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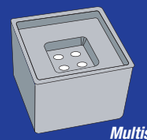
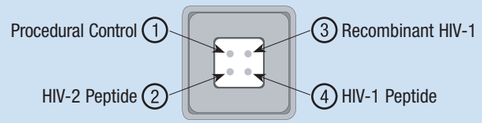
Multisite Evaluation of Bio-Rad Multispot HIV-1/HIV-2 Rapid Test to Detect and Differentiate HIV-1 from HIV-2 and to be a Stand-Alone HIV-1 Multi-Test Algorithm Based on Separate Recombinant and Peptide Antigen Spots

C. Bentsen[1], P. Wells[1], L. Staswick[1], E. Martin[1], B. Branson[2], N. Constantine[3], C. Cole[1] and P. Coleman[1]
[1] Bio-Rad Laboratories, Redmond, WA; [2] Centers for Disease Control and Prevention, Atlanta, GA; [3] University of Maryland, Baltimore, MD


Multispot Cartridge

Direct correspondence to:
Christopher Bentsen M.S., RAC
RA/QA and Clinical Affairs
Bio-Rad Laboratories
6565 185th Ave NE
Redmond, WA 98052
christopher_bentsen@bio-rad.com



Abstract

Background/Objective: Rapid HIV tests provide timely and accurate test results. Algorithms for HIV confirmation using combinations of rapid, simple assays have been proposed by the WHO. The utility of Multispot for differentiating HIV-1 from HIV-2 and as a stand-alone multi-test assay for HIV-1 was analyzed based on results from its separate recombinant HIV-1, synthetic HIV-1 peptide, and synthetic HIV-2 peptide antigen spots.

Methods: A total of 3146 fresh plasma and 2325 fresh serum samples from 3146 subjects was tested at 7 locations in the U.S.; matched serum and plasma samples from 801 known HIV-1 positive patients; 620 serum samples and 1441 plasma samples from high-risk subjects; and 905 matched serum and plasma samples from low-risk subjects. A panel of 203 worldwide HIV-1 positive samples was also tested. In addition, a total of 201 frozen serum and plasma samples from known HIV-2 positive patients was tested, as well as 500 frozen serum samples collected from West Africa. The intensity of reactivity for each Multispot HIV-1 and HIV-2 antigen spot was graded on a scale of 0 to 4, and compared to the intensity of the Procedural Control Spot.

Results: 1903 samples (801 known HIV-1 positive serum, 801 known HIV-1 positive plasma, 70 plasma from high-risk subjects, 28 serum from high-risk subjects, and 203 worldwide HIV-1 positive samples) were positive by HIV-1 Western blot and 207 samples were positive by HIV-2 Western blot. All were detected by Multispot (100% sensitivity for both HIV-1 and HIV-2). Multispot specificity, based on blot-negative samples from both low and high-risk subjects, was 1494/1495 (99.93%) with fresh serum samples and 2272/2274 (99.91%) with fresh plasma samples. Multispot's ability to differentiate HIV-1 from HIV-2 was evaluated in 1071 samples positive by HIV-1 Western blot only (870 U.S. plasma samples and 201 worldwide samples) and 109 samples positive by HIV-2 Western blot only. Multispot correctly identified 1070/1071 (99.91%) samples as HIV-1 and 107/109 (98.16%) samples as HIV-2; the 3 remaining samples were HIV dually reactive on Multispot. Spot intensity was evaluated in fresh-paired serum/plasma samples from 871 patients known to be HIV-1 positive or HIV-1 Western blot positive. 832 patients (96%) showed strong (3+ to 4+) reactivity to **both** HIV-1 antigen spots; the remaining 39 HIV-1 positive patients were positive on both of the HIV-1 antigen spots, but with 1+ to 2+ reactivity on one or both spots. The 4 Multispot false-positive samples from 3 subjects in the specificity study had only weak (1+) reactivity on one or both of the HIV-1 antigen spots. Strong reactivity on both Multispot HIV-1 antigens provides 100% PPV with positive HIV-1 Western blot results or known HIV-1 infection.

