Wake Forest Baptist Medical Center	**Geenius (BioRad) HIV 1/2 Western Blot** **MS-13**	**Dept:**	705 Micro
		Effective Date:	09/07/17
		Revised Date:	01/09/19
		Contact:	Microbiology Manager
Name & Title: Dr. Gregory Pomper		**Date:**	
Signature:			

1) General Procedure Statement:

- **Purpose:** This procedure is to serve as a guide for trained personnel in the Clinical Microbiology Laboratory to perform the test described herein. This procedure should be used in conjunction with proper training and only by qualified technologists.

- **Responsible Department/Scope:**
  i. Procedure owner/implementer: Dr. Elizabeth Palavecino.
  ii. Procedure prepared by: Christy Hernandez, MT(ASCP)
  iii. Who performs procedure: Clinical Microbiology Laboratory personnel.

2) Procedure:

Summary

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 reactive on a 4th generation HIV assay should undergo supplemental testing with an immunoassay that differentiates HIV-1 from HIV-2 antibodies.

Principle

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 bands. When there are no HIV antibodies, there are no pink/purple bands in the test area. The sample continues to migrate through the membrane and a pink/purple band 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.

Specimen

The Geenius™ HIV 1/2 Supplemental Assay has been validated on serum samples at WFBMC.

Serum samples collected by standard laboratory procedure may be used in the test. Be sure that the tube of serum is well mixed after collection and before testing. Use a laboratory pipette to withdraw **5 µL** of the sample (note: SST tubes are acceptable). Perform the test following the Assay Procedure instructions below.

Serum samples may be stored at 2-8°C for up to 7 days or up to 48 hours at room temperature (18-30°C). For long-term storage, the serum 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.

Specimen Shipping

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

Equipment and Materials

Materials required but sold separately

- Geenius™ Reader and dedicated software
- Geenius™ HIV 1/2 Controls : Each package contains a Positive Control vial, a Negative Control vial

Materials required but not provided

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

Materials Provided

Geenius™ HIV 1/2 Supplemental Assay **Cat. No. 72461 (20 Tests)** Store kit at 2 to 30°C (36 to 86°F).		
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 (Contains bovine and goat sera, with preservatives: < 0.1% sodium azide, 0.125% gentamicin sulfate and 0.125% streptomycin sulfate.)	Ready to Use
Microtubes (20 pipettes)	15 µL Microtubes - Capillary plastic pipettes (no anti-coagulant), for collection and testing of whole blood samples.	Ready to Use

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 30ºC, 64 to 86ºF). If the cassette, samples, controls and/or kit components have been refrigerated, bring to room temperature (18 to 30°C, 64 to 86°F) 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 86ºF), 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.)
5. A clean new pipette or pipette tip should be used with each sample. Caution should be used when opening sample near cassette to eliminate possible cross-contamination from aerosol.

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.

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.

Quality Control

Internal Quality Control

Each Geenius™ HIV 1/2 Supplemental Assay cassette has a control band that 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 band will appear in the Control (C) area to indicate the cassette is working properly (Refer to Calculation and Interpretation of Results).

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 sample and follow the directions in the Refer to Calculation and Interpretation of Results. 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 **with every batch of patient samples** and under the following circumstances:

- 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).

Lot-to-Lot Quality Control
For each new lot or shipment run a previous known positive and negative patient sample or an external positive and negative control. Record results on New Lot QC Log.

Updating New Lots (Cassettes) in Geenius Software
See Geenius User Manual Chapter 5 "Kit Lot Management," page 45. Note that you must "declare" the control lot numbers for each new cassette lot.

Updating New Lots (Controls) in Geenius Software
See Geenius User Manual Chapter 6 "Control Management," page 51. Note that you must update new control lot numbers on current cassette lots. See Section 6.1, "Declare the Controls," page 52.

