Geenius™ HIV 1/2 Supplemental Assay

Instructions For Use

A Qualitative Assay for the Confirmation and Differentiation of Individual Antibodies to HIV-1 and HIV-2 in Whole Blood, Serum, or Plasma Specimens.

For *In Vitro* Diagnostic Use 72461 • 20 Tests

CONTENTS

These Instructions For Use must be read completely before performing the test. Failure to follow these instructions may give inaccurate test results. Users of this test should follow the CDC Universal Precautions for prevention of transmission of human immunodeficiency virus, hepatits B virus, and other bloodborne pathogens.[1]

1 - INTENDED USE

The Geenius™ HIV 1/2 Supplemental Assay is a single-use immunochromatographic assay 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 (EDTA, heparin, and sodium citrate).

The Geenius HIV 1/2 Supplemental Assay is intended for use as an aid in the diagnosis of infection with HIV-1 and/or HIV-2. It is intended for use as an additional, more specific test to confirm the presence of antibodies to HIV-1 and HIV-2 for specimens found to be repeatedly reactive by diagnostic screening procedures. The assay may also be used to confirm the presence of antibodies to HIV-1 and/or HIV-2 in pediatric subjects (i.e., children as young as 2 years of age).

The results of the Geenius HIV 1/2 Supplemental Assay are read and interpreted only by the Geenius Reader with dedicated software.

RESTRICTIONS

- Sale of the Geenius™ HIV 1/2 Supplemental Assay is restricted to clinical laboratories that have an adequate quality assurance program, including planned systematic activities to provide adequate confidence that requirements for quality will be met and where there is assurance that operators will receive and use the instructional materials.
- The Geenius™ HIV 1/2 Supplemental Assay is approved for use only by an agent of a clinical laboratory.
- The Geenius™ HIV 1/2 Supplemental Assay is not approved for testing of specimens from blood, plasma, cell, or tissue donors that are repeatedly reactive on HIV-1/2 donor screening assays.

CLIA COMPLEXITY: Moderate

2 - SUMMARY AND EXPLANATION OF THE TEST

Acquired immunodeficiency syndrome (AIDS) is caused by viruses transmitted by sexual contact, exposure to blood (including sharing contaminated needles and syringes) or certain blood products, or transmitted from an infected mother to her fetus or child during the perinatal period.[2] Additionally, transmission of these viruses can occur through tissue transplantation.[3] Human Immunodeficiency Virus Type 1 (HIV-1) has been isolated from patients with AIDS and AIDS-related complex (ARC).[4-6] HIV-1 was thought to be the sole causative agent of these syndromes until 1986, when a second type of Human Immunodeficiency Virus (HIV-2) was isolated and also reported to cause AIDS.[7-8] Since the initial discovery, hundreds of cases of HIV-2 infection have been documented worldwide, including cases of AIDS related to HIV-2.[9] In the United States, there have been more than 80 cases of infection with HIV-2 reported, including three potential blood donors.[10-16]

This second immunodeficiency virus is similar to, but distinct from, HIV-1. Both viruses have similar morphology and lymphotropism,[16] and the modes of transmission appear to be identical.[9,18] The HIV-1 and HIV-2 genomes exhibit about 60% homology in conserved genes such as gag and pol,

and 39-45% homology in the envelope genes.[19] Serologic studies have also shown that the core proteins of HIV-1 and HIV-2 display frequent cross-reactivity whereas the envelope proteins are more type-specific.[20]

Within the two major HIV types, there is significant variation, as well. By analyzing sequences of representative strains, HIV-1 has been divided into four groups: group M (for major), including at least 9 subtypes, 3 sub-subtypes of A, and 2 sub-subtypes of F (A1, A2, A3, B, C, D, F1, F2, G, H, J, and K); group O (for outlier); group N (for non-M, non-O), and group P.[21-25] Similarly, the HIV-2 strains have been classified into at least five subtypes (A through E).[26] Some HIV-1 variants share ≤50% homology in their envelope genes with the sequences of more common prototype strains.

Despite some degree of immunological cross-reactivity between types and subtypes of HIV, reliable detection of the more divergent strains may only be achieved by incorporating specific sequences into the assay design. In one study, detection of HIV-2 positive samples by licensed HIV-1 antibody kits ranged from 60% to 91%, depending on the test used.[27] Detection of HIV-1 Group O samples by HIV-1 and HIV-1/HIV-2 assays varied from 0% to 100% in studies with U.S.-licensed and European test kits.[28,29]

The Geenius HIV 1/2 Supplemental Assay is an immunochromatographic test that incorporates highly conserved recombinant proteins and synthetic peptides representing HIV-1 and HIV-2 proteins. The Geenius HIV 1/2 Supplemental Assay is simple and easy to use for the detection and differentiation of individual antibodies to HIV-1 and HIV-2 in serum, plasma or whole blood.[36]

The Geenius HIV 1/2 Supplemental Assay can be used in accordance with current CDC recommendations for Laboratory Testing for the Diagnosis of HIV Infection.[38] Per the CDC recommended algorithm, specimens initially reactive on a 4th generation HIV assay should undergo supplemental testing with an immunoassay that differentiates HIV-1 from HIV-2 antibodies.

3 – BIOLOGICAL PRINCIPLES OF THE TEST

The Geenius HIV 1/2 Supplemental Assay cassette contains antibody binding protein A, which is conjugated to colloidal gold dye particles, and HIV-1 and HIV-2 antigens, 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 causes the specimens and reagents to flow laterally and facilitates the binding of antibodies to the antigens. In a reactive sample, the antibodies are captured by the antigens immobilized in the Test area.

The protein A-colloidal gold binds to the captured antibodies, causing development of pink/purple lines. When there are no HIV antibodies, there are no pink/purple lines in the Test area. The sample continues to migrate through the membrane and a pink/purple line develops in the control (C) area, which contains Protein A. This built-in procedural control provides evidence that the test was performed properly and that the sample and reagents have migrated through the cassette.

