Summary of Safety and Effectiveness

I. General Information

Reference No.: PMA BP 040046

Applicant: Bio-Rad Laboratories
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Phone: 425 881-8300
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Generic Name: Rapid HIV-1 and HIV-2 Antibody Test

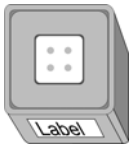
Product Trade Name: Multispot HIV-1/HIV-2 Rapid Test

Date of Notice of Approval to the Applicant: November 12, 2004

II. Indications For 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.

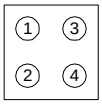
III. Brief Description of Test



The Multispot HIV-1/HIV-2 is an enzyme immunoassay for the detection and differentiation of Human Immunodeficiency Virus Types 1 and 2 (HIV-1 and HIV-2) in fresh or frozen human serum and plasma. This assay uses microscopic particles coated with the antigens that represent a portion of HIV-1 or HIV-2. The microparticles are immobilized on a reaction membrane of the HIV-1/HIV-2 Cartridge in the form of Test Spots. The reaction membrane also contains a Reactive Spot that serves as a “control spot” for monitoring the performance of the test procedure and as a sample addition monitor.

Samples to be tested are diluted in Specimen Diluent, mixed, and poured into the prefilter in the Cartridge. After the specimen has been completely absorbed, the prefilter is removed, and the membrane is washed. The Conjugate, containing alkaline phosphatase-labeled goat anti-human IgG (H+L), is then added to the Cartridge. If HIV-specific antibodies are present in the specimen, the Conjugate will bind to the antibody-antigen complex. Unbound Conjugate is removed by a wash step. Next, Development Reagent is added to the Cartridge. A purple color develops on the corresponding Test Spots in proportion to the amount of antibody to HIV-1 or HIV-2 that has been bound to the coated microparticles. A purple color will also develop in the Reactive 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 a purple color in the Reactive Spot and Test Spots.

(1) Reactive Spot (3) HIV-1 Test Spot



(2) HIV-2 Test Spot (4) HIV-1 Test Spot

Components of the Multispot HIV-1/HIV-2 Rapid Test are listed below:

1. HIV-1/HIV-2 Cartridge: Base container with a reaction membrane spotted with HIV-1 and HIV-2 peptides and an HIV-1 recombinant protein; an absorbent pad; a removable specimen pre-filter; foil-sealed
2. Positive Control Serum: Heat-inactivated serum containing anti-HIV-1 and anti-HIV-2 immunoglobulin. Preservative: ProClin 300.
3. Negative Control Serum: Human serum, nonreactive for antibodies to HIV-1 and HIV-2. Preservative: ProClin 300.
4. Specimen Diluent: Diluent for specimen and Controls. Preservative: ProClin 150 and Thimerosal.
5. Conjugate: Anti-human IgG (H+L) (goat) alkaline phosphatase conjugated solution. Preservative: ProClin 150.
6. Wash Solution: Contains TRIS, Urea, and Nitro blue Tetrazolium. Preservative: ProClin 150.
7. Development Reagent: 3-Indoxyl phosphate
8. Stop Solution: 0.1N H_2SO_4
9. Disposable Transfer Pipets
10. Eyedropper

IV. 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.
- The Multispot HIV-1/HIV-2 Rapid Test is not approved for use to screen blood or tissue donors.

V. Warnings

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.
4. Bring all reagents to room temperature (20-30°C) before use.

VII. Limitations of the Test

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.
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.

VII. Alternative Practices and Procedures

The detection of antibodies to HIV-1 in humans is primarily performed using laboratory-based assays for serum, plasma, oral fluid or urine. The majority of these tests use principles similar to that of the Multispot HIV-1/HIV-2 Rapid Test; utilizing peptides, recombinant antigens, isolated proteins or viral lysate immobilized onto a solid phase support to capture antibodies in a patient sample. In many cases, the detection of the captured antibodies is accomplished using an

instrument to reveal a colored or chemiluminescent endpoint from enzymatic reactions. All reactive screening test results require supplemental, more specific testing.

The Multispot HIV-1/HIV-2 Rapid Test differs from traditional laboratory-based testing in that it requires no extra instrumentation, and alkaline phosphatase-labeled goat anti-human IgG conjugate is used for colorimetric detection and qualitative visual interpretation of test results.

VIII. Potential Adverse Affects Effects of the Device on Health

There have been no adverse effects of the Multispot HIV-1/HIV-2 Rapid Test indicated during studies performed to date.

IX. Summary of Preclinical Studies

A. Reactivity with Sensitivity and Seroconversion Panel

1. 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.

2. 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 1. 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 1
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 2 and 3. Multispot HIV-1/HIV-2 was able to detect antibodies to HIV-1 similar to the licensed EIA.

Table 2
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 3
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

B. 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 4.

Table 4
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).

C. 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%.

D. Animal studies

No animal studies were performed during evaluation of the Multispot HIV-1/HIV-2 Rapid Test.

XI. Summary of Clinical Studies

A. Sensitivity for HIV-1

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 5.

Table 5
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%).

2. 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 6.

Table 6
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%).

B. 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 7. 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 7
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 7, 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%).

C. 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 8.

Table 8
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.

C. Specificity

1. 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-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 5) 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 9.

Table 9
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 negative by HIV-1 EIA and those positive 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 is calculated to be 1494/1495 or 99.93% (95% CI = 99.79 – 100.00%).

2. 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 nonreactive 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 6) were added to the low-risk population for calculation of total specificity for plasma specimens, as shown in Table 10.

Table 10
Specificity in Low and High-Risk Populations
Fresh Plasma

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

[a] Includes all samples negative by HIV-1 EIA and those positive 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 is calculated to be 2272/2274 or 99.91% (95% CI = 99.77 – 100.00%).

XII. Conclusions Drawn from the Studies

Risk/Benefit Analysis

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. Additionally, transmission of the viruses can occur through tissue transplantation. Human Immunodeficiency Virus Type 1 (HIV-1) has been isolated from patients with AIDS and AIDS-related complex (ARC). 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. Since the initial discovery, hundreds of cases of HIV-2 infection have been documented worldwide. In the United States, there have been more than 80 cases of infection with HIV-2 reported, including two blood donors.

Recent advances in the treatment of HIV infection have increased the value of early diagnosis and medical intervention. Rapid testing for HIV provides same-day test results in clinic settings so that patients can receive immediate counseling. The Bio-Rad Laboratories Multispot HIV-1/HIV-2 Rapid Test is both sensitive and highly specific for the detection of circulating antibodies to HIV-1 and/or HIV-2 in fresh or frozen serum or plasma. It can also differentiate HIV-1 antibodies from HIV-2 antibodies, and can be performed both rapidly and reliably without instrumentation.

Safety

There were no adverse reactions observed in any of the clinical or pre-clinical studies that were conducted. All operators performed testing in accordance with the training provided.