

Laboratory Procedure Manual

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

Matrix: **Serum/Plasma**

Method: **Bio-Rad Multispot HIV-1/HIV-2 Rapid
Test**

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_I	LBXHIV1	HIV-1
	LBXHIV2	HIV-2

Bio-Rad Multispot HIV-1/HIV-2 Rapid Test

1. Summary of Test Principle and Clinical Relevance- EIA

The Multispot HIV-1/HIV-2 Rapid Test is a single use qualitative immunoassay to detect and to differentiate circulating antibodies to human immunodeficiency virus types 1 and 2 (HIV-1, HIV-2) in fresh or frozen human serum and plasma. This rapid HIV-1/HIV-2 test kit is intended as an aid in the diagnosis of infection with HIV-1 and/or HIV-2 in fresh or frozen human serum or plasma. This test is suitable for use in multi-test algorithms designed for statistical validation of an HIV screening test result or as part of an HIV-1/HIV-2 diagnostic testing algorithm that includes differentiation of HIV-1 and HIV-2 antibodies.

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. In one study, detection of HIV-2-positive samples by HIV-1 antibody kits ranged from 60% to 91%, depending on the test used.²⁵ The Multispot HIV-1/HIV-2 Rapid Test incorporates highly conserved recombinant and synthetic peptide sequences representing HIV-1 and HIV-2 envelope proteins.²⁶⁻³² The Multispot HIV-1/HIV-2 Rapid Test is designed to detect antibodies to HIV-1 and HIV-2 in serum or plasma rapidly and reliably without

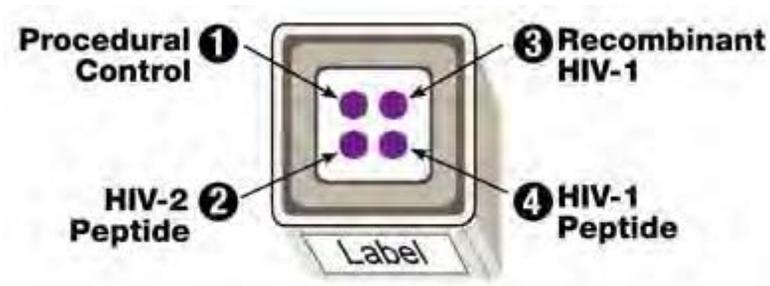
instrumentation. This test is suitable for use in multi-test algorithms designed for statistical validation of rapid HIV test results or as part of an HIV-1/HIV-2 diagnostic testing algorithm that includes differentiation of HIV-1 and HIV-2 antibodies³³⁻³⁹.

Biological Principles of the Procedure

The Multispot HIV-1/HIV-2 Rapid Test is based on the principle of ImmunoConcentration™.⁴⁰ The Multispot HIV-1/HIV-2 Cartridge contains a removable specimen prefilter, the reaction membrane, and an absorbent pad. All of the liquids added to the Cartridge are absorbed by the pad and contained within the Cartridge. When the test is completed, the entire Cartridge can be decontaminated by standard laboratory practices (see Precautions for Users) and properly discarded.

Microscopic particles are separately coated with the antigens that represent portions of the transmembrane envelope proteins HIV-1 and HIV-2, respectively. The microparticles are immobilized on the reaction membrane of the Multispot HIV-1/HIV-2 Cartridge and form the Test Spots. The reaction membrane also contains a Procedural Control Spot that serves as a control spot to ensure that the entire test procedure was properly executed. Samples to be tested are diluted in Specimen Diluent and then added to the prefilter in the Cartridge. After the diluted specimen has been completely absorbed, the prefilter is removed. If antibodies against HIV-1 and/or HIV-2 are present in the specimen, they bind to the antigens on the microparticles in the specific spots on the cartridge membrane. The Conjugate, which contains alkaline phosphatase-labeled goat anti-human IgG (H+L chain specific), is then added to the Cartridge. The Conjugate binds to the human antibody-antigen complexes that are immobilized in the spots on the cartridge membrane. Unbound Conjugate is removed by a wash step.

Next, Development Reagent is added to the Cartridge. A purple color develops on the Test Spots in proportion to the amount of antibodies against HIV-1 and/or HIV-2 that have been bound to the antigen-coated microparticles and detected by the Conjugate. A purple color will also develop on the Procedural Control Spot when the test has been performed correctly. Color development is stopped by the addition of Stop Solution. The membrane is examined visually for the presence of purple color on the Procedural Control Spot and on the Test Spots.



- ① Procedural Control: Anti-human IgG (goat)
- ② HIV-2 Peptide: Peptide representing the immunodominant epitope of the HIV-2virus gp36 envelope glycoprotein
- ③ Recombinant HIV-1: Recombinant gp41 (HIV-1 envelope glycoprotein) expressed in *E.coli* (gp41)
- ④ HIV-1 Peptide: Peptide representing the immunodominant epitope of the HIV-1virus gp41 envelope glycoprotein

2. Safety Precautions

1. This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Handle appropriately with the requisite Good Laboratory Practices including lab coat, eye/face protection and disposable gloves (synthetic, non-latex gloves are recommended) while handling kit reagents and patient samples. Wash hands thoroughly after performing the test.
2. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
3. Do not pipette by mouth.
4. This product contains dry natural rubber in the dropper bulb used with the Specimen Diluent bottle.
5. Dispose of all specimens and materials used to perform the test as biohazardous waste. Laboratory chemical and biohazardous wastes must be handled and discarded in accordance with all local, regional, and national regulations. For additional information on biosafety requirements, refer to CDC recommendations for Universal Precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens.⁴¹
6. Complete hazard information and precautions are located in the Safety Data Sheet (SDS) available at bio-rad.com or upon request.

