

Laboratory Procedure Manual

Analyte: **HIV-1/HIV-2 Differentiation Assay**

Matrix: **Serum/Plasma**

Method: **Bio-Rad Geenius HIV-1/HIV-2
Supplemental Assay**

as performed by: *HIV Laboratory Branch
Division of HIV/AIDS Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD, and TB
Prevention*

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Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each protocol before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

File Name	Variable Name	SAS Label
HIV_K_R	LBXHIV1	HIV-1
	LBXHIV2	HIV-2

Bio-Rad Geenius HIV-1/HIV-2 Supplemental Assay

1. Summary of Test Principle and Clinical Relevance- Immunoassay

The Geenius" HIV 1/2 Supplemental Assay (Geenius) 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).

Geenius is a moderate complexity assay and is intended for use as an addition, more specific test to confirm the presence of antibodies to HIV-1 and/or 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).

Geenius is not approved for testing of blood donors.

The results of the Geenius are read and interpreted only by the Geenius Reader with a dedicated software.

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.¹ Additionally, transmission of the viruses can occur through tissue transplantation.² HIV-1 has been isolated from patients with AIDS and AIDS-related complex (ARC).³⁻⁵ HIV-1 was thought to be the sole causative agent of these syndromes until 1986, when a second type of HIV (HIV-2) was isolated and also reported to cause AIDS.⁶⁻⁷ Since the initial discovery, hundreds of cases of HIV-2 infection have been documented worldwide, including cases of AIDS related to HIV-2.⁸ In the United States, there have been more than 80 cases of infection with HIV-2 reported, including three potential blood donors.⁹⁻¹⁴

HIV-2 is similar to, but distinct from, HIV-1. Both viruses have similar morphology and lymphotropism,¹⁵ and the modes of transmission appear to be identical.^{8,16} 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.¹⁷ 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.¹⁸

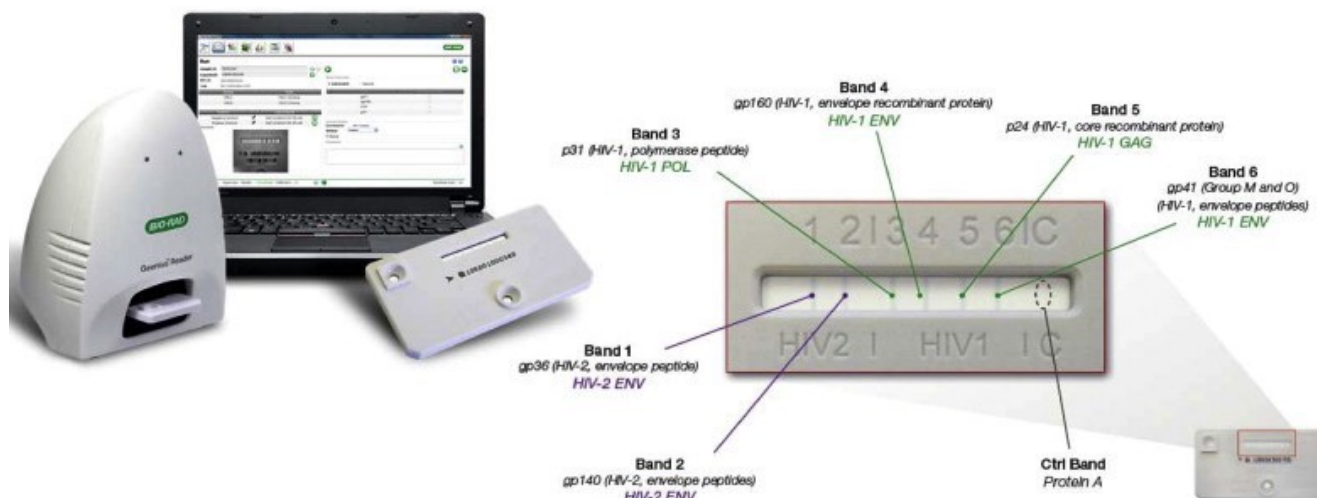
Within the two major HIV types, there is significant variation as well. By analyzing sequences of representative strains, HIV-1 has been divided into four groups: group M (for major), including at least 9 subtypes, 3 sub-subtypes of A, and 2 sub-subtypes of F (A1, A2, A3, B, C, D, F1, F2, G, H, J, and K); group O (for outlier); group N (for non-M, non-O), and group P.¹⁹⁻²³ Similarly, HIV-2 strains have been classified into at least five subtypes (A through E).²⁴ Some HIV-1 variants share $\leq 50\%$ homology in their *env* genes with those *env* sequences of more common prototype strains.

