Multi-site laboratory evaluation of a dual HIV/syphilis point-of-care rapid test for simultaneous detection of HIV and syphilis antibodies

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Summary

This study was a multi-site laboratory based evaluation of the performance of SD BIOLINE® HIV/Syphilis Duo test using previously characterized sera from six countries around the world. The sensitivity and specificity of the Duo test were consistently high across sites.



Background

- Syphilis is a curable disease, yet an estimated 1.4 million pregnant women are syphilis infected each year.
- Recently the World Health Organization (WHO) has called for the dual elimination of MTCT HIV and syphilis.
- Screening for HIV and syphilis are highly recommended as part of a comprehensive dual elimination strategy.
- The advent of integrated dual diagnostic tests for HIV and syphilis could provide a major breakthrough, facilitating integration of screening of syphilis into HIV prevention programs such as prevention of MTCT of HIV and targeted screening for high-risk populations.
- Test developers have created rapid tests that can detect multiple infections in the same specimen using a single device. The SD BIOLINE HIV/Syphilis Duo test [17] is a qualitative detection method using a solid phase immunochromatographic assay.
- This study was a multisite laboratory-based evaluation of the performance of the SD BIOLINE HIV/Syphilis Duo test using previously characterized serum samples from six countries.

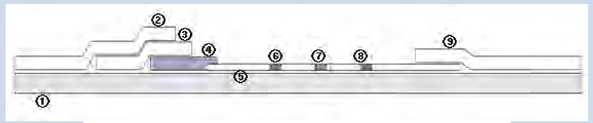


Image Key

1. Plastic cassette
2. Sample pad 2
3. Sample pad 1
4. Gold conjugation pad
5. Nitrocellulose membrane
6. Test line for HIV antibodies
7. Test line for Syphilis antibodies
8. Control line
9. Absorbent pad



Methods

Study Sites and Population

- A total of 6 study sites around the world participated in the evaluation study from 2012 to 2013: laboratories in Ghana, Togo, Kenya, Mexico, Laos, and Myanmar.
- Specimens included stored sera which had previously been tested for syphilis and HIV infection according to local guidelines.

Test for Evaluation

- The SD BIOLINE® Duo HIV/Syphilis rapid point-of-care test uses a solid phase immunochromatographic assay to detect IgG, IgM and IgA antibodies to HIV specific antigens (HIV-1 gp41, sub O, HIV-2 gp36) and recombinant *Treponema (T.) pallidum* antigens (17KDa) in human serum.
- The recombinant HIV-1/2 antigen – colloid gold conjugate, 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.

Comparison Testing

- For gold standard testing each country used a combination of *Treponema pallidum* particle agglutination assay (TPPA) or *Treponema pallidum* Hemaglutination Assay (TPHA) and Rapid plasma reagin (RPR) for detection of syphilis infection and enzyme immunoassay (EIA), Western Blot, and rapid tests for the detection of HIV infection. The exact tests used for characterization of serum samples are displayed in Table 1.

Data analysis

- We calculated the sensitivity and specificity of test performance at each individual site using the exact binomial method to calculate 95% confidence intervals (CI).
- Analyses were conducted using SAS v9.3 (Cary, NC).

Table 1. Country, Reference tests, and algorithms for HIV and *Treponema pallidum* characterization in sera specimens.

Country	Reference Tests for HIV	Reference Tests for *Treponema pallidum*
Ghana	HIV I/II Genscreen (Biorad, France) First response HIV 1-2-0 (PMC, India)	RPR (Omega Diagnostics, Scotland) TPHA (Omega Diagnostics, Scotland)
Togo	Vironostika HIV1/2 Uniform II plus (Biomérieux BV, Boxtel, The Netherlands) Inno-lia (Innogenetics N.V. Belgium)	RPR Carbon (CYPRESS diagnostics, Langdorp, Belgium) TPHA (CYPRESS diagnostics, Langdorp, Belgium)
Myanmar	HIV Ag/Ab ELISA (Murex, UK) HIV ½ Stat-Pak (Chembio, New York, USA)	Omega TPHA (Omega, UK)
Kenya	Determine RDT (Alere Medical Co. Ltd, Japan) Unigold RDT (Trinity Biotech plc, Bray, Ireland) Vironostika HIV1/2 Uniform II plus (Biomérieux BV, Boxtel, The Netherlands)	RPR Kit, (BD Macrovue RPR kit, Maryland) TPHA Kit (Randox Laboratories Ltd, UK)
Laos	Anilab HIV ELISA Standard Diagnostics HIV 1/2 3.0 rapid test	RPR Syphilis Test SD Syphilis TPHA liquid (Human Diagnostics, Wiesbaden, Germany)
Mexico	VITROS ECi/ECiQ Immunodiagnostic System Anti HIV 1 + 2 (Johnson & Johnson, UK) Vironostika HIV1/2 Uniform II plus (Biomérieux BV, Boxtel, The Netherlands) New LAV Blot I (Bio-Rad, CA, USA)	IMMUTREP® -USR (Omega Diagnostics, Scotland, UK) FTA- ABS Trepo Spot IF (BioMerieux, Marcy l'Etoile, France)

Results

- Summarized results for each study site for HIV *T. pallidum* antibody test performance can be seen in Table 2a and Table 2b.
- In total 2336 HIV characterized specimens and 2059 syphilis characterized specimens were used to evaluate this HIV/Syphilis Duo kit across the six countries.
- The combined sensitivity and specificity for testing HIV status was 99.91% (95% CI: 99.51%, 100%) and 99.67% (95% CI: 99.16, 99.91%), respectively. For the detection of antibodies to syphilis, the combined sensitivity and specificity was 99.67% (95% CI: 98.82%, 99.96%) and 99.72% (95% CI: 99.29%, 99.92%), respectively.