Handling precautions:

1. Do not use any kit components beyond their stated expiration date.
2. Do not mix components from different lots.
3. Do not use the components in any other type of test kit as a substitute for the components in this test kit.
4. Use the Multispot HIV-1/HIV-2 Cartridge and disposable Transfer Pipets only once and then dispose of as described in Safety Precautions. Do not reuse these kit components.
5. Exercise care in opening and reusing reagent bottles to avoid microbial contamination of the reagents.
6. Prior to running the assay, verify that the prefilter is seated firmly on top of the Cartridge by pressing down firmly and evenly.
7. Always hold each reagent bottle vertically and allow each reagent to fall freely from the dropper tip. Do not touch the dropper tip to any surface; this may contaminate the reagent.
8. Avoid contact of the Stop Solution with any oxidizing agent. Do not allow Stop Solution to come into contact with metals.
9. Handle the Negative and Positive Control Serums in the same manner as patient specimens.
10. Inadequate adherence to package insert instructions may result in erroneous results.
11. When removing the Transfer Pipets from the bag, avoid touching the tips of the pipets.
12. The test should be performed with Cartridges that are placed on a flat surface.

13. Adequate lighting is required to read test results.

WARNINGS FOR USERS

For In Vitro Diagnostic Use

1. The package insert must be read completely before performing the test. Failure to follow the insert may give inaccurate test results.
2. The Bio-Rad Multispot HIV-1/HIV-2 Rapid Test has been approved for use with serum and plasma specimens only. Use of this test kit with specimens other than those specifically approved for use with this test kit may result in inaccurate test results.
3. Bring all reagents to room temperature (20-30°C) before use.
4. The following is a list of potential chemical hazards contained in some kit components (refer to product REAGENTS chart):



a. WARNING: Some reagents contain 0.1% ProClin 150 or 0.5% ProClin 300:

H317: May cause an allergic skin reaction.

P280: Wear protective gloves/protective clothing/eye protection/face protection.

P302 + P352: IF ON SKIN: Wash with plenty of soap and water.

P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention.

P501: Dispose of contents and container in accordance to local, regional, national and international regulations.

ProClin 300 (0.1% ProClin 150 and 0.5% ProClin 300) are biocidal preservatives that are irritating to eyes and skin, may be detrimental if enough is ingested, and may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.



b. WARNING: Some reagents contain 0.1% Sodium Azide [NaN₃]:

H303: May be harmful if swallowed.

H313: May be harmful in contact with skin.

P312: Call a POISON CENTER or doctor/physician if you feel unwell.

Sodium azide may react with lead and copper plumbing to form metal azides that are highly explosive. If disposed of in the sink, flush plumbing with a large volume of water to prevent azide buildup.

c. The dilute 0.1 N sulfuric acid (H₂SO₄) Stop Solution may be detrimental if swallowed and by contact, particularly to eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wastes can typically be neutralized to pH 6-8 for disposal if trained and equipped to do so, however always dispose of dilute acidic / corrosive solutions in accordance with local, regional, national and international regulations. Do not pour water into this product.

5. Users of this test should follow the CDC Universal Precautions for prevention of transmission of HIV, hepatitis B virus, and other bloodborne pathogens.⁴¹
6. The Multispot HIV-1/HIV-2 Rapid Test contains human blood components. No known test method can offer complete assurance that infectious agents are absent. Therefore, all human blood derivatives, reagents and human specimens should be handled as if capable of transmitting infectious disease, following recommended Universal Precautions for bloodborne pathogens as defined by OSHA, Biosafety Level 2 guidelines from the current CDC/NIH Biosafety in Microbiological and Biomedical Laboratories⁴², WHO Laboratory Biosafety Manual⁴³, and/or local, regional and national regulations. The following human blood derivatives are found in this kit:
 - a. The Positive Control Serum has been heat-treated to inactivate HIV viruses and has been tested and found nonreactive for Hepatitis B Surface Antigen (HBsAg) and antibodies (Ab) to Hepatitis C virus (HCV).
 - b. The human source material used in the preparation of the Negative Control Serum has been tested and found nonreactive for Hepatitis B Surface Antigen (HBsAg), antibodies to Hepatitis C virus (HCV Ab), and antibodies to HIV (HIV-1/HIV-2 Ab).

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 bleach, 70- 80% ethanol or isopropanol, an iodophor (such as 0.5% Wescodyne™ Plus), or a phenolic, etc.) and wiped dry.

Spills containing acid should be appropriately absorbed (wiped up) or neutralized, wiped dry and then the area wiped with one of the chemical disinfectants; material used to absorb the spill may require biohazardous waste disposal.

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

3. Computerization; Data System Management

HIV antibody differentiation results are manually entered into a Microsoft Excel result file spreadsheet. After a run is complete and any additional corrections by the analyst are made, the Excel result file is finalized. Data is 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

Fresh or frozen serum or plasma collected by standard phlebotomy procedures may be used in the test. The minimally acceptable volume of specimen available for performing the test is 40 μ L. Approximately 30 μ L is used for running each test. No clinically significant effect has been detected in assay results of serum or plasma samples with increased levels of hemoglobin, protein, albumin, lipids, or bilirubin. **Performance of this assay has not been evaluated on patient samples that have been heat-inactivated.**

The following anticoagulants have been evaluated and found to be acceptable for use with this test: EDTA, sodium citrate, sodium heparin, and SST tubes. Samples that are collected into anticoagulant tubes should be filled as labeling indicates to avoid improper dilution. **Use of other anticoagulants has not been evaluated and may give incorrect results.**

Specimens may be stored at 2-8°C for 7 days or at room temperature (20-30°C) for up to 48 hours. For long-term storage, the specimens should be frozen (-20°C or colder). Specimen may be frozen and thawed up to 5 times.