Despite some degree of immunological cross-reactivity between types and subtypes of HIV, reliable detection of antibodies derived from the more divergent strains may only be achieved by incorporating type-specific protein sequences into the assay design.

Biological Principles of the Procedure

The Geenius™ HIV 1/2 Supplemental Assay (Geenius™) is a single-use immunochromatographic assay for the confirmation and differentiation of individual antibodies to HIV-1 and HIV-2. The assay incorporates highly conserved recombinant proteins and synthetic peptides representing HIV-1 and HIV-2 proteins (figure) and can be used for the detection and differentiation of individual antibodies to HIV-1 and HIV-2 in serum, plasma or whole blood.

Geenius™ is formatted as a dual-path lateral flow membrane-based assay in a cassette-designed platform. The membrane is coated with HIV-1 and HIV-2 antigens in the Test area (Bands 1-6 in Figure). Fibrous pads attached to the membrane support material contain colloidal gold particles conjugated to Protein A, an antibody binding protein. The sample is applied to the Sample + Buffer well and, after the solution has migrated onto the test strip, additional buffer is added to well 2. The buffer moves the solution laterally over the membrane and facilitates the binding of any HIV-specific antibodies to the HIV antigens. In a reactive sample, the antibodies are captured by the antigens immobilized in the Test area and the protein A-colloidal gold conjugate binds to the captured antibodies, causing the development of pink/purple lines. When HIV antibodies are not present, no pink/purple lines will be observed in the Test area. The sample continues to migrate through the membrane and a pink/purple line develops in the control (C) area, which also contains Protein A. This built-in procedural control provides evidence that the test was performed properly and that both the sample and reagents have effectively migrated through the membrane and cassette.



Results must be read 15-20 minutes using the Geenius™ reader (b) after adding the last buffer in well 2. A minimum of 15 minutes and a maximum of 30 minutes are needed to report the results.

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.²⁵⁻³⁰ 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 5 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.³¹

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.

WARNINGS FOR USERS

For In Vitro Diagnostic Use

1. The Instructions For Use (IFU) 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)

3. **Computerization; Data System Management**

After a run is complete and any additional corrections by the analyst are made, the Excel result file is finalized. HIV antibody differentiation results are manually entered into a Microsoft Excel result file spreadsheet. Data is saved as csv file and transmitted electronically to Westat's ISIS computer system weekly and transferred from there to NCHS.

4. **Specimen Collection, Storage, and Handling Procedures; Criteria for Specimen Rejection**

The Geenius assay can be performed on venous or fingerstick whole blood, serum, or plasma specimens. Fresh or frozen serum or plasma collected by standard phlebotomy procedures may be used in the test. The following anticoagulants may be used for collecting plasma specimens: EDTA, heparin or sodium citrate. **Use of other anticoagulants has not been evaluated and may give incorrect results.**

The minimally acceptable volume of specimen available for performing the test is 30 μ L. Approximately 5 μ L is used for running each test. **Performance of this assay has not been evaluated on patient samples that have been heat-inactivated.**

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). 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.

If specimens are to be shipped, they should be packed in compliance with local regulation 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.

If sample is outside of specified collection, storage or handling conditions listed above then the sample is rejected, or tested and reported with an exception clause.

5. **Procedures for Microscopic Examinations; Criteria for Rejection of Inadequately Prepared Slides**

Not applicable for this procedure

6. **Equipment and Instrumentation, Materials, Reagent Preparation, Calibrators (Standards), and Controls**

A. Reagents

Geenius™ HIV-1/2 Supplemental Assay

Product No. 72461 (20 Tests)

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

Kits must be stored at 2 to 30° C

B. Reagent Preparation

All solutions and reagents are ready to use as supplied. The cassettes and Buffer should be stored at 2 to 30°C (36 to 86° F). If the samples and/or kit components have been refrigerated (2-8°C), bring all reagents to room temperature (18 to 30°C) prior 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 reagents are stable until their printed expiration dates. Do not use beyond the stated expiration date.

