Multispot HIV-1/HIV-2 Rapid Test

Rapid Enzyme Immunoassay to be used as a diagnostic aid for the detection and differentiation of HIV-1 and HIV-2 antibodies in serum or plasma.

For *In Vitro* Diagnostic Use **25228** **50 Tests**

CONTENTS

This package insert must be read completely before performing the test. Failure to follow the insert may give inaccurate test results. Users of this test should follow the CDC Universal Precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens.[1]

Complexity: Moderate

1. NAME AND INTENDED USE

The Multispot HIV-1/HIV-2 Rapid Test is a single use qualitative immunoassay to detect and 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 rapid HIV test results. When multiple rapid HIV tests are available, this test should be used in appropriate multi-test algorithms.

RESTRICTIONS

- **Sale of the Multispot HIV-1/HIV-2 Rapid Test is restricted to clinical laboratories that have an adequate quality assurance program, including planned systematic activities to provide adequate confidence that requirements for quality will be met and where there is assurance that operators will receive and use the instructional materials.**
- **The Multispot HIV-1/HIV-2 Rapid Test is approved for use only by an agent of a clinical laboratory.**
- **Test subjects must receive the "Subject Information" prior to specimen collection, and appropriate information when test results are provided.**
- **The Multispot HIV-1/HIV-2 Rapid Test is not approved for use to screen blood, plasma, cell, or tissue donors.**

2. SUMMARY AND EXPLANATION OF THE TEST

Acquired Immunodeficiency Syndrome (AIDS) is caused by viruses transmitted by sexual contact, exposure to blood (including sharing contaminated needles and syringes) or certain blood products, or transmitted from an infected mother to her fetus or child during the perinatal period.[2] Additionally, transmission of the 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 (Human Immunodeficiency Virus Type 2 or 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[9]. In the United States, there have been more than 80 cases of infection with HIV-2 reported, including two blood donors.[10-15]

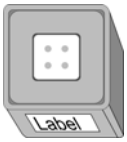
This second immunodeficiency virus (HIV-2) is similar to, but distinct from, HIV-1. Both viruses have similar morphology and lymphotropism,[16] and the modes of transmission appear to be identical.[9,17] The HIV-1 and HIV-2 genomes exhibit about 60% homology in conserved genes such as *gag* and *pol*, and 39 – 45% homology in the envelope genes.[18] 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.[19]

Within the two major HIV types, there is significant variation, as well. By analyzing sequences of representative strains, HIV-1 has been divided into three groups: group M (for major), including at least ten subtypes (A through J); group O (for outlier); and group N (for non-M, non-O).[20-22] Similarly, the HIV-2 strains have been classified into at least five subtypes (A through E).[23] 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 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.[24] The Multispot HIV-1/HIV-2 Rapid Test incorporates highly conserved recombinant and synthetic peptide sequences representing HIV-1 and HIV-2 envelope proteins.[25-31] 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.

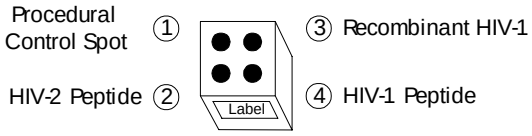
3. BIOLOGICAL PRINCIPLE OF THE TEST



The Multispot HIV-1/HIV-2 Rapid Test is based on the principle of Immuno-Concentration™.[32] The 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 Section 6, Precautions for Users) and properly discarded.

Microscopic particles are separately coated with the antigens that represent portions of the transmembrane proteins HIV-1 and HIV-2, respectively. The microparticles are immobilized on the reaction membrane of the HIV-1 and 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-2 virus gp36 envelope glycoprotein
③ Recombinant HIV-1:	Recombinant gp41 (HIV-1 envelope glycoprotein) expressed in *E. coli* (gp41 rDNA)
④ HIV-1 Peptide:	Peptide representing the immunodominant epitope of the HIV-1 virus gp41 envelope glycoprotein

4. 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 serum 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 • 0.01% Thimerosal	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_2SO_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	Use in Specimen Diluent bottle.

5. WARNINGS FOR USERS

For *In Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert may give inaccurate test results.
2. **This kit 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. Users of this test should follow the CDC Universal Precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens.[1]
4. Bring all reagents to room temperature (20-30°C) before use.

6. PRECAUTIONS FOR USERS

Safety Precautions:

1. Some reagents contain 0.1% ProClin® 150 or 0.5% ProClin® 300.
   Xi.Irritant
   R43: May cause sensitization by skin contact.
   S24-35-37: Avoid contact with skin. This material and its container must be disposed of in a safe way. Wear suitable gloves.
2. Some reagents contain 0.1% Sodium azide or ≤26% propylene glycol.

