HIV-2 positive specimens, when compared to their more diluted forms (1:10 to 1:100000). The equivalence of results between non diluted and diluted samples shows the absence of hook effect.

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