Title: Laboratory Evaluation of a Dual Path Platform Assay for Rapid Point-of-Care HIV and Syphilis Testing

Running Title (50/54 characters and spaces): Evaluation of an Assay for HIV and Syphilis Testing

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Abstract (72/75 words)

We assessed the laboratory performance of the Chembio Dual Path Platform® HIV-Syphilis rapid immunodiagnostic test and electronic reader for HIV and *Treponema pallidum* antibody detection in 450 previously characterized serum specimens. For HIV antibody detection, visual and electronic reader sensitivity was 100% and specificity was 98.7%. For *Treponema pallidum* antibody detection, test sensitivity was 94.7% and specificity was 100.0% for visual interpretation; the electronic reader’s sensitivity was 94.7% and specificity was 99.7%.
Rapid multiplex point-of-care tests are increasingly available means to screen for syphilis and HIV infections (1-4). Dual screening tests for antibodies to HIV and *Treponema pallidum* have been evaluated in sera and venipuncture whole blood showing excellent sensitivity and specificity (5-7). We assessed the laboratory performance of the Chembio Dual Path Platform® (DPP®) HIV-Syphilis rapid test and Chembio digital electronic reader in stored serum samples collected from a cohort of men who have sex with men (MSM) and transwomen at high risk for HIV and syphilis infection.

Serum specimens were collected and stored at −20°C between 2013 to 2014 from MSM and transwomen who were recruited into an ongoing cohort study in Lima, Peru (8). HIV positivity was determined with the Genscreen Ultra HIV Ag-Ab test (Bio-Rad, Hercules, CA), a fourth-generation enzyme immunoassay (EIA) (11). Positive EIA tests were confirmed with Western blot (New Lav Blot I; Bio-Rad, Hercules, CA). Specimens also underwent rapid plasma reagin (RPR) testing, using the BD Macro-Vue™ RPR (rapid plasma reagin) Card Test Kit (BD, Franklin Lakes, NJ) (12), and *Treponema Pallidum* Particle Agglutination (TPPA) testing (Serodia, Fujirebio Diagnostics Inc., Tokyo, Japan), used according to manufacturer's instructions (13).

The Chembio DPP® HIV-Syphilis test is a single-use, visual and qualitative immunochromatographic dual rapid test for the detection of antibodies to HIV types 1 and 2 and *Treponema pallidum* in human sera, plasma, venous or fingerprick whole blood samples (20). A red control line confirms test validity. Visual observation of a red line in the HIV and/or syphilis detection zone was interpreted as reactive (14).
Immediately after visual interpretation, tests were analyzed using the small, battery powered Chembio electronic reader, designed specifically to complement Chembio’s DPP® technology. The electronic reader scans the DPP® test cartridge and displays a numerical value based on the test line intensity. If the electronic reader value is higher than the set cut-off value, the sample is reported as positive; the test is reported as negative if the measured value is lower than the cut-off value.

We estimated the sensitivity, specificity, calculated 95% confidence intervals (CIs) using the exact binomial method and calculated the concordance between the visual results of the Chembio DPP® HIV-Syphilis rapid test and the results of the reference tests using Cohen’s kappa coefficient. Specimens were defined as HIV positive based on Western blot test results. Specimens were defined as Treponema pallidum antibody positive based on TPPA test results. For electronic reader data, we estimated the sensitivity, specificity, calculated 95% confidence intervals (CIs) using the exact binomial method and evaluated the performance of different cut-off values.

Of the 450 specimens, 100 were confirmed by Western blot to be HIV-1 positive only, 99 were positive for Treponema pallidum antibodies by TPPA only, of which 79 (80%) had RPR titers between 1:1 and 1:64; and 51 were positive for both HIV and Treponema pallidum antibodies by Western blot and TPPA. Of the dual antibody reactive specimens, 72% (37/51) had RPR titers between 1:1 and 1:64; the remaining 200 specimens tested negative for HIV and syphilis antibodies.

Additionally, positive and negative controls were used with tested specimens.
For visual interpretation of HIV antibody reactivity, the test showed 155 positive and 295 negative results (Table 1). There were 4 false-positive (DPP®, positive, Western blot negative) and no false-negative results. The sensitivity of the HIV antibody component was 100% (95% CI, 97.6%–100.0%) and the specificity was 98.7% (95% CI, 96.6%–99.6%). The kappa coefficient for correlation between the reference HIV-1 Western blot test and the Chembio DPP® HIV-Syphilis rapid test results was 0.98 (95% CI, 0.96–1.0).

For visual interpretation of Treponema pallidum antibody reactivity, the test showed 142 positive and 308 negative results (Table 2). Of those, there were eight false-negative (DPP®, negative, TPPA positive) and no false-positive results. The sensitivity of the Treponema pallidum antibody component was 94.7% (95% CI, 89.8%–97.7%) and specificity was 100.0% (95% CI, 98.8%–100.0%). The kappa coefficient for correlation between the reference TPPA syphilis test and the Chembio DPP® HIV-Syphilis rapid test results was 0.96 (95% CI, 0.93–0.99).

