Laboratory Evaluation of Three Rapid Diagnostic Tests for Dual Detection of HIV and *Treponema pallidum* Antibodies

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The performance of three research-use-only, dual HIV and syphilis rapid diagnostic tests (RDTs) was evaluated for 150 patient serum samples and compared to reference HIV and *Treponema pallidum* antibody detection methods. The RDTs performed comparably, with sensitivities of 93 to 99% and specificities of 97 to 100%. The kappa statistic between the RDTs was 0.95.

In the United States, HIV and syphilis (caused by *Treponema pallidum*) coinfection is increasingly common, with an estimated median HIV seroprevalence in men with syphilis of 27.5% and in women with syphilis of 12.4% (1). Men who have sex with men (MSM) have particularly high rates of HIV and syphilis coinfection, documented to be 47 to 72% in some areas of the United State (2–6). Coinfection with syphilis can increase the transmission of HIV by both increasing viral shedding through open ulcers (7, 8) and by increasing patient viral load (9, 10).

Reference methods used by most major clinical laboratories in the United States for the diagnosis of HIV include enzyme immunoassays (EIAs) for the qualitative detection of antibodies to HIV-1 and HIV-2. EIA-reactive specimens are typically confirmed with an HIV-1 antibody Western blot assay. In 2014, the Centers for Disease Control and Prevention (CDC) issued new guidance for HIV diagnostic testing, which included primary testing by a combination immunoassay that detects both HIV-1 and HIV-2 antibodies and the HIV-1 p24 antigen. Specimens reactive by the screening assay undergo supplemental testing with an immunoassay that differentiates HIV-1 and HIV-2 antibodies (11). Specimens that are reactive on initial antigen/antibody combination immunoassays and nonreactive or indeterminate in the HIV-1/HIV-2 antibody differentiation immunoassay are then tested with an FDA-approved HIV-1 RNA nucleic acid test (NAT) (11). Reference methods for diagnosis of syphilis include primary screening by nontreponemal tests, such as the rapid plasma reagin (RPR), and confirmation with a treponemal-specific test, such as the *T. pallidum* particle agglutination (TP-PA) assay. Alternatively, many laboratories have adopted a “reverse algorithm,” whereby a *T. pallidum*-specific immunoassay (e.g., enzyme immunoassay) is the screening test and a nontreponemal test, such as the RPR, is performed on EIA-reactive samples to determine the stage of the disease and monitor treatment (12).

In the United States, HIV tests are also commonly administered in both clinical and nonclinical community-based organizations, through the use of the Clinical Laboratory Improvement Amendments (CLIA)-waived rapid diagnostic tests (RDTs) for HIV, which detect HIV-1 and HIV-2 antibodies. The advantage of such testing is that results are immediately available at the point of care, which provides early diagnosis of HIV infection and improved linkage to care (13–15). In contrast, FDA-approved, CLIA-waived point-of-care tests for the diagnosis of syphilis are not yet available in the United States, although such tests are available in other countries (16). At the time of this writing, one rapid *T. pallidum* test has obtained FDA clearance and is awaiting the CLIA waiver (Syphilis Health Check [Diagnostics Direct, Youngstown, OH]). As is the case for HIV, rapid diagnosis and treatment of syphilis is critical to reducing transmission. The availability of rapid, CLIA-waived syphilis tests will allow immediate evaluation and treatment of patients who test positive for syphilis and the potential for screening in nonmedical settings. The bulk of the syphilis epidemic in the United States is among MSM, and the largest increase in primary and secondary syphilis cases between 2009 and 2012 was in MSM aged 25 to 29 years (17). However, sexually active MSM, and in particular young MSM, do not seek HIV and syphilis screening at the frequencies recommended by the CDC. As such, the availability of CLIA-waived, rapid, dual testing has the potential to reduce both syphilis and HIV rates among this at-risk population. While evaluation of point-of-care testing with RDTs for HIV or syphilis has been performed in various settings, the use of dual RDTs for both HIV and syphilis has not been fully evaluated.