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

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

MULTISPOT HIV-1/HIV-2 Rapid Test

Product No. 25228 (50 Tests)

Component	Contents	Preparation
1 • Multispot HIV-1/HIV-2 Cartridge (50)	•Foil-sealed base container with specimen prefilter; Membrane with 1 Procedural Control Spot and 3 Test Spots	Remove foil seal before use
2 • Positive Control Serum 1 dropper bottle (1 mL)	• Heat-inactivated human serum/plasma containing anti-HIV-1 and anti-HIV-2 immunoglobulin; Nonreactive for HBsAg and antibody to HCV • 0.1% Sodium azide • 0.5% ProClin™ 300	Dilute in Specimen Diluent as described
3 • Negative Control Serum 1 dropper bottle (1 mL)	• Human serum; Nonreactive for HBsAg and antibody to HIV and HCV • 0.1% Sodium azide • 0.5% ProClin™ 300	Dilute in Specimen Diluent as described
4 • Specimen Diluent 1 dropper bottle (25 mL)	• Diluent for specimens and Controls • 0.1% ProClin™ 150 • 0.125% ProClin™ 300	Dispense with dropper provided
5 • Conjugate 1 dropper bottle (9.5 mL)	• Anti-human IgG (H+L) (goat) alkaline phosphatase conjugated solution • 0.1% ProClin™ 150	Ready to use as supplied
6 • Wash Solution 2 dropper bottles (2 x 85 mL)	• TRIS • Urea • Propylene glycol • Nitroblue tetrazolium • 0.1% ProClin™ 150	Ready to use as supplied
7 • Development Reagent 1 dropper bottle (8.5 mL)	• 3-Indoxyl phosphate	Ready to use as supplied
8 • Stop Solution 1 dropper bottle (55 mL)	• 0.1 N H ₂ SO ₄ (sulfuric acid)	Ready to use as supplied
9 • Disposable Transfer Pipets (60)	• Polyethylene transfer pipets	Ready to use as supplied
10 • Eyedropper (1)	• Polyethylene eyedropper and cap with rubber bulb; Contains Dry Natural Rubber, a potential sensitizer	Use in Specimen Diluent bottle

B. Reagent Preparation

All solutions and reagents are ready to use as supplied. Store kit at 2-8°C or room temperature (20-30°C). If stored at 2-8°C, bring all reagents to room temperature before use, and return entire kit to 2-8°C when not in use. The kit may be used up to kit expiration date when stored at 2-8°C or for up to 3 months if stored at room temperature. When stored at room temperature, change the expiration date to three months after start of room temperature storage (do not change the date if it less than 3 months before kit). Do not freeze test components.

C. Materials Provided

- Package insert (1)
- Subject Information Notice (1) - The Notice in the kit box may be copied as needed.
- Customer letter (1)

D. Materials Required But Not Provided

1. Disposable glass or polypropylene test tubes (do not use polystyrene) to prepare diluted specimens and controls (for example: 12 x 75 mm tubes)

2. Test tube racks
3. Absorbent pads or paper towels
4. Biohazard bags with closures
5. 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™.
6. Disposable gloves.
7. Laboratory timer.
8. Precision pipettors that deliver 30 µL and 300 µL (optional for addition of specimen and Specimen Diluent). Precision pipettors that deliver 10 µL and 90 µL as needed for dilutional testing of dually positive samples.
9. Indelible laboratory marker.

7. Calibration and Calibration Verification Procedures

Procedural Control

Each Multispot HIV-1/HIV-2 Cartridge has a built-in procedural control, the Procedural Control Spot, which is used to determine validity of the assay. The Procedural Control Spot must be reactive (a definite purple spot) on each Cartridge for the results of that Cartridge to be valid.

Quality Control

Using individual Multispot HIV-1/HIV-2 Cartridges as described in the Assay Procedure above, run 1 Positive Control Serum and 1 Negative Control Serum (both provided in the kit) under the following circumstances to monitor proper test performance:

- A new operator uses the kit, prior to performing testing of specimens.
- A new test kit lot is used.
- A new shipment of kits is used.
- The temperature used during storage of the kit falls outside of 2-30°C (35.6-86°F).
- The temperature of the test area falls outside of 20-30°C (68-86°F).
- According to intervals defined by the testing facility.

Results are read by examining the membrane and comparing the location of colored spots on the membrane to the diagram below. **Position the Multispot HIV-1/HIV-2 Cartridge with the ID label facing the user.** The appearance of any purple color in any of the Test Spots, regardless of intensity, must be considered as presence of that Spot.

Expected results are as follows:



Negative Control
Note placement of Cartridge ID label

Only the Procedural Control Spot shows purple color development. The 3 Test Spots show no color development.



Positive Control

The Procedural Control Spot, both HIV-1 Test Spots, and the HIV-2 Test Spot show purple color development.

Preliminary Statements

- Once testing has been started, it should be completed without interruption.
- Do not use more than ten (10) Multispot HIV-1/HIV-2 Cartridges in a batch, since using more Cartridges may make it difficult to complete the testing without interruption. Larger numbers of specimens can be tested by running several batches of up to 10 Cartridges.
- The eyedropper used to dispense Specimen Diluent is packaged separately from the bottle of Specimen Diluent. The first time a kit is used, remove the eyedropper from the packaging and insert it into the bottle of Specimen Diluent. Discard the original cap and use the eyedropper as the cap for the bottle. Two full eyedroppers dispenses approximately 300 μ L of Specimen Diluent.
- A 30 μ L precision pipettor can be used for addition of the sample to the Specimen Diluent. The disposable Transfer Pipets supplied in the kit dispense approximately 30 μ L per drop.
- The Cartridges should be placed on a flat surface during the assay procedure to ensure proper flow of specimen and reagents through the membrane.
- All solutions must be completely absorbed (no standing liquid) into the Cartridge membrane before proceeding to the next step in the Assay Procedure.

Assay Procedure

1. Bring kit and specimens to room temperature (20-30°C) before beginning testing. It is essential that all kit components are at room temperature before use.
2. Place the required number of Cartridges on a flat surface with the patient ID label facing toward the operator. Peel away the foil seals and discard them. Label the Cartridges to correspond with the test tubes and the specimens to be tested.

Note: Verify that the blue prefilter and gray top support are seated securely in the base of the Cartridge by pressing down firmly and evenly on both pieces. The prefilter must be present in order to use the Cartridge for testing.

3. Label a test tube for each specimen or control to be tested.
4. Invert the Specimen Diluent bottle ten times to thoroughly mix just prior to drawing the reagent.
5. Add two full eyedroppers of Specimen Diluent to each specimen and control tube.