Xn.Harmful

R21/22: Harmful in contact with skin and if swallowed.

S24/25-28-35-36: Avoid contact with skin and eyes. After contact with skin, wash immediately with plenty of water. This material and its container must be disposed of in a safe way. Wear suitable protective clothing. Sodium azide forms lead or copper azides in laboratory plumbing and may explode on percussion, such as hammering. To prevent formation of lead or copper azide, flush drains thoroughly with water after disposing of solutions containing sodium azide.

3. This kit contains ≤0.01% Thimerosal (≤50 ppm Mercury w/v). Mercury compounds are sensitizers and may be considered reproductive toxicants and environmental pollutants by government agencies at certain concentrations/quantities. Handle appropriately and dispose of according to local, regional, and national regulations. [R43-61]
4. The Multispot HIV-1/HIV-2 Rapid Test contains human blood components. The Positive Control has been heat-treated to inactivate viruses. The human source material used in the preparation of the Negative Control has been tested and found nonreactive for Hepatitis B Surface Antigen (HBsAg), antibodies to Hepatitis C virus (HCV Ab), and antibodies to Human Immunodeficiency Virus (HIV-1/HIV-2 Ab). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as though capable of transmitting infectious diseases, using universal precautions recommended for bloodborne pathogens, as defined by local regulations.
5. Do not pipette by mouth.
6. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Stop Solution is a diluted acid. Wipe up spills immediately. Flush the area of the spill with water. If the Stop Solution contacts the skin or eyes, flush with copious amounts of water and seek medical attention. Never pour water into this product.
9. **Spills should be decontaminated with an effective disinfectant.** Disinfectants known to inactivate the virus include (but are not limited to) a solution of 10% bleach (0.5% solution of sodium hypochlorite), 70% ethanol, or 0.5% Wescodyne™ Plus (EPA Reg. #959-16-52). The 10% bleach should be made fresh daily. Allow 60 minutes for decontamination.
   NOTE: DO NOT AUTOCLAVE MATERIALS THAT CONTAIN BLEACH.
10. **Dispose of all specimens and materials used to perform the test as biohazardous waste. The preferred method of disposal is autoclaving for at least one hour at ≥121°C. Disposable materials may be incinerated. 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.[1]**
11. **This Product Contains Dry Natural Rubber in the dropper bulb used with the Specimen Diluent bottle.**

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 Controls 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.

7. REAGENT PREPARATION AND STORAGE

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 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 less than 3 months expiration remains on the kit). Do not freeze test components.

8. SPECIMEN COLLECTION, PREPARATION, AND STORAGE

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 µl. Approximately 30 µl is used for running each test. **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, and sodium heparin. 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^{0}C) for up to 48 hours. For long-term storage, the specimens should be frozen (-20°C or colder). Specimens 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.

9. MULTISPOT HIV-1/HIV-2 RAPID TEST PROCEDURE

Materials Provided

See REAGENTS section on page 5. Additional Materials Provided which are included in the kit:

- Package insert (1)
- Subject Information Notice (50)
- Customer letter (1)

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 75mm 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 10 µl and 90 µl as needed for dilutional testing of dually positive samples.

Preliminary Statements

1. **Once testing has been started, it should be completed without interruption.**
2. 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.
3. 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 eyedroppers-full dispenses approximately 300 µl of Specimen Diluent.
4. The disposable Transfer Pipets supplied in the kit dispense approximately 30 µl per drop.
5. The Cartridges should be placed on a flat surface during the assay procedure to ensure proper flow of specimen and reagents through the membrane.
6. **All solutions must be completely absorbed into the Cartridge membrane before proceeding to the next step in the Assay Procedure.**

Assay Procedure

1. **Bring all of the reagents and specimens to room temperature** (20-30°C) before beginning testing. It is very important that the reagents (especially the Multispot HIV-1/HIV-2 Cartridges) be at room temperature before they

are used. Remove from the box all of the reagent bottles and the Cartridges that will be used. Allow them to sit at room temperature for at least one to two hours to warm up.

2. **Label a test tube for each specimen or control to be tested.**

3. **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 prefilter is seated firmly on top of the Cartridge by pressing down firmly and evenly.

4. **Add two eyedroppers-full 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 eyedropper-full.

5. Using a separate 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 Transfer Pipet to touch any part of the tube or the Specimen Diluent in the tube. Discard the used Transfer Pipet into the biohazard waste**.**

6. 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 (see Note in Step 5 above). Do not allow the tip of the dropper to touch any part of the tube**.**

7. **Mix each diluted specimen and control (when run) thoroughly. Mix gently to avoid foaming.**

8. **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.