C. Materials Provided

- None

D. Materials Required But Not Provided

1. Absorbent pads or paper towels
2. Biohazard bags with closures
3. Household bleach (5% or 8% sodium hypochlorite), diluted to a minimum concentration of 10% bleach (0.5% sodium hypochlorite). Alternative disinfectants include 70% ethanol or 0.5% Wescodyne™
4. Disposable gloves, lab coat, safety eyewear
5. Laboratory timer
6. Precision pipettors that deliver 5 µL
7. Indelible laboratory marker

7. Calibration and Calibration Verification Procedures

Procedural Control

Each Geenius 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.

Quality Control

Geenius HIV 1/2 Controls are available separately for use with the Geenius 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 specimens and follow the directions in the Interpretation of Test Results. It is the responsibility of each facility using the Geenius 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.

Results must be interpreted with the Geenius Reader (Ref: 72465) and the dedicated software.

The following table indicates the criteria employed by the Geenius software to interpret the HIV-1 result and the HIV-2 result and provide a “Final Assay Interpretation”. The detection and differentiation features are managed by proprietary algorithm. The cassettes should not be interpreted by visual inspection.

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

The software detects the presence or absence of Bands 1- 6 and the Control band in the cassette; determines the presence or absence of antibodies to HIV-1 and/or HIV-2; and generates both the HIV-1 and HIV-2 results that are used in combination to determine the Final Assay Interpretation.

The Final Assay Interpretation should always be reported to the ordering health care provider and it refers only to the results for the Geenius Assay, and it is not intended to indicate a clinical diagnosis.

Table 1. Reportable Geenius results

Final Assay Interpretation = Final Specimen Status	HIV-1 Result	HIV-2 Result	Notes for the Laboratory
HIV Antibody NEGATIVE	Ab nonreactive	Ab nonreactive	No HIV-1 or HIV-2 bands were detected. The sample is non-reactive for HIV
HIV-1 INDETERMINATE	Ab indeterminate	Ab nonreactive	HIV-1 band(s) were detected but did not meet the criteria for HIV-1 Positivity. No HIV-2 bands were detected.
HIV-2 INDETERMINATE	Ab nonreactive	Ab indeterminate	One HIV-2 band was detected but did not meet the criteria for HIV-2 Positivity. No HIV-1 bands were detected.
HIV INDETERMINATE	Ab indeterminate	Ab indeterminate	HIV-1 and HIV-2 bands were detected but did not meet the criteria for HIV-1 Positivity or HIV-2 Positivity.
HIV-1 POSITIVE	Ab reactive	Ab nonreactive	HIV-1 bands were detected and met the criteria for HIV-1 Positivity. No HIV-2 bands detected. Antibodies to HIV-1 confirmed in the
HIV-1 POSITIVE	Ab reactive	Ab indeterminate	HIV-1 bands were detected and met the criteria for HIV-1 Positivity. One HIV-2 band was detected but did not meet the criteria for HIV-2 Positivity. Antibodies to HIV-1 confirmed in the sample. HIV-2 indeterminate result is likely due to cross-reactivity of HIV-1 antibodies on HIV-2 antigens
HIV-2 POSITIVE	Ab nonreactive	Ab reactive	HIV-2 bands were detected and met the criteria for HIV-2 Positivity. No HIV-1 bands detected. Antibodies to HIV-2 confirmed in the
HIV-2 POSITIVE	Ab indeterminate	Ab reactive	HIV-1 band(s) were detected and did not meet the criteria for HIV-1 Positivity. HIV-2 bands were detected and met the criteria for HIV-2 Positivity. Antibodies to HIV-2 confirmed in the sample. HIV-1 indeterminate result is likely due to cross-reactivity of HIV-2 antibodies on HIV-1 antigens
HIV-2 POSITIVE with HIV-1 cross-reactivity	Ab reactive (cross-reactivity)	Ab reactive	HIV-1 bands were detected and met the criteria for HIV-1 Positivity. HIV-2 bands were detected and met the criteria for HIV-2 Positivity. Antibodies to HIV-2 confirmed in the sample. HIV-1 band intensities are reactive but below a specified index. The HIV-1 Ab reactive result is likely due to cross-reactivity of HIV-2 antibodies on
HIV POSITIVE Untypable	Ab reactive	Ab reactive	HIV-1 bands were detected and met the criteria for HIV-1 Positivity. HIV-2 bands were detected and met the criteria for HIV-2 Positivity. Antibodies to HIV-1 and HIV-2 confirmed in the sample. Further testing is indicated.