Note: With the eyedropper in the Specimen Diluent, hold vertically and squeeze the bulb completely, draw Specimen Diluent up into the eyedropper, and gently expel all of the

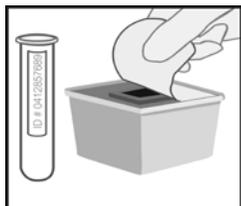
Specimen Diluent into the test tube. Repeat this sequence to deliver the second full eyedropper.

6. Using a precision pipet with a separate pipet tip for each sample, add 30 μ L of specimen to the Specimen Diluent. Alternatively, using a separate kit-provided Transfer Pipet for each specimen, draw up a small amount of specimen. While holding the pipet vertically over the appropriate dilution tube, add one drop to the tube.

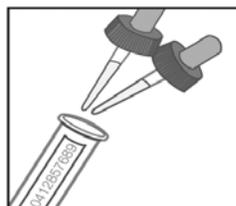
Note: The drop should fall freely into the Specimen Diluent, not onto the side of the tube. If the drop does fall onto the side of the tube, make sure that the entire drop drains down into the Specimen Diluent. If the drop does not drain into the Specimen Diluent, discard the tube and prepare a new dilution. Do not allow the tip of the pipet to touch any part of the tube or the Specimen Diluent in the tube. Discard the used pipet tip or Transfer Pipet into the biohazardous waste.

7. Test Positive and Negative Control Serums as described in the QC section. When preparing Positive and Negative Control Serums, hold the dropper bottles vertically over the tubes labeled for controls and squeeze gently.
Add one drop of each control to the appropriately labeled tube. The drop should fall freely into the Specimen Diluent. Do not allow the tip of the dropper to touch any part of the tube.
8. Mix each diluted specimen and control thoroughly. Mix gently to avoid foaming.
9. Pour the contents of each tube into the specimen prefilter of each corresponding pre-labeled Cartridge, using a separate Cartridge for each tube. Wait two minutes, after which the solution must be completely absorbed through the prefilter into the Cartridge.
10. Remove and discard the prefilter into the biohazardous waste.
11. Fill the central well of each Cartridge with Wash Solution by holding the bottle vertically and squeezing gently. Do not touch bottle to solution in Cartridge well. Wait for the Wash Solution to be absorbed completely before proceeding.
12. Add three drops of Conjugate to the central well of each Cartridge by holding the bottle vertically and squeezing gently. Do not touch bottle tip to solution in Cartridge well. Wait two minutes.
13. Fill the central well of each Cartridge with Wash Solution by holding the bottle vertically and squeezing gently. Do not touch bottle to solution in Cartridge well. Wait for the Wash Solution to be fully absorbed before proceeding.
14. Repeat previous step so that each Cartridge is washed twice. Wait for the Wash Solution to be absorbed completely before proceeding.
15. Add three drops of Development Reagent to the central well of each Cartridge by holding the bottle vertically and squeezing gently. Wait five minutes.
16. Fill the central well of each Cartridge with Stop Solution by holding the bottle vertically and squeezing gently. Wait for the Stop Solution to be absorbed completely before reading results.
17. Read test results according to Test Result Appearance and Interpretation, (Rapid Testing) or (Antibody Differentiation Test in a Diagnostic Testing Algorithm), either immediately or anytime up to 4 hours after completing the test. An elevated background can appear over time with some specimens; therefore, reading results within 1 hour is optimal.

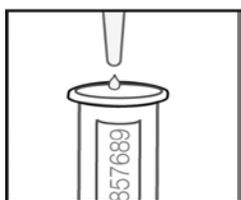
Figure 1. Multispot Test procedure



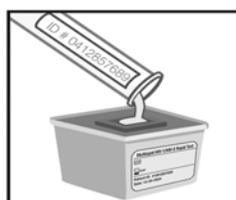
1. Remove foil; press prefilter down. Label cartridge and specimen or control tubes.



2. Add 2 full droppers of Specimen Diluent to each microtiter tube.



3. Add one drop of each sample or control to each labeled tube using a transfer pipette. Mix well. Avoid foaming.



4. Pour each sample into the prefilter of the labeled cartridge. Wait 2 minutes.



5. Remove and discard prefilter.



6. Fill the central well of each cartridge with Wash Solution.



7. Once absorbed, add 3 drops of Conjugate. Wait 2 minutes.



8. Fill well with Wash Solution and let absorb. Repeat.



9. Add 3 drops of Development Reagent. Wait 5 minutes.



10. Fill well with Stop Solution. Allow to absorb and read results.

Dilutional Procedure for HIV Differentiation – Rapid HIV-1/HIV-2 Testing

The following procedure is used to differentiate samples that demonstrate purple color development in the HIV-2 Spot as well as in one or both of the HIV-1 Spots.

1. Dilute the specimen 1:10 (using a calibrated pipettor, add 90 μ L of Negative Control Serum and 10 μ L of sample to a separate test tube; or, alternatively, 135 μ L of Negative Control Serum and 15 μ L of sample). Mix well.
2. Test the diluted sample as in steps 3-16 in the Assay Procedure section. Use the 1:10 diluted sample in place of the undiluted sample in step 6 of the Assay Procedure.
3. Read the results according to the criteria in Test Result Appearance and Interpretation.
 - If the results are nonreactive at this dilution, the specimen should be interpreted as **“Preliminary Positive for antibodies to HIV (undifferentiated).”**
 - If the Procedural Control Spot is reactive and the Test Spots are reactive for only HIV-1 or HIV-2, the result can be reported as Preliminary Positive for antibodies to the specific HIV type identified.
 - If one or both of the HIV-1 Spots and the HIV-2 Spot are still reactive, continue testing as follows.
4. Dilute the 1:10 diluted specimen again by 10-fold in Negative Control Serum, following the procedure in step 1 above (the final dilution is 1:100).
5. Test the diluted sample as in steps 3-16 in the Assay Procedure section. Use the 1:100 diluted sample in place of the undiluted sample in step 6 of the Assay Procedure.
6. Read the results according to the criteria above in Test Result Appearance and Interpretation.
 - If the Procedural Control Spot is reactive and the Test Spots are reactive for only HIV-1 or HIV-2, the result can be reported as Preliminary Positive for antibodies to the specific HIV type identified.
 - If the dual HIV reactivity does not disappear at the 1:100 dilution, or if the HIV-1 and HIV-2 spots both become nonreactive at the same dilution, the specimen should be interpreted as **“Preliminary Positive for antibodies to HIV (undifferentiated).”**

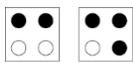
RAPID HIV-1/HIV-2 TESTING

Nonreactive:



Only the Procedural Control Spot shows purple color development. The 3 Test Spots show no color development. Test result is interpreted as negative for HIV-1 and HIV-2 antibodies.