9. **Remove and discard the prefilter into the biohazardous waste.**

10. **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****.*

11. **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.

12. **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 before proceeding.***

13. **Repeat step 12** so that each Cartridge is washed twice**.** ***Wait for the Wash Solution to be absorbed completely before proceeding****.*

14. **Add three drops of Development Reagent** to the central well of each Cartridge by holding the bottle vertically and squeezing gently. ***Wait five minutes.***

15. **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.***

16. Read test results, according to the Test Result Appearance and Interpretation section below, either immediately or anytime up to 24 hours after completing the test.

10. QUALITY CONTROL – VALIDATION OF RESULTS

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 (purple) 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
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.

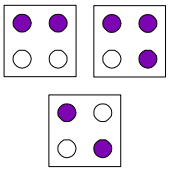
11. TEST RESULT APPEARANCE AND INTERPRETATION

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. Follow the CDC guidelines for counseling, testing, and referral when informing test subjects of these HIV test results and their interpretation.[33]

Nonreactive: Report results as described in the CDC guidance for reporting test results and interpretation.[33]

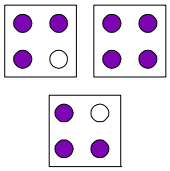
Only the Procedural Control Spot shows purple color development. The 3 Test Spots show no color development. Test result is interpreted as negative for HIV-1 and HIV-2 antibodies.

Reactive - Report results as described in the CDC guidance for reporting test results and interpretation.[33]



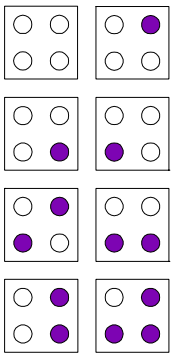
HIV-1 Reactive:
The Procedural Control Spot shows purple color development and the recombinant HIV-1 spot and/ or the HIV-1 Peptide spot show purple color development. Test result is interpreted as Preliminary Positive for HIV-1 antibodies.

HIV-2 Reactive:
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.



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.

Invalid



If no color develops in the Procedural Control Spot, regardless of color development anywhere else on the membrane, the results are invalid.

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.

Note: The appearance of any purple color in any spot must be considered as presence of that spot.

Dilutional Procedure for HIV Differentiation
The following procedure is used to differentiate samples that demonstrate purple color development in the HIV-2 spot as well as one or both of the HIV-1 spots.

1. Dilute the specimen 1:10 (using a calibrated pipettor, add 90 µl of Negative Control and 10 µl of sample to a separate test tube). Mix well.
2. Test the diluted sample as in steps 3-15 in the Assay Procedure section. Use the 1:10 diluted sample in place of the undiluted sample in Step 5 of the Assay Procedure.
3. Read the results and interpret according to the criteria above in Test Result Appearance and Interpretation.
4. 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.
5. If one or both of the HIV-1 spots and the HIV-2 spot are still reactive, dilute the 1:10 diluted specimen again by 10-fold in Negative Control, following the procedure in step 1 above (the final dilution is 1:100).
6. Test the diluted sample as in steps 3-15 in the Assay Procedure section. Use the 1:100 diluted sample in place of the undiluted sample in step 5 of the Assay Procedure. 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, the specimen should be interpreted as "Preliminary Positive for antibodies to HIV (undifferentiated)". Follow CDC guidelines for counseling, testing, and referral when informing test subjects of these HIV test results and their interpretation.[33]

12. LIMITATIONS OF THE PROCEDURE

1. 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.
2. 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.
3. The following anticoagulants have been evaluated and found to be acceptable for use with this test: EDTA, sodium citrate, and sodium heparin. **Use of other anticoagulants has not been evaluated and may give incorrect results.**
4. Performance of this assay has not been evaluated on patient samples that have been heat-inactivated.
5. Polystyrene tubes should not be used to prepare specimens for this test.
6. 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 antibody to HIV-1 or HIV-2 is present.
7. 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.
8. 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.
9. 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.[34]
10. 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. Clinical correlation is indicated with appropriate counseling, medical evaluation, and possibly additional testing to decide whether a diagnosis of HIV infection is accurate.
11. 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 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 (although they are reported as Preliminary Positive for antibodies to the specific HIV type identified).
12. The intensity of the Test Spot does not correlate with antibody titer of the specimen.
13. 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.

13. EXPECTED PERFORMANCE CHARACTERISTICS

Sensitivity for Antibodies to HIV-1

Sera

The reactivity of the Multispot HIV-1/HIV-2 Rapid Test was evaluated at two geographically diverse locations in the U.S. with 801 fresh serum samples from known HIV-1-positive individuals, and at three geographically diverse locations in the U.S. with 620 prospective fresh sera from patients at high risk for HIV-1 infection. The results of testing with the Multispot HIV-1/HIV-2 Rapid Test, a licensed EIA, and Western blot are shown below in Table 1.