In this study, we evaluated the performance of three commercially available, research-use-only (RUO) HIV/*T. pallidum* antibody dual RDTs by using remnant, deidentified sera from 150 people who were previously tested by routine methods. Twenty-five specimens were obtained from the San Francisco Department of Public Health (and had been previously characterized to be positive for HIV and syphilis antibodies); HIV and syphilis testing was confirmed at UCLA prior to the start of the study. The remaining 125 serum specimens were from the UCLA Clinical Microbiology Laboratory and selected based on the results of routine HIV and syphilis serologic testing. HIV testing was performed using the Siemens Advia Centaur HIV 1/O/2 enzyme immunoassay (HIV EIA; Siemens, Tarrytown, NY); all positives were confirmed by Western blotting, using the GS HIV-1 Western blot kit (Bio-Rad, Hercules, CA). RPR testing was performed using the Macro-Vue 18-mm circle card test (Becton Dickinson, Sparks, MD). Presence of *T. pallidum* antibodies was confirmed by using

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The results of the RDTs for HIV were compared to those via routine testing (EIA and Western blotting). The results of the RDTs for T. pallidum were compared to the TP-PA test results. Specimens that yielded discordant or difficult-to-interpret (faint) results were repeated using all reference methods and all 3 RDTs, in parallel. Data were summarized using descriptive statistics, including sensitivity and specificity, with 95% confidence intervals (CI) calculated by using the exact binomial distribution method. The kappa statistic was used to describe concordance between the three RDTs. Statistical analyses were performed using Microsoft Excel. All protocols were approved by the UCLA Institutional Review Board.

Among 150 samples included in this study, 29 (19.3%) were negative for T. pallidum and HIV, 24 (16%) were positive for T. pallidum but negative for HIV, 35 (23.3%) were positive for HIV but negative for T. pallidum, and 62 (41.3%) were positive for both HIV and T. pallidum by the reference methods. All HIV EIA-positive results were confirmed by a positive HIV-1 Western blot assay (data not shown). RPR titers for the 86 specimens positive by the TP-PA assay ranged from non reactive (n = 28) to a 1:512 titer (mean titer of reactive specimens, 1:8).

The performance of the RDTs is listed in Table 1. Sensitivity for HIV antibody detection by the RDTs was 98 to 99% and specificity was 94 to 100%, compared to the Siemens Advia HIV EIA. Similarly, detection of T. pallidum antibodies was excellent for all three methods, ranging from 93 to 95% sensitivity and 97 to 100% specificity, compared to the TP-PA assay (Table 2). The kappa coefficient between the three RDTs was 0.95 (95% CI, 0.80 to 1.0) for the HIV component and 0.93 (95% CI, 0.78 to 1.0) for the T. pallidum component.

Repeat testing did not resolve any false-negative or false-positive results observed with the RDTs. All false-negative T. pallidum antibody results (n = 7) were from HIV-positive specimens (Table 3). Two of these were from specimens with high (≥1:8) RPR results. Two false-positive T. pallidum results were observed, both only with the MedMira assay and again in HIV-positive specimens (Table 3). Four false-positive HIV results were observed; three of these were with the MedMira assay (Table 4), two of which had

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ChemBio DPP HIV-syphilis assay</th>
<th>SD Bioline HIV/syphilis duo</th>
<th>MedMira Muliplo rapid syphilis/HIV antibody test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen</td>
<td>Whole blood, serum, or plasma</td>
<td>Whole blood, serum, or plasma</td>
<td>Whole blood, serum, or plasma</td>
</tr>
<tr>
<td>Time to detection (min)</td>
<td>25</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>Equipment</td>
<td>Requires a timer</td>
<td>Requires a timer</td>
<td>None</td>
</tr>
<tr>
<td>Shelf life</td>
<td>24 mos at room temp</td>
<td>24 mos at room temp</td>
<td>18 mos at room temp</td>
</tr>
<tr>
<td>HIV component</td>
<td>Recombinant HIV-1 and HIV-2 antigens (not specified)</td>
<td>Recombinant HIV-1 gp41, sub-O antigens</td>
<td>Recombinant HIV-2 gp36 antigen</td>
</tr>
<tr>
<td>T. pallidum component</td>
<td>Recombinant antigen (not specified)</td>
<td>Recombinant antigen (17 kDa)</td>
<td>Recombinant antigens (15 kDa, 17 kDa, 47 kDa)</td>
</tr>
<tr>
<td>Method</td>
<td>Solid-phase immunochromatographic assay</td>
<td>Solid-phase immunochromatographic assay</td>
<td>Vertical flow immunoassay</td>
</tr>
<tr>
<td>Antibodies detected</td>
<td>IgM and IgG antibodies to HIV and T. pallidum antigens</td>
<td>IgG, IgM, and IgA antibodies to HIV and T. pallidum antigens</td>
<td>IgM and IgG antibodies to HIV and T. pallidum peptides</td>
</tr>
</tbody>
</table>

The reference HIV antibody method was the Siemens Advia Centaur HIV 1/02 enzyme immunoassay and the GS HIV-1 Western blot kit, and the T. pallidum antibody reference method was the Serodia TP-PA test.