Reactive:



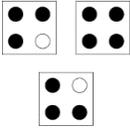
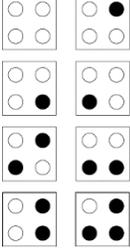
HIV-1 Reactive – Preliminary Positive:

The Procedural Control Spot shows purple color development and the recombinant HIV-1 Spot and/or the HIV-1 Peptide Spot show purple color development. Test result is interpreted as Preliminary Positive for HIV-1 antibodies.



HIV-2 Reactive – Preliminary Positive:

The Procedural Control Spot shows purple color development. The HIV-2 Peptide Spot shows purple color development. Test result is interpreted as Preliminary Positive for HIV-2 antibodies.

	<p>HIV Reactive (Undifferentiated) – Preliminary Positive: The Procedural Control Spot shows purple color development. The HIV-2 Peptide Spot shows purple color development as well as one or both HIV-1 Spots. In this case, the specimen may be tested by additional methods which allow for differentiation between HIV-1 and HIV-2. See dilutional procedure.</p>
	<p>Invalid – Do Not Report Any Results If no color develops in the Procedural Control Spot, regardless of color development anywhere else on the membrane, the results are invalid.</p> <p>If the background on the membrane is dark and interferes with the interpretation of the spots, the results are invalid. In addition, if there are stray purple marks or discoloration that interfere with reading the spots, the assay should be repeated. Repeat the assay, and if results are still invalid collect a fresh sample or test by another method.</p> <p>If there is a halo of coloration that appears around all of the Test Spots and the Procedural Control Spot, no results should be reported. An abnormal halo is typically larger in size and lighter in color than the Procedural Control Spot. The halo appears around each of the Test and Control Spots and may be apparent on the membrane regardless of purple color development associated with positive results. Samples exhibiting this unusual reaction should be tested by another methodology to determine HIV status of the sample.</p>

USE OF MULTISPOT AS AN ANTIBODY DIFFERENTIATION TEST IN A DIAGNOSTIC TESTING ALGORITHM

Test Result Appearance and Interpretation – Diagnostic Testing Algorithm that Includes Differentiation between HIV-1 and HIV-2 Antibodies:

Place the Cartridges with the patient ID label facing toward the operator prior to reading test results. Examine the Cartridge membrane and compare the location of colored spots to the diagram below. The appearance of a definite purple color in any of the Test Spots must be considered as presence of that Spot. Follow guidelines for using the assay in an HIV testing algorithm.

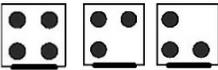
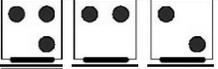
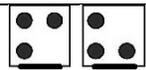
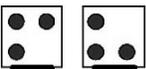
	<p>Nonreactive: Only the Procedural Control Spot shows purple color development. The 3 Test Spots show no color development. Test result is interpreted as negative for HIV-1 and HIV-2 antibodies. Additional testing is recommended, including HIV nucleic acid testing (NAT).</p>
<p>Reactive:</p>	

	<p>HIV-1 POSITIVE: The Procedural Control Spot shows purple color development and both the recombinant HIV-1 Spot and the HIV-1 Peptide Spot show purple color development. Test result is interpreted as Positive for HIV-1 antibodies</p>
	<p>HIV-2 POSITIVE: The Procedural Control Spot shows purple color development. The HIV-2 Peptide Spot shows purple color development. Test result is interpreted as Positive for HIV-2 antibodies.</p>
	<p>HIV Reactive (Undifferentiated) : The Procedural Control Spot shows purple color development. The HIV-2 Peptide Spot shows purple color development as well as one or both HIV-1 Spots. In this case, the specimen may be tested by additional methods which allow for differentiation between HIV-1 and HIV-2. See dilutional procedure which follows.</p>
	<p>HIV-1 INDETERMINATE: The Procedural Control Spot shows purple color development and either the recombinant HIV-1 Spot or the HIV-1 Peptide Spot shows purple color development but not both HIV-1 Spots. Test result is interpreted as Indeterminate for HIV-1 antibodies and testing for HIV nucleic acid is recommended.</p>
	<p>Invalid – Do Not Report Any Results If no color develops in the Procedural Control Spot, regardless of color development anywhere else on the membrane, the results are INVALID. (See examples.) If the background on the membrane is dark and interferes with the interpretation of the spots, the results are invalid. In addition, if there are stray purple marks or discoloration that interferes with reading the spots, the assay should be repeated. Repeat the assay, and if results are still invalid collect a fresh sample or test by another method.</p>
	<p>If there is a halo of coloration that appears around all of the Test Spots and the Procedural Control Spot, no results should be reported. An abnormal halo is typically larger in size and lighter in color than the Procedural Control Spot. The halo appears around each of the Test and Control Spots and may be apparent on the membrane regardless of purple color development associated with positive results. Samples exhibiting this unusual reaction should be tested by another methodology to determine HIV status of the sample.</p>

**Test Result Interpretation – Dilutional Testing for Diagnostic Testing Algorithm
 1:10 Dilutional Testing**

Initial Result	1:10 Dilution Result	Interpretation/Action
	  	HIV-1 POSITIVE
		HIV-2 POSITIVE
	 	Retest at 1:100 dilution
		Retest at 1:100 dilution
		HIV POSITIVE (undifferentiated)
 	 	HIV-1 INDETERMINATE
		HIV-2 POSITIVE
	 	Retest at 1:100 dilution
		HIV POSITIVE (undifferentiated)

