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 human 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 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.

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 Notice" prior to specimen collection, and appropriate information when test results are provided, unless this test is used as part of a multi-test diagnostic algorithm.**
- **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.

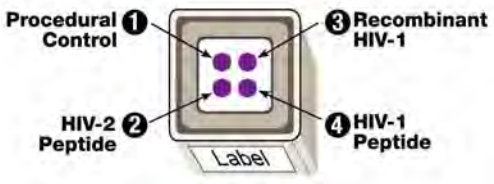
Despite some degree of immunological cross-reactivity between types and subtypes of HIV, reliable detection of antibodies derived from the more divergent strains may only be achieved by incorporating type- specific protein sequences into the assay design. In one study, detection of HIV-2 positive samples 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. 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 antibodies.[32-38]

3 - BIOLOGICAL PRINCIPLE OF THE TEST

The Multispot HIV-1/HIV-2 Rapid Test is based on the principle of ImmunoConcentration™.[39] 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 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-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 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_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; Contains Dry Natural Rubber, a potential sensitizer	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. 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 [NaN_3]:**
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 (H_2SO_4) 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 human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens.[1]
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 derivates, 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 Laboratories*[(40)], WHO *Laboratory Biosafety Manual*[(41)], 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 to Hepatitis C virus (HCV Ab).

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 Human Immunodeficiency Virus (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.

6 - PRECAUTIONS FOR USERS

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. Wear appropriate protective clothing, 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.[1]**
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.

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. 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). 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 (1) - The Notice in the kit box may be copied as needed.
- 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 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.

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 full eyedroppers dispenses approximately 300 µL of Specimen Diluent.
4. A 30µL 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 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 (no standing liquid) into the Cartridge membrane before proceeding to the next step in the Assay Procedure.**

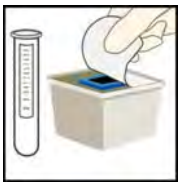
Assay Procedure

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 30µL of of specimen to the Specimen Diluent. Alternatively, 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 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.

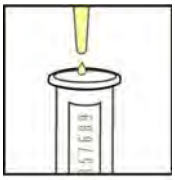
   Add one drop of each control to the appropriately labeled tube. The drop should fall freely into the Specimen Diluent (see Note in Step 6 above). Do not allow the tip of the dropper to touch any part of the tube.
8. **Mix each diluted specimen and control (when run) thoroughly. Mix gently to avoid foaming.**
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 step 13** 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, Section 11 (Rapid Testing) or Section 14 (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.



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

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



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

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.

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 (a definite purple spot) on each Cartridge for the results of that Cartridge to be valid.

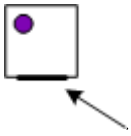
Quality Control

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

Note placement of Cartridge ID label.

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

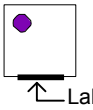
11 - TEST RESULT APPEARANCE AND INTERPRETATION – RAPID HIV-1/HIV-2 TESTING

Note: For interpretation of the assay when used as the differentiation assay in a diagnostic testing algorithm, refer to Section 14 - USE OF MULTISPOT AS THE ANTIBODY DIFFERENTIATION TEST IN A DIAGNOSTIC TESTING ALGORITHM

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.[42]

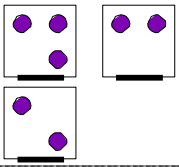
Interpretation for Rapid HIV-1/HIV-2 Testing:

Nonreactive - Report results as described in the CDC guidance for reporting test results and interpretation.[42]



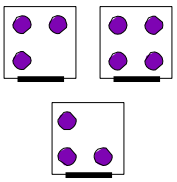
Only the Procedural Control Spot shows purple color development. The 3 Test Spots show no color development. Test result is interpreted as negative for HIV-1 and HIV-2 antibodies.

Reactive (Preliminary Positive) - Report results as described in the CDC guidance for reporting test results and interpretation.[42]



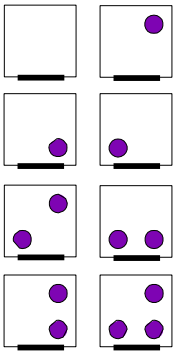
HIV-1 Reactive - Preliminary Positive:
The Procedural Control Spot shows purple color development and the recombinant HIV-1 Spot and/or the HIV-1 Peptide Spot show purple color development. Test result is interpreted as Preliminary Positive for HIV-1 antibodies.