4 - REAGENTS

Component	Description	Preparation
Cassette (20)	Cassette with nitrocellulose membrane containing HIV-1 and HIV-2 antigens in Test area, protein A in Control area and protein A-colloidal gold conjugate in Buffer well area	Ready to use
Buffer (5 ml)	Diluent with preservative (< 0.1% sodium azide, 0.125% gentamicin sulfate and 0.125% streptomycin sulfate)	Ready to use
Microtubes (20 pipettes)	15µL Microtubes - Capillary plastic pipettes (no anti-coagulant)	Ready to use

Storage: Store kit at 2 to 30°C (36 to 86°F)

5 – WARNINGS FOR USERS

For *In Vitro* Diagnostic Use

1. These Instructions For Use must be read completely before performing the test. Failure to follow these instructions may give inaccurate test results.
2. Use of this test kit with sample types other than those specifically approved for use with this device may result in inaccurate test results.
3. This test should be performed at room temperature (18 to 30°C, 64 to 86°F). If the pouch is stored refrigerated, bring it to room temperature before use.
4. In the event that the test kit is stored at temperatures outside the temperature range of 2 to 30°C (36 to 86°F), the Geenius HIV 1/2 Controls (Ref: 72339) should be used to ensure the assay is performing properly. (Note that if this occurs, the Geenius HIV 1/2 Controls should be included in every test run that is performed using test kit lots that have been stored in that area)

6 – PRECAUTIONS FOR USERS

Safety Precautions

1. Handle the samples and materials contacting samples as if capable of transmitting infection.
2. Wear protective clothing, including lab coat, eye/face protection and disposable gloves (synthetic, non-latex gloves are recommended) while handling kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
3. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
4. 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, EPA Registration #4959-16-52], or a phenolic, etc.), and wiped dry.[30-33]

   Spills containing acid should be appropriately absorbed (wiped up) or neutralized, the area flushed with water and wiped dry; materials used to absorb the spill may require biohazardous waste disposal. Then the area should be decontaminated with one of the

chemical disinfectants.

NOTE: DO NOT PLACE SOLUTIONS CONTAINING BLEACH INTO THE AUTOCLAVE.

5. 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.
6. For additional information refer to: Centers for Disease Control (CDC): Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HIV and Recommendations for Post exposure Prophylaxis[34].

Handling Precautions

1. The Geenius™ HIV 1/2 Supplemental Assay Cassette is for single use only.
2. Do not use the test cassettes or kit reagent beyond their stated expiration dates.
3. Do not use the test cassette if the cassette pouch does not contain a desiccant packet. Discard the test cassette and use a new cassette from a pouch that contains a desiccant.
4. Do not use any test cassette if its pouch has been perforated. Do not open the cassette's sealed foil pouch until just prior to use
5. Do not mix components from different lot numbers of kits.

7 – REAGENT PREPARATION AND STORAGE

All components of the Geenius HIV 1/2 Supplemental Assay are ready to use as supplied. The Geenius HIV 1/2 Supplemental Assay cassettes and Buffer should be stored at 2 to 30°C. If the samples and / or kit components have been refrigerated, bring to room temperature (18 to 30º C) prior to testing.

Do not open cassette pouches until performing a test. Do not freeze pouches. The Buffer should not be removed from its original bottle. When stored as indicated, test cassettes and reagent are stable until their printed expiration dates. Do not use beyond the stated expiration date.

8 – SPECIMEN COLLECTION, PREPARATION, AND STORAGE

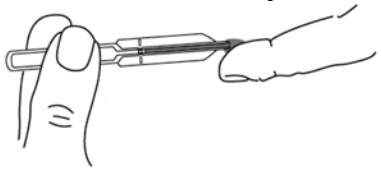
The Geenius HIV 1/2 Supplemental Assay can be performed on venous or fingerstick whole blood, serum, or plasma samples.

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. Avoid squeezing the fingertip to accelerate bleeding as this may dilute the blood with excess tissue fluid. Collect 15µL of the sample from the second drop, touching the disposable Microtube pipette provided to the drop of blood until the pipette is full. Follow the procedure below.

Step 1:

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



Step 2:

Fingerstick whole blood should be tested immediately after collection.

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 + Buffer well and squeeze the bulb.

Perform the test following the Assay Procedure instructions below.

Venous Whole Blood

Draw blood following laboratory procedure for obtaining venous blood. Collect the blood in a tube containing EDTA, heparin or sodium citrate. Be sure the tube of blood is well mixed before sampling. Use a laboratory pipette to withdraw 15µL of the blood. Perform the test following the Assay Procedure instructions below.

DO NOT FREEZE WHOLE BLOOD. 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.

Serum or Plasma

Serum or plasma samples collected by standard laboratory procedure may be used in the test. The following anticoagulants may be used for collecting plasma samples: EDTA, heparin or sodium citrate. Be sure that the tube of serum or plasma is well mixed after collection and before testing. Use a laboratory pipette to withdraw 5µL of the sample. Perform the test following the Assay Procedure instructions below.

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. It is also recommended to centrifuge thawed specimens to remove gross particulate matter. Serum and plasma samples may be stored at 2-8 C for up to 7 days and up to 48 hours at room temperature (18-30°C).

Specimen Shipping

If specimens are to be shipped, they should be packed in compliance with regulations covering the transportation of etiologic agents. Serum, and plasma specimens can be shipped at ambient conditions (18-30°C) for up to 2 days or samples can be shipped refrigerated with cold packs or wet ice.