Table 1
Detection of HIV-1 Antibody in Serum Samples

Population	# of Samples Tested	Multispot Reactive	Licensed HIV-1 EIA Repeatedly Reactive	Licensed HIV-1 Western Blot Positive
HIV-1 Known Positive, U.S. Fresh Sera	801	801	801	801
HIV-1 High-Risk Fresh Sera	620	28	29[a]	28
Total	**1421**	**829**	**830**	**829**

[a] One specimen was Negative on HIV-1 Western blot.

Of the 829 confirmed HIV-1-positive serum samples from known HIV-1 positive individuals and from individuals at high risk for HIV-1 infection, all 829 were reactive when tested on the Multispot HIV-1/HIV-2 Rapid Test. Based on these studies, 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%).

Plasma

The reactivity of the Multispot HIV-1/HIV-2 Rapid Test was evaluated at two geographically diverse locations in the U.S. with 801 fresh plasma samples from known HIV-1 positive individuals, and at four geographically diverse locations in the U.S. with 1441 prospective fresh plasma from patients at high risk for HIV-1 infection. The results of testing with the Multispot HIV-1/HIV-2 Rapid Test, a licensed EIA, and Western blot are shown below in Table 2.

Table 2
Detection of HIV-1 Antibody in Plasma Samples

Population	#of Samples Tested	Multispot Reactive	Licensed HIV-1 EIA Repeatedly Reactive	Licensed HIV-1 Western Blot Positive
HIV-1 Known Positive, U.S. Fresh Plasma	801	801	801	801
HIV-1 High-Risk Fresh Plasma	1441	70	72[a]	70
Total	**2242**	**871**	**873**	**871**

[a] One specimen was Indeterminate and one specimen was Negative on HIV-1 Western blot.

Of the 871 confirmed HIV-1 positive plasma samples from known HIV-1 positive individuals and from individuals at high risk for HIV-1 infection, all 871 were reactive when tested on the Multispot HIV-1/HIV-2 Rapid Test. Based on these studies, 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 ability of the Multispot HIV-1/HIV-2 Rapid Test to detect antibodies to HIV-2 in samples known to be positive for HIV-2 is presented in Table 3. Samples were frozen sera (N=61) and frozen plasma (N=140) and were collected in Africa (N=163), the United States (N=13) and unknown locations (N=25). All samples tested were positive on a research use HIV-2 Western blot, and repeatedly reactive on both a licensed HIV-2 EIA and on a licensed HIV-1/HIV-2 EIA. In addition, the ability of Multispot HIV-1/HIV-2 to detect HIV-2 antibodies in specimens collected

prospectively from individuals in an HIV-2 endemic area was evaluated on 500 frozen serum specimens previously collected in Sierra Leone, Africa.

Table 3
Detection of HIV-2 Antibody in Serum/Plasma Samples

Population	# of Samples Tested	HIV-2 Western Blot (Research Use) Positive	
		Multispot Reactive	Licensed HIV-2 EIA and HIV-1/HIV-2 EIA Repeatedly Reactive
HIV-2 Known Positive	201	201[a]	201
HIV-2 Endemic Population	500	6[b]	6[b]
Total	**701**	**207**	**207**

[a] Two specimens were identified as positive for both HIV-1 and HIV-2 based on results of Western blot and PCR testing.
[b] Western blot testing identified 2 of these specimens as positive for both HIV-1 and HIV-2.

As shown in Table 3, of the 207 confirmed HIV-2 positive specimens (i.e., HIV-2 Western blot positive) from known HIV-2 positive individuals and from individuals in an HIV-2 endemic population, all 207 were reactive when tested on the Multispot HIV-1/HIV-2 Rapid Test. Based on the results from these studies, 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

The ability of Multispot to differentiate HIV-1 and HIV-2 antibodies was determined by evaluating the samples that were identified by Western blot testing as positive for HIV-1 or HIV-2, as shown below in Table 4.

Table 4
Differentiation of HIV-1 and HIV-2 Antibodies in Western Blot Positive Samples

HIV Status[a]	Number of Specimens	Multispot Test Result Interpretation[b]			% Correct
		HIV-1	HIV-2	HIV-1/HIV-2	
HIV-1	1071	1070	0	1	99.91%
HIV-2	109	0	107	2	98.16%

[a] HIV-1 status was determined based on a positive result on a licensed HIV-1 Western blot. HIV-2 status was determined based on a positive result on a research use HIV-2 Western blot, with a corresponding negative or indeterminate result on a licensed HIV-1 Western blot.
[b] Interpretation was based on initial Multispot test results if reactive for HIV-1 or HIV-2 only, or on the result from testing of diluted specimens that were reactive for both HIV-1 and HIV-2 on initial test results.

