Development of a Rapid HIV-1 Confirmatory Test
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Abstract

In the United States, only the Western blot and Immuno Fluorescent Antibody Assay (IFA) are approved by the FDA for confirmatory HIV testing. Both of these tests are labor intensive, expensive, slow to provide results and require skilled readers. The World Health Organization has recommended an algorithm for confirmation using combinations of simplified tests with different HIV antigens. We developed a rapid lateral flow, immunochromatographic assay with synthetic peptides and native protein antigens representing env (p24, gp120, and gp160), gag (p24) and pol (p51/66), applied on the same nitrocellulose strip. The sample is applied to a disposable cassette which then flows onto the test strip, hydrates and mixes with a reporter conjugate, to which IgG antibodies in the specimen bind and immobilize the colorimetric reporter at the site of that band on the strip. At completion (~30 min.), the device can be read visually or by using a portable optical reader, which detects the intensity of the test bands.

Preliminary results using colloidal gold as the reporter with 4 positive and 2 negative serum specimens demonstrated detectable banding patterns comparable to results of the Western blot. Visible bands remained detectable with 1:1600 dilutions of HIV-positive sera, suggesting sensitivity is sufficient to detect the lower level of antibodies present in oral fluid.

A confirmatory lateral flow assay for HIV is feasible, and can be optimized for specimens with low antibody concentrations, such as oral fluid.

Discussion

Background

• Only two confirmatory methods are FDA approved: Western blot and IFA.
• Western blot (WB) uses inactivated viral antigens, electrophoretically separated by molecular weight.
• These protein bands are transferred to nitrocellulose strip.
• In WB, a multi-step procedure, IgG antibodies to specific HIV-1 proteins in the sample bind to the protein bands on nitrocellulose.
• WB procedure is complex, requires specialized equipment, technical expertise, and 4 - 24 hours.
• A rapid, lateral flow, HIV-1 confirmatory test based on similar principles could provide results in < 30 min.

Method

• We developed a lateral flow assay with multiple viral proteins or peptides immobilized as separate bands on a nitrocellulose membrane.
• Materials representing env (p24, gp120, gp160), gag (p24) and pol (p51/66) were immobilized on nitrocellulose.
• The optimal order for the different bands on the nitrocellulose was determined empirically.

Results

Table 1: Comparison of rapid lateral flow test with Western Blot.
The banding pattern in the lateral flow assay completely matched the Western blot.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Western blot result</th>
<th>Rapid Lateral Flow result</th>
</tr>
</thead>
<tbody>
<tr>
<td>0729902</td>
<td>p24 p51/66 gp120 gp160</td>
<td>p24 p51/66 gp120 gp160</td>
</tr>
<tr>
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</tr>
<tr>
<td>13964</td>
<td>x x x x x</td>
<td>x x x x x</td>
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</tbody>
</table>

Table 2: Comparison of the number of steps.

<table>
<thead>
<tr>
<th>Western blot Test</th>
<th>Rapid Lateral Flow test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Mix the sample</td>
<td></td>
</tr>
<tr>
<td>2. Add 20ul of the</td>
<td></td>
</tr>
<tr>
<td>3. Read the result</td>
<td></td>
</tr>
<tr>
<td>4. Total time is</td>
<td></td>
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</table>

Figure 1: Test device with lateral flow strip.
The disposable test device will have two openings on top. One for sample application and one for reading the result. The strip will have a sample pad and a conjugate pad that contains the reporter on one end and an absorbent pad on the other end.

Figure 2: Pictorial representation of the lateral flow test.
Step 1: Mix 5ul sample in 1ml of sample diluent.
Step 2: Add 200ul of the mixture to the sample pad. Conjugate will bind to specific HIV-1 proteins in the sample. It flows across the various HIV antigens, antibodies specific to a particular antigen band will bind.
Step 3: At the completion of the test (~30 min.), the device can be read.

Figure 3: Dilutional sensitivity
Two HIV-1 positive and two negative serum specimens were run at dilutions of 1:400, 1:800, 1:1600, 1:3200 and 1:6400. All five bands were detectable for both the positive specimen down to 1:1600 dilution.

Conclusions

• A confirmatory rapid lateral flow assay for HIV is feasible, and can be optimized for specimens with low antibody concentrations.
• The lateral flow assay is much faster and less labor intensive compared to the currently approved Western blot or IFA confirmatory tests.

REFERENCES