9 – GEENIUS HIV 1/2 SUPPLEMENTAL ASSAY PROCEDURE

Materials Provided
See Reagents Section

Accessories Available upon Request

- Geenius HIV 1/2 Controls : Each package contains a Positive Control vial, a Negative Control vial, and 5 µL Microtube pipettes

Materials Required but sold separately

- Geenius Reader and dedicated software

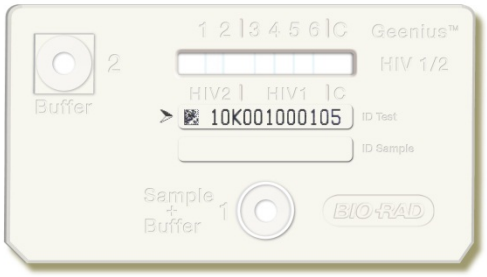
Materials required but not provided

- Clock, watch or other timing device
- Pipettor capable of delivering 5µL and 15µL of sample
- Pipettor(s) capable of delivering 60 µL and 150 µL Buffer (optional)
- Disposable gloves
- Biohazard disposal containers

Assay Procedure

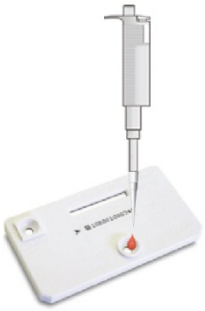
1. Remove the Geenius HIV 1/2 Supplemental Assay cassette from its pouch and place it on a flat surface. **NOTE**: Do not use the cassette if the desiccant packet is missing from the pouch; discard the cassette and open a new test cassette. The desiccant does not need to be removed from the pouch. Label the cassette with sample ID or test number. Note that the Geenius HIV 1/2 Supplemental Assay cassette has six (6) blue colored lines in the Test Window; If any of the 6 colored lines are absent, DO NOT USE. Discard the cassette and use a new test cassette.

Figure 1



2. Using a Microtube plastic pipette or laboratory pipette, dispense 5 µL of serum / plasma or 15 µL of whole blood to the center of the Sample + Buffer Well 1 of the cassette (see Figure 2 below).

Figure 2



3. Immediately following the addition of the sample **(but no longer than 5 minutes)**, use the dropper bottle to add 2 drops or a calibrated laboratory pipette to add 60 µL of Buffer into the Sample +Buffer Well 1, (see Figure 3 below).

Figure 3

4. 

Wait 5 to 7 minutes.
Wait until the blue lines in the cassette window completely disappear **(minimum and maximum wait times of 5-7 minutes respectively)** before going to the next step.
If some blue lines remain after 7 minutes, discard the cassette and use a new one.

NOTE: A slight bluish-greenish color may remain on the membrane, but none of the actual colored lines should be seen at this point.

Use the dropper bottle to add 5 drops or a laboratory pipette to add 150 µL of Buffer to Buffer Well 2 (see Figure 4 below).

Figure 4

5. 

Read the test result 15-20 minutes after adding the Buffer to Buffer Well 2.

In some cases test lines may appear in less than 15 minutes; however, a minimum of 15 minutes is needed to report results.

Do not read a Geenius cassette with the presence of any background color. Test results must be read with the Geenius Reader. Do not read results more than 30 minutes after the addition of the Buffer to Buffer Well 2.
Refer to the Geenius Reader User Manual for instructions regarding the operation of the Geenius Reader.

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

10 - QUALITY CONTROL – VALIDATION OF RESULTS

Internal Quality Control
Each Geenius HIV 1/2 Supplemental Assay cassette has a control line which is used to determine validity of the assay and confirm that sample has been added to the cassette. When the test has been performed correctly, a pink/purple line will appear in the Control (C) area to indicate the cassette is working properly (Refer to Interpretation of Test Results section of this product insert).

External Quality Control
Geenius HIV 1/2 Controls are available separately for use with the Geenius HIV 1/2 Supplemental Assay to verify the performance of the test. The Positive Control will produce a positive test result for both HIV-1 and HIV-2. The Negative Control will produce a negative test result. Run the controls as described in the Assay Procedure section for a serum / plasma sample and follow the directions in the Interpretation of Test Results section of this product insert. It is the responsibility of each facility using the Geenius HIV 1/2 Supplemental Assay to establish an adequate quality assurance program to ensure the performance of the device under specific locations and conditions of use.

Test the Geenius HIV 1/2 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 to 30°C (36 to 86°F) (Note that if this occurs, the Geenius HIV 1/2 Controls should be included in every test run that is performed using test kit lots that have been stored in that area).
- If the temperature of the testing area falls outside of 18 to 30°C (64 to 86°F).
- At periodic intervals as indicated by the user facility.

11 - INTERPRETATION OF TEST RESULTS

Results must be interpreted with the Geenius Reader (Ref: 72465) and the dedicated software. Refer to the Geenius Reader User Manual for instructions regarding the operation of the Geenius Reader.

The Geenius™ HIV 1/2 Supplemental 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 envelope peptide)	HIV-2 ENV
Band 2:	gp140 (HIV-2 envelope peptides)	HIV-2 ENV
Band 3:	p31 (HIV-1 polymerase peptide)	HIV-1 POL
Band 4:	gp160 (HIV-1 envelope 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 envelope peptides)	HIV-1 ENV

Control band: Protein A

Note: A pink/purple line should always appear in the Control (C) area, whether or not a band appears in the Test area. If there is no distinct pink/purple line visible in the Control (C) area, then the test is INVALID. A test that is INVALID cannot be interpreted. It is recommended that the test be repeated with a new cassette.

Assay Interpretation by the Geenius™ Software

The Geenius Software detects the presence or absence of Bands 1-6 above, determines the presence or absence of antibodies to HIV-1 and/or HIV-2, and generates an "HIV-1 Result" that is Positive, Indeterminate, or Negative, and an "HIV-2 Result" that is Positive, Indeterminate, or Negative. The following table indicates the criteria employed by the Geenius Software to interpret the HIV-1 Result and HIV-2 Result and provide an "Assay Interpretation."