HIV-1:
In the HIV-1 known positive and high-risk populations, there were 1071 samples that were HIV-1 positive by Western blot (1001 from known positive U.S. and worldwide populations and 70 from high risk populations). Multispot identified 1070 of the 1071 samples as HIV-1 reactive only (1070/1071 = 99.91%; 95% CI of 99.68 – 100.00%). The remaining sample, which was HIV-2 Western blot indeterminate, was dually reactive (undifferentiated) on Multispot HIV-1/HIV-2.

Of the 801 samples from known HIV-1 positive U.S. individuals, all were positive by HIV-1 Western blot and all were reactive with the Multispot HIV-1/HIV-2 Rapid Test. 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%).

HIV-2:
In the known HIV-2 positive population, there were 109 samples that were HIV-2 positive only by Western blot, and 92 samples were also positive by HIV-1 Western blot. Multispot identified 107 of these 109 samples as reactive for HIV-2 only (107/109 = 98.16%; 95% CI of 95.14 – 100.00%). The 2 remaining samples, which were indeterminate on HIV-1 Western blot, were dually reactive (undifferentiated) on Multispot.

Of the 201 samples from known HIV-2 positive individuals, all were positive by HIV-2 Western blot and all were reactive with the Multispot HIV-1/HIV-2 Rapid Test. One hundred ninety (190) of these 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.[35]

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 negative.

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

Sensitivity was also assessed by testing 10 commercial seroconversion panels and 3 low/mixed titer sensitivity panels. The results of seroconversion panel testing, in comparison to results with a licensed HIV-1/HIV-2 EIA and a licensed HIV-1 Western Blot, are shown in Table 5. 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.

Table 5
HIV-1 Seroconversion Panels, N=10

Panel ID		Day Since 1st Bleed	Multispot Result	Licensed HIV-1/HIV-2 EIA
L	PRB912-01	0	Negative	NR
	PRB912-02	9	**HIV-1**	**RR**
	PRB912-03	14	**HIV-1**	**RR**
V	PRB922-01	0	Negative	NR
	PRB922-02	4	**HIV-1**	NR
	PRB922-03	7	**HIV-1**	**RR**
	PRB922-04	11	**HIV-1**	**RR**
AB	PRB927-01	0	Negative	NR
	PRB927-02	28	Negative	NR
	PRB927-03	33	**HIV-1**	**R**
	PRB927-04	35	**HIV-1**	**R**
	PRB927-05	40	**HIV-1**	**R**
AD	PRB929-01	0	Negative	NR
	PRB929-05	21	Negative	NR
	PRB929-06	25	Negative	NR
	PRB929-07	28	**HIV-1**	**R**
AI	PRB934-01	0	Negative	NR
	PRB934-02	7	**HIV-1**	**RR**
	PRB934-03	11	**HIV-1**	**RR**
AP	PRB940-01	0	Negative	NR
	PRB940-03	11	Negative	NR
	PRB940-04	15	**HIV-1**	**RR**
	PRB940-05	18	**HIV-1**	**RR**
	PRB940-06	22	**HIV-1**	**RR**
	PRB940-07	25	**HIV-1**	**RR**
	PRB940-08	29	**HIV-1 & HIV-2**	**RR**
AQ	PRB941-01	0	Negative	NR
	PRB941-03	9	Negative	NR
	PRB941-04	18	**HIV-1**	NR
	PRB941-05	21	**HIV-1**	NR
	PRB941-06	25	**HIV-1**	**RR**
AT	PRB944-01	0	Negative	NR
	PRB944-04	9	Negative	NR
	PRB944-05	14	**HIV-1**	**R**
	PRB944-06	16	**HIV-1**	**R**
AU	PRB945-01	0	Negative	NR
	PRB945-04	13	Negative	NR
	PRB945-05	15	Negative	NR
	PRB945-06	20	**HIV-1**	**R**
SV	SV-0401-A	0	Negative	NR
	SV-0401-E	14	Negative	NR
	SV-0401-F	18	**HIV-1**	**RR**
	SV-0401-G	22	**HIV-1**	**RR**

NR = Nonreactive, RR = Repeatedly Reactive, R = Reactive (single test)

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, are shown in Tables 6 and 7. Multispot HIV-1/HIV-2 was able to detect antibodies to HIV-1 similar to the licensed EIA.