1:100 Dilutional Testing

Initial Result	1:10 Dilution Result	1:100 Dilution Result	Interpretation/Action
			HIV-1 POSITIVE
			HIV-2 POSITIVE
			HIV POSITIVE (undifferentiated)
			HIV POSITIVE (undifferentiated)
			HIV POSITIVE (undifferentiated)
 	 	 	HIV-1 INDETERMINATE
			HIV-2 POSITIVE
			HIV POSITIVE (undifferentiated)
			HIV POSITIVE (undifferentiated)

8. Method Performance Specifications

Sensitivity for Antibodies to HIV-1

The sensitivity of the Multispot HIV-1/HIV-2 Rapid Test for antibodies to HIV-1 with serum specimens is calculated to be 100% (95% CI = 99.94 – 100.00%).

The sensitivity of the Multispot HIV-1/HIV-2 Rapid Test for antibodies to HIV-1 with plasma specimens is calculated to be 100% (95% CI = 99.94 – 100.00%).

Sensitivity for Antibodies to HIV-2

The sensitivity of the Multispot HIV-1/HIV-2 Rapid Test for antibodies to HIV-2 is calculated to be 100% (95% CI = 99.76 – 100%).

HIV-1 and HIV-2 Differentiation

Seven hundred ninety-nine (799) of the 801 samples (99.8%) were detected as HIV-1 reactive only on Multispot HIV-1/HIV-2, and the remaining 2 samples were dually reactive (undifferentiated) on Multispot HIV-1/HIV-2. Multispot identified 799 of the 801 known HIV-1 positive samples as HIV-1 reactive only (799/801 = 99.75%; 95% CI of 99.34 – 100.00%).

One hundred ninety (190) of 201 known HIV-2 specimens (94.5%) were detected as HIV-2 reactive only on Multispot HIV-1/HIV-2. Nine were reactive for both HIV-1 and HIV-2 and two were identified by Multispot as HIV-1 reactive.

Note: Samples reactive for both HIV-1 and HIV-2 may resolve to HIV-1 due to the lower titer of the HIV-2 antibody as compared to the HIV-1 antibody. Dual infections with both HIV-1 and HIV-2 viruses are unusual but may occur in individuals from HIV-2 endemic countries.

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

A total of 79 frozen serum and 124 frozen plasma specimens from various worldwide geographic locations outside of the U.S. were tested on Multispot HIV-1/HIV-2. HIV-1 subtypes represented included subtypes A, B, C, D, E, F, and G. All 203 specimens from this worldwide panel were reactive on Multispot HIV-1/HIV-2. In addition, 12 HIV-1 Serotype Group O frozen plasma samples were tested on Multispot HIV-1/HIV-2. Ten (10) samples were from Cameroon, one was from Spain, and one was from the United States. Eleven (11) of the 12 HIV-1 Group O serotype samples were reactive when tested on Multispot HIV-1/HIV-2, and one was nonreactive.

Reactivity with Seroconversion and Sensitivity (Low and Mixed Titer) Panels

Multispot HIV-1/HIV-2 detected the presence of antibody to HIV-1 in specimens from ten Seroconversion Panels as early as, or earlier than, a licensed HIV-1/HIV-2 EIA. The results of testing Multispot HIV-1/HIV-2 on 2 low titer panels and 1 mixed titer panel, in comparison to a licensed HIV-1/HIV-2 EIA, were assessed as well. Multispot HIV-1/HIV-2 was able to detect antibodies to HIV-1 similar to the licensed EIA.

Specificity

The specificity of the Multispot HIV-1/HIV-2 Rapid Test using serum specimens in these studies is calculated to be 1494/1495 or 99.93% (95% CI = 99.79 – 100.00%).

The specificity of the Multispot HIV-1/HIV-2 Rapid Test using plasma specimens in these studies is calculated to be 2272/2274 or 99.91% (95% CI = 99.77 – 100.00%).

Place the Cartridges with the patient ID label facing toward the operator prior to reading test results. Examine the Cartridge membrane and compare the location of colored spots to the diagram below. The appearance of any purple color must be considered as presence of that Spot.

9. Reportable Range of Results

Reportable results are expressed as positive or negative.

10. Quality Control (QC) Procedures

Procedural Control

Each Multispot HIV-1/HIV-2 Cartridge has a built-in procedural control, the Procedural Control Spot, which is used to determine validity of the assay. The Procedural Control Spot must be reactive (a definite purple spot) on each Cartridge for the results of that Cartridge to be valid.

Quality Control

Using individual Multispot HIV-1/HIV-2 Cartridges as described in the Assay Procedure, run 1 Positive Control Serum and 1 Negative Control Serum (both provided in the kit) under the following circumstances to monitor proper test performance:

- A new operator uses the kit, prior to performing testing of specimens.
- A new test kit lot is used.
- A new shipment of kits is used.
- The temperature used during storage of the kit falls outside of 2-30°C.
- The temperature of the test area falls outside of 20-30°C.
- According to intervals defined by the testing facility. The HIV Reference Laboratory will include the Positive and Negative Control with each run.

Results are read by examining the membrane and comparing the location of colored spots on the membrane to the diagram below. Position the Multispot HIV-1/HIV-2 Cartridge with the ID label facing the user. The appearance of any purple color in any of the Test Spots, regardless of intensity, must be considered as presence of that Spot.

Expected results are as follows:

Figure 2



Negative Control

Only the Procedural Control Spot shows purple color development. The 3 Test Spots show no color development.



Positive Control

The Procedural Control Spot, both HIV-1 Test Spots, and the HIV-2 Test Spot show purple color development.