Conclusions: Multispot is a sensitive and specific rapid test for the detection of HIV-1 and HIV-2 antibodies, and can reliably differentiate HIV-1 infection from HIV-2. In addition, Multispot could serve as a multi-test algorithm for HIV-1 in a single device when there is strong (3+ to 4+) reactivity to **both** HIV-1 antigen spots.

BIO-RAD

Assay Procedure

1. Bring all of the reagents and specimens to room temperature (20-30°C) before beginning testing.
2. Label a test tube for each specimen or control to be tested.
3. Place the required number of Multispot Cartridges on a flat surface with the patient ID label facing toward the operator. Peel away the foil seals and discard them. Label the Multispot Cartridges to correspond with the specimens to be tested.
4. Add two eyedroppers-full of Specimen Diluent to each specimen and control tube.
5. Using a separate transfer pipet for each specimen, draw up a small amount of specimen. While holding the pipet vertically over the appropriate dilution tube, add one drop to the tube.
6. Add one drop of each control to the appropriately labeled tube.
7. Mix each diluted specimen and control thoroughly.
8. Pour the contents of each tube into the specimen prefilter of each corresponding pre-labeled Multispot Cartridge, using a separate Cartridge for each tube. *Wait two minutes.*
9. Remove and discard the prefilter into the biohazardous waste.
10. Fill the central well of each Cartridge with Wash Solution by holding the bottle vertically and squeezing gently. Wait for the Wash Solution to be absorbed completely.
11. Add three drops of Conjugate Reagent to the central well of each Cartridge. *Wait two minutes.*
12. Fill the central well of each Cartridge with Wash Solution. Wait for the Wash Solution to be absorbed before proceeding.
13. Repeat step 12 so that each Cartridge is washed twice. Wait for the final Wash Solution to be absorbed.
14. Add three drops of Development Reagent to the central well of each Cartridge. *Wait five minutes.*
15. Fill the central well of each cartridge with Stop Solution. Wait for the Stop Solution to be absorbed completely.
16. Read the results.

Dilutional Procedure for HIV Differentiation

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

1. Dilute the specimen 1:10 in Negative Control. Mix well.
2. Test the diluted sample, using the 1:10 diluted sample in place of the undiluted sample in the Assay Procedure.
3. Read the results.
4. If the Procedural Control Spot is reactive and the Test Spots are reactive for only HIV-1 or HIV-2, the result can be reported as Preliminary Positive for antibodies to the specific HIV type identified.
5. If one or both of the HIV-1 spots and the HIV-2 spot are still reactive, dilute the 1:10 diluted specimen again by 10-fold in Negative Control (final dilution is 1:100).
6. Test the diluted sample, using the 1:100 diluted sample in place of the undiluted sample in the Assay Procedure. If the Procedural Control Spot is reactive and the Test Spots are reactive for only HIV-1 or HIV-2, the result can be reported as Preliminary Positive for antibodies to the specific HIV type identified.

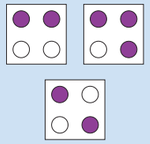
If the dual HIV reactivity does not disappear at the 1:100 dilution, the specimen should be interpreted as "Preliminary Positive for antibodies to HIV (undifferentiated)."

Test Result Appearance and Interpretation

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

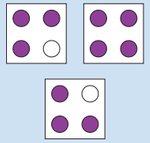
Only the Procedural Control Spot shows purple color development. The 3 Test Spots show no color development. Test result is interpreted as negative for HIV-1 and HIV-2 antibodies.

Reactive: Report results as described in the CDC guidance for reporting test results and interpretation.



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

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



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

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

Sensitivity

HIV-1: 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 Multispot for antibodies to HIV-1 with serum specimens is calculated to be 100% (95% CI = 99.94-100.00%). 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 Multispot. Based on these studies, the sensitivity of Multispot for antibodies to HIV-1 with plasma specimens is calculated to be 100% (95% CI = 99.94-100.00%).

HIV-2: 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 Multispot. Based on the results from these studies, the sensitivity of Multispot for antibodies to HIV-2 is calculated to be 100% (95% CI = 99.76-100%).

