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

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Abstract

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 atypical presentations 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 were infected with human immunodeficiency virus (HIV).(3) HIV and syphilis infection screening should be offered to every pregnant women to prevent adverse outcomes of pregnancy that include still birth, 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 co-infected patients, syphilis can increase transmission of HIV by increasing viral shedding at the site of genital ulcers(11, 12) and increases HIV viral load(13, 14).

Syphilis infection is generally diagnosed using two serologic laboratory-based tests; however, over the past 10 years, the advent of syphilis rapid tests have 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) These 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 STD clinics in Lima, Peru were used for the evaluation. The specimen had been collected and serum separation was conducted in the field. The sera were then transported on ice to the laboratory, in less than 4 hours. Serum was frozen and stored at -30°C the same day of collection. The stored specimens were thawed and tested for HIV infection using 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. Each positive enzyme immunoassay test was confirmed using a Western Blot test (NEW LAV BLOT I, Bio-Rad, France). Those that were positive on both the enzyme immunoassay and the Western Blot were considered HIV positive. Additionally, if enzyme immunoassay was positive and the Western Blot was indeterminate then Western blot was performed at a later date on a follow-up specimen and if positive the specimen was considered HIV positive. For the *Treponema pallidum* antibody comparison, *Treponema Pallidum* Particle Agglutination (SERODIA-TPPA, Fujirebio Diagnostics, Inc., Japan) was used. Rapid plasma reagin (RPR) tests (BD Macro-Vue RPR, Beckon-Dickenson, 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, 47 kDa) and synthetic HIV peptides to gp36, gp41, gp120

and HIV-1 group O. 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 manufacturer instructions by trained laboratory personnel. First, 3 drops of a Universal Buffer then 1 drop of serum specimen was applied to the center of the test cartridge which contains the test membrane. An InstaGold cap was placed onto the test cartridge and an additional 12 drops of the Universal Buffer was 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 Universal Buffer 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 positive result and a *T. pallidum* dot indicated that the specimen was positive for *T. pallidum* antibody.

We estimated sensitivity and specificity and calculated the 95% confidence interval (CI) using the exact binomial method. We determined the concordance between the test under evaluation and the reference tests using Cohen's Kappa statistic. All analyses were conducted using SAS v9.3 (Cary, NC, USA).

A total of 200 serum specimens were tested using the Multiplo rapid TP/HIV antibody test. Of the 200 tests, 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 Mulitplo test gave 74 true positive results, 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%, 100%) and the specificity estimate was 91.9% (95% CI: 85.7%, 96.1%). The Kappa coefficient for correlation between reference HIV test results and the Multiplo HIV test result was .90 (95% CI: .83, .96).

For the *T. pallidum* antibody component, of the 193 valid test results, the test produced 104 true positive results, 6 false positive, 6 false negative, and 77 true negative *T. pallidum* results [Table 2]. Of the 6 *T. pallidum* false negative Multiplo *T. pallidum* test results, all 6 had reactive RPR tests, with RPR titers of four 1:1, one 1:2, and one 1:4. In addition, all 6 false positive *T. pallidum* Multiplo test results were RPR non-reactive. The sensitivity and specificity estimates were 94.6% (95% CI: 88.5%, 98.0%) and 92.8% (95% CI: 84.9%, 97.3%), respectively. The Kappa coefficient for the *T. pallidum* component was .87 (95% CI: .80, .94).

We evaluated a dual rapid immunodiagnostic test for HIV and syphilis infection in a laboratory setting in Lima, Peru using characterized serum specimens. Our evaluation showed high performance of this dual rapid test. Other dual tests for HIV and syphilis are also being

manufactured and show great promise which show sensitivity estimates ranging from 97% to 100% and specificity estimates ranging from 99% to 100%. (19, 21) 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 2 tests didn't 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 test are developed for use with real-time clinical specimens, 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.

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Table 1. Laboratory performance for detection of HIV antibodies using a dual HIV/syphilis rapid immunodiagnostic test (N=200).

		Number of samples		Total	Sensitivity (95% CI)	Specificity (95% CI)	Kappa Coefficient (95% CI)
		Ref test +	Ref test -		100%	91.9%	.90
	Multiplo Test +	74	10	84	(95.1%, 100%)	(85.7%, 96.1%)	(.83, .96)
	Multiplo Test -	0	114	114			
Total		74	124	198			

2 tests gave invalid result (no control line).

Table 2. Laboratory performance for detection of *Treponema pallidum* antibodies using a dual HIV/syphilis rapid immunodiagnostic test (N=200).

		Number of samples		Total	Sensitivity (95% CI)	Specificity (95% CI)	Kappa Coefficient (95% CI)
T. pallidum Component		Ref test +	Ref test -		94.6%	92.8%	.87
	Multiplo Test +	104	6	110	(88.5%, 98.0%)	(84.9%, 97.3%)	(.80, .94)
	Multiplo Test -	6	77	83			
Total		110	83	193			

2 tests gave invalid result (no control line).

5 tests gave invalid results for the *Treponema pallidum* component

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