Laboratory Evaluation of a Dual Rapid Immunodiagnostic Test for HIV and Syphilis Infection

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New dual tests for HIV and syphilis have been developed. Our study aimed to evaluate the laboratory performance of a dual rapid immunodiagnostic test for HIV and syphilis. Our evaluation showed high performance of this dual rapid test, which should be considered for implementation to increase screening coverage and efficiency.

Syphilis is a curable disease, yet 10 million persons worldwide have new syphilis infections each year (1). Syphilis frequently has an atypical presentation that may be difficult to differentiate from other sexually transmitted infections (STIs), making effective diagnostics essential for the identification of infection (2). In 2012, over 35 million people had prevalent human immunodeficiency virus (HIV) (3). HIV and syphilis infection screening should be offered to every pregnant woman to prevent adverse outcomes of pregnancy that include stillbirth, prematurity, neonatal death, or mother-to-child transmission of syphilis and/or HIV infection (4–6).

Syphilis infection during pregnancy is associated with over a 2.7-fold-increased risk of mother-to-child HIV transmission (7). Additionally, syphilis infection, like other genital ulcer diseases, may facilitate HIV acquisition and transmission (8–10). In coinfected patients, syphilis can increase transmission of HIV by increasing viral shedding at the site of genital ulcers (11, 12) and increasing the HIV load (13, 14).

Syphilis infection is generally diagnosed by using two serologic laboratory-based tests; however, over the past 10 years, the advent of syphilis rapid tests has allowed for point-of-care screening (15, 16). In order to increase screening coverage, new dual tests for HIV and syphilis have been developed (17–19). Those dual tests will allow for syphilis, a disease with less advocacy and donor funding, to become part of HIV prevention programs (20). Our study aimed to evaluate the laboratory performance of a dual rapid immunodiagnostic test for HIV and syphilis.

Stored serum specimens collected from men who have sex with men and transgender women presenting to one of two sexually transmitted disease clinics in Lima, Peru, were used for the evaluation. The specimens were collected and serum separation was conducted in the field. The serum samples were then transported on ice to the laboratory, in less than 4 h. Serum was frozen and stored at −30°C the same day of collection. The stored specimens were thawed and tested for HIV infection by using a fourth-generation enzyme immunoassay (Genscreen Ultra HIV Ag-Ab; Bio-Rad, France) for the simultaneous qualitative detection of HIV p24 antigen and antibodies to gp41 and gp36 of HIV type 1 (HIV-1 groups M and O) and HIV type 2 in human serum or plasma. Each positive enzyme immunoassay test was confirmed using a Western blot test (New Lav Blot I; Bio-Rad, France). Those samples that were positive in both the enzyme immunoassay and the Western blot assay were considered HIV positive. Additionally, if the enzyme immunoassay result was positive and the Western blot assay result was indeterminate, then Western blotting was performed at a later date on a follow-up specimen; if this result was positive, that specimen was considered HIV positive. For the Treponema pallidum antibody comparison, a Treponema pallidum particle agglutination test (Serodia-TPPA; Fujirebio Diagnostics, Inc., Japan) was used. Rapid plasma reagin (RPR) tests (BD MacroVue RPR; Becton Dickinson, NJ) were also conducted on all specimens.

The Multiplo Rapid TP/HIV antibody test (Medmira Inc., Halifax, Nova Scotia, Canada) is made up of a test cartridge that contains an immunoreactive test membrane comprised of T. pallidum recombinant antigens (15 kDa, 17 kDa, and 47 kDa in size) and synthetic HIV peptides for gp36, gp41, gp120, and HIV-1 group O antigen. In addition, the test membrane has a procedural and reagent control line that contains an optimized amount of protein.

The Multiplo dual test was performed following the manufacturer’s instructions by trained laboratory personnel. First, 3 drops of a buffer solution and then 1 drop of serum specimen were applied to the center of the test cartridge, which contained the test membrane. An InstaGold cap was placed onto the test cartridge, and an additional 12 drops of the buffer solution were added to the top. The InstaGold cap, which contains a proprietary protein A/protein L colloidal gold conjugate, reacts with antibodies present in the sample. Once the solution was absorbed, the cap was removed, 3 drops of additional buf-
fer solution were added, and the captured antibodies were visualized on the test membrane. The test was read immediately by one trained laboratorian. A red control line was visualized on valid tests. A visible HIV line indicated an HIV antibody component of the analysis. Of the 198 samples that yielded a Multiplo HIV test result, 84 were HIV positive by reference tests. Of the 193 specimens that yielded a T. pallidum test result, 110 were positive for T. pallidum antibody.

We estimated sensitivity and specificity and calculated 95% confidence intervals (CIs) by using the exact binomial method. We determined the concordance between the test under evaluation and the reference tests by using Cohen’s kappa statistic. All analyses were conducted using SAS v9.3 (SAS Institute, Cary, NC).

