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

Abstract

Background/Objective: Rapid HIV tests provide timely and accurate identification of HIV infection. The Multispot HIV-1/HIV-2 (HIV-1/HIV-2) assay is a multi-test assay for the detection of antibodies to HIV (undifferentiated). This study evaluates the Multispot assay in a blinded setting.

Methods: A total of 3146 fresh plasma and 2235 fresh serum samples were tested in 301 centers in 76 countries for HIV-1 and HIV-2. Of these, 871 HIV-1 positive and 871 HIV-2 positive samples were tested, as well as 500 frozen serum samples collected from West Africa. The intensity of reactivity for each HIV-1 and HIV-2 antigen spot was graded on a scale of 0 to 4. The specificity of Multispot using serum specimens in these studies is calculated to be 100% (95% CI = 99.94-100.00%).

Results: Eight hundred thirty-two (832) patients (871 HIV-1 positive patients, 149 positive plasma and 383 positive serum samples) were tested. The specificity of Multispot using plasma specimens in these studies is calculated to be 100% (95% CI = 99.94-100.00%).

Conclusion: Multispot is a sensitive and specific rapid test for the detection of HIV-1 and HIV-2 antibodies, and can reliably differentiate HIV infection from HIV-2 infection. In addition, Multispot could serve as a Stand-Alone HIV-1 Multi-Test Algorithm for the detection and differentiation of HIV-1 and HIV-2 antibodies.

Assay Procedure

1. Bring all of the reagents and specimens to room temperature (20-30°C) before beginning testing.
2. Label a test strip or control strip to be tested.
3. Place the required number of Multispot Cartridges on a flat surface.
4. Add two drops of specimen to each Multispot Antigen Spot and gently incubate.
5. Using a separate pipet for each specimen, draw up a small amount of specimen. Hold the pipet vertically over the appropriate dilution tube, add one drop to the tube.
6. Add two drops of a Negative Control to each of the corresponding Multispot Antigen Spots.
7. Mix each diluted specimen and control thoroughly.
8. Pour the contents of each tube into the specimen prefilled multispot cartridge, using a separate cartridge for each tube. Wait five minutes.
9. Remove and discard the prefilled 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 drops of Conjugate Reagent to the central well of each cartridge.
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 Wash Solution to be absorbed completely.
14. Add three drops of Development Reagent to the central well of each cartridge.
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 spots as well as one or both of the HIV-1 spots.

1. Dilute the specimen 1:10 in Negative Control Mix. Wait 10 minutes.
2. Place the diluted sample in a separate Cartridge for each tube. Wait 15 minutes.
3. Add the appropriate volume of each of five reagents to the diluted sample in the Assay Procedure. Wait for the Stop Solution to be absorbed completely.
4. Read the results.

Sensitivity

HIV-1: Of the 829 confirmed HIV-1 positive samples that were HIV-1 reactive on Multispot, 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 was 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 829 were reactive when tested on Multispot. Based on these studies, the sensitivity of Multispot for antibodies to HIV-1 with plasma samples 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 at high risk for HIV-2 infection, all 207 were reactive when tested on Multispot. Based on these results, the sensitivity of Multispot for antibodies to HIV-2 was calculated to be 100% (95% CI = 99.94-100.00%).

HIV-1/HIV-2 Group O: Twelve (12) HIV-1/HIV-2 Group O samples known to be HIV-2 positive 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/HIV-2 Group O 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 as HIV-2 positive by HIV-1 Western blot testing as positive or HIV-1 as shown in Table 1.

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

<table>
<thead>
<tr>
<th>HIV Status</th>
<th>HIV-1 Antibody</th>
<th>HIV-2 Antibody</th>
<th>HIV-1/HIV-2 Antibody</th>
<th>% Correct</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 positive</td>
<td>100%</td>
<td>0%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>HIV-2 positive</td>
<td>0%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>HIV-1/HIV-2</td>
<td>50%</td>
<td>50%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 2: Multisite Multispot HIV-1/HIV-2 Multi-Test Algorithm

<table>
<thead>
<tr>
<th>Patient Type</th>
<th>Specimen</th>
<th>HIV-1 Antigen Spots</th>
<th>HIV-2 Antigen Spots</th>
<th>HIV-1/HIV-2 Antigen Spots</th>
<th>Patient Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 positive</td>
<td>Plasma</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>HIV-1 positive</td>
</tr>
<tr>
<td>HIV-2 positive</td>
<td>Plasma</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>HIV-2 positive</td>
</tr>
<tr>
<td>HIV-1 positive</td>
<td>Serum</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>HIV-1 positive</td>
</tr>
<tr>
<td>HIV-2 positive</td>
<td>Serum</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>HIV-2 positive</td>
</tr>
</tbody>
</table>

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

Summary and Conclusions

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

Specificity: Of the 2742 plasma samples correctly identified by Multispot for antibodies to HIV-1, 99.93% of the 2742 plasma samples were correctly identified by Multispot for antibodies to HIV-1.

Stand-Alone HIV-1 Multi-Test Algorithm

During the evaluation of Multispot, the spot intensity was graded and read 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 Positive Control Spot

4 = purple color reaction at least as intense as the Positive Control Spot

The following procedures are used to differentiate positive samples:

- HIV-1: Of the 829 confirmed HIV-1 positive samples that were HIV-1 reactive on Multispot, all 829 were reactive when tested on the Multispot HIV-1/Western blot. The specificity of Multispot using fresh serum samples and plasma samples from known HIV-2 positive patients was tested, as well as matched serum and plasma samples from 801 known HIV-1 positive individuals and from individuals at high risk for HIV-2 infection.

- 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 at high risk for HIV-2 infection, all 207 were reactive when tested on Multispot. Based on these results, the sensitivity of Multispot for antibodies to HIV-2 was calculated to be 100% (95% CI = 99.94-100.00%).

- HIV-1/HIV-2 Group O: Twelve (12) HIV-1/HIV-2 Group O samples known to be HIV-2 positive 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/HIV-2 Group O samples were reactive when tested on Multispot, and one was nonreactive.