Table 6
HIV-1 Low Titer Panels

	Panel ID	Multispot Result	Licensed HIV-1/HIV-2 EIA
PRB106	01	**HIV-1**	**R**
	02	Negative	**R**
	03	**HIV-1**	**R**
	04	**HIV-1**	**R**
	05	**HIV-1**	**R**
	06	Negative	NR
	07	**HIV-1**	**R**
	08	**HIV-1**	NR
	09	**HIV-1**	**R**
	10	**HIV-1**	**R**
	11	**HIV-1**	**R**
	12	**HIV-1**	**R**
	13	**HIV-1**	**R**
	14	**HIV-1**	**R**
	15	**HIV-1**	**R**
PRB107	01	Negative	NR
	02	**HIV-1**	NR
	03	**HIV-1**	NR
	04	**HIV-1**	**R**
	05	Negative	NR
	06	**HIV-1**	**R**
	07	**HIV-1**	NR
	08	Negative	**R**
	09	Negative	NR
	10	**HIV-1**	**R**
	11	**HIV-1**	**R**
	12	Negative	NR
	13	Negative	NR
	14	**HIV-1**	**R**
	15	**HIV-1**	**R**

NR = Nonreactive, R = Reactive (single test)

Table 7
HIV-1 Mixed Titer Panel (PRB203)

Panel ID	Multispot Result	Licensed HIV-1/HIV-2 EIA
PRB203-01	**HIV-1**	**RR**
PRB203-02	**HIV-1**	**RR**
PRB203-03	Negative	NR
PRB203-04	**HIV-1**	NR
PRB203-05	**HIV-1**	**RR**
PRB203-06	**HIV-1**	**RR**
PRB203-07	**HIV-1**	**RR**
PRB203-08	**HIV-1**	**RR**
PRB203-09	**HIV-1**	**RR**
PRB203-10	**HIV-1**	**RR**
PRB203-11	**HIV-1**	**RR**
PRB203-12	**HIV-1**	**RR**
PRB203-13	**HIV-1**	**RR**
PRB203-14	**HIV-1**	NR
PRB203-15	**HIV-1**	**RR**
PRB203-16	**HIV-1**	**RR**
PRB203-17	**HIV-1**	**RR**
PRB203-18	**HIV-1**	**RR**
PRB203-19	**HIV-1**	**RR**
PRB203-20	Negative	NR
PRB203-21	**HIV-1**	**RR**
PRB203-22	**HIV-1**	NR
PRB203-23	**HIV-1**	**RR**
PRB203-24	**HIV-1**	**RR**
PRB203-25	**HIV-1**	**RR**

NR = Nonreactive, R = Repeatedly Reactive

Specificity

Sera

The specificity of Multispot HIV-1/HIV-2 with serum samples was evaluated in both low and high-risk populations for HIV infection. Samples in the three low-risk populations were obtained from a regional blood donor center (N=505) and from 2 low prevalence areas (N=200 and N=199) in geographically distinct areas of the United States. One specimen from the low risk population was confirmed positive for HIV infection and was excluded from the specificity analysis, giving a total of 903 specimens. An additional 592 HIV antibody-negative samples collected from individuals of unknown HIV serostatus in the population of 620 individuals at high risk for HIV described above in the Sensitivity section (Table 1) were added to the low risk population for calculation of total specificity for serum specimens. These added 592 samples were from 3 clinical sites and were nonreactive by HIV-1 EIA and negative by HIV-1 Western blot. The results of testing using Multispot HIV-1/HIV-2 compared to results with the reference test are shown in Table 8.

Table 8
Specificity in Low and High-Risk Populations
Fresh Sera

Test Group	Total Samples Negative by Reference Test[a]	Multispot Reactive	Multispot Nonreactive
Low Risk	903	1	902
High Risk	592	0	592
Totals	**1495**	**1**	**1494**

[a] Includes all samples nonreactive by HIV-1 EIA and those reactive by HIV-1 EIA that were negative on HIV-1 Western blot

Of the 1495 samples from individuals at low risk and high risk for HIV infection that were negative for antibodies to HIV by reference testing, 1494 were nonreactive on Multispot HIV-1/HIV-2. One (1) serum sample that was reactive for HIV-1 on Multispot was nonreactive on HIV-1/HIV-2 EIA and HIV-2 EIA, and negative by HIV-1 Western blot.

Combining the data from the studies of low-risk fresh serum samples and the high-risk fresh serum samples that were negative for antibodies to HIV by the reference test, 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%).

