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Laboratory Evaluation of a Point-of-Care Downward-Flow Assay for Simultaneous Detection of Antibodies to *Treponema pallidum* and Human Immunodeficiency Virus

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Combining the detection of syphilis and HIV antibodies into one point-of-care test integrates syphilis screening into already existing HIV screening programs, which may be particularly beneficial in settings such as antenatal care. Using the INSTI Multiplex downward-flow immunoassay, we tested 200 stored serum samples from high-risk patients enrolled in a longitudinal study on HIV infection and syphilis in Peruvian men who have sex with men and transgender women. This rapid assay detected HIV and *Treponema pallidum* serum antibodies with sensitivities of 100% (95% confidence interval [CI], 95.9% to 100%) and 87.4% (95% CI, 81.4% to 92.0%), respectively, and specificities of 95.5% (95% CI, 89.9% to 98.5%) and 97.0% (95% CI, 84.2% to 99.9%), respectively ($n = 200$). The sensitivity for syphilis antibody detection was higher in patients with a rapid plasma reagin titer of ≥1:8 (97.3%) than in those with a titer of ≤1:4 (90%) or a nonreactive titer (66.7%).

While syphilis, an infection caused by the spirochete *Treponema pallidum*, is a curable disease, it is estimated that there are 5.6 million new syphilis infections in adults annually (1) compared to approximately 2 million new HIV infections (2). Although new syphilis infections are more common, HIV screening has become a priority in low- and middle-income countries. Syphilis frequently presents atypically, which can make it difficult to clinically differentiate from other sexually transmitted infections; thus, effective diagnostic tests are crucial for correctly identifying and managing syphilis infections (3). Highly accurate diagnostic tests for HIV infection and syphilis exist, but they are often inaccessible to people living in areas with the highest burden of disease. Point-of-care tests provide an opportunity to reach those populations through tests that are inexpensive, easy to perform, and rapid, which enables same-day testing and treatment. Combining the detection of syphilis and HIV antibodies into one point-of-care test integrates syphilis screening into already existing HIV screening programs, which might be particularly beneficial in settings such as antenatal care (4).

The INSTI Multiplex downward-flow immunoassay (bioLytical Laboratories, Inc., Canada) is for the simultaneous detection of HIV and *T. pallidum* antibodies. Results can be read in less than 1 min. Previous studies of the INSTI HIV-1/HIV-2 antibody test found it to be highly sensitive, specific, and easy to use (5–11). The INSTI Multiplex assay was developed recently, and no published data on its accuracy, feasibility, or acceptability exist yet.

Using the INSTI Multiplex, we tested 200 stored (−80°C) serum samples from high-risk patients enrolled in a longitudinal study on HIV infection and syphilis in Peruvian men who have sex with men and transgender women (12). Genital lesions are a sign of primary syphilis, and 14 of the 200 serum samples were from patients who had primary syphilis, confirmed by *T. pallidum* DNA detection using PCR (12). The sera were tested for HIV and *T. pallidum* antibodies when the samples were first collected. The reference standard for HIV antibody detection was a 4th-generation enzyme immunoassay (EIA) (Genscreen ULTRA HIV Ag-Ab; Bio-Rad, France), followed by a confirmatory Western blot test (NEW LAV BLOT I; Bio-Rad, France) for those with a reactive EIA. The reference standard for *T. pallidum* antibody detection was a *T. pallidum* particle agglutination (TP-PA) titer of ≥1:80 (SERODIA-TPPA; Fujirebio Diagnostics, Japan). Sera were also tested using the rapid plasma reagin (RPR) test (BD Macro-Vue RPR; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) with serial 2-fold dilutions to determine the RPR titer. An RPR titer of ≥1:8 is indicative of a recent infection and a greater risk for active infection (13). The INSTI Multiplex was performed by trained laboratory personnel according to manufacturer instructions. We calculated the sensitivity and specificity for each infection, stratifying *T. pallidum* antibody results by RPR titer, and calculated 95% confidence intervals (CIs) using the binomial method.