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HIV-1 RESULT	**HIV-2 RESULT**	**ASSAY INTERPRETATION**
Negative	Negative	**HIV NEGATIVE**
Indeterminate	Negative	**HIV-1 INDETERMINATE**[a]
Negative	Indeterminate	**HIV-2 INDETERMINATE**[b]
Indeterminate	Indeterminate	**HIV INDETERMINATE**[c]
Positive	Negative	**HIV-1 POSITIVE**
Positive	Indeterminate	**HIV-1 POSITIVE**
Negative	Positive	**HIV-2 POSITIVE**.
Indeterminate	Positive	**HIV-2 POSITIVE**
Positive	Positive	**HIV-2 POSITIVE with HIV-1 cross-reactivity:** Antibody to HIV-2 confirmed in the sample. HIV-1 positivity (with only one HIV-1 envelope band, gp160 or gp41), is due to cross-reactivity and precludes confirmation of HIV-1*. *Note: Differentiation features managed by proprietary algorithm.
Positive	Positive	**HIV POSITIVE Untypable (undifferentiated):** Antibodies to HIV-1 and HIV-2 confirmed in the sample. This may occur in an HIV-2 positive sample with significant cross-reactivity to HIV-1, or may be due to co-infection with both HIV-1 and HIV-2 (rare)*. *Note: Differentiation features managed by proprietary algorithm.

[a] HIV-1 band(s) detected but did not meet the criteria for HIV-1 Positive

[b] HIV-2 band(s) detected but did not meet the criteria for HIV-2 Positive

[c] HIV band(s) detected but did not meet the criteria for HIV-1 Positive or HIV-2 Positive

12 - LIMITATIONS OF THE TEST

1. The Geenius HIV 1/2 Supplemental Assay must ONLY be used with whole 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 EDTA heparin or sodium citrate, may not yield accurate results. For serum samples, collect blood without anticoagulant.
2. The instructions in this product insert must be followed in order to obtain accurate results with the Geenius HIV 1/2 Supplemental Assay.
3. If results are read earlier than 15 minutes or later than 30 minutes after the addition of Buffer to Buffer Well 2, the results may be erroneous.
4. The Geenius HIV 1/2 Supplemental Assay **must** be interpreted using the Geenius Reader and Software.
5. A Geenius HIV 1/2 Supplemental Assay test result that is INVALID should not to be reported and the sample(s) should be retested with a new cassette.
6. A positive assay result interpretation using the Geenius HIV 1/2 Supplemental Assay confirms the presence of specific antibodies to HIV-1 and/or HIV-2 in the sample. HIV and AIDS-related conditions are clinical syndromes caused by HIV-1 and HIV-2 and their diagnosis can only be established clinically.
7. False negative results may occur in individuals infected with HIV-1 and/or HIV-2 who are receiving highly active antiretroviral therapy (HAART).
8. For a positive result, the intensities of the test lines do not necessarily correlate with the titer of antibody in the sample.
9. A negative or 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. It is recommended that testing be repeated on a specimen freshly drawn after 2-4 weeks[35]
10. A person who has antibodies to HIV-1 or HIV-2 is presumed to be infected with the virus, however, 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. It is recommended that testing be repeated on a specimen freshly drawn after 2-4 weeks[35]
11. Assay Interpretation Limitations:
    - A Geenius HIV 1/2 Supplemental Assay cassette that contains smudges or background in the band area that may interfere with test interpretation should not be read. The sample should be retested with a new Geenius HIV 1/2 Supplemental Assay cassette.
    - An “Indeterminate” interpretation does not exclude the possibility of early seroconversion of the test subject or a cross-reaction with other retroviruses**.** The homology between HIV-1 and HIV-2 viruses can lead to cross reactivity between anti-HIV-1 and anti-HIV-2 antibodies. It is recommended that testing be repeated on a specimen freshly drawn after 2-4 weeks[35]
    - Samples which meet the HIV-1 Positive criteria may in some rare cases show cross reactivity on one of the HIV2 envelope bands. In most of the cases, this profile that confirms an HIV1 infection does not exclude the rare possibility of a secondary HIV-2 seroconversion (co-infection).
    - Samples which meet the HIV-2 Positive criteria can show cross reactivity on one or more HIV-1 bands. In most cases, an HIV-1 indeterminate profile associated with an HIV-2 positive profile is a true HIV-2 only infection. However it does not exclude the possibility of a secondary HIV-1 seroconversion (co-infection).
    - Samples which meet both HIV-1 and HIV-2 Positive criteria, but are reactive with only one detected envelope band (gp160 or gp41), are generally HIV-2 positive samples

which show HIV-1 cross reactivity. This represents 54% of the cases in the clinical study of 200 samples characterized as HIV-2 only infections. Such profiles do not exclude the rare possibility of HIV-1 and HIV-2 co-infection.

- Samples with reactivity to all 4 envelope bands (all of the HIV-1 env and HIV-2 env bands) have all been HIV-2 positive samples with HIV-1 reactivity that cannot be differentiated (HIV Untypable or Undifferentiated). Such samples represent 6% of the cases in the clinical study of 200 samples that have been characterized as positive for HIV-2 only. Such profiles do not exclude the possibility of HIV-1 and HIV-2 co-infection, which are rare. Only one (1) plasma sample of the total of 1,043 samples from 299 patients with known HIV-1 infections was found to be HIV Untypable or Undifferentiated.
- HIV-2 Indeterminate test results for samples from persons without any risk factors for HIV-2 infections should be confirmed by retesting with a new Geenius HIV 1/2 Supplemental Assay cassette before reporting.

13 - PERFORMANCE CHARACTERISTICS

SPECIFICITY

Low Risk Population

Four hundred and twenty (420) samples prospectively collected from one hundred and twenty (120) individuals at low risk for HIV infection (military recruits, soldiers, and civilians) were tested with the Geenius HIV 1/2 Supplemental Assay. Results are presented in Table 1.