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.
- b. Do not report any results from invalid runs

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

1. For a preliminary positive result, when used as a rapid HIV-1/HIV-2 test, clinical correlation is indicated with appropriate counseling, medical evaluation, and possibly additional testing (for example, Western blot or indirect immunofluorescence assay) to decide whether a diagnosis of HIV infection is accurate.
2. The Assay Procedure and the Test Result Appearance and Interpretation must be followed closely when testing for the presence of antibodies to HIV-1 or HIV-2 in plasma or serum from individual subjects. Failure to follow the procedure may give inaccurate results.
3. The test was designed to test individual specimens of fresh or frozen serum or plasma. Data regarding test kit interpretation were derived from testing individual samples. Insufficient data are available to interpret tests performed on other body specimens, pooled blood or processed plasma, and products made from such pools. Testing of these specimens is not recommended.
4. The following anticoagulants have been evaluated and found to be acceptable for use with this test: EDTA, sodium citrate, sodium heparin and SST tubes. **Use of other anticoagulants has not been evaluated and may give incorrect results.**
5. Performance of this assay has not been evaluated on patient samples that have been heat-inactivated.
6. Polystyrene tubes should not be used to prepare specimens for this test.
7. AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically. Testing alone cannot be used to diagnose AIDS, even if the recommended investigation of reactive specimens suggests a high probability that the antibody to HIV-1 or HIV-2 is present.
8. A nonreactive result for an individual subject indicates absence of detectable HIV antibodies. However, a nonreactive test result does not preclude the possibility of exposure to or infection with HIV-1 and/or HIV-2.
9. Nonreactive results can occur if the quantity of marker present in the sample is below the detection limits of the assay, or if the marker that is detected is not present during the stage of disease in which a sample is collected.
10. The risk of any asymptomatic person with a reactive serum or plasma developing AIDS or an AIDS-related condition is not known, as the course of HIV infections may vary among individual patients and may be altered by antiretroviral therapy. However, in a prospective study, AIDS developed in 51% of homosexual men after 10 years of infection.⁴³
11. A person who has antibodies to HIV-1 is presumed to be infected with the virus, except 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.
12. Specimens which are reactive for antibodies to both HIV-1 and HIV-2 on initial testing should be retested, according to the dilutional test protocol, to identify potential cross-reaction and differentiate between HIV-1 and HIV-2. Results of dilutional testing, when used for rapid HIV-1/HIV-2 testing, should be reported as Preliminary Positive for antibodies to the specific virus type identified in the dilutional testing. Specimens that are dually reactive when tested undiluted but only reactive for one virus type at the 1:100 dilution may be dually positive; these

samples are reported as Preliminary Positive for antibodies to the specific HIV type identified, when used for rapid HIV-1/HIV-2 testing.

13. The intensity of the Test Spot does not correlate with antibody titer of the specimen.
14. Samples reactive for both HIV-1 and HIV-2 may resolve to HIV-1 at higher dilutions due to the lower avidity of the HIV-2 antibody as compared to the HIV-1 antibody.
15. **The Multispot HIV-1/HIV-2 Rapid Test cannot be used as part of a diagnostic testing algorithm for both the initial testing and the differentiation testing of the same sample.**

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 $\leq -20^{\circ}\text{C}$.

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, negative or borderline/indeterminate result. Assay controls are monitored for proper performance on each run.

References

1. Centers for Disease Control: Provisional Public Health Service inter-agency recommendations for screening donated blood and plasma for antibody to the virus causing acquired immunodeficiency syndrome. **Morbidity and Mortality Weekly Rep** 34:5-7, 1985.
2. Delmonico FL, Snyderman DR: Organ donor screening for infectious diseases. **Transplantation** 65(5):603-610, 1998.
3. Barre-Sinoussi F, Chermann JC, Rey F, et al: Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). **Science** 220:868-871, 1983.
4. Gallo RC, Salahuddin SZ, Popovic M, et al: Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. **Science** 224:500-503, 1984.
5. Coffin J, Haase A, Levy JA, et al: What to call the AIDS virus? **Nature** 321:10, 1986.
6. Clavel F, Guetard D, Brun-Vezinet F: Isolation of a new human retrovirus from West African patients with AIDS. **Science** 233:343-346, 1986.
7. Clavel F, Manshino K, Chameret S, et al: Human immunodeficiency virus type 2 infection associated with AIDS in West Africa. **New Engl J Med** 316:1180-1185, 1987.
8. Schim van der Loeff MF and Aaby P: Towards a better understanding of the epidemiology of HIV- 2. **AIDS** 13 (Suppl. A):S69-S84, 1999.
9. Centers for Disease Control: AIDS due to HIV-2 infection - New Jersey. **Morbidity and Mortality Weekly Rep** 37:33-35, 1988.
10. Hoff R, Weiblen BJ, Schwerzler M, et al: Specific antibodies to HIV-2 detected in an anonymous newborn blood specimen from Massachusetts. **Fourth Consensus Conference on Testing for Human Retroviruses**, March 1989.
11. Ayanian JZ, Maguire JH, Marlink RG, et al: HIV-2 infection in the United States. **New Engl J Med** 320:1422-1423, 1989.
12. O'Brien TR, George JR, Holmberg SD: Human immunodeficiency virus type 2 infection in the United States. **JAMA** 267:2775-2779, 1992.
13. Centers for Disease Control: Update: HIV-2 infection among blood and plasma donors-- United States, June 1992-June 1995. **Morbidity and Mortality Weekly Rep** 44:603-606, 1995.
14. Sullivan PS, Fleming PL: Surveillance for HIV-2 in the United States: Update and recommendations for future surveillance. Presented at the Association of Public Health Laboratories Conference, Charlotte, NC, March 6-9, 2000.
15. Brun-Vezinet F, Katlama C, Roulot D, et al: Lymphadenopathy associated virus type 2 in AIDS and AIDS-related complex. **Lancet** 1:128-132, 1987.
16. Quinn TC, Zaccarias FRK, St. John RK: AIDS in the Americas: an emerging public health crisis. **New Engl J Med** 320:1005-1007, 1989.
17. Guyader M, Emerman M, Sonigo P, et al: Genome organization and transactivation of the human immunodeficiency virus type 2. **Nature** 326:662-669, 1987.
18. Cabrian K, Shriver K, Goldstein L, et al: Human immunodeficiency virus type 2: a review. **J Clinical Immunoassay** 11:107-114, 1988.
19. Janssens W, Buvé A, Nkengasong JN: The puzzle of HIV-1 subtypes in Africa. **AIDS** 11:705-712, 1997.