For each of the 200 INSTI Multiplex assays, a purple control dot appeared, indicating a valid test. The sensitivity and specificity for the detection of HIV antibodies were 100% (95% CI, 95.9% to 100%) and 95.5% (95% CI, 89.9% to 98.5%), respectively. With TP-PA as the reference standard, the overall sensitivity and spec-

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TABLE 1 Sensitivities for the detection of *Treponema pallidum* antibodies stratified by RPR titer[a]

RPR titer (no. of samples)	Sensitivity (%) of INSTI Multiplex compared to a reactive TP-PA[b] test (95% CI)
Nonreactive (31)	66.7 (48.6–83.3)
1:1 (33)	87.9 (71.8–96.6)
1:2 (33)	97.0 (84.2–99.9)
1:4 (34)	85.3 (68.9–95.0)
≤1:4 (100)	90.0 (82.4–95.1)
≥1:8 (37)	97.3 (85.8–99.9)
All TP-PA reactive sera (168)	87.4 (81.4–92.0)

[a] A higher titer indicates a more recent infection. RPR, rapid plasma reagin.
[b] TP-PA, *Treponema pallidum* particle agglutination.

TABLE 2 Performance of TP-PA and INSTI Multiplex for the detection of *Treponema pallidum* antibodies in patients with primary syphilis

Sample	*T. pallidum* PCR result[a]	TP-PA result[b]	RPR titer[c]	INSTI Multiplex *T. pallidum* line result
A	Positive	Nonreactive	Nonreactive	Nonreactive
B	Positive	Nonreactive	Nonreactive	Nonreactive
C	Positive	Reactive	1:1	Reactive
D	Positive	Reactive	1:2	Reactive
E	Positive	Reactive	1:4	Nonreactive
F	Positive	Reactive	1:4	Reactive
G	Positive	Reactive	1:8	Reactive
H	Positive	Reactive	1:8	Reactive
I	Positive	Reactive	1:16	Reactive
J	Positive	Reactive	1:16	Reactive
K	Positive	Reactive	1:16	Reactive
L	Positive	Reactive	1:32	Reactive
M	Positive	Reactive	1:64	Reactive
N	Positive	Reactive	1:64	Reactive

[a] "Positive" indicates that *T. pallidum* DNA was detected in swabs from genital lesions.
[b] "Reactive" indicates the presence of antibodies to *T. pallidum*. TP-PA, *Treponema pallidum* particle agglutination.
[c] A higher titer indicates a more recent infection. RPR, rapid plasma reagin.

ificity for the detection of *T. pallidum* antibodies were 87.4% (95% CI, 81.4% to 92.0%) and 97.0% (95% CI, 84.2% to 99.9%), respectively. Table 1 lists the sensitivities for the detection of *T. pallidum* antibodies stratified by RPR titer.

Of 14 serum samples from patients with *T. pallidum* PCR-positive primary syphilitic lesions, 12 tested positive by TP-PA, yielding a sensitivity of the TP-PA for primary syphilis of 85.7% (95% CI, 57.2% to 98.2%). Eleven of the 14 primary syphilis samples tested positive for *T. pallidum* by the INSTI Multiplex, yielding a *T. pallidum* sensitivity of 78.6% (95% CI, 49.2% to 95.3%). Table 2 lists the performances of TP-PA and the INSTI Multiplex for the detection of *T. pallidum* antibodies in primary syphilis cases.