Preliminary Statements

- Once testing has been started, it should be completed without interruption.
- Do not use more than ten (10) Geenius cassettes in a batch.
- The Cartridges should be placed on a flat surface during the assay procedure to ensure proper flow of specimen and reagents through the membrane.

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.
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 (Figure below).



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.
4. Wait until the blue lines in the cassette window completely disappear (minimum and maximum wait times of 5 to 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 to add 5 drops or laboratory pipette to add 150 μL of Buffer to Buffer Well 2 (Figure below).
5. Read the test result 15-20 minutes after adding the Buffer to Buffer Well 2. A minimum of 15 minutes is needed to report results and results must be read within 30 minutes of the addition of Buffer to Buffer Well 2. **NOTE:** do not read the cassette with the presence of background color. Test results must be read with the Geenius reader.

6. Discard the used pipette tips, cassettes, and any other test materials into a biohazard container.

8. Method Performance Specifications

Sensitivity for Antibodies to HIV-1

The sensitivity of the Geenius Test for antibodies to HIV-1 with serum specimens (n= 299) is calculated to be 99.33% (95% CI = 97.58- 99.82%).

The sensitivity of the Geenius Test for antibodies to HIV-1 with EDTA plasma specimens (n= 151) is calculated to be 99.34% (95% CI = 96.34 – 99.88%) and in heparin plasma (n= 148) 99.32% (95% CI= 96.27- 99.88%).

The sensitivity of the Geenius Test for HIV-1 antibodies in pediatric population is calculated to be 100% (95% CI= 91.22- 100%).

Sensitivity for Antibodies to HIV-2

Of 200 HIV-2 antibody positive samples, 38 were HIV-2 positive, 54% HIV-2 with HIV-1 cross-reactivity, 6% were HIV positive untypable, 1.5% HIV-2 indeterminate, and 0.5% HIV indeterminate.

HIV-1 and HIV-2 co-infection

The reactivity of the Geenius Test in three samples from patients know to be co-infected with both HIV-1 and HIV-2 viruses is 100%.

Reactivity of Multispot HIV-1/HIV-2 on Worldwide Specimens and on HIV-1 Group O Serotype Samples

The reactivity of the Geenius Test for 136 HIV-1 Group M subtype samples is calculated to be 100% (95% CI= 97.25- 100%).

The reactivity of the Geenius Test for 15 HIV-1 Group O samples is calculated to be 86.66%. Two amples were HIV-1 indeterminate, while 13 were HIV-1 positive with the Geenius Test.

Reactivity with HIV-1 Seroconversion Panels

Of the 230 seroconversion panel plasma specimens tested, 68.26% had detectable HIV-1 RNA. The Geenius Test found 45.22% (95% CI= 37.64- 53.04%) Positive compared to 41/40% (95% CI= 33.98- 49.23%) reactive on a rapid HIV-1/2 differentiation assay and 45/22% Positive compared to 35/67% (95% CI= 28.59- 48.43%) Positive on HIV-1 Western blot.

Specificity

Four hundred and twenty (420) samples (serum, EDTA and heparin plasma, fingerstick whole blood, EDTA and heparin whole blood) 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. 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. The overall Indeterminate rate in the low risk population was 3.13% (13/416) for all matched sample types combined and no sample tested Geenius positive.

In a panel of 100 retrospective samples that were false reactive on FDA licensed or approved HIV tests were tested with Geenius. The overall Indeterminate rate in this population was 6% (6/100) and no sample tested Geenius positive.

