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Field Evaluation of a Dual Rapid Diagnostic Test for HIV and Syphilis in Lima, Peru

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Abstract

Objectives—Screening for HIV and syphilis in key populations is recommended by the World Health Organization 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 persons at high risk of HIV and syphilis infections.

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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.

Author roles: 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

Contributor statement: 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.

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 4th-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% confidence intervals (CI).

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%,93.5%) and specificity 98.8% (95%CI: 96.5%,99.8%). For detection of HIV infection, the sensitivity of the dual test was 99.1% (95%CI: 94.8%,100.0%) and specificity 99.4% (95%CI: 97.7%,99.9%).

Conclusion—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.

MESH Key words

Point of Care Technology; HIV; Syphilis; Diagnostic Tests; Global Health; Peru

BACKGROUND

Screening for human immunodeficiency virus (HIV) and syphilis amongst groups at high risk of infection as well as pregnant women is highly recommended for by the World Health Organization 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 percent of MSM in Peru are living with HIV infection, compared to just 0.4 percent in Peru's adult population as a whole.[1] Syphilis infection has also been found to be associated with HIV infection in those populations.[23] In co-infected 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 *T. 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 center 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 center 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 4th-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 *Treponema pallidum* antibody comparison, *Treponema Pallidum* Particle Agglutination (SERODIA-TPPA, Fujirebio Diagnostics, Inc., Japan) was used qualitatively. Rapid plasma reagin (RPR) (BD Macro-Vue™ RPR, Becton, Dickinson and Co., Franklin Lakes, NJ) results were also available for all participants to assist with clinical diagnosis. RPR titer 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 (20μL), the drop of blood was added into the test 'sample well' followed by 3 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, Korea) is a qualitative detection method using a solid phase immunochromatographic assay. The recombinant HIV-1/2 antigen, recombinant *Treponema 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 *Treponema pallidum* antigen (TpN17) in human whole blood. There is a built-in control colored 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 clinician. A reference standard was used to determine the visual intensity of the color of the bands [Figure 1]. The test was read by two readers separately after 20 minutes, per the manufacturer's instructions, and again at 60 minutes to determine if the test result remained consistent. Participants received their HIV and syphilis results based on the clinic regular testing protocols, two 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% confidence intervals (CI). We also analyzed the results by RPR titer, ≤1:4 and >1:4 because a titer of more than 1:4 is likely to be a recent infection. Cohen's kappa statistic was calculated to determine the concordance between the reference test and the test under evaluation, the two test readers' result interpretation at 20 minutes, as well as the concordance between the reading at 20 minutes with the reading at 60 minutes. All analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC).

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 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 titers >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 color 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 [Table 1 and 2]. The sensitivity for the HIV component of the dual HIV/syphilis test was 99.1% (95% Confidence interval (CI): 94.8%, 100.0%). The specificity of the HIV component was 99.4% (95% CI: 97.7%, 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%, 93.5%) and 98.8% (95% CI: 96.5%, 99.8%), respectively. Among those with higher RPR titers (treponemal antibody positive and RPR>1:4), the sensitivity for the *Treponema pallidum* component of the test was 94.3% (95% CI: 84.3%, 98.8%).

The intensity of the color 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 color band was 64% (Interquartile range (IQR): 65) and the median value for the positive *T. pallidum* color band was 8% (IQR: 23).

Of the 408 specimens that were read separately by two lab technicians, the Kappa coefficient was .99 (95% CI: .98, 1.00) for the HIV component of the test, and for the *T. pallidum* component of the test the Kappa coefficient was 1.00 (95% CI: 1.00, 1.00). At 60 minutes after inoculation of the tests, the result was read 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 minutes between the two reading times. Of those that became reactive after 60 minutes, 3 of those that had given a negative result for *T. pallidum* at 20 minutes 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 Kappa coefficient between the reading at 20 minutes and the reading at 60 minutes for the HIV and *T. pallidum* components were .98 (95% CI: .96, 1.0) and .97 (95% CI: .95, 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 multi-site 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 minutes after inoculation but we evaluated the results also after 60 minutes to detect whether they were the same or had changed since the first reading at 20 minutes. Most results remained consistent, but some became positive over time. Of the results for the *T. pallidum* color band, some became positive over the 60 minutes 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 color bands that indicate positive results as part of the data analysis. The color 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.[511–17] This field evaluation show comparable results to other field studies for single rapid tests in the current published literature.[511–17] Laboratory studies show better performance than field studies because they allow for higher levels of control over experimental variables.[18] 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.[23] In addition, worldwide 1.4 million pregnant women are syphilis infected and 80% of those will have adverse pregnancy outcomes as a result.[19] By integrating syphilis into HIV screening programs 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.[20–22] 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.[689]

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 color bands indicating a positive result tended to be lighter for detection of antibodies to *T. pallidum* when compared to HIV, an automated reader may have utility to improve the sensitivity using high optical resolution that can detect presence of a color band beyond what the human eye is capable of discerning.[23] A limitation of treponemal rapid tests for syphilis is that treponemal antibodies can persist for life even following curative treatment. In some settings confirmatory testing may be warranted. In contrast, in some settings the benefits of early treatment may outweigh the risks of unnecessary treatment.

