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ORIGINAL ARTICLE

Field evaluation of a dual rapid diagnostic test for HIV infection and syphilis in Lima, Peru

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Mention of any meeting(s) where the information has previously been presented: preliminary results were shared in a poster presentation at the CDC Prevention Conference 2014 in Atlanta, Georgia, and will be shared at AIDS 2014 in Melbourne, Australia.

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ABSTRACT

Objectives Screening for HIV and syphilis in key populations is recommended by the WHO to reduce the morbidity, mortality and transmission associated with undiagnosed and untreated infections. Rapid point-of-care tests that can detect multiple infections with a single fingerprick whole blood specimen using a single device are gaining popularity. We evaluated the field performance of a rapid dual HIV and syphilis test in people at high risk of HIV and syphilis infections.

Methods Participants included men who have sex with men and transgender women recruited in Lima, Peru. Reference standard testing for detection of HIV and syphilis infections, conducted using blood samples from venipuncture, included *Treponema pallidum* particle agglutination and fourth-generation HIV enzyme immunoassay for which positive results had a confirmation HIV Western blot test. For the evaluation test, SD BIOLINE HIV/Syphilis Duo test (Standard Diagnostics, Korea), a fingerprick blood specimen was used. Sensitivity and specificity were calculated and the exact binomial method was used to determine 95% CIs.

Results A total of 415 participants were recruited for the study. The dual test sensitivity for detection of *T. pallidum* infection was 89.2% (95% CI 83.5% to 93.5%) and specificity 98.8% (95% CI 96.5% to 99.8%). For detection of HIV infection, the sensitivity of the dual test was 99.1% (95% CI 94.8% to 100%) and specificity 99.4% (95% CI 97.7% to 99.9%).

Conclusions This high performing dual test should be considered for the use in clinical settings to increase uptake of simultaneous testing of HIV and syphilis and accelerate time to treatment for those who need it.

BACKGROUND

Screening for HIV and syphilis among groups at high risk of infection as well as pregnant women is highly recommended for by the WHO to reduce the morbidity and mortality associated with undiagnosed and untreated infections. In Peru and Latin America, men who have sex with men (MSM) and transgender women represent the groups at highest risk of new HIV infections.[1] Recent estimates suggest that 12.4% of MSM in Peru are living with HIV infection, compared with just 0.4% in Peru's adult population as a whole.[1] Syphilis infection has also been found to be associated with HIV infection in those populations.[2 3] In coinfected patients, syphilis can increase transmission of HIV by increasing viral shedding and viral load.[4]

Rapid point-of-care tests for syphilis should be used to accelerate worldwide syphilis screening and subsequent treatment.[5] Several immunochromatographic syphilis tests for detection of *Treponema pallidum* are in use around the world.[5] Recently, test developers have created rapid point-of-care tests that can detect multiple infections with a single specimen using a single device.[6–9] The use of those dual rapid tests for HIV and syphilis as screening tools in sexual health clinics could help prevent HIV and syphilis transmission. The SD BIOLINE HIV/Syphilis Duo test (Standard Diagnostics, Korea) is a lateral flow immunochromatographic assay. Laboratory evaluations of this test have shown high performance;[10] however, field studies using whole blood fingerprick specimens are essential to understand how the test will perform in real-world settings. The aim of this study was to evaluate the field performance of the dual test.

METHODS

Study population/study sites

Participants were recruited between July 2013 and March 2014 at two clinical sites, the Alberto Barton Clinic, a public sexually transmitted infection health centre located in Callao, the main port of Peru and their regular attendees consist of MSM and transgender women; and the Epicentro Clinic, a gay men's community health centre in Southern Lima that targets health services to MSM and transgender women. Consecutive MSM and transgender women that presented to one of the two clinics, consented to participate, and were 18 years of age or older were included in the study sample. Some participants were aware of their HIV and/or syphilis serostatus and some were unknown. Reference tests for comparison to the dual test results were conducted at the Laboratory of Sexual Health at the Faculty of Sciences of Universidad Peruana Cayetano Heredia.