Using Chembio’s electronic reader with the default cut-off value of 10 for HIV antibody detection, the sensitivity was 100.0% (95% CI, 97.6%–100.0%), specificity was 98.7% (95% CI, 96.6%–99.6%), and the kappa coefficient was 0.98 (95% CI, 0.96–1.0) (Table 1). By changing the cut-off value to 21, the specificity was 100.0% (95% CI, 98.8%–100.0%), sensitivity was 100.0% (95% CI, 97.6%–100.0%), and the kappa coefficient for correlation was 1.0. No statistical difference was found when comparing a cut-off value of 10 against visual interpretation (p-value >0.1).

Using Chembio’s electronic reader with the default cut-off value of 10 for Treponema pallidum antibody detection, the sensitivity was 94.7% (95% CI, 89.8%–97.7%).
specificity was 99.7% (95% CI, 98.2–100.0%), and the kappa coefficient was 0.95 (0.92–0.98) (Table 2). Various cut-off values for the *Treponema pallidum* antibody component showed that the cut-off value of 10 demonstrated the best performance. No statistical difference was found when comparing a cut-off value of 10 against visual interpretation (p-value >0.1).

Repeat laboratory testing was done on specimens with discordant test results. All four initially false-positive specimens for HIV antibodies were found to be negative on repeat testing with the Chembio DPP® HIV-Syphilis rapid test, with both visual interpretation and the electronic reader.

Of the eight specimens with false-negative treponemal antibody results, repeat Chembio DPP® HIV-Syphilis rapid tests were weakly positive for four samples on visual observation; 4 remained negative. The electronic reader classified two of those four weakly reactive on visual interpretation as positive.

We conducted a laboratory-based performance study of the Chembio DPP® HIV-Syphilis rapid test and electronic reader with characterized, stored serum samples in Lima, Peru. For visual interpretation, we found an outstanding performance of the test. Based on varying the electronic reader cut-off values, the cut-off value of 21 had a 100% specificity and sensitivity for HIV antibody detection. For *Treponema pallidum* antibody detection, the electronic reader, with the default cut-off value of 10, had the best sensitivity and specificity.

Our results are comparable to other laboratory evaluations previously conducted with different point-of-care tests in sera and whole blood for HIV and treponemal antibody
detection, which also obtained high performance values for sensitivity and specificity (1, 5-7, 15-17). In our evaluation, the Chembio DPP® HIV-Syphilis rapid test's sensitivity to detect HIV antibodies was somewhat higher than its sensitivity to detect syphilis antibodies in concurrence with several other studies (5, 6, 15, 16, 18).

Our study had some limitations. We used specimens from a single population in Lima, Peru, that did not include any pregnant women or low-risk populations. The population had a high risk for HIV and syphilis acquisition, so our values for the test's sensitivity and specificity might not be representative. The tested specimens were stored sera instead of the ideal samples—fingerprick whole blood specimens. Since evaluation of stored serum specimens seems to be highly accurate, further studies are warranted using fingerprick whole blood specimens to evaluate field performance of the Chembio DPP® HIV-Syphilis dual platform rapid test and electronic reader.

Acknowledgements

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References


Table 1. Laboratory performance for the detection of HIV antibodies using a dual HIV/syphilis rapid immunodiagnostic test, Lima, Peru, 2015 (N=450)

**Visual result**

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<tr>
<th>Number of samples</th>
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<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Kappa Coefficient (95% CI)</th>
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<tr>
<td>WB test +</td>
<td>151</td>
<td>100.0% (97.6%-100.0%)</td>
<td>98.7% (96.6%-99.6%)</td>
<td>.96-1.0</td>
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<tr>
<td>WB test -</td>
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<tr>
<td><strong>DPP® Chembio +</strong></td>
<td>155</td>
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<tr>
<td>DPP® Chembio -</td>
<td>295</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>151</td>
<td>100.0% (97.6%-100.0%)</td>
<td>98.7% (96.6%-99.6%)</td>
<td>.96-1.0</td>
</tr>
</tbody>
</table>

**Electronic reader device (cut off = 10)**

<table>
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<th>Number of samples</th>
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<td>.96-1.0</td>
</tr>
</tbody>
</table>
CI: Confidence Interval
WB test: Western blot test
DPP®: Dual Path Platform®
Table 2. Laboratory performance for the detection of *Treponema pallidum* antibodies using a dual HIV/syphilis rapid immunodiagnostic test, Lima, Peru, 2015 (N=450)

**Visual result**

<table>
<thead>
<tr>
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<th>Total</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Kappa Coefficient (95% CI)</th>
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<td>142</td>
<td>94.7% (89.8%-97.7%)</td>
<td>100.0% (98.2%-100.0%)</td>
<td>.96 (.93-.99)</td>
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<tr>
<td><strong>Total</strong></td>
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**Electronic reader device (cut off = 10)**

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<th>Sensitivity (95% CI)</th>
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<th>Kappa Coefficient (95% CI)</th>
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<td>94.7% (89.8%-97.7%)</td>
<td>99.7% (98.2%-100.0%)</td>
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<tr>
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<td>300</td>
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CI: Confidence Interval

TPPA test: Treponema Pallidum Particle Agglutination test

DPP®: Dual Path Platform®