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.



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 which follows.

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 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 of the Test Spots, regardless of intensity, must be considered as presence of that Spot.

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 µL of Negative Control Serum and 10 µL of sample to a separate test tube; or, alternatively, 135 µL of Negative Control Serum and 15 µL 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 above 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)."**

Follow CDC guidelines for counseling, testing, and referral when informing test subjects of these HIV test results and their interpretation.[42]

12. LIMITATIONS OF THE PROCEDURE

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. EXPECTED PERFORMANCE CHARACTERISTICS – RAPID HIV-1/HIV-2 TESTING

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

		HIV-2 Western Blot (Research Use) Positive	
Population	**# of Samples Tested**	**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.[44]

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

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, RR = 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 positive). 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 – USE OF MULTISPOT AS AN ANTIBODY DIFFERENTIATION TEST IN A DIAGNOSTIC TESTING ALGORITHM

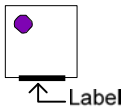
When the Multispot HIV-1/HIV-2 Rapid Test is used as an HIV-1/HIV-2 antibody differentiation assay in a diagnostic testing algorithm for HIV, as recommended by the Clinical Laboratory Standards Institute (CLSI)[32], follow the previous instructions in Sections 5 – 10 and Section 12 to perform the test. The following instructions for result appearance and interpretation are used in place of the instructions in Section 11 that describe use of the assay as a rapid HIV-1/HIV-2 test.

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.[32]

Interpretation for Diagnostic Testing Algorithm that Differentiates HIV-1 and HIV-2 Antibodies:

Nonreactive

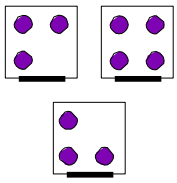


Only the Procedural Control Spot shows purple color development. The 3 Test Spots show no color development. Test result is interpreted as negative for HIV-1 and HIV-2 antibodies. Additional testing is recommended, including HIV nucleic acid testing (NAT).

Reactive

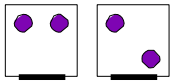
HIV-1 POSITIVE:
The Procedural Control Spot shows purple color development and **both** the recombinant HIV-1 Spot and the HIV-1 Peptide Spot show purple color development. Test result is interpreted as Positive for HIV-1 antibodies

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



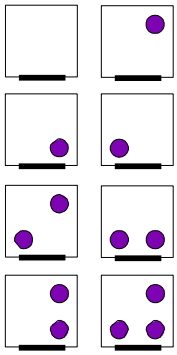
HIV POSITIVE (Undifferentiated):
The Procedural Control Spot shows purple color development. The HIV-2 Peptide Spot shows purple color development as well as one or both HIV-1 Spots. In this case, the specimen may be tested by additional methods which allow for differentiation between HIV-1 and HIV-2. See diutional procedure which follows.

Indeterminate



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.

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.

Note: The appearance of a definite purple color in any of the Test Spots must be considered as presence of that Spot.

Dilutional Procedure for Diagnostic Testing Algorithm that Differentiates HIV–1 and HIV-2 Antibodies

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 µL of Negative Control Serum and 10 µL of sample to a separate test tube; or, alternatively, 135 µL of Negative Control Serum and 15 µL 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 below in Test Result Interpretation – Dilutional Testing for Diagnostic Testing Algorithm.