Comparison tests/reference standard tests

The venipuncture blood specimens were transported to the reference laboratory for serum separation and comparison testing. The reference test for comparison to the HIV component of the dual rapid test was the 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 (HIV-2) in human serum or

plasma. A confirmation Western blot test was conducted (NEW LAV BLOT I, Bio-Rad, France) for all specimens that were positive on the enzyme immunoassay, as is done routinely in this setting, and those that were positive on both the enzyme immunoassay and the Western blot were considered HIV infected. For the *T. pallidum* antibody comparison, *T. pallidum* particle agglutination (SERODIA-TPPA, Fujirebio Diagnostics, Japan) was used qualitatively. Rapid plasma reagin (RPR) (BD Macro-Vue RPR, Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) results were also available for all participants to assist with clinical diagnosis. RPR titre levels were determined using serial dilutions.

Test under evaluation

Participants underwent a fingerstick blood specimen collection and a venipuncture blood specimen collection. The fingerstick specimen was used on site to conduct the evaluation dual test. The participant's finger was pricked with a lancet, a capillary pipette was used to collect one drop of blood (20 μL), the drop of blood was added into the test 'sample well' followed by three drops of assay diluent solution. The test was performed and read according to manufacturer's instructions. The SD BIOLINE HIV/Syphilis Duo dual test (Standard Diagnostics) is a qualitative detection method using a solid phase immunochromatographic assay. The recombinant HIV-1/2 antigen, recombinant *T. pallidum* antigens, colloid gold conjugate, the specimen sample and sample diluents move along the membrane chromatographically to the test region and form a visible line as the antigen–antibody–antigen gold particle complex forms. The test qualitatively detects antibodies to all isotypes (IgG, IgM, IgA) specific to HIV-1 including subtype-O, HIV-2 and specific IgM and IgG antibodies to recombinant *T. pallidum* antigen (TpN17) in human whole blood. There is a built-in control coloured band mechanism in the test. The control band should always appear if the test procedure is performed properly and the test assay diluent has been applied successfully.

After the dual tests were each inoculated with the specimen, they were visually read by the site trained clinic laboratory personnel in a private space out of view of the study participant and clinical health professionals. A reference standard was used to determine the visual intensity of the colour of the bands (figure 1). The test was read by two readers separately (not checked for blinding) after 20 min, per the manufacturer's instructions, and again at 60 min to determine if the test result remained consistent. Participants received their HIV and syphilis results based on the clinic regular testing protocols, 2 weeks after recruitment. Results from the dual test were not reported to participants and were not used for clinical management.

Data analysis

Sensitivity and specificity were calculated and the exact binomial method was used to determine 95% CIs. We also analysed the results by RPR titre, ≤1:4 and >1:4 because a titre of >1:4 is likely to be a recent infection. Cohen's κ statistic was calculated to determine the concordance between the reference test and the test under evaluation, the two test readers' result interpretation at 20 min, as well as the concordance between the readings at 20 and 60 min. All analyses were performed using SAS V.9.3 (SAS Institute, Cary, North Carolina, USA).

Ethics

Ethical approval for this study was granted by the Ethics Committee at Universidad Peruana Cayetano Heredia with approval ID 61522. Written informed consent was obtained from all participants.

RESULTS

The participants included in this evaluation were 415 MSM and transgender women. All of the participants had reference standard and evaluation tests for HIV and 413 had reference standard and evaluation testing conducted for the presence of antibodies to *T. pallidum*. Of the participants, 105 (25.3%) were HIV infected and 167 (40.2%) had evidence of antibodies to *T. pallidum*, of which, 143 (85.6%) had reactive RPR tests and 53 (31.7%) had RPR titres >1:4. Of the participants that had reference testing completed for both HIV and *T. pallidum*, 64 (15.5%) had positive results for both HIV infection and antibodies to *T. pallidum*.

The quality control colour band was present for all (100%) participants on the dual test. For the HIV component of the test, there were two false positive results and one false negative result (tables 1 and 2). The sensitivity for the HIV component of the dual HIV/syphilis test was 99.1% (95% CIs 94.8% to 100%). The specificity of the HIV component was 99.4% (95% CI 97.7% to 99.9%). For the *T. pallidum* component of the test, there were three false positive results and 18 false negative results. The sensitivity and specificity for the *T. pallidum* component of the test were 89.2% (95% CI 83.5% to 93.5%) and 98.8% (95% CI 96.5% to 99.8%), respectively. Among those with higher RPR titres (treponemal antibody positive and RPR>1:4), the sensitivity for the *T. pallidum* component of the test was 94.3% (95% CI 84.3% to 98.8%).

The intensity of the colour bands indicating a positive test result on the dual HIV/syphilis test were recorded by the study readers using a reference standard (table 3). The median value for the positive HIV colour band was 64% (IQR: 65) and the median value for the positive *T. pallidum* colour band was 8% (IQR: 23).