Table 1: Specificity of Geenius HIV 1/2 Supplemental Assay in a Low Risk Population

Matched Sample Type	Number	Geenius HIV 1/2 Supplemental Assay		
		NEG	IND	POS
Serum	120	115	5[a] (4.17%)	0
Fingerstick	60	57	3[b] (5.00%	0
Whole Blood EDTA	58*	56	2[c] (3.45%))	0
Plasma EDTA	60	60	0	0
Whole Blood Heparin	58*	55	3[d] (5.17%)	0
Plasma Heparin	60	55	5[e] (8.33%)	0

* Two (2) whole blood EDTA and 2 whole blood heparin samples had invalid results and were excluded from analysis.
[a] Of the 5 indeterminate serum samples, 3 were HIV-2 indeterminate and 2 were HIV-1 indeterminate.
[b] Of the 3 indeterminate fingerstick samples, 2 were HIV-2 indeterminate and 1 was HIV-1 indeterminate.
[c] Of the 2 indeterminate whole blood EDTA plasma samples, 1 was HIV-2 indeterminate, and 1 was HIV-1 indeterminate.
[d] Of the 3 indeterminate whole blood heparin samples, 1 was HIV-2 indeterminate and 2 were HIV-1 indeterminate.
[e] Of the 5 indeterminate heparin plasma samples, 3 were HIV-2 indeterminate, 1 was HIV-1 indeterminate, and 1 was HIV Indeterminate.

The overall Indeterminate rate in the low risk population was 4.33% (18/416) for all matched sample types combined.

Note: All samples from the 120 prospective low risk subjects were negative on an FDA licensed HIV-1/HIV-2 EIA reference test, and would not normally be tested using the Geenius HIV 1/2 Supplemental Assay.

False Reactive Sample Panel
A panel of one hundred (100) retrospective samples that were false reactive on FDA licensed or approved HIV tests were tested with the Geenius HIV 1/2 Supplemental Assay. Results are presented in Table 2.

Table 2: Specificity of Geenius HIV 1/2 Supplemental Assay in False Reactive Samples

Assay	Number of False Reactives Tested	Geenius™ HIV 1/2 Supplemental Assay		
		NEG	IND	POS
HIV Ag/Ab Combo	50	49	1[a] (2.00%)	0
HIV 1/2 EIA	43	40	3[b] (6.98%)	0
HIV 1/2 Rapid Test	7	5	2[c] (28.57%)	0
TOTAL	100	94	6 (6.00%)	0

[a] One (1) false reactive sample was HIV-1 indeterminate .
[b] Of three (3) false reactive samples, one (1) was HIV-1 indeterminate, one (1) was HIV-2 Indeterminate, and one (1) was HIV Indeterminate .
[c] Two (2) HIV-1/2 rapid test false reactive samples were HIV-1 indeterminate.

No sample in this population tested positive on the Geenius HIV 1/2 Supplemental Assay. The overall Indeterminate rate in this population was 6% (6/100).

Medical Conditions Unrelated to HIV Infection
A panel of 140 retrospective samples representing 14 categories of medical conditions unrelated to HIV infection were tested with the Geenius HIV 1/2 Supplemental Assay. Results are presented in Table 3.

Table 3: Medical Conditions Unrelated to HIV Infection

Unrelated Medical Condition	Number Tested	Geenius HIV 1/2 Supplemental Assay		
		NEG	IND	POS
Autoimmune disease patients	10	10	0	0
Dialysis patients	10	9	1[a]	0
EBV infection	10	10	0	0
HBsAg infection	10	10	0	0
HCV infection	10	8	2[a]	0
Hemophilia patients	10	10	0	0
High rheumatoid factor	10	9	1[a]	0
HTLV I/II antibody positive	10	10	0	0
Multiparous (pregnant) females	10	10	0	0
Multiple transfusions	10	10	0	0
Post-Influenza vaccine recipients*	10	10	0	0
Pre-Influenza vaccine recipients *	10	10	0	0
Vaccinia vaccine samples	10	10	0	0
Yeast (Candida) reactive	10	8	2[a]	0
TOTAL	140	(134/140) 95.71%	(6/140) 4.29%	(0/140) 0.00%

*The 10 pre-Influenza vaccine and 10 post-Influenza vaccine specimens tested in the study were matched.
[a] HIV-2 Indeterminate.

The overall indeterminate rate was 4.29% (6/140). Of the 140 unrelated medical condition samples, 139 were negative on an FDA licensed HIV-1/HIV-2 screening assay (historical data) and one was not tested.

Note: All of these specimens were non-reactive on an FDA licensed HIV-1/HIV-2 EIA test, and would not normally be tested using the Geenius HIV 1/2 Supplemental Assay.

In a previous cross-reactivity study performed in Europe, a panel of 231 potentially cross-reactive samples representing 29 different disease states was tested on the Geenius HIV 1/2 Supplemental Assay. Of the 231 different samples, 219 specimens tested negative and 12 specimens from 10 different medical conditions tested HIV-1 or HIV-2 Indeterminate due to reactive bands at trace level (HTLV (2/10), HCV (1/10), HAV IgG (1/10), HBs Ag (1/10), CMV IgG (1/10), Rubella IgG (1/10), RF (1/10), Scleroderma (1/2), Cirrhosis (1/5) and Malaria (2/16)). The overall indeterminate rate was 5.20% (12/231).

Pediatric Sample Population

The specificity of the Geenius HIV 1/2 Supplemental Assay with normal pediatric samples was determined by testing ten (10) normal pediatric (ages 2-10) samples.

Of the ten samples, nine were Negative and one was HIV-1 Indeterminate on the Geenius HIV 1/2 Supplemental Assay. The ten HIV low risk pediatric samples were negative on an FDA-approved HIV-1/2 Ag/Ab Combo EIA (historical data).

SENSITIVITY

HIV Positive Population

One thousand forty three (1043) samples prospectively collected from two hundred ninety nine (299) known HIV-1 positive/AIDS patients were tested with the Geenius HIV 1/2 Supplemental Assay. Results are presented in Table 4.

