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Dual rapid test for HIV and syphilis: A laboratory evaluation of the diagnostic accuracy of the Standard Q HIV/Syphilis Combo Test☆

Claire C. Bristow [a,*], Silver Keith Vargas Rivera [b], Lourdes B. Ramos Cordova [b], Luz Jazmín Qquellon Palacios [b], Kelika A. Konda [c], Jeffrey D. Klausner [c,d]

[a] Division of Infectious Diseases and Global Public Health, Department of Medicine, University of California San Diego, La Jolla, CA, USA
[b] Universidad Peruana Cayetano Heredia, Lima, Peru
[c] Division of Infectious Diseases, Department of Medicine, University of California Los Angeles, Los Angeles, CA, USA
[d] Department of Epidemiology, Fielding School of Public Health, University of California Los Angeles, Los Angeles, CA, USA

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ABSTRACT

We evaluated the Standard Q HIV/Syphilis Combo Test (SD Biosensor, South Korea), a dual rapid test using stored sera (N = 400) in a laboratory setting in Lima, Peru. The sensitivity and specificity for HIV antibody detection was 100.0% (95% CI: 98.2–100.0%) and 99.5% (95% CI: 97.2–100.0%), respectively. For treponemal antibody detection the sensitivity and specificity was 97.5% (95%CI:94.3–99.2%) and 100.0% (95%CI:98.2–100.0%), respectively.

1. Background

Syphilis, a sexually transmitted infection (STI) caused by the bacterium *Treponema pallidum*, is a significant global health problem with approximately 6 million new cases per year (Newman et al., 2015). However, while syphilis is curable, both male and female rates of primary and secondary syphilis have increased in every region, in every age group and every race/ethnicity group in the U.S. (Centers for Disease Control and Prevention (CDC), 2017), and countries across the world are seeing large increases in syphilis in key populations such as men who have sex with men (MSM) (Abara et al., 2016; Chen et al., 2017; Kojima and Klausner, 2018). HIV co-infection with STIs is common (Kalichman et al., 2011; Rieg et al., 2008). Approximately one-half of all MSM diagnosed with syphilis are also co-infected with HIV (Centers for Disease Control and Prevention (CDC), 2017).

Routine screening and subsequent timely treatment are mainstays of HIV and STI control programs. Dual rapid tests for HIV and syphilis antibody detection are available and recommended by the World Health Organization (World Health Organization (WHO), 2017). Those tests can be conducted at the point-of-care and can be done using a drop of blood from a fingerprick. Rapid tests may make screening more feasible and accessible (Gliddon et al., 2017; Tucker et al., 2013). Dual rapid tests for HIV and syphilis allow for the simultaneous screening for the two infections using one specimen and one test device. We aimed to estimate the test accuracy of the Standard Q HIV/Syphilis Combo Test (SD Biosensor, South Korea), a new dual rapid test using stored sera in a laboratory setting in Lima, Peru. The Standard Q HIV/Syphilis Combo uses a lateral flow format similar to other rapid tests on the market, however it tests for and distinguishes between antibodies to HIV-1, HIV-2 and syphilis. The test is not yet commercially available but is under evaluation for CE marking, World Health Organization Pre-qualification and Registration in Brazil, Peru and Paraguay.

2. Methods

2.1. Specimen collection and reference testing

Stored serum specimens collected from a cohort of MSM and transgender women attending sexually transmitted disease clinics in Lima, Peru, were used for the evaluation (N = 400). Those specimens were

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* Corresponding author. Tel.: +1-415-250-2681; fax: +1-858-534-7566.
E-mail address: cbristow@ucsd.edu (C.C. Bristow).

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Table 1
Laboratory performance for detection of HIV antibodies using Standard Q HIV/Syphilis Combo Test (N = 400).

HIV	Number of samples		Total	Sensitivity (95% CI)	Specificity (95% CI)	Concordance: Kappa Coefficient (95% CI)
	Ref test +	Ref test -				
Standard Q Pos	200	1	201	100.0% (98.2–100.0%)	99.5% (97.2–100.0%)	0.995 (0.985–1.000)
Standard Q Neg	0	199	199			
Total	200	200	400			

Reference (ref) testing was conducted with a Western blot immunoassay kit (new Lav blot I; Bio-Rad, France) and 3rd generation rapid test or 4th generation EIA positive. HIV negative specimens were those that were EIA and rapid test negative.
No Standard Q tests gave invalid results.

stored between 2013 and 2016 as part of a cohort study that has been described elsewhere (Kojima et al., 2017). Initial blood collection and serum separation (at speed of 3500 RPM × 10 minutes) were conducted at clinical sites (Hettich® EBA 20 centrifuge). Two aliquots of serum were collected, one for HIV and syphilis testing and the second as back up. Samples were stored at −20 °C and then transported to the Laboratory of Sexual Health at University Peruana Cayetano Heredia in Lima, Peru within 3 hours where they were stored at −80 °C until thawed for the evaluation.