Procedure

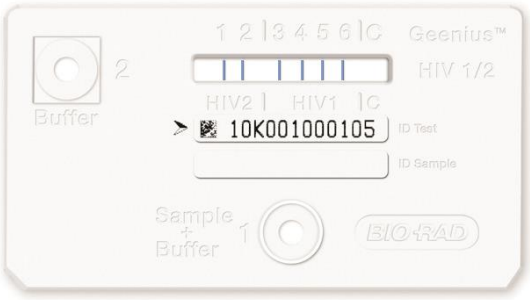
NOTE: If the patient has been tested in the WFBMC Clinical Microbiology Laboratory previously, and was reported as Reactive for HIV1, the HIV Western Blot does not have to be performed. Report the HIV result as: PREWB which translates to "Western Blot not performed because patient has been previously confirmed as reactive by Western Blot at WFBMC."

NOTE: A HIV Western Blot test cannot be ordered as a separate test. It is a reflex test for HIV EIA reactive samples (reactive times two) by either the Centaur or a Rapid HIV. Samples that require Western Blot testing are placed in the Serology refrigerator in a rack labeled HIV Western Blot. A note is left for third shift indicating that a Western Blot needs to be performed that night.

Procedure – Stepwise

1. If the cassette, samples, controls and/or kit components have been refrigerated, bring to room temperature (18 to 30ºC, 64 to 86ºF) before use.
2. Turn on the laptop and open the Geenius software. The Geenius reader should automatically power on and connect. This is indicated by a green LED indicator on the reader.
3. In the software, enter the username and password then click the green checkmark.

4. 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.



WARNING: The cassette should not be picked up or tilted during the testing procedure, including during the incubation steps.

Testing should be performed on a flat and level surface.

5. Using a laboratory pipette, dispense 5 µL of serum or control to the center of the Sample + Buffer Well 1 of the cassette.

6. Immediately following the addition of the sample **(but no longer than 5 minutes)**, use the dropper bottle to add 2 drops of Buffer into the Sample + Buffer Well 1. **NOTE:** When

dispensing Buffer into the cassette Sample + Buffer Well 1, it is essential that the dropper be held vertically. Buffer drops should fall freely for the tip, onto the membrane in the center of the well, to ensure the full amount is delivered. Do not touch tip of dropper bottle to cassette well, as this may prevent the required amount from being delivered.

7. **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 any blue lines, or any portion of the blue lines, remain after 7 minutes from dispensing Buffer into Well 1, the cassette is invalid, and a new cassette must be used to repeat the assay.

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 of Buffer to Buffer Well 2. **See NOTE in step 6.**

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

After adding Buffer to Well 2, and before reading the cassette, wait until all fluid has migrated across test strip and no streaks or background remains (at least 15 minutes). If any background or streaks remain, allow migration to continue up to 30 minutes. **Do not read results more than 30 minutes after the addition of the Buffer to Buffer Well 2.**

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

Do not read a Geenius™ cassette that contains smudges or background in the band Test area that may interfere with test interpretation. The sample should be retested with a new Geenius HIV1/2 Supplemental Assay cassette.

Test results must be read with the Geenius™ Reader. Refer to the Geenius Reader User Manual for detailed instructions regarding the operation of the Geenius reader.

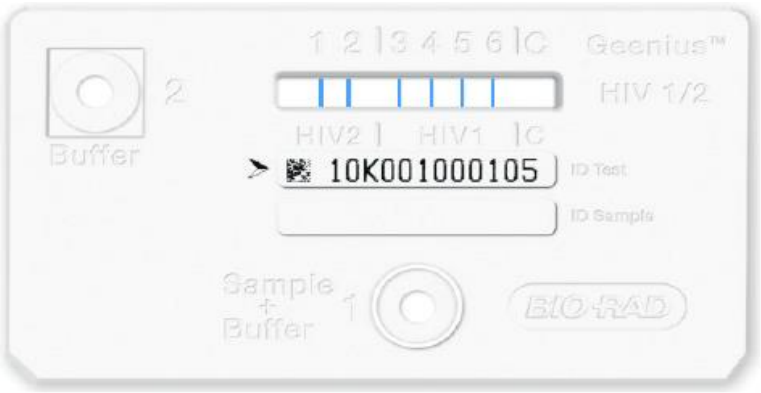
Reading the Cassette:

1. Open the Geenius software, click the Run tab.
2. Insert the cassette into the reader up to the stop with the arrow pointing towards the reader.
3. You'll get a message saying "Sample ID required." Click the green check ("OK").
4. Enter/scan the sample ID then click the green check ("run").
5. The reader will read the cassette. Click the green double arrow ("next") to save and prepare to read the next cassette.
6. When all samples have been read, click the Results tab.
7. Click the check box next to each sample and control. Click print summary icon.
8. Place the printed results (after Supervisory review) in the Western Blot results notebook and turn off the laptop.

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

Interpretation of Results

Results must be interpreted with the Geenius™ Reader (REF 92465) 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 bands in the test area that 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 band 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 band 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, (and the Control band), 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."

HIV-1 RESULT	**HIV-2 RESULT**	**ASSAY INTERPRETATION**
Negative	Negative	**HIV Non-Reactive by WB.** Non-Reactive for HIV 1&2 antibodies. Cannot rule out acute infection. Recommend follow up testing for viral load (HIV1 Ultraquant) if clinically or epidemiologically indicated.
Indeterminate	Negative	**HIV-1 INDETERMINATE by Western Blot** [a]
Negative	Indeterminate	***HIV-2 INDETERMINATE by Western Blot** [b]
Indeterminate	Indeterminate	**HIV INDETERMINATE by Western Blot** [c]
Positive	Negative	**HIV-1 Reactive, confirmed by Western Blot**
Positive	Indeterminate	**HIV-1 Reactive, confirmed by Western Blot**
Negative	Positive	***HIV-2 Reactive, confirmed by Western Blot***
Indeterminate	Positive	***HIV-2 Reactive, confirmed by Western Blot***
Positive	Positive	***HIV-2 Reactive with HIV-1 cross-reactivity:** Antibody to HIV2 confirmed in the sample. HIV1 positivity (with only one HIV-1 envelope band, gp160 or gp41), due to cross-reactivity; precludes confirmation of HIV1.
Positive	Positive	***HIV Reactive Untypeable (undifferentiated):** Antibodies to HIV1 and 2 confirmed. May occur in HIV2 reactive sample with significant cross-reactivity to HIV1; or co-infection with both HIV1&2 (rare). Further testing is indicated.

[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*
Consult supervisor before reporting any HIV-2 reactive/indeterminate results

LIMITATIONS OF THE TEST

1. The Geenius™ HIV 1/2 Supplemental Assay must ONLY be used with serum at WFBMC. 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 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 diagnoses 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 bands 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 specimens that are reactive on the initial antigen/antibody combination immunoassay and have interpretation of negative or indeterminate with the HIV-1/HIV-2 antibody differentiation immunoassay should be tested with an FDA-approved HIV-1 nucleic acid test (NAT). At WFBH, a HIV ultraquant by PCR is recommended.
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.
    - Samples that meet the HIV-1 Positive criteria may, in some rare cases, show cross reactivity on one of the HIV-2 envelope bands. In most of the cases, this profile that

confirms an HIV-1 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 that 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 envelope and HIV-2 envelope 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, or the possibility of HIV-1 positive samples with significant cross-reactivity of HIV-2 antigens.
- 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.

3) Review/Revision/Implementation:

All procedures must be reviewed at least every 2 years.

- All new and procedures that have major revisions must be signed by the Department Chairman.
- All reviewed procedures and procedures with minor revisions can be signed by the designated section medical director.

4) Related Procedures:

- HIV Antigen/Antibody Combo (Chemistry)
- Bio-Rad Geenius User Manual

5) References:

Bio-Rad: Geenius HIV ½ Supplemental Assay Package Insert. Redmond, WA, September, 2017.

6) Attachments: None.

7) Revised/Reviewed Dates and Signatures:

Review/Revision Date	Signature