Table 4: Sensitivity of Geenius HIV 1/2 Supplemental Assay in Prospective Known HIV-1 / AIDS Positive Patients

Matched Sample Type	Number Tested	Geenius HIV 1/2 Supplemental Assay Results					Rapid HIV 1/2 Supplemental / Differentiation Assay	HIV-1 Western Blot	FDA Licensed (3rd Gen) HIV-1/HIV-2 EIA
		POS	IND	NEG	Sensitivity	Wilson 95 % CI			
Serum	299	297	2[a]	0	99.33% (297/299)	97.59% - 99.82%	*99.00% (296/299)	**99.00% (296/299)	100% (299/299)
Fingerstick	148[c]	148	0	0	100% (148/148)	97.46% - 100%	NA	NA	NA
Whole Blood EDTA	150[d]	150	0	0	100% (150/150)	97.50% - 100%	NA	NA	NA
EDTA Plasma	151	150	1[b]	0	99.34% (150/151)	96.34% - 99.88%	NA	NA	NA
Whole Blood Heparin	147[e]	146	0	1[b]	99.32% (146/147)	96.24% - 99.88%	NA	NA	NA
Heparin Plasma	148[f]	147[g]	1[b]	0	99.32% (147/148)	96.27% - 99.88%	NA	NA	NA

[a] Two (2) AIDS patient serum samples were HIV-1 indeterminate on the Geenius HIV 1/2 Supplemental Assay.
[b] Of the 2 AIDS patient samples that had HIV-1 indeterminate results for serum, 1 had an HIV-1 indeterminate EDTA plasma sample and the second AIDS patient had a negative whole blood heparin sample and an HIV-1 indeterminate heparin plasma sample.
[c] For the fingerstick samples,152 samples were collected, 4 were invalid and were excluded from the analysis. Of the 148 fingerstick results 59 were from HIV-1 positive patients and 89 were from AIDS patients.
[d] For the whole blood EDTA, 151 samples were collected , 1 sample was invalid and was excluded from analysis.
[e] For the whole blood Heparin, 150 samples were collected, 3 test results were invalid and 1 was double enrolled and was excluded.
[f] For the plasma Heparin, 150 samples were collected, 2 test results were invalid and 1 was double enrolled and was excluded.
[g] The result for one (1) heparin plasma sample from an AIDS patient was HIV Positive – Untypable (undifferentiated).
* Three (3) samples were indeterminate on the Rapid HIV 1/2 Supplemental Differentiation Assay , including the 2 AIDS patient serum samples that were indeterminate on the Geenius Supplemental Assay.
** Three(3) samples were indeterminate on the HIV-1 Western blot, including the 2 AIDS patient serum samples that were indeterminate on the Geenius Supplemental Assay.

All 299 serum samples from the HIV positive/AIDS patients were repeatedly reactive when tested on a third generation FDA licensed HIV-1/HIV-2 EIA. Three (3) of these serum samples were HIV-1 indeterminate on either an FDA approved Rapid HIV-1/HIV-2 Supplemental and Differentiation assay or a FDA licensed HIV-1 Western blot. Therefore the sensitivity of these comparator assays was 99.00% (296/299) for this population.

CDC Stage 3 AIDS Patients

Seven hundred twenty three (723) prospectively collected samples from two hundred twelve (212) known AIDS patients, categorized as CDC Stage 3, were tested with the Geenius HIV 1/2 Supplemental Assay. Results are presented in Table 5.

Table 5: Sensitivity of Geenius HIV 1/2 Supplemental Assay in Prospective Known CDC Stage 3 AIDS Patients

Sample Type	Number Tested	POS	IND	NEG	Sensitivity	95% Wilson CI	**Rapid HIV-1 /2 Supp. / Diff. Test Results**	**HIV-1 Western Blot**	**FDA Licensed (3rd Gen) HIV-1/HIV-2 EIA**
Serum	212	210	2 [c]	0	99.06% (210/212)	96.62% - 99.74%	*98.58% (209/212)	*98.58% (209/212)	100.0% (212/212)
Fingerstick	89	89	0	0	100% (89/89)	95.85% - 100%	NA	NA	NA
Whole Blood EDTA	88 [a]	88	0	0	100% (88/88)	95.81% - 100%	NA	NA	NA
EDTA Plasma	89	88	1 [d]	0	98.88% (88/89)	93.90% - 99.80%	NA	NA	NA
Whole Blood Heparin	122 [b]	121	0	1 [d]	99.18% (121/122)	95.50% - 99.86%	NA	NA	NA
Heparin Plasma	123 [e]	122 [f]	1 [d]	0	99.19% (122/123)	95.53% - 99.86%	NA	NA	NA

[a] For whole blood EDTA, 89 samples were collected, 1 test result was invalid and was excluded.

[b] For whole blood heparin, 124 samples were collected, 1 test result was invalid and 1 was double enrolled and was excluded.

[c] Two (2) patient serum samples were HIV-1 indeterminate.

[d] Of the 2 patient samples that had HIV-1 indeterminate results for serum, 1 had an HIV-1 indeterminate EDTA plasma sample. The second had a negative whole blood heparin sample and an HIV- 1 indeterminate heparin plasma sample.

[e] For plasma heparin, 124 samples were collected, 1 was double enrolled and was excluded.

[f] The result for one (1) heparin plasma sample from an AIDS patient was HIV Positive – Untypable (undifferentiated).

* Three (3) samples were indeterminate on either the Rapid HIV 1/2 Supplemental Differentiation Assay or the HIV-1 Western Blot, including the two samples that were indeterminate on the Geenius Supplemental Assay.

Two (2) CDC Stage 3 AIDS patients, (diagnosed in 2002 and 2004 respectively) had indeterminate results on the Geenius HIV 1/2 Supplemental Assay.

All 212 serum samples from the AIDS patients were reactive when tested on a third generation FDA licensed HIV-1/HIV-2 EIA. Three (3) samples were HIV-1 indeterminate on either an FDA approved Rapid HIV-1/HIV-2 Supplemental and Differentiation assay or a FDA licensed HIV-1 Western blot. Therefore the sensitivity of the two comparator assays in this population was 98.58% (209/212).