At clinic sites, samples were tested for HIV with a third generation rapid test (Determine HIV ½ Alere Determine, Israel) and syphilis infection was evaluated by using a 3rd generation treponemal antibody rapid test (Determine Syphilis Alere Determine, Israel).

In the laboratory the stored specimens were thawed and tested for HIV antibodies and antigen using a fourth-generation enzyme immunoassay (EIA, Genscreen Ultra HIV Ag-Ab; BioRad, 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. Specimens with a reactive result on the HIV rapid test or the HIV EIA underwent a confirmatory Western Blot test (New Lav Blot I; Bio-Rad, France). Those samples that were positive on the HIV rapid test, HIV EIA and the Western blot assay were considered HIV positive for this analysis. HIV negative specimens were those that had negative HIV third-generation rapid test and a negative fourth-generation EIA. Western blot testing was not done on specimens that were both rapid test and EIA-negative.

For the *Treponema pallidum* antibody evaluation, a *Treponema pallidum* particle agglutination test (TPPA, Serodia-TPPA; Fujirebio Diagnostics, Inc., Japan) was performed. Rapid plasma reagin (RPR) tests (BD Macro-Vue RPR; Becton Dickinson, NJ) were also conducted on all specimens. Treponemal positive specimens were those that were TPPA reactive (at 1:80) and treponemal negative specimens as those that were TPPA non-reactive. All reference testing was performed prior to specimen storage and was not repeated during the present evaluation of the Standard Q HIV/Syphilis Combo test.

All reference testing was performed according to manufacturer directions.

2.2. Test under evaluation

The Standard Q HIV/Syphilis Combo test (SD Biosensor, South Korea) is a rapid immunochromatographic assay that contains a test membrane pre-coated with recombinant HIV-1 gp41 protein/recombinant HIV-1 subtype O, recombinant HIV-2 gp36 protein, and recombinant TP17 protein. The test includes a control line that should always appear if the test procedure is performed properly. The test components allow simultaneous detection of antibodies to HIV-1/2 and syphilis in one single device and provide discrimination between HIV-1 and HIV-2 antibodies with the three-line region, “H1”, “H2”, “SYP” for HIV-1, HIV-2 and syphilis, respectively.

The Standard Q HIV/Syphilis Combo test was performed according the manufacturer's instructions at the Laboratory of Sexual Health at the University Peruana Cayetano Heredia in Lima, Peru. First, 10 μL of serum were added to the sample well of the test device. Then, 3 drops of assay diluent were added into the sample well. After 15 minutes, the test was read independently by two trained laboratory personnel blinded to the reference results. An HIV-1 reactive result was represented by a line on the “H1” region, an HIV-2 reactive result was represented by a line on the “H2” region and a syphilis reactive result was represented by a line on the “SYP” region.

2.3. Data analysis

We estimated the sensitivity, specificity of the Standard Q HIV/Syphilis Combo test and used the exact binomial method to determine 95% confidence intervals (CIs). In addition, we stratified the data by HIV infection status and RPR titer (1:1, 1:2, 1:4, ≥1:8) and calculated the sensitivity of the Standard Q test for detection of treponemal antibody within groups. We calculated concordance between the Standard Q test and reference tests using Cohen's Kappa statistic. We calculated the inter-reader reliability using percent agreement. All analyses were conducted using Stata v14 (Texas, USA).

Table 2
Laboratory performance for detection of Treponemal antibodies using Standard Q HIV/Syphilis Combo Test (N = 400).

Treponema pallidum	Number of samples		Total	Sensitivity (95% CI)	Specificity (95% CI)	Concordance: Kappa Coefficient (95% CI)
	Ref test +	Ref test -				
Standard Q Pos	195	0	195	97.5% (94.3–99.2%)	100.0% (98.2–100.0%)	0.975 (0.953–0.997)
Standard Q Neg	5	200	205			
Total	200	200	400			

Reference (ref) testing was conducted with *Treponema pallidum* particle agglutination (TPPA) assay (Serodia-TPPA; Fujirebio diagnostics Inc., Japan).
No Standard Q tests gave invalid results.