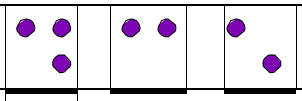
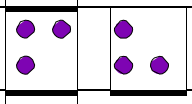
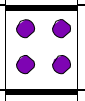
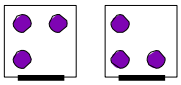
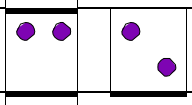
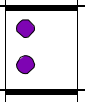
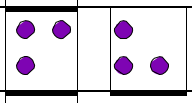
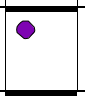
- If the Procedural Control Spot is reactive and the Test Spots are nonreactive at this dilution, the specimen should be interpreted as **"POSITIVE for antibodies to HIV (undifferentiated)."**
- If the Procedural Control Spot is reactive and the Test Spots are reactive for only HIV-1 or HIV-2, the result can be reported as POSITIVE for antibodies to the specific HIV type identified. For dilutional testing, a result is considered positive for HIV-1 if at least one of the HIV-1 spots is reactive. It is not required to have both HIV-1 spots as reactive in dilutional testing as long as both were reactive when tested undiluted. Refer to the table below for interpretation criteria.
- If a sample is reactive for one HIV-1 spot and the HIV-2 spot on initial testing and is only reactive for the HIV-1 spot on dilutional testing, that sample should be interpreted as HIV-1 INDETERMINATE.
- 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 below in Test Result Interpretation – Dilutional Testing for Diagnostic Testing Algorithm.
   - 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 POSITIVE for antibodies to the specific HIV type identified. For dilutional testing, a result is considered positive for HIV-1 if at least one of the HIV-1 spots is reactive. It is not required to have both HIV-1 spots as reactive in dilutional testing as long as both were reactive when tested undiluted. Refer to the table below for interpretation criteria.
   - 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 **"POSITIVE for antibodies to HIV (undifferentiated)."**
   - If a sample is reactive for one HIV-1 spot and the HIV-2 spot on initial testing and testing at a 1:10 dilution, and is only reactive for the HIV-1 spot on dilutional testing, that sample should be interpreted as HIV-1 INDETERMINATE.

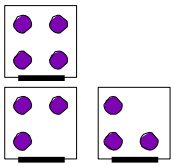
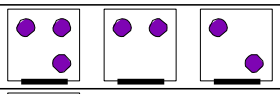
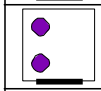
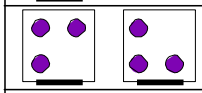
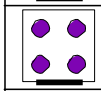
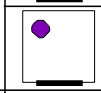
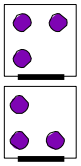
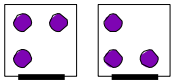
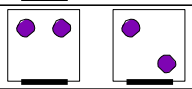
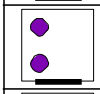
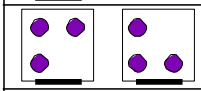
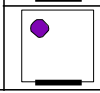
Follow CDC guidelines for counseling, testing, and referral when informing test subjects of these HIV test results and their interpretation.[42]

Test Result Interpretation – Dilutional Testing for Diagnostic Testing Algorithm

1:10 Dilutional testing:

Initial Result	1:10 Dilutional 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 Dilutional Result	1:100 Dilutional Result	Interpretation
			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)

15. PERFORMANCE CHARACTERISTICS OF MULTISPOT IN A DIAGNOSTIC TESTING ALGORITHM

The CDC and several public health laboratories have evaluated the use of the Multispot HIV-1/HIV-2 Rapid Test as the HIV-1/HIV-2 antibody differentiation assay in a diagnostic testing algorithm, after repeatedly reactive results were obtained from an HIV-1/2 antibody immunoassay or HIV antigen/antibody combination assay. The studies were conducted on specimens that had been submitted for clinical testing. Results of the algorithm using Multispot were compared to test results from an HIV-1 Western blot, an HIV-1 qualitative RNA assay, or both. Some specimens were also tested with an HIV-2 EIA or HIV-2 qualitative RNA assay. Results of these studies documenting performance of the Multispot as the HIV-1/HIV-2 antibody differentiation assay in the diagnostic testing algorithm are summarized here.

Study 1: In a CDC study that included use of the Multispot HIV-1/HIV-2 Rapid Test as the differentiation assay in a diagnostic testing algorithm, 830 specimens were tested, of which 416 were from individuals with established HIV infection and 414 were HIV negative. The interpretation of Multispot results in this study was based on the rapid testing criteria, and not the criteria described above for use of the assay as an antibody differentiation test in a diagnostic testing algorithm. The Multispot sensitivity observed in this study was 99.52% with a 95% confidence internal of 98.26 – 99.62%. The observed specificity for Multispot in this study was 99.03% with a 95% confidence interval of 97.54 – 99.61%. The specificity observed in this study may have been higher if the diagnostic algorithm interpretive criteria were used (requiring two HIV-1 spots for a reactive test result). This study demonstrated improved sensitivity for detecting acute HIV-1 infections when Multispot was used in the alternative algorithm, while maintaining the ability to accurately detect established HIV-1 infections.[33]