Plasma

The specificity of Multispot HIV-1/HIV-2 with plasma samples was evaluated in both low and high-risk populations for HIV infection. Samples were obtained from a regional blood donor center (N=505) and from 2 low prevalence areas (N=200 plasma and N=199 plasma) in geographically distinct areas of the United States. One specimen from the low-risk population was confirmed positive for HIV infection and was excluded from the specificity analysis, giving a total of 903 specimens. An additional 1371 HIV-1 antibody-negative fresh plasma samples collected from individuals of unknown HIV serostatus in a population at high risk for HIV (taken from the high-risk population described in the Sensitivity section above, Table 2) were added to the low-risk population for calculation of total specificity for plasma specimens, as shown in Table 9.

Table 9
Specificity in Low and High-Risk Populations
Fresh Plasma

Test Group	Total Samples Negative by Reference Test[a]	Multispot Reactive	Multispot Nonreactive
Low Risk	903	2	901
High Risk	1371	0	1371
Totals	**2274**	**2**	**2272**

[a] Includes all samples nonreactive by HIV-1 EIA and those reactive by HIV-1 EIA that were negative on HIV-1 Western blot

Of the 2274 samples from individuals at low risk and high risk for HIV infection that were negative for antibodies to HIV by reference testing, 2272 were nonreactive on Multispot HIV-1/HIV-2. Two (2) plasma samples that were reactive for HIV-1 on Multispot were nonreactive on HIV-1/HIV-2 EIA or HIV-2 EIA, and negative by HIV-1 Western blot.

Combining the data from the studies of low-risk fresh plasma samples and the high-risk fresh plasma samples that were negative for antibodies to HIV by the reference test, 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%).

Interfering Substances and Unrelated Medical Conditions

The Multispot HIV-1/HIV-2 Rapid Test was evaluated in studies of samples with potentially interfering substances, with various anticoagulants, and from individuals with unrelated medical conditions to determine any effect on test sensitivity and specificity.

Potentially interfering substances and anticoagulants tested, and the number of specimens tested, are as follows: hemolyzed (20), icteric (20), lipemic (20), elevated albumin (20), SST serum (10), EDTA plasma (10), heparin plasma (10), and citrated plasma (10). The sensitivity and specificity of Multispot was not affected by the presence of these interfering substances or anticoagulants, with the exception of one icteric specimen whose test results were uninterpretable on repeated testing due to high background.

Performance of Multispot HIV-1/HIV-2 was evaluated on a series of 227 unspiked specimens from individuals with unrelated medical conditions. In addition, two aliquots of each specimen were spiked with an HIV-1 or an HIV-2 positive specimen to give a level of reactivity in the low positive range. Results from the testing of these unspiked and HIV-1 and HIV-2 spiked specimens are shown in Table 10.

Table 10
Unrelated Medical Conditions

Unrelated Medical Condition	Unspiked Aliquots with Negative Results	HIV-1 Spiked Aliquots with HIV-1 Results	HIV-2 Spiked Aliquots with HIV-2 Results
Anti-HAV	12/12	10/10	10/10
Anti-HCV	12/12	10/10	10/10
Anti-EBV	12/12	10/10	10/10
Anti-HSV	12/12	10/10	9/10[e]
Anti-CMV	14/14	10/10	10/10
Anti-HTLV-I	9/10[a]	10/10	10/10[f]
Anti-HTLV-II	11/12[b]	9/10[c]	9/10[c]
Anti-Rubella	12/12	10/10	10/10
Anti-Toxoplasmosis	11/12[b]	10/10	10/10
Cancers	10/10	9/10[c]	10/10
Cirrhosis	10/10	10/10	10/10
Elevated IgG	10/10	9/10[c]	9/9
Elevated IgM	10/10	10/10	10/10
HBsAg +	15/15	10/10	10/10[f]
Rheumatoid Factor +	10/10	10/10	10/10
RPR +	10/10	8/10[d]	10/10
Multiparous	12/12	10/10	10/10
Multi-Transfused	12/12	10/10	10/10
Systemic Lupus	9/10[b]	10/10	10/10
VZV+	10/10	10/10	9/10[c]
TOTALS	**223/227 (98.2%)**	**195/200 (97.5%)**	**196/199 (98.5%)**

[a] One un-spiked sample in this group was falsely reactive for HIV-2.

[b] One un-spiked sample in this group was falsely reactive for HIV-1.

[c] One spiked sample in this group was falsely nonreactive

[d] Two spiked samples in this group were falsely nonreactive.

[e] One sample in this group spiked with HIV-2 was HIV-1 reactive.

[f] One sample in this group, spiked with HIV-2, was dually reactive for HIV-1 and HIV-2.