HIV-2 Positive Samples

Sensitivity Performance with Known HIV-2 Positive Samples

Two hundred (200) known HIV-2 antibody positive samples obtained from individuals from different geographic locations (161 from Ivory Coast, 20 from Guinea Bissau, and 19 from USA) were tested with the Geenius HIV 1/2 Supplemental Assay.

Of the two hundred (200) known HIV-2 antibody positive samples, 38.50% (77/200) were interpreted as only HIV-2 Positive, 54.00% (108/200) were interpreted as HIV-2 with HIV-1 cross reactivity, 6.00% (12/200) were interpreted as HIV Untypable (undifferentiated), and 1.50% (3/200) were interpreted as HIV-2 indeterminate.

All samples from the known 200 HIV-2 positive subjects were positive on a third generation FDA licensed HIV-1/HIV-2 EIA reference test (historical data).

HIV-1 and HIV-2 Co-infected Patient Samples

Sensitivity Performance with Known HIV-1 and HIV-2 Co-infected Patient Samples

Three (3) samples from patients known to be co-infected with both HIV-1 and HIV-2 viruses were obtained from France and were tested with the Geenius HIV 1/2 Supplemental Assay.

The reactivity of the Geenius HIV 1/2 Supplemental Assay with the three (3) samples was 100%, All the samples were found to be HIV Positive Untypable (undifferentiated), which means that they were found positive for both HIV-1 and HIV-2 antibodies.

Pediatric Sample Population

Sensitivity Performance with Known HIV-1 Positive Pediatric Samples

The reactivity of the Geenius HIV 1/2 Supplemental Assay in positive pediatric patients was determined by testing forty (40) known HIV-1 antibody positive pediatric samples (ages 2-20).

The reactivity of the Geenius HIV 1/2 Supplemental Assay with the HIV-1 positive pediatric samples was 100% HIV-1 positive (40/40), with a 95% CI of 91.22% to 100%. All 40 samples were HIV-1 positive.

The forty (40) HIV-1 positive pediatric samples were all repeatedly reactive on an FDA approved HIV 1/2 Ag/Ab Combo EIA and positive on an HIV-1 Western Blot (historical data).

HIV-1 Group M Subtype Samples

Sensitivity Performance with Known HIV-1 Group M Subtype Positive Samples

The reactivity of the Geenius HIV 1/2 Supplemental Assay with HIV-1 Group M subtype samples was determined by testing one hundred and thirty six (136) HIV-1 antibody positive Group M subtype specimens (A, A1, B, C, D, F, F2, G, A/E, A/G, H, J, K, U, CRFs) collected from individuals in Cameroon.

The reactivity of the Geenius HIV 1/2 Supplemental Assay for the 136 HIV-1 Group M Subtype samples tested was 100% (136/136) HIV positive (135 HIV-1 positive and 1 HIV positive untypable/undifferentiated), with a 95% confidence interval of 97.25% to 100%.

HIV-1 Group O Subtype Samples
Sensitivity Performance with Known HIV-1 Group O Subtype Positive Samples
Fifteen (15) specimens known to be positive for antibodies to HIV-1 Group O were tested with the Geenius HIV 1/2 Supplemental Assay.

The Geenius HIV 1/2 Supplemental Assay was HIV-1 Positive for 13 and HIV-1 Indeterminate for 2 of the 15 known positive HIV-1 Group O samples. None of the specimens was found to be Negative.

PERFORMANCE PANELS

HIV-1 Incidence / Prevalence Panel
An HIV-1 Incidence / Prevalence panel containing seven (7) known HIV-1 positive incidence (new infections) members and eight (8) known HIV-1 positive prevalence (long-standing infections) members was tested with the Geenius HIV 1/2 Supplemental Assay.

The Geenius HIV 1/2 Supplemental Assay was reactive with 100% (15/15) of the HIV-1 incidence / prevalence panel members with a 95% confidence interval of 79.57% - 100%. All 15 panel members were HIV-1 positive.

HIV-1 / HIV-2 Performance Panel
An HIV-1 / HIV-2 Performance Panel containing seven (7) HIV-1 positive and seven (7) HIV-2 positive panel members was tested with the Geenius HIV 1/2 Supplemental Assay.

The Geenius HIV 1/2 Supplemental Assay gave correct results for the seven HIV-1 panel members ("HIV-1 Positive") and five of the HIV-2 panel members ("HIV-2 Positive") for all three lots tested. One HIV-2 panel member was HIV-2 Indeterminate on all three lots tested. Additionally, one HIV-2 panel member was HIV-2 Positive on two of three lots tested and HIV-2 Indeterminate on the remaining lot. None of the panel members was found to be Negative on any lot tested.

HIV-1 Seroconversion Panels
Twenty six (26) commercially available seroconversion panels were tested with the Geenius HIV 1/2 Supplemental Assay. The reactivity with the two hundred and thirty (230) specimens in the panels is presented in Table 6.

Table 6: Reactivity in HIV-1 Seroconversion Panels
Note: The number of positive panel members found to be repeatedly reactive or positive is listed for each test.

