JCM Accepts, published online ahead of print on 8 October 2014
J. Clin. Microbiol. doi:10.1128/JCM.02468-14

LABORATORY EVALUATION OF THREE RAPID DIAGNOSTIC TESTS FOR THE DUAL DETETION OF HIV AND TREPONEMA PALLIDUM ANTIBODIES

Romney M. Humphries[#1], Jennifer S Woo[1], Jun Ho Chung[1], Anita Sokovic[2] Claire C. Bristow[2], Jeffrey D. Klausner[2]

Department of Pathology and Laboratory Medicine[1], Department of Infectious Diseases[2], David Geffen School of Medicine at UCLA, Los Angeles, California, USA

RUNNING HEAD: EVALUATION OF HIV AND SYPHILIS RAPID DIAGNOSTIC TESTS

#Address correspondence to Romney Humphries PhD., rhumphries@mednet.ucla.edu

*Present address: Brentwood Annex, 11633 San Vicente Blvd, Los Angeles, CA 90049

ABSTRACT

The performance of three research use only, dual HIV and syphilis rapid diagnostic tests (RDTs) was evaluated for 150 patient serum samples, as compared to reference HIV and *Treponema pallidum* antibody detection methods. RDTs performed comparably, with sensitivity of 93-99% and specificity of 97-100%. Kappa statistic between the RDTs was 0.95.

MANUSCRIPT

In the U.S., HIV and syphilis (caused by *Treponema pallidum*) co-infection is increasingly common, with the estimated median HIV seroprevalence in men with syphilis to be 27.5% and in women with syphilis to be to be 12.4% (1). Men who have sex with men (MSM) have particularly high rates of HIV and syphilis co-infection, documented to be 47 - 72% in some areas of the U.S. (2-6). Co-infection with syphilis can increase the transmission of HIV, by both increasing viral shedding through open ulcers (7, 8) and by increasing patient viral load (9, 10).

Reference methods used by most major clinical laboratories in the in U.S. for the diagnosis of HIV include enzyme immunoassays (EIAs) for the qualitative detection of antibodies to HIV-1 and HIV-2. EIA-reactive specimens are typically confirmed with an HIV-1 antibody Western Blot. In 2014, the Centers for Disease Control and Prevention issued new guidance for HIV diagnostic testing, which includes primary testing by a combination immunoassay that detects both HIV-1 and HIV-2 antibodies and the HIV-1 p24 antigen. Specimens reactive by the screening assay undergo supplemental testing with an immunoassay that differentiates HIV-1 and HIV-2 antibodies (11). Specimens that are reactive on initial antigen/antibody combination immunoassays and nonreactive or indeterminate on the HIV-1/HIV-2 antibody differentiation immunoassay are then tested with an FDA-approved HIV-1 RNA nucleic acid test (NAT) (11). Reference methods for diagnosis of syphilis include primary screening by non-treponemal tests, such as the rapid plasma reagin (RPR), and confirmation with a treponemal-specific test, such as the *T. pallidum* particle agglutination (TP-PA) assay. Alternatively, many laboratories have adopted a ‘reverse algorithm’, whereby a *T. pallidum*-specific

immunoassay (e.g. enzyme immunoassay) is the screening test and a non-treponemal test, such as the RPR, is performed on EIA-reactive samples to stage the disease and monitor treatment (12).