**Table 1** Comparison of the three HIV/T. pallidum antibody RDTs used in this study

**Table 2** Laboratory performance levels of three rapid diagnostic tests for the dual detection of HIV and T. pallidum antibodies, compared to reference methods

<table>
<thead>
<tr>
<th>RDT</th>
<th>HIV antibody % sensitivity (95% CI)</th>
<th>% specificity (95% CI)</th>
<th>T. pallidum antibody % sensitivity (95% CI)</th>
<th>% specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD Bioline</td>
<td>97.9 (92.0–99.6)</td>
<td>100 (91.5–100)</td>
<td>93.0 (84.8–97.1)</td>
<td>100 (92.9–100)</td>
</tr>
<tr>
<td>ChemBio</td>
<td>98.9 (93.6–99.9)</td>
<td>98.1 (88.6–99.9)</td>
<td>95.3 (87.9–98.5)</td>
<td>100 (92.9–100)</td>
</tr>
<tr>
<td>MedMira</td>
<td>97.9 (92.0–99.6)</td>
<td>94.2 (83.1–98.5)</td>
<td>94.1 (86.3–97.8)</td>
<td>96.9 (88.2–99.5)</td>
</tr>
</tbody>
</table>

The reference HIV antibody method was the Siemens Advia Centaur HIV 1/02 enzyme immunoassay and the GS HIV-1 Western blot kit, and the T. pallidum antibody reference method was the Serodia TP-PA test.

**Table 3** HIV and syphilis discordant results observed in the RDTs

**Table 4** False-positive T. pallidum results observed with the RDTs
syphilis antibodies
testing with an appropriate HIV RNA NAT is recommended (11). If positive, the diagnosis is confirmed, and if negative, additional
differential test for those patients with a positive HIV RDT. If
clinical use by the U.S. FDA since 2002. The CDC recommends a
qualitatively comparable.

methods, with excellent sensitivity and specificity. Ease of use was
performance by the RDTs that was comparable to the reference
similarly difficult to interpret. Overall, our evaluation showed
(tives), 10 SD tests (6.7%; all true positives), and 6 ChemBio tests
actions were noted for 16 MedMira tests (10.7%; two false posi-
tives), 10 SD tests (6.7%; all true positives), and 6 ChemBio tests
(4%; all true positives). Repeat testing yielded results that were
similarly difficult to interpret. Overall, our evaluation showed
performance by the RDTs that was comparable to the reference
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qualitatively comparable.

In the United States, several HIV RDTs have been approved for
clinical use by the U.S. FDA since 2002. The CDC recommends a
second specimen be collected and tested by an HIV 1/2 immuno-
differential test for those patients with a positive HIV RDT. If
positive, the diagnosis is confirmed, and if negative, additional
testing with an appropriate HIV RNA NAT is recommended (11). In
contrast, the clinical experience with syphilis RDTs is limited in
the United States, as only one manufacturer has recently received
FDA clearance for their syphilis RDT. The management and fol-
low-up testing for patients that test positive with a syphilis RDT
remains to be defined but will likely include confirmation with
laboratory-based treponemal tests and nontreponemal testing for
disease staging and monitoring of treatment. Nonetheless, global
experience with syphilis RDTs has shown excellent results. A sys-
tematic review of multiple syphilis RDTs used in 15 studies from
over 22,000 whole-blood, plasma, or fingerstick specimens
showed sensitivity (median of 86%; interquartile range, 75% to
94%) and specificity (99%; interquartile range, 98% to 99%) that
were comparable with nontreponemal screening tests character-
istics (18). A more recent meta-analysis further reported perform-
ance levels that were estimated to be comparable to those for
laboratory-based treponemal tests for these rapid treponemal
tests (16).