Panel ID	Number of Panel Members Tested	HIV-1 RNA Positive Panel Members	Automated (4th Gen) HIV Ag/Ab Combo EIA	FDA Licensed (3rd Gen) HIV 1/2 EIA	Geenius HIV-1/HIV-2 Supplemental Assay Results	Rapid HIV-1 /2 Supp. / Diff. Test Results	HIV-1 WB Results
001	9	6	6	5	3	2	3
002	13	7	5	4	2	2	3
003	10	6	5	3	2	2	2
004	8	6	3	2	0	0	0
005	7	4	5	5	5	3	4
006	8	3	2	2	0	0	0
007	3	1	1	1	1	1	1
008	14	11	5	5	3	3	2
009	6	4	4	3	2	3	2
010	10	5	3	3	1	2	0
011	27	17	16	14	14	13	13
012	25	18	17	14	13	9	11
013	6	4	3	2	2	2	2
014	5	4	5	2	1	2	0
015	8	4	4	3	1	0	0
016	6	2	2	2	1	2	0
017	6	5	4	2	2	2	2
018	9	5	4	4	4	4	1
019	8	8	7	6	5	5	1
020	6	5	4	2	1	1	0
021	6	5	3	2	0	0	0
022	4	4	3	3	0	0	1
023	6	5	4	0	1	1	2
024	7	5	2	1	1	0	0
025	7	7	7	0	4	4	4
026	6	6	5	3	2	2	2
Total	**230**	**157**	**129 / 157**	**93 / 157**	**71 / 157**	**65 / 157**	**56 / 157**
% HIV-1 RNA Positives detected			82.17%	59.24%	45.22%	41.40%	35.67%
95% Confidence Interval			75.43% - 87.36%	51.42% - 66.61%	37.64% - 53.04%	33.98% - 49.23%	28.59% - 48.43%

*Historical data on the Rapid HIV 1/2 Supplemental and Differentiation Assay was evaluated using a new diagnostic algorithm interpretation approved by FDA in March 2013.

The Geenius HIV 1/2 Supplemental Assay results were compared to previously known results obtained with the comparator assays shown in Table 1 above**.** The HIV Ag/Ab Combo EIA, the HIV 1/2 EIA, and the Rapid HIV-1/2 supplemental/differentiation test are FDA-approved tests.

Of the 230 seroconversion panel specimens tested, 68.26 % (157/230) had detectable HIV-1 RNA. The Geenius HIV 1/2 Supplemental Assay found 45.22% (71/157, 95% CI 37.64% - 53.04%) Positive compared to 41.40% (65/157, 95% CI 33.98% - 49.23%) reactive on a Rapid HIV-1/2 supplemental/differentiation assay. Also in this study the Geenius HIV 1/2 Supplemental Assay found 45.22% Positive compared to 35.67% (56/157, 95% CI 28.59% - 48.43%) Positive on the HIV-1 Western Blot.

REPRODUCIBILITY

A 17 member reproducibility panel for the Geenius HIV 1/2 Supplemental Assay was prepared at Bio-Rad Laboratories and provided to 3 sites for testing. Three clinical lots of the Geenius HIV 1/2 Supplemental Assay were used in the evaluation.

The 17 member reproducibility panel included 5 serum members, 5 EDTA plasma members, 5 heparin plasma members and two (2) Geenius HIV 1/2 Supplemental Assay kit controls. The reproducibility panel was tested on the Geenius HIV 1/2 Supplemental Assay following the instructions for use. Each panel member was tested twice a day (AM and PM), for 5 days on 3 kit lots of the Geenius HIV 1/2 Supplemental Assay, at each of 3 sites, for a total of 90 replicates per panel member at all three sites combined (5 days x 2 per day x 3 lots x 3 sites = 90 replicates per panel member). Each Geenius HIV 1/2 Supplemental Assay test result was read and interpreted using the Geenius HIV 1/2 Supplemental Assay Reader and Software.

The total percent (%) agreement of the Geenius HIV 1/2 Supplemental Assay results was calculated for each of the 17 reproducibility panel members as the number of results that were correct compared to the known sample status, along with the 95% confidence interval. Results were reported as Positive, Indeterminate, or Negative. The results are shown in Table 7. This study demonstrated that the Bio-Rad Geenius HIV 1/2 Supplemental Assay is highly reproducible.

.

Table 7: Reproducibility Results

Panel Member	Panel Description	Replicates*	Total Results	% Agreement	95 % CI
1	HIV-1 antibody positive serum	90	90/90 HIV-1 Positive	100%	95.91% - 100%
2	HIV-1 antibody positive EDTA plasma	89	89/89 HIV-1 Positive	100%	95.86% - 100%
3	HIV-1 antibody positive heparin plasma	90	90/90 HIV-1 Positive	100%	95.91% - 100%
4	HIV-1 indeterminate serum	89	85/89 HIV-1 Indeterminate	95.51%	89.01% - 98.24%
5	HIV-1 indeterminate EDTA plasma	87	84/87 HIV-1 Indeterminate	96.55%	90.35% - 98.82%
6	HIV-1 indeterminate heparin plasma	90	85/90 HIV-1 Indeterminate	94.44%	87.65% - 97.60%
7	HIV-2 indeterminate serum	86	80/86 HIV-2 Indeterminate	93.02%	85.60% - 96.76%
8	HIV-2 indeterminate EDTA plasma	88	76/88 HIV-1 Indeterminate	86.36%	77.66% - 92.02%
9	HIV-2 indeterminate heparin plasma	89	84/89 HIV-1 Indeterminate	94.38%	87.51% - 97.58%
10	HIV-2 antibody positive serum	90	90/90 HIV-2 Positive	100%	95.91% - 100%
11	HIV-2 antibody positive EDTA plasma	90	88/90 HIV-2 Positive	97.78%	92.26% - 99.39%
12	HIV-2 antibody positive heparin plasma	89	89/89 HIV-2 Positive	100%	95.86% - 100%
13	HIV non-reactive serum	90	89/90 HIV Negative	98.89%	93.97% - 99.80%
14	HIV non-reactive EDTA plasma	90	88/90 HIV Negative	97.78%	92.26% - 99.39%
15	HIV non-reactive heparin plasma	90	89/90 HIV Negative	98.89%	93.97% - 99.80%
16	Kit Positive control serum	90	90/90 HIV-1/2 Positive	100%	95.91% - 100%
17	Kit Negative control serum	90	89/90 HIV Negative	98.89%	93.97% - 99.80%

*Replicate values for each panel member that are less than 90 are due to invalid test results excluded from analysis.

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