Table 2a. Laboratory performance for detection of HIV antibodies using a dual HIV/syphilis test in previously characterized sera samples in 6 laboratory sites.

Country	Year	N	True Positive	False Positive	False Negative	True Negative	Sensitivity Estimate (exact 95% CI)	Specificity Estimate (exact 95% CI)
Ghana	2012	400	250	0	0	150	100% (98.54%, 100%)	100% (97.57%, 100%)
Togo	2013	310	203	0	0	107	100% (98.20%, 100%)	100% (96.61%, 100%)
Myanmar	2013	245	114	1	0	130	100% (96.82%, 100%)	99.24% (95.82%, 99.98%)
Kenya	2013	698	345	0	1	352	99.71% (98.40%, 99.99%)	100% (98.96%, 100%)
Mexico	2013	527	158	0	0	369	100% (97.69%, 100%)	100% (99.01%, 100%)
Laos	2013	156	53	3	0	100	100% (93.28%, 100%)	97.09% (91.72%, 99.40%)
OVERALL	-	2336	1123	4	1	1208	99.91% (99.51%, 100%)	99.67% (99.16%, 99.91%)

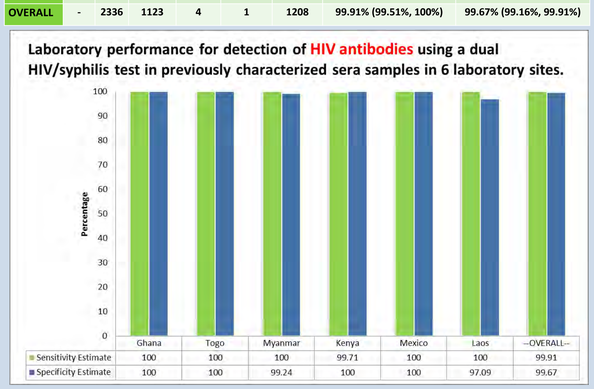
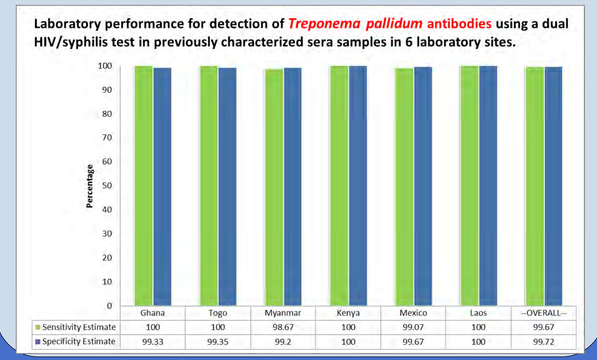


Table 2b. Laboratory performance for detection of *Treponema pallidum* antibodies using a dual SD BIOLINE HIV/syphilis test in previously characterized sera samples in 6 laboratory sites.

Country	Year	N	True Positive	False Positive	False Negative	True Negative	Sensitivity Estimate (exact 95% CI)	Specificity Estimate (exact 95% CI)
Ghana	2012	400	250	1	0	149	100% (98.54%, 100%)	99.33% (96.34%, 99.98%)
Togo	2013	241	88	1	0	152	100% (95.89%, 100%)	99.35% (96.41%, 99.98%)
Myanmar	2013	200	74	1	1	124	98.67% (92.79%, 99.97%)	99.20% (95.62%, 99.98%)
Kenya	2013	698	85	0	0	613	100% (95.75%, 100%)	100% (99.40%, 100%)
Mexico	2013	414	106	1	1	306	99.07% (94.90%, 99.98%)	99.67% (98.20%, 99.99%)
Laos	2013	106	6	0	0	100	100% (54.07%, 100%)	100% (96.38%, 100%)
OVERALL	-	2059	609	4	2	1444	99.67% (98.82%, 99.96%)	99.72% (99.29%, 99.92%)



Discussion

- We completed a study combining laboratory performance evaluations from six different laboratories across the world.
- The sensitivity and specificity of the dual HIV/syphilis test using serum was consistently high across six countries.
- Point-of-care tests can result in accelerated linkage to treatment and care by decreasing the time to result and can be used in settings with limited laboratory access.
- Dual rapid tests with multiple analytes provide additional advantages by testing for multiple infections and there is further efficiency in using a single device and a single specimen.
- Multiplex diagnostic testing can increase uptake of screening for often ignored infections like syphilis.
- These findings of very high performance are consistent with the performance reported for single point-of-care tests for HIV and syphilis.
- Other dual rapid tests for HIV and syphilis have been developed and evaluated and are showing promising results, such as the Multiplo Rapid TP/HIV Antibody Test (Medmira, Canada) and the DPP® HIV-Syphilis Assay (Chembio, New York, USA).
- This study was conducted in sera in laboratory settings. The test, however, is optimally used as a point-of-care test in whole blood finger stick specimens. Additional evaluation should be considered in field settings.
- While there were some differences in the gold standard test algorithms, there was no apparent impact on test performance.
- A major strength of this study was that it showed consistently high performance in all six settings around the world that have differing rates of infection and human antibody profiles.



Acknowledgements

Study was supported in part by standard diagnostics, we thank them for their support. We would also like to acknowledge the laboratories and staff involved in this evaluation: Department of Clinical Microbiology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana; Kenya Medical Research Institute, Nairobi, Kenya; Centre National de Référence des Tests VIH, Laboratoire de Microbiologie, Lomé Togo; National Health Laboratory, Yangon, Myanmar; NMCH, Vientiane Capital, Laos; and Departamento de Enfermedades Emergentes y Urgencias, InDRE, Mexico. In addition, we would like to thank the Klausner Research and Education Fund for their support of this project.

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