FINAL REPORT

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Laboratory Evaluation of a Dual Rapid Test for HIV and Syphilis:
Standard Q HIV/Syphilis Combo (SD BIOSENSOR)

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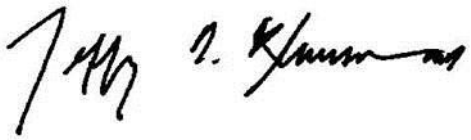

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A retrospective evaluation of the Standard Q HIV/Syphilis Combo Test using stored sera

Specimen collection, clinic testing and reference testing

Stored serum specimens collected from a cohort of men who have sex with men and transgender women attending sexually transmitted disease clinics in Lima, Peru, were used for the evaluation.

Initial blood collection and serum separation (3500rpm x 10 min) were conducted at clinical sites. Two aliquots of serum were collected, one for HIV and syphilis diagnosis and the other as back up. Samples were stored at -20°C and then transported maintaining cold chain to the Laboratory of Sexual Health at UPCH 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 specimens were tested for HIV infection 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 HIV enzyme immunoassay underwent a confirmatory Western Blot test (New Lav Blot I; Bio-Rad, France). Those samples that were positive on the HIV rapid test, HIV enzyme immunoassay and the Western blot assay were considered HIV positive for this analysis.

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. HIV negative specimens were those that had negative HIV third-generation rapid test and a negative forth-generation EIA. The treponemal negative specimens were TPPA negative.

Test under evaluation.

The Standard Q HIV/Syphilis Combo test (SD Biosensor, Korea) is a rapid inmunochromatographic assay that contains a test membrane pre-coated with recombinant HIV-1 gp41 protein / recombinant HIV-1 subtype O gp41, recombinant HIV-2 gp36 protein, recombinant TPP17 protein and monoclonal anti-HIV-1 / monoclonal anti-syphilis respectively. The test includes a control line that should always appear if the test procedure is performed properly. Its components allow simultaneous detection of 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. First, 10µL 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 and recorded separately by two trained laboratory personnel blinded to the reference results. A control line appeared on all tests. An HIV-1 positive result was represented by a line on the “H1” region, an HIV-2 positive result was represented by a line on the “H2” region and a syphilis positive result was represented by a line on the “SYP” region. In case of presence of lines “H1” and “H2”simultaneosly, the line with the stronger intensity should be considered as positive.

Data analysis.

We estimated the sensitivity, specificity of the Standard Q HIV/Syphilis Combo test and used the exact binomial method determine 95% confidence intervals (CIs). In addition, we assessed the sensitivity of the Standard Q test for detection of treponemal antibody by HIV status and by RPR titer (RPR nonreactive, 1:1, 1:2, 1:4, ≥1:8). 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 v.14 (Texas, USA).

Results.

The overall percent agreement between the two Standard Q HIV/Syphilis Combo Test readers was 100%.

Sensitivity and specificity of the Standard Q test 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.

Among those specimens from HIV infected individuals, 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.

The treponemal sensitivity increased with increased RPR titer

Conclusion.

The Standard Q HIV/Syphilis Combo test provided highly accurate results on stored será.

Tables of results.

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)	Inter-reader reliability: 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 and rapid test negative.
No Standard Q tests gave invalid results

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

Trep	Number of samples		Total	Sensitivity (95% CI)	Specificity (95% CI)	Inter-reader reliability: 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.

Trep	Number of samples		Total	Sensitivity (95% CI)	Specificity (95% CI)	Inter-reader reliability: 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

Table 4. Sensitivity of the Standard Q HIV/Syphilis Combo Test in TPPA positive samples (n=200) stratified by RPR titer.		
	Standard Q	
RPR titer	Sensitivity (95% CI)	TP/(TP+FN)
Non-Reactive	N.A.	N.A.
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