20. Charneau P, Borman AM, Quillant C, et al: Isolation and envelope sequence of a highly divergent HIV-1 isolate: definition of a new HIV-1 group. **Virology** 205:247-253, 1994.
21. Simon F, Maucière P, Rogues P, et al: Identification of a new human immunodeficiency virus type 1 distinct from group M and group O. **Nature Medicine** 4:1032-1037, 1998.
22. Meloni S T, et al. Distinct human immunodeficiency virus type 1 subtype A virus circulating in West Africa: Sub-subtype A3. *J Virology* 78(22):12438-12445, 2004.
23. Plantier JC, Leoz M, Dickerson JE, De Oliveira F, Cordonnier F, Lemée V, Damond F, Robertson DL, Simon F. A new human immunodeficiency virus derived from gorillas. *Nat Med.* 8:871-2. 2009.
24. Gao, F, Yue L, Robertson DL, et al: Genetic diversity of human immunodeficiency virus type 2: evidence for distinct subtypes with differences in virus biology. **J Virology** 68:7433-7447, 1994.
25. George JR, Rayfield M, Philips S, et al: Efficacies of U.S. FDA-licensed HIV-1 screening enzyme immunoassays for detecting antibodies to HIV-2. **AIDS** 4:321-326, 1990.
26. Starcich BR, Hahn BH, Shaw GM, et al: Identification and characterization of conserved and variable regions in the envelope gene of HTLV-III/LAV, the retrovirus of AIDS. **Cell** 45:637-648, 1986.
27. Wang JJG, Steel S, Wisniewolski R, Wang CY: Detection of antibodies to human T-lymphotrophic virus type III by using a synthetic peptide of 21 amino acid residues corresponding to a highly antigenic segment of gp41 envelope protein. **Proc Nat Acad Sci USA** 83:6159-6163, 1986.
28. Cosand WL: Synthetic antigen for the detection of AIDS-related disease. U.S. Patent #4,629,783, 1986.
29. Fenouillet E, Sorensen A-M, Lacroix M, Coutellier A, Herson S, Fretz-Foucault C, Gluckman J-C: Early and specific diagnosis of seropositivity to HIVs by an enzyme-linked immunosorbent assay using env-derived synthetic peptides. **AIDS** 4:1137-41, 1990.
30. Gnann JW, McCormick, Mitchell S, Nelson J, Oldstone MBA: Synthetic peptide immunoassay distinguishes HIV type 1 and HIV type 2 infections. **Science** 237:1346-1349, 1987.
31. Gnann JW, Nelson JA, Oldstone MBA: Fine mapping of an immunodominant domain in the transmembrane glycoprotein of human immunodeficiency virus. **J Virol** 61:2639-2641, 1987.
32. Alizon M, Wain-Hobson S, Montagnier L, Sonigo P: Genetic variability of the AIDS virus: nucleotide sequence analysis of two isolates from African patients. **Cell** 46:63-74, 1986.
33. Rosenberg, EC, Brennan CA, et al. Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infections; Approved Guideline Clinical and Laboratory Standards Institute, M53-A. Vol 31. No 13. 2011.
34. Masciotra S, McDougal JS, Feldman J, Sprinkle P, Wesolowski L, Owen SM. Evaluation of an alternative HIV diagnostic algorithm using specimens from seroconversion panels and persons with established HIV infections. **J Clin Virol.** 2011 Dec;52 Suppl 1:S17-22.
35. Centers for Disease Control, Interpretation and Use of the Western Blot Assay for Serodiagnosis of Human Immunodeficiency Virus Type 1 Infections. **Morbidity and Mortality Weekly Report** 38(S-7): 1-7, 1989.

36. Nasrullah M, Ethridge SF, Delaney KP, Wesolowski LG, Granade TC, Schwendemann J, Boromisa RD, Heffelfinger JD, Owen SM, Branson BM. Comparison of alternative interpretive criteria for the HIV-1 Western blot and results of the Multispot HIV-1/HIV-2 Rapid Test for classifying HIV-1 and HIV-2 infections. **J Clin Virol** 2011 Dec.;S23-S27.
37. Styer LM, Sullivan TJ, Parker MM. Evaluation of an alternative supplemental testing strategy for HIV diagnosis by retrospective analysis of clinical HIV testing data. **J Clin Virol.** 2011 Dec;52 Suppl 1:S35-40.
38. Torian LV, Forgione LA, Punsalang AE, Pirillo RE, Oleszko WR. Comparison of Multispot EIA with Western blot for confirmatory serodiagnosis of HIV. **J Clin Virol.** 2011 Dec;52 Suppl 1:S41-44.
39. Wesolowski LG, Delaney KP, Hart C, Dawson C, Owen SM, Candal D, Meyer WA 3rd, Ethridge SF, Branson BM. Performance of an alternative laboratory-based algorithm for diagnosis of HIV infection utilizing a third generation immunoassay, a rapid HIV-1/HIV-2 differentiation test and a DNA or RNA-based nucleic acid amplification test in persons with established HIV-1 infection and blood donors. **J Clin Virol.** 2011 Dec;52 Suppl 1:S45-9.
40. Valkirs GE, Barton R: ImmunoConcentration™ - A new format for solid phase immunoassays. **Clin Chem.** 31: 1427-1431, 1985.
41. Centers for Disease Control: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens in health-care settings. **Morbidity and Mortality Weekly Rep** 37:377-388, 1988.
42. US Department of Health and Human Services. **Biosafety in Microbiological and Biomedical Laboratories.** 5th ed. Washington, DC: US Government Printing Office, HHS publication No. (CDC) 21-1112. December 2009.
43. World Health Organization. **Laboratory Biosafety Manual.** 3rd ed. Geneva: World Health Organization. 2004.