Overall, in the 227 unrelated medical condition (UMC) samples, 223 were nonreactive in Multispot. Falsely reactive results were observed in 1 sample each from specimens containing antibodies to HTLV-I, HTLV-II, toxoplasmosis, and SLE. Of the 200 UMC samples spiked with low levels of HIV-1 antibodies, 195 were reactive for HIV-1 and 5 were falsely nonreactive (1 anti-HTLV-II Ab positive, 1 cancer patient, 1 with elevated IgG, and 2 RPR postiive). Of the 199 UMC samples spiked with low levels of HIV-2 antibodies, 196 were reactive for HIV-2 and 3 were falsely nonreactive (1 each positive for antibodies to HSV, HTLV-II, and VZV).

Multispot HIV-1/HIV-2 Reproducibility Testing

The reproducibility of Multispot HIV-1/HIV-2 was evaluated at 5 sites with a panel of 7 specimens tested by 9 operators on 3 days on 3 lots at each site. A total of 6 kit lots were evaluated in this study. The intensity of each spot was scored, and the overall interpretation for each specimen was determined based on the scoring pattern. A total of 566 tests were performed (81 replicates of 7 panel members, minus one sample vial with inadequate volume for testing). The results from all of the sites demonstrate that for strong reactive HIV-1 and HIV-2 specimens and negative specimens, the reproducibility of the Multispot HIV-1/HIV-2 was 100%. The reproducibility of weakly reactive specimens was also acceptable, ranging from 90.1 – 100% agreement on specimens that were prepared by dilution of a strong reactive sample, and 98.8 - 100% agreement on HIV dual reactive specimens. In summary, overall reproducibility on all 566 tests was 98.0%.

14. BIBLIOGRAPHY

1. 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.
2. 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.
3. Delmonico FL, Snydman DR: Organ donor screening for infectious diseases. **Transplantation** 65(5):603-610, 1998.
4. 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.
5. 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.
6. Coffin J, Haase A, Levy JA, et al: What to call the AIDS virus? **Nature** 321:10, 1986.
7. Clavel F, Guetard D, Brun-Vezinet F: Isolation of a new human retrovirus from West African patients with AIDS. **Science** 233:343-346, 1986.
8. 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.
9. 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.
10. Centers for Disease Control: AIDS due to HIV-2 infection - New Jersey. **Morbidity and Mortality Weekly Rep** 37:33-35, 1988.
11. 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.
12. Ayanian JZ, Maguire JH, Marlink RG, et al: HIV-2 infection in the United States. **New Engl J Med** 320:1422-1423, 1989.
13. O'Brien TR, George JR, Holmberg SD: Human immunodeficiency virus type 2 infection in the United States. **JAMA** 267:2775-2779, 1992.
14. 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.
15. 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.
16. 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.
17. Quinn TC, Zacarias FRK, St. John RK: AIDS in the Americas: an emerging public health crisis. **New Engl J Med** 320:1005-1007, 1989.
18. Guyader M, Emerman M, Sonigo P, et al: Genome organization and transactivation of the human immunodeficiency virus type 2. **Nature** 326:662-669, 1987.
19. Cabrian K, Shriver K, Goldstein L, et al: Human immunodeficiency virus type 2: a review. **J Clinical Immunoassay** 11:107-114, 1988.
20. Janssens W, Buvé A, Nkengasong JN: The puzzle of HIV-1 subtypes in Africa. **AIDS** 11:705-712, 1997.
21. 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.
22. Simon F, Mauclère 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.

23. 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.

24. 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.

25. 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.

26. 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.

27. Cosand WL: Synthetic antigen for the detection of AIDS-related disease. U.S. Patent #4,629,783, 1986.

28. 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.

29. 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.

30. 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.

31. 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.

32. Valkirs GE, Barton R: Immunoconcentration™ - A new format for solid phase immunoassays. **Clin Chem**. 31: 1427-1431, 1985.

33. Centers for Disease Control: Revised Guidelines for HIV Counseling, Testing, and Referral and Revised Recommendations for HIV Screening of Pregnant Women. **Morbidity and Mortality Weekly** Rep 50:32-35, 2001

34. Rutherford GW, Lifson AR, Hessol NA, et al: Course of HIV infection in a cohort of homosexual and bisexual men: an 11 year follow-up study. **Br Med J** 301: 1183-1188, 1990.

35. Walther-Jallow L, Andersson S, Da Silva Z: High Concordance between Polymerase Chain Reaction and Antibody Testing of Specimens from Individuals Dually Infected with HIV Types 1 and 2 in Guinea-Bissau, West Africa. **AIDS Research and Human Retroviruses** 15: 957-962, 1999.

Bio-Rad Laboratories
Redmond, WA 98052, U.S. A.
For Customer Orders or Technical Service call:
1-800-2BIORAD (1-800-224-6723

Revised: December 2004
503851