Dual RDTs for HIV and syphilis infection have been less well
evaluated in either laboratory or clinical settings. Recently, a mul-
tisite laboratory evaluation from 6 countries demonstrated excel-
ent sensitivity and specificity of the SD Bioline HIV/Syphilis Duo
RDT. The sensitivity and specificity of the HIV antibody test com-
ponent (n = 2,336 specimens tested) were reported to be 99.9% and
99.7%, respectively. For the T. pallidum antibody component
(n = 2,059 specimens tested), the sensitivity and specificity were
99.7% and 99.7%, respectively (19). These values are comparable
to those obtained in the present study.

Limitations to our study include a relatively small number of
patient specimens evaluated. Another limitation includes the use
of patient serum, as opposed to fingerstick whole-blood speci-
cimens, which would be used for point-of-care testing. Further-
more, the performance levels of these tests may have been higher

repeatedly faint reactions for HIV. Two specimens yielded false-
negative HIV reactions (Table 4). One of these specimens was
negative by all three RDTs, whereas specimen SF5 was negative by
the MedMira and SD RDTs but positive by the Chembio RDT
(Table 4). Faint HIV reactions were observed in 2 MedMira tests
(1.3%; both false positive), 3 SD tests (2%; all true positives), and
2 Chembio tests (1.3%; one false positive). Faint T. pallidum re-
actions were noted for 16 MedMira tests (10.7%; two false posi-
tives), 10 SD tests (6.7%; all true positives), and 6 ChemBio tests
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<table>
<thead>
<tr>
<th>Specimen</th>
<th>Reference method</th>
<th>T. pallidum component of RDT</th>
<th>HIV Western blot</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID</td>
<td>TP-PA</td>
<td>MedMira</td>
<td>SD</td>
</tr>
<tr>
<td>96</td>
<td>−</td>
<td>Faint +</td>
<td>−</td>
</tr>
<tr>
<td>16</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>40</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>SF23</td>
<td>+</td>
<td>−</td>
<td>Faint +</td>
</tr>
<tr>
<td>SF5</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>SF8</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

<sup>a</sup> Shaded boxes indicate discordant results.

<sup>b</sup> Faint results were considered positive.

<sup>c</sup> NR, not reactive.

TABLE 4 Characteristics of specimens with discordant HIV antibody results by one or more RDTs for the dual detection of HIV and syphilis antibodies

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Reference method</th>
<th>HIV component of RDT&lt;sup&gt;a&lt;/sup&gt;</th>
<th>HIV Western blot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP-PA</td>
<td>MedMira</td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>HIV EIA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>−</td>
<td>Faint +&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−</td>
</tr>
<tr>
<td>16</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>40</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>SF23</td>
<td>+</td>
<td>−</td>
<td>Faint +</td>
</tr>
<tr>
<td>SF5</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>SF8</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

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<sup>b</sup> Faint reactivity was considered positive.

<sup>c</sup> NR, not reactive.
in our study than what would be observed in the real world, as testing was performed in a controlled laboratory setting by a small number of highly skilled technicians. Future research should include field evaluations of dual HIV/syphilis rapid tests.

While RDTs are not intended to replace standard reference methods, the development of quality RDTs could have an enormous impact on public health initiatives, by providing earlier identification of patients infected with HIV and/or *T. pallidum.* The fact that syphilis is a cofactor in HIV transmission and HIV infection affects the clinical presentation of syphilis (9, 10), and coupled with the high rate of HIV and syphilis coinfection among MSM in the United States, it underscores the need for both HIV and syphilis testing at the point of care. Since no HIV/syphilis dual RDTs remain categorized as research use only in the United States, such testing requires use of two RDTs. The recent FDA clearance of the Syphilis Health Check RDT makes such testing now feasible. In regions of the United States where coinfection rates are high, or in areas where testing can be targeted to high-risk patient populations, the availability of such testing may not only improve detection of syphilis infection, in addition to HIV, but also prevent further transmission by immediate treatment (18).

In summary, our study is the first to evaluate the sensitivity and specificity of three commercial combination HIV and syphilis RDTs in parallel, and we demonstrated comparable performance to reference methods for all three RDTs. Further use of these RDTs in the clinical setting may more adequately determine their performance as point-of-care tests. However, until the manufacturers submit data to the FDA for clearance of these products, this testing will not be available in the United States.

ACKNOWLEDGMENTS

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REFERENCES