In the U.S., HIV tests are also commonly administered in both clinical and non-clinical community based organizations, through the use of CLIA-waived rapid diagnostic tests (RDTs) for HIV, which detect HIV-1 and HIV-2 antibodies. The advantage of such testing is that results are immediately available at the point-of-care, which provides early diagnosis of HIV infection, and improved linkage to care (13-15). In contrast, FDA-approved, CLIA waived point-of-care tests for the diagnosis of syphilils are not yet available in the U.S., although these are available in other countries (16). At the time of this writing, one rapid *T. pallidum* test has obtained FDA clearance and is awaiting CLIA waiver [Syphilis Health Check (Diagnostics Direct, Youngstown, OH, USA)]. As is the case for HIV, rapid diagnosis and treatment of syphilis is critical to reducing transmission. The availability of rapid, CLIA-waived syphilis tests will allow immediate evaluation and treatment of patients who test positive for syphilis, and the potential for screening in non-medical settings. The bulk of the syphilis epidemic in the U.S. is among MSM, and the largest increase in primary and secondary syphilis between 2009 and 2012 was in MSM aged 25-29 (17). However, sexually active MSM, and in particular young MSM, do not seek HIV and syphilis screening at the frequencies recommended by the CDC. As such, the availability of CLIA-waived, rapid, dual testing has the potential to reduce both syphilis and HIV rates among this at-risk population. While evaluation of point-of-care testing with RDTs for HIV or syphilis has been

performed in various settings, the use of dual RDTs for both HIV and syphilis has not been fully evaluated.

In this study, we evaluated the performance of three commercially available, research-use-only (RUO), HIV / *T. pallidum* antibody dual RDTs using remnant, de-identified sera from 150 patients that were previously tested by routine methods. Twenty-five specimens were obtained from the San Francisco Department of Public Health (previously characterized to be positive for HIV and syphilis antibodies); HIV and syphilis testing was confirmed at UCLA prior to the start of the study. The remaining 125 serum specimens were from UCLA Clinical Microbiology Laboratory, and selected based on the results of routine HIV and syphilis serologic testing. HIV testing was performed using the Siemens Advia Centaur HIV 1/O/2 enzyme immunoassay (HIV EIA, Siemens, Tarrytown, NY, USA); all positives were confirmed by western blot, using the GS HIV-1 Western Blot kit (BioRad, Hercules, CA). RPR testing was performed using the Macro-Vue™ 18 mm Circle Card Test (Becton Dickinson, Sparks, MD). Presence of *T. pallidum* antibodies was confirmed using the Serodia TP-PA test (Fujirebio Diagnostics, Inc., Malvern, PA, USA). All specimens were stored at -70°C prior to testing by the RDTs.

The three RUO dual HIV / Syphilis RDTs evaluated were the MedMira Multiplo TP/HIV (MedMira Inc, Halifax, Nova Scotia, Canada), Standard Diagnostics (SD) BIOLINE HIV/Syphilis Duo (Standard Diagnostics Inc, Gyeonggi-do, Republic of Korea), and Chembio DPP® HIV-Syphilis Assay (ChemBio Diagnostics Inc, Medford, New York, USA). The SD and Chembio tests are solid phase immunochromatographic assays, whereas the MedMira test is a vertical flow qualitative immunoassay. All three assays

96 are single-use RDTs for the dual, qualitative detection of HIV-1, HIV-2 and *T. pallidum*
97 antibodies. None of the RDTs differentiate HIV-1 from HIV-2. Comparison of the RDTs
98 evaluated in this study is presented in Table 1. All three dual RDTs evaluated in the
99 present study can be stored at room temperature, require no laboratory equipment
100 (other than a timer), are easy to perform, rapid, and relatively easy to interpret.

101 Specimens were tested by all 3 RDTs in parallel, following the manufacturer's
102 instructions, by a trained laboratory technician blinded to the reference results. Any
103 discernible reactivity in the RDTs, even those with faint reactions, was considered
104 positive as recommended by the manufacturers' package insert. The results of the RDTs
105 for HIV was compared to that of routine testing (EIA and Western Blot). The results of
106 the RDTs for *T. pallidum* was compared to the TP-PA test. Specimens that yielded
107 discordant or difficult-to-interpret (faint) results were repeated by all reference methods
108 and all 3 RDTs, in parallel. Data were summarized using descriptive statistics, including
109 sensitivity and specificity, with 95% confidence intervals (CI) calculated by the exact
110 binomial distribution method. The Kappa statistic was used to describe concordance
111 between the three RDTS. Statistical analyses were performed in Microsoft Excel. All
112 protocols were approved by the UCLA Institutional Review Board.

113 Among 150 samples included in this study, 29 (19.3%) were negative for *T