Of the 408 specimens that were read separately by two lab technicians, the κ coefficient was 0.99 (95% CI 0.98 to 1.0) for the HIV component of the test, and for the *T. pallidum* component of the test the κ coefficient was 1.0 (95% CI 1.0 to 1.0). At 60 min after inoculation of the tests, the result was read

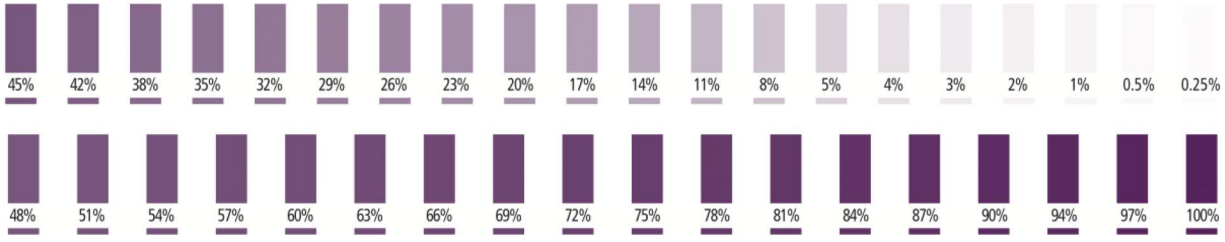

Figure 1 Standard for band intensity for SD BIOLINE HIV/Syphilis Duo test. The colour bands on this reference standard were compared with positive results on the dual test and the per cent intensity was recorded by two trained laboratory staff.

Table 1 Field performance for detection of HIV antibodies using a dual HIV/syphilis test

	Number of samples		Total	Sensitivity (95% CI)	Specificity (95% CI)	κ Coefficient* (95% CI)
HIV component	Ref test +	Ref test −		99.1%	99.4%	0.89
Dual test +	104	2	106	(94.8% to 100%)	(97.7% to 99.9%)	(0.85 to 0.94)
Dual test −	1	308	309			
Total	105	310	415			

*Cohen's κ statistic was calculated to determine the concordance between the reference test and the test under evaluation.

again by the first lab technician at which time some of the results became reactive; four of the HIV results and five of the *T. pallidum* results became positive in the 40 min between the two reading times. Of those that became reactive after 60 min, three of those that had given a negative result for *T. pallidum* at 20 min and gave a positive result were TPPA positive. All of the HIV results that became reactive to positive were negative on the reference tests. The κ coefficient between the reading at 20 min and the reading at 60 min for the HIV and *T. pallidum* components were 0.98 (95% CI 0.96 to 1.0) and 0.97 (95% CI 0.95 to 1.0), respectively.

DISCUSSION

In this study, we compared the field performance of a dual test to reference standard tests. The HIV and syphilis components of the dual test were evaluated separately. The HIV component and the *T. pallidum* component of the dual test had similarly very high specificities, while the sensitivity for the HIV component was higher than the sensitivity for the *T. pallidum* component. A recent multisite laboratory evaluation of this dual test also documented high performance.[10] In addition, the concordance was very high between results interpreted by two laboratory technicians.

The manufacturer stipulates that the test should be read at 20 min after inoculation, but we evaluated the results also after 60 min to detect whether they were the same or had changed since the first reading at 20 min. Most results remained consistent, but some became positive over time. Of the results for the *T. pallidum* colour band, some became positive over the 60 min that were also TPPA positive, which suggests that the band indicating a positive result of the evaluation test was visually undetectable at first became detectable. We included a visual intensity recording of the colour bands that indicate positive results as part of the data analysis. The colour band intensity tended to be darker for detection of antibodies to HIV than for detection of antibodies to *T. pallidum*; the lighter bands may contribute to the lower sensitivity for the *T. pallidum* component of the test.

Though multiple studies have evaluated the performance of various rapid tests, those devices detect either HIV or syphilis infection but not both simultaneously.[5 11–17] This field evaluation show comparable results to other field studies for single rapid tests in the current published literature.[5 11–17] Laboratory studies show better performance than field studies because they allow for higher levels of control over experimental variables.[10] However, a field study such as ours has the benefit of real-world conditions and, therefore, more adequately demonstrates the dual test's practical value as a screening tool.

Because HIV and syphilis share common risk factors and have comparable modes of transmission, the prevention and treatment of these infections can be addressed with similar strategies. In addition, syphilis infection has been found to be associated with HIV in high-risk groups.[2 3] Worldwide 1.4 million pregnant women are syphilis infected and 80% of those will have adverse pregnancy outcomes as a result.[18] By integrating syphilis into HIV screening programmes we can increase uptake of syphilis testing, reduce the prevalence of syphilis infections, and save the lives of babies around the world. This dual test is particularly timely given the WHO and UNAIDS recommendations for a dual strategy for the prevention of HIV and syphilis as well as the WHO policy on sexually transmitted infections testing for key populations.[19–21] Therefore, combining the delivery and implementation of HIV and syphilis services, beginning with dual rapid tests for screening, could be useful for improving testing and treatment in clinical settings.

Although this study focused on the SD BIOLINE HIV/Syphilis Duo test, other dual rapid tests that allow for the simultaneous diagnosis of HIV-1/2 and syphilis have been developed, including the Chembio DPP HIV–Syphilis assay and the MedMira multiplo rapid TP/HIV antibody test.[6 8 9]

There are limitations to consider. Because the dual test is qualitative, it is possible that our readers interpreted some lines as positive that others could have called negative and vice versa. The colour bands indicating a positive result tended to be lighter for detection of antibodies to *T. pallidum* when compared with HIV, an automated reader may have utility to improve the sensitivity using high optical resolution that can detect the presence of a colour band beyond what the human eye is capable of discerning.[22] A limitation of treponemal rapid tests for syphilis is that treponemal antibodies can persist for life even following curative treatment. In some settings additional testing may be warranted. In contrast, in some settings the benefits of early treatment may outweigh the low risks and costs of unnecessary treatment.

Table 2 Field performance for detection of *Treponema pallidum* antibodies using a dual HIV/syphilis test

	Number of samples		Total	Sensitivity (95% CI)	Specificity (95% CI)	κ Coefficient* (95% CI)
T. pallidum component	Ref test +	Ref test −		89.2%	98.8%	0.98
Dual test +	149	3	153	(83.5% to 93.5%)	(96.5% to 99.8%)	(0.96 to 1.0)
Dual test −	18	243	261			
Total	167	246	413			

*Cohen's κ statistic was calculated to determine the concordance between the reference test and the test under evaluation.

Table 3 Visual intensity of the colour of the bands indicating positive results for the dual test HIV/syphilis test band intensity

	N	Median %	IQR	Minimum %	Maximum %
HIV band colour intensity	106	64	65	0.3	100
Treponema pallidum band colour intensity	150*	8	23	0.3	97

*Intensity was not recorded by clinical staff for three of the tests that were syphilis positive on the dual test.

In conclusion, our results show good clinical performance of a dual test for HIV and syphilis and provide support for the implementation of this and potentially other dual tests for screening in clinical settings. Following the WHOs recommendations for HIV and syphilis screening to reduce the morbidity and mortality associated with untreated infections, it may be possible to include dual rapid tests for HIV and syphilis as part of screening strategies targeting key populations.

Key messages

- The SD BIOLINE HIV/Syphilis Duo test is a rapid test for the simultaneous detection of HIV and syphilis infection.
- The HIV component and the *Treponema pallidum* component of the dual test had similarly very high specificities (around 99%).
- The sensitivity for the HIV component (99%) was higher than the sensitivity for the *T. pallidum* component (89%).
- This rapid test can be used in any setting for screening of HIV infection and syphilis.

Handling editor Jackie A Cassell

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Contributors CCB performed the data analysis, wrote the manuscript and provided support with study implementation. SRL provided oversight for the study and laboratory work. SRL also provided critical review of the manuscript. EH performed the literature review and assisted with manuscript preparation. BJB provided analytic guidance and critical review. LBR coordinated the study and data management. SKV and JAF conducted the laboratory work and assisted with data management. CFC and JDK conceived of the study and provided oversight. All authors read, revised and approved the final manuscript. CCB assisted with training of study staff, assisted with study implementation, performed the data analysis and wrote the manuscript. SRL provided study management and training of study staff. EH assisted with manuscript writing and data analysis. BJB provided review and revisions to the manuscript. LBR was the study coordinator and data manager. SKV and JAF conducted laboratory testing and assisted with study coordination. CFC is the study PI and provided study oversight. JDK is the co-PI and also provided study oversight and provided feedback and revision to the manuscript.

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Competing interests None declared.

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