A total of 200 serum specimens were tested using the Multiplo Rapid TP/HIV antibody test. Of the 200 specimens tested, 198 gave a valid control line and were included in the analysis; an additional 5 tests had a T. pallidum dot that did not absorb the sample and buffer fluid and were excluded from the T. pallidum component of the analysis. Of the 198 samples that yielded a Multiplo HIV test result, 84 were HIV positive by reference tests. Of the 193 specimens that yielded a T. pallidum test result, 110 were positive for T. pallidum antibody on the reference test.

The HIV component of the Multiplo test gave 74 true-positive results and 10 false-positive, 0 false-negative, and 114 true-negative results (Table 1). The sensitivity estimate of the HIV component was 100% (95% CI, 95.1% to 100%), and the specificities was 91.9% (95% CI, 85.7% to 96.1%). The kappa coefficient for correlation between the reference HIV test result and the Multiplo HIV test result was 0.90 (95% CI, 0.83 to 0.96).

For the T. pallidum antibody component, of the 193 valid test results, the test produced 104 true-positive results and 6 false-positive, 6 false-negative, and 77 true-negative T. pallidum results (Table 2). Of the 6 specimens with T. pallidum false-negative test results, all 6 were reactive by RPR tests, with RPR titers of 1:1 for four specimens, 1:2 for one, and 1:4 for one. In addition, all 6 false-negative T. pallidum Multiplo test results were RPR nonreactive. The sensitivity and specificity estimates were 94.6% (95% CI, 88.5% to 98.0%) and 92.8% (95% CI, 84.9% to 97.3%), respectively. The kappa coefficient for the T. pallidum component was 0.87 (95% CI, 0.80 to 0.94).

We evaluated a dual rapid immunodiagnostic test for HIV and syphilis infection in a laboratory setting in Lima, Peru, by using characterized serum specimens. Our evaluation showed a high performance level for this dual rapid test. Other dual tests for HIV and syphilis are also being manufactured and have shown great promise, with estimated sensitivities ranging from 97% to 100% and estimated specificities ranging from 99% to 100% (18, 19). One of the causes for differences in performance is the use of different reference tests, a consideration when interpreting and comparing different studies. Dual tests are increasingly available in countries outside the United States.

This study was subject to some limitations. Due to the study sample size, we had somewhat wide confidence intervals for performance estimates. Additional evaluations should be performed to better estimate the true test performance. Additionally, 3.5% of tests under evaluation did not perform adequately; five had an unreadable T. pallidum result and two tests did not give control lines and were therefore deemed invalid.

Demonstrating the performance of new diagnostic tests with laboratory specimens is the first important step in test evaluation; however, because rapid tests are developed for use with clinical specimens at the point of care, further evaluation in the field with whole-blood specimens is needed.

The World Health Organization has called for the dual elimination of HIV and syphilis through harmonized strategies to reduce adverse outcomes of pregnancy and prevent the continued transmission of infections (6). Dual HIV and syphilis point-of-care rapid testing has immense public health importance to identify, treat, and prevent the spread of these infections.

### Table 1: Laboratory performance for detection of HIV antibodies by using a dual HIV/syphilis rapid immunodiagnostic test

<table>
<thead>
<tr>
<th>Multiplo HIV result</th>
<th>No. of samples with reference HIV test result of:</th>
<th>Total no. of samples</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Kappa coefficient (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>74</td>
<td>10</td>
<td>84</td>
<td>100% (95.1–100%)</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>0</td>
<td>114</td>
<td>114</td>
<td>(84.9–97.3%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>74</strong></td>
<td><strong>124</strong></td>
<td><strong>198</strong></td>
<td><strong>0.90 (0.83–0.96)</strong></td>
</tr>
</tbody>
</table>

*For the 200 specimens tested, two gave an invalid result (no control line) and therefore were not included in the analysis.

### Table 2: Laboratory performance for detection of Treponema pallidum antibodies by using a dual HIV/syphilis rapid immunodiagnostic test

<table>
<thead>
<tr>
<th>Multiplo T. pallidum result</th>
<th>No. of samples with reference T. pallidum result of:</th>
<th>Total no. of samples</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Kappa coefficient (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>104</td>
<td>6</td>
<td>110</td>
<td>94.6% (88.5–98.0%)</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>6</td>
<td>77</td>
<td>83</td>
<td>(84.9–97.3%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>110</strong></td>
<td><strong>83</strong></td>
<td><strong>193</strong></td>
<td><strong>0.87 (0.80–0.94)</strong></td>
</tr>
</tbody>
</table>

*Of the 200 samples tested, 2 gave an invalid result (no control line) and were not analyzed further. In addition, 5 tests gave invalid results for the Treponema pallidum component and therefore were not included in the results.
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REFERENCES