Study 2: The Multispot HIV-1/HIV-2 Rapid Test was evaluated retrospectively by CDC using test results from a public health laboratory to assess the ability to accurately differentiate HIV-1 and HIV-2 infections in individuals known to be infected with HIV. Multispot was positive in 1788/1790 specimens that were HIV-1 Western blot positive by the CDC criteria[34], and negative in 2/1790. Six specimens in this study were confirmed as HIV-2 by a research-use HIV-2 Western blot and DNA NAT, of which four were positive on Multispot for HIV-2 only and two were undifferentiated. Multispot correctly identified HIV-1 infection in specimens that were not identified as positive by Western blot, including at least 15% (15/96) of the specimens that were HIV-1 Western blot indeterminate and 1% (2/249) of those that were negative by Western blot. Two HIV-1 Western blot indeterminate results had discordant results between Multispot and follow-up test results: one specimen was undifferentiated on Multispot and HIV negative on follow-up testing and one specimen was negative on Multispot and confirmed HIV-1 positive on follow-up testing. The appearance of test results (reactivity for one or both HIV-1 spots) and the results of dilutional testing were not reported in this study. No information was provided on the interpretive criteria used in this study (original diagnostic criteria [requiring only one HIV-1 spot for a positive interpretation] or the diagnostic algorithm interpretive criteria [requiring two HIV-1 spots for a positive interpretation]), nor were the results of dilutional testing. This study demonstrated that the Multispot HIV-1/HIV-2 Rapid Test can correctly identify HIV-2 infected individuals and that the performance of the Multispot HIV-1/HIV-2 is comparable or exceeds that of an HIV-1 Western blot when used in the HIV diagnostic testing algorithm.[35]

Study 3: A separate study to assess the use of Multispot as an HIV-1/HIV-2 differentiation assay for confirmation of repeatedly reactive EIA results included the testing of the 38,257 specimens, of which 1578 were identified as HIV-1 positive in the testing algorithm based either on a positive HIV-1 Western blot (n=1546), detectable HIV-1 RNA (n=29), or follow-up specimen results (n=3). Using the interpretative criteria that require only one HIV-1 spot to be reactive for a positive interpretation, 1575/1578 (99.8%) of specimens were classified as HIV-1 reactive by Multispot and three were non-reactive or undifferentiated by Multispot. Of the 1562 specimens that were reactive for both HIV-1 spots, two identified as HIV-1 positive on Multispot were Western blot indeterminate with envelope reactivity (including gp41 or gp160) indicating the samples were likely positive, although false positive results could not be ruled out. Thirteen specimens out of the 1578 were reactive for only one HIV-1 spot (interpretation of Multispot indeterminate), of which 11 were true positives as confirmed by HIV-1 Western blot (n=4), NAT (n=6) or follow-up specimen testing (n=1).[36] This study demonstrated that the performance of the Multispot HIV-1/HIV-2 Rapid Test is comparable to HIV-1 Western blot when used in the HIV diagnostic testing algorithm.

Study 4: A study was performed at a public health laboratory to compare the Multispot HIV-1/HIV-2 Rapid Test to Western blot for use in a confirmatory testing algorithm for HIV. Multispot identified 8670/8678 HIV-1 Western blot positive specimens giving a sensitivity of 99.91% based on positive Western blot as the gold standard. An additional 26 specimens were positive by Multispot, of which 3 specimens were negative on Western blot and were identified as positive for HIV-1, and 23/63 specimens were indeterminate on Western blot and were identified as positive for HIV-1 (11 specimens) or positive for HIV-2 (12 specimens).[37] This study demonstrated that using the Multispot HIV-1/HIV-2 Rapid Test in the HIV diagnostic testing algorithm identified additional specimens as HIV-1 positive or HIV-2 positive that were indeterminate on HIV-1 Western blot.

Study 5: The performance of the diagnostic testing algorithm that uses the Multispot HIV-1/HIV-2 Rapid Test to differentiate HIV-1 from HIV-2 was evaluated in a study that included 2090 HIV-1 Western blot positive specimens and 1508 blood donors that were HIV negative by immunoassay and HIV-1 NAT. The observed Multispot sensitivity when used as a rapid test was 99.95% with a 95% confidence internal of 99.73 – 100% compared to HIV-1 Western blot n=2090). The specificity was 99.40% with a 95% confidence interval of 98.87 – 99.73% (n=1508).[38] This study demonstrated that the performance of the Multispot HIV-1/HIV-2 Rapid Test is comparable to HIV-1 Western blot when used in the HIV diagnostic testing algorithm.

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