We found the INSTI Multiplex assay to be highly sensitive and specific for the detection of antibodies to HIV. However, the specificity of 95.5% indicates that confirmatory testing may be warranted for positive HIV results in the INSTI test. The assay was less sensitive for the detection of *T. pallidum* antibodies, but nearly one-third of the 200 samples had a nonreactive RPR titer, and less than one-fifth had an RPR titer of ≥1:8. While the INSTI Multiplex had lower sensitivity for samples with an RPR titer of ≤1:4, it was highly sensitive for the detection of *T. pallidum* antibodies among those with an RPR titer of ≥1:8. If the goal of point-of-care testing is to identify those cases of recent syphilis with an RPR titer of ≥1:8, the high sensitivity of the INSTI Multiplex for these specimens is encouraging. Although the INSTI Multiplex test had a lower sensitivity for the detection of *T. pallidum* antibodies in the primary stage of syphilis than it did for the later stages of syphilis, it had a sensitivity for primary syphilis similar to that of TP-PA. The inability to identify cases before syphilis antibodies are generated during primary syphilis is a limitation of all antibody-based tests and represents a missed opportunity to identify cases of primary syphilis. An additional limitation of treponemal antibody-based tests is that treponemal antibodies may persist for life, even after the infection has been treated. Follow-up testing would be ideal to avoid overtreatment, but the benefits of treatment at the point of care, especially in high-risk populations, may outweigh the potential risks of overtreatment. Therefore, rapid treponemal antibody-based tests such as the INSTI Multiplex remain useful tools at the point of care.

Previous studies evaluated dual tests for antibodies to HIV and *T. pallidum*, including the MedMira Multiplo (MedMira; Halifax, Nova Scotia, Canada), the SD BIOLINE HIV/Syphilis Duo test (Standard Diagnostics; Gyeonggi-do, Republic of Korea), and the Chembio DPP HIV-syphilis assay (Chembio Diagnostics; Medford, NY) (7, 14–23). These dual tests demonstrated sensitivities and specificities for HIV antibody detection ranging from 93.8% to 100% and 91.9% to 100%, respectively. With the exception of a low outlier of 46.4% sensitivity for the Chembio DPP HIV-syphilis assay in one study (18), these tests demonstrated sensitivities and specificities for *T. pallidum* antibody detection ranging from 81.0% to 99.7% and 92.8% to 100%, respectively. Our results are within those ranges. We found a sensitivity of *T. pallidum* antibody detection for specimens with an RPR titer of ≥1:8 of 97.3%, which is in the high end of that range. Conversely, the authors who found the Chembio DPP's sensitivity for detecting *T. pallidum* antibodies to be 46.7% noted that only 11% of their samples were "high titer" (18). Therefore, the sensitivity of tests for *T. pallidum* antibody detection seems to be associated with the frequency of high-titer RPRs in the sample population.

Although previous studies of dual tests have not looked at primary versus later stages of syphilis, one study of four point-of-care antibody tests for *T. pallidum* alone did (24). It found a lower sensitivity for detecting *T. pallidum* antibodies in primary syphilis specimens (80.4% to 90.2%) than for detecting later stages of syphilis (94.3% to 98.6%). The sensitivity of 78.6% that we found for the INSTI Multiplex is similar to the sensitivities of those syphilis point-of-care tests in specimens from patients with primary syphilis.

Given that this is the first independent evaluation of the INSTI Multiplex, further research should be done to assess the assay's accuracy in other settings, as well as its feasibility and acceptability in clinical and field settings. Ours was a laboratory evaluation using frozen sera which had been stored and tested before, and it is possible that thawing and refreezing these samples diminished the likelihood of detecting antibodies to HIV or syphilis. The study should be repeated using whole-blood finger-prick specimens, as the assay would be used in the field. The small number of samples from patients with primary syphilis may have affected our ability to draw a strong conclusion on the performance of the test among

those with primary syphilis. Further research with a larger sample is needed to demonstrate the accuracy of the INSTI Multiplex in cases of primary syphilis.

In conclusion, the INSTI Multiplex demonstrated very good performance in the detection of antibodies to HIV and syphilis. The sensitivity was higher in syphilis patients with a higher RPR titer. The sensitivity of the INSTI Multiplex for detecting antibodies to *T. pallidum* in cases of primary syphilis was similar to that of TP-PA, the standard laboratory treponemal antibody-based test. Our results show the INSTI Multiplex to be a promising option for implementation of dual testing of HIV and syphilis at the point of care.

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