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 World Health Organization's 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.

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References

1. UNAIDS. UNAIDS report on the global AIDS epidemic 2013. 2013. http://wwwunaidsorg/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_enpdf
2. Sanchez J, Lama JR, Peinado J, et al. High HIV and ulcerative sexually transmitted infection incidence estimates among men who have sex with men in Peru: awaiting for an effective preventive

intervention. Journal of acquired immune deficiency syndromes. 2009; 51(Suppl 1):S47–51. [PubMed: 19384102]

3. Sanchez J, Lama JR, Kusunoki L, et al. HIV-1, sexually transmitted infections, and sexual behavior trends among men who have sex with men in Lima, Peru. Journal of acquired immune deficiency syndromes. 2007; 44(5):578–85. [PubMed: 17279049]
4. Quinn TC, Wawer MJ, Sewankambo N, et al. Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group The New England journal of medicine. 2000; 342(13):921–9.
5. Tucker JD, Bu J, Brown LB, et al. Accelerating worldwide syphilis screening through rapid testing: a systematic review. The Lancet infectious diseases. 2010; 10(6):381–6. [PubMed: 20510278]
6. Inc. CDS. Chembio DPP® HIV-Syphilis assay. 2013. http://chembiocom/wp-content/uploads/2013/10/MS-12-021-HIV-Syphilis-Sell-Sheet-Rev-3pdf
7. Inc SD. SD BIOLINE HIV/Syphilis Duo. 2013. https://ensurinvmedcom/ensur/broker/ensurbrokeraspx?code=LEF063EN&cs=;27202615
8. Inc M. MedMira multiplo rapid syphilis/HIV antibody test. 2013. http://wwwmedmiracom/images/uploads/MPSIABPM0001EN_Rev_1_1pdf
9. Esfandiari, J. Performance Evaluation of the Chembio DPP® HIV-Syphilis Assay: A Novel. Point-of-care Rapid HIV ½ and Syphilis Abstract presented at: 2012 HIV Diagnostics Conference 2012; 12 December 2012; Atlanta, GA. https://custom.cvent.com/ADE0EB81B3184D618E2FB8340F1EC28E/files/29f3717707a44f91859f65feb4cefec6.pdf
10. Bristow CC, Adu-Sarkodie Y, Ondondo RO, et al. Multisite Laboratory Evaluation of a Dual Human Immunodeficiency Virus (HIV)/Syphilis Point-of-Care Rapid Test for Simultaneous Detection of HIV and Syphilis Infection. Open Forum Infect Dis. 2014; 1(1) ofu015.
11. Benzaken AS, Sabido M, Galban E, et al. Field performance of a rapid point-of-care diagnostic test for antenatal syphilis screening in the Amazon region, Brazil. International journal of STD & AIDS. 2011; 22(1):15–8. [PubMed: 21364061]
12. Bergman J, Gratrix J, Plitt S, et al. Feasibility and Field Performance of a Simultaneous Syphilis and HIV Point-of-Care Test Based Screening Strategy in at Risk Populations in Edmonton, Canada. AIDS research and treatment. 2013; 2013:819593. [PubMed: 24527210]
13. Kagulire SC, Opendi P, Stamper PD, et al. Field evaluation of five rapid diagnostic tests for screening of HIV-1 infections in rural Rakai, Uganda. International journal of STD & AIDS. 2011; 22(6):308–9. [PubMed: 21680664]
14. Montoya PJ, Lukehart SA, Brentlinger PE, et al. Comparison of the diagnostic accuracy of a rapid immunochromatographic test and the rapid plasma reagin test for antenatal syphilis screening in Mozambique. Bulletin of the World Health Organization. 2006; 84(2):97–104. [PubMed: 16501726]
15. Nessa K, Alam A, Chawdhury FA, et al. Field evaluation of simple rapid tests in the diagnosis of syphilis. International journal of STD & AIDS. 2008; 19(5):316–20. [PubMed: 18482961]
16. Tucker JD, Bien CH, Peeling RW. Point-of-care testing for sexually transmitted infections: recent advances and implications for disease control. Current opinion in infectious diseases. 2013; 26(1):73–9. [PubMed: 23242343]
17. West B, Walraven G, Morison L, et al. Performance of the rapid plasma reagin and the rapid syphilis screening tests in the diagnosis of syphilis in field conditions in rural Africa. Sexually transmitted infections. 2002; 78(4):282–5. [PubMed: 12181468]
18. Bristow CCA-S, Y. Ondondo RO, Bukusi EA, Anoumou Dagnra C, Oo KY, Htoo Pe EH, Khamsay C, Thi Houng L, Vázquez Campuzano R, Estes J, Klausner JD. Multi-site laboratory evaluation of a dual HIV/syphilis point-of-care rapid test for simultaneous detection of HIV and syphilis. Open Forum Infectious Diseases. 2014
19. Newman L, Kamb M, Hawkes S, et al. Global estimates of syphilis in pregnancy and associated adverse outcomes: analysis of multinational antenatal surveillance data. PLoS medicine. 2013; 10(2):e1001396. [PubMed: 23468598]
20. UNICEF East Asia and Pacific Regional Office. Elimination of new paediatric HIV infections and congenital syphilis in Asia-Pacific, 2011–2015. 2011. http://wwwuniceforg/eapro/PPTCT_CF_and_ME_guide_17Aug11pdf