In a panel of 140 retrospective samples, representing 14 categories of medical conditions unrelated to HIV infection were tested with Geenius. Of the 140, 139 were negative on an FDA licensed HIV test and one was not tested. The overall Indeterminate rate was 2.86% (4/140) and no sample tested Geenius positive.

9. Reportable Range of Results

Reportable results are listed in Table 1.

10. Quality Control (QC) Procedures

Procedural Control

Each Geenius cassette has a built-in procedural control, the Control band that is used to determine validity of the assay and confirm that the sample has been added to the cassette. When a pink/purple band appear in the Control line means that the cassette and reaction worked properly.

Quality Control

Using individual Geenius cassettes as described in the Assay Procedure, run one HIV-1/2 Positive Control and one Negative Control, both provided in the kit. Test both 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 testing areas falls outside of 18 to 30°C (64 to 86°F)
- If the temperature of the test storage area falls outside of 2 to 30°C (36 to 86° F)
- At periodic intervals as indicated by the user facility. The HIV Reference Laboratory will include the Positive and Negative Control with each run.

Expected results are as follows:

HIV-1/2 Positive control: HIV untypable

HIV Negative control: HIV antibody negative

11. Remedial Action If Calibration or QC Systems Fail To Meet Acceptable Criteria

- a. Repeat the test if any of the controls do not meet expected reactivities until QC in the Geenius reader is shown as a green dot or passed.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

- False negative results may occur in individuals infected with HIV-1 and/or HIV-2 who are receiving highly active antiretroviral therapy (HAART).
- For a positive result, the intensities of the test bands do not necessarily correlate with the titer of antibody in the sample.
- 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.
- 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 not be infected with HIV. It is recommended that testing be repeated on a specimen freshly drawn after 2-4 weeks.
- An assay cassette that contains smudges or background in the band area that may interfere with the test interpretation should not be read. Retest with a new assay cartridge.
- Indeterminate interpretations do not exclude the possibility of early seroconversion of the test subject or a cross-reaction with other retroviruses. Retest on freshly drawn specimen 2-4 weeks later.
- HIV-1 positive samples may show cross reactivity on one of the HIV-2 envelope bands, this does not exclude the rare possibility of a secondary HIV-2 positive seroconversion (co-infection).
- HIV-2 positive samples can show cross reactivity on one or more HIV-1 bands, this does not exclude the possibility of a secondary HIV-1 seroconversion (co-infection).
- Samples that are HIV-1 and HIV-2 positive, but are reactive with only one detected envelope band (gp160 or gp41), are generally HIV-2 positive with HIV-1 cross reactivity. This does not exclude the rare possibility of HIV-1 and HIV-2 co-infection.
- Samples with reactivity to all 4 envelope bands (HIV-1 and HIV-2) have all been HIV-2 positive samples with HIV-1 reactivity that cannot be differentiated (HIV Untypable or Undifferentiated). This does not exclude the possibility of HIV-1 and HIV-2 co-infection.
- 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 cassette before reporting.

13. Reference Ranges (Normal Values)

A normal sample is negative for HIV antibodies.

14. Critical Call Results (Panic Values)

Not applicable to this assay method.

15. Specimen Storage and Handling during Testing

Specimens are stored at $\leq -20^{\circ}\text{C}$ until testing. After an aliquot of the thawed sample has been removed for testing, the residual is refrozen and stored at $\leq -80^{\circ}\text{C}$.

16. Alternate Methods for Performing Test or Storing Specimens if Test System Fails

If the analytical system fails, store specimens at $\leq -20^{\circ}\text{C}$ until the system is investigated and the problem is resolved.

17. Test Result Reporting System; Protocol for Reporting Critical Calls (If Applicable)

Not applicable to this assay method.

18. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

Standard record keeping involves using the computerized database and the hard copy results themselves to track specimens. Records are maintained indefinitely. Only numerical identifiers (e.g., case ID numbers) should be used. All personal identifiers should be available only to the medical supervisor or project coordinator to safeguard confidentiality.

For the NHANES study, residual serum is retained at $\leq -70^{\circ}\text{C}$ for 1 year and then returned to NCHS serum bank.

19. Summary Statistics and QC Graphs

Qualitative assays are assays with a positive, indeterminate or negative result. Assay controls are monitored for proper performance on each run.

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