HIV-1 Group O: Twelve (12) HIV-1 Serotype Group O frozen plasma samples were tested on Multispot. 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, and one was nonreactive.

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 in Table 1.

Table 1: Differentiation of HIV-1 and HIV-2 Antibodies in W. 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-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.

Specificity

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

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

Stand-Alone HIV-1 Multi-Test Algorithm

During the evaluation of Multispot, the spot intensity was graded and recorded according to the following criteria:

0 – no color reaction
1+ – any trace or weak (faint purple) color reaction
2+ – definite light purple color reaction
3+ – purple color reaction less intense than the Procedural Control Spot
4+ – purple color reaction at least as intense as the Procedural Control Spot

Multispot results are defined as strongly reactive when there is a color intensity of 3+ to 4+.

The individual HIV-1 spot intensity patterns were evaluated to determine their correlation with HIV-1 Western blot results. The results from fresh-paired serum/plasma samples from 871 patients that were previously known to be positive for HIV-1 antibodies or were HIV-1 Western blot positive in this clinical trial are shown in Table 2.

Table 2: HIV-1 Known Positive Population, HIV-1 Western Blot only Positive

Multispot pattern	# of patients	Percentage
Both HIV-1 spots 3+ to 4+	832	96%
One or both HIV-1 spots 1+ to 2+	39	4%
Total	871	100%

Eight hundred thirty-two (832) patients of 871 (96%) showed strong (3+ to 4+) reactivity to **both** HIV-1 antigen spots (locations 3 and 4); results for the remaining 39 HIV-1 positive patients were positive on both of the HIV-1 antigen spots, but with 1+ to 2+ reactivity on one or both spots. All samples with strong reactivity on **both** Multispot HIV-1 antigen spots were HIV-1 Western blot positive.

There were 3146 non-infected patients in the low and high-risk groups in this study. Of these, there were 3 patients that were HIV-1 Western blot negative and Multispot false positive. The spot intensity on these 3 patients was only weak (1+) reactivity on one or both of the HIV-1 antigen spots and no reactivity on the remaining spots. These results are presented in Table 3.

Table 3: Multispot False Positive Reactivity

Patient	Sample Type	Spot Reaction Pattern			
		Procedural Control Spot	HIV-2 Peptide	HIV-1 Recombinant	HIV-1 Peptide
1	Plasma	4	0	0	1
2	Plasma	4	0	1	0
3	Plasma	4	0	1	1
	Serum	4	0	1	0

Summary and Conclusions

Sensitivity: In a population of 829 confirmed HIV-1 positive serum samples, the sensitivity of Multispot was 100%. In a population of 871 confirmed HIV-1 positive plasma samples, the sensitivity of Multispot was 100%.

Specificity: A total of 1494/1495 serum were correctly identified by Multispot for a specificity of 99.93%. A total of 2272/2274 plasma were correctly identified by Multispot for a specificity of 99.91%.

HIV-1 and HIV-2 Differentiation: Multispot correctly identified 1070 of the 1071 samples (99.91%) that were known HIV-1 positive as HIV-1 reactive only. Multispot correctly identified 107 of the 109 samples (98.16%) that were HIV-2 Western blot only positive as HIV-2 reactive only.

Stand-Alone HIV-1 Multi-Test Algorithm: Of the 871 patients that were known positive for HIV-1 antibodies, 832 (96%) showed strong (3+ to 4+) reactivity to **both** HIV-1 antigen spots. Of the 3146 that were negative for HIV-1 antibodies, all (100%) were negative or only weakly (1+) reactive on one or both of the HIV-1 antigen spots. Strong reactivity on **both** Multispot HIV-1 antigens provides 100% PPV with positive HIV-1 Western blot results or known HIV-1 infection.

Conclusions: Multispot is a sensitive and specific rapid test for the detection of HIV-1 and HIV-2 antibodies, and can reliably differentiate HIV-1 infection from HIV-2. In addition, Multispot could serve as a multi-test algorithm for HIV-1 in a single device when there is strong (3+ to 4+) reactivity to **both** HIV-1 antigen spots.