21. WHO. Prevention and treatment of HIV and other sexually transmitted infections among men who have sex with men and transgender people Recommendations for a public health approach 2011. Available at: http://wwwwhoint/hiv/pub/guidelines/msm_guidelines2011/en/2011

22. WHO. Elimination of mother-to-child transmission (EMTCT) of HIV and syphilis: Global guidance on criteria and processes for validation. Available at: http://wwwwhoint/reproductivehealth/publications/rtis/9789241505888/en/2014

23. Mudanyali O, Dimitrov S, Sikora U, et al. Integrated rapid-diagnostic-test reader platform on a cellphone. Lab on a chip. 2012; 12(15):2678–86. [PubMed: 22596243]

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 *T. 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.

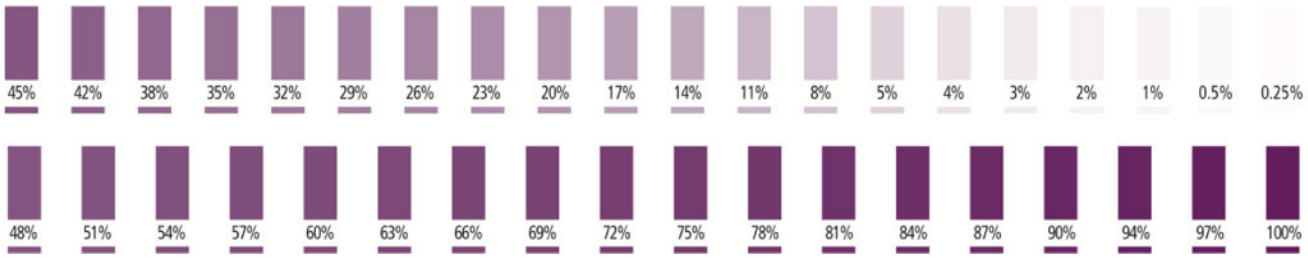

Figure 1. Standard for band intensity for SD BIOLINE HIV/Syphilis Duo test

*The color bands on this reference standard were compared to positive results on the dual test and the percent 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*)	Kappa Coefficient** (95% CI*)
HIV Component	Ref test +	Ref test −		99.1%	99.4%	.89
Dual Test +	104	2	106	(94.8%, 100%)	(97.7%, 99.9%)	(.85, .94)
Dual Test −	1	308	309			
total	105	310	415			

*CI= Confidence interval

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

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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*)	Kappa Coefficient** (95% CI*)
T. pallidum Component	Ref test +	Ref test −		89.2%	98.8%	.98
Dual Test +	149	3	153	(83.5%, 93.5%)	(96.5%, 99.8%)	(.96, 1.00)
Dual Test −	18	243	261			
total	167	246	413			

*CI = Confidence interval

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

Table 3

Visual intensity of the color of the bands indicating positive results for the dual test HIV/syphilis test band intensity

	N	Median %	Interquartile Range	Minimum %	Maximum %
HIV band color intensity	106	64	65	0.3	100
T. pallidum band color intensity	150*	8	23	0.3	97

*Note: Intensity was not recorded by clinical staff for 3 of the tests that were syphilis positive on the dual test.