Table 3
Laboratory performance for detection of Treponemal antibodies using Standard Q HIV/Syphilis Combo Test (N = 200) among HIV infected.

Treponema pallidum	Number of samples		Total	Sensitivity (95% CI)	Specificity (95% CI)	Concordance: Kappa Coefficient (95% CI)
	Ref test +	Ref test -				
Standard Q Pos	97	0	97	97.0% (91.5%- 99.4%)	100.0% (96.4–100.0%)	0.970 (0.936–1.000)
Standard Q Neg	3	100	103			
Total	100	100	200			

Reference (ref) testing was conducted with *Treponema pallidum* particle agglutination (TPPA) assay (Serodia-TPPA; Fujirebio diagnostics Inc., Japan).
No Standard Q tests gave invalid results.

2.4. Ethical review

The institutional review board at the Universidad Peruana Cayetano Heredia approved the protocol under protocol number 102076.

3. Results

A total of 400 sera specimens were used for the evaluation, 100 non-reactive for both HIV and treponemal antibodies, 100 HIV antibody reactive but non-reactive for treponemal antibodies, 100 treponemal antibody reactive but HIV non-reactive, and 100 reactive for both HIV and treponemal antibodies. All 400 sera specimens gave a result on the Standard Q HIV/Syphilis Combo test. The overall percent agreement between the two Standard Q HIV/Syphilis Combo Test readers was 100%.

The sensitivity and specificity of the Standard Q HIV/Syphilis Combo test for HIV antibody detection was 100.0% (95% CI: 98.2–100.0%) and 99.5% (95% CI: 97.2–100.0%), respectively [Table 1]. For treponemal antibody detection the sensitivity and specificity was 97.5% (95% CI: 94.3–99.2%) and 100.0% (95% CI: 98.2–100.0%), respectively [Table 2]. Among those HIV reactive specimens, the sensitivity and specificity for treponemal antibody detection was 97.0% (95% CI: 91.5%- 99.4%) and 100.0% (95% CI: 96.4–100.0%), respectively [Table 3]. The treponemal sensitivity was 100% (95% CI: 93.2–100.0%) for those at RPR titers greater than or equal to 1:8 [Table 4].

4. Discussion

We assessed the performance of the Standard Q HIV/Syphilis Combo test using 400 stored serum specimens. The test was highly accurate. The sensitivity for detection of HIV antibody was 100% and over 97% for the detection of treponemal antibody. The specificity was 99.5% for HIV antibody detection and was 100% for treponemal antibody.

HIV and syphilis continue to cause morbidity and mortality around the globe (Kojima and Klausner, 2018; UNAIDS, 2016). Strategies to improve the uptake and reach of routine screening are urgently needed. Rapid dual testing is one strategy that may have impact. A recent meta-analysis of 18 studies of the diagnostic accuracy of various dual HIV and syphilis tests found that the laboratory performance of dual tests tended to be high, the sensitivity of HIV antibody detection ranged from 98% to 100%, and specificity from 92% to 100% (Gliddon et al., 2017). The meta-analysis also found that for syphilis antibody detection, reported sensitivities ranged from 93% to 100% and specificity values ranged from 93% to 100%. Our findings are within that same range. Because that meta-analysis found that there was a reduction in diagnostic accuracy in field settings compared to laboratory settings for syphilis (Gliddon et al., 2017), the Standard Q HIV/Syphilis Combo test should be evaluated in field settings using fingerprick whole blood specimens.

In conclusion, the Standard Q HIV/Syphilis Combo test provided highly accurate HIV and treponemal antibody results on stored sera.

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Table 4
Sensitivity of the Standard Q HIV/Syphilis Combo Test in *Treponema pallidum* particle agglutination positive samples (n = 200) stratified by RPR titer.

Rapid plasma reagin titer	Standard Q	
	Sensitivity (95% CI)	Standard Q positive positive/total positive
1:1	98.1% (90.1-100.0%)	53/54
1:2	96.2% (86.8-99.5%)	50/52
1:4	95.2% (83.8–99.4%)	40/42
≥1:8	100.0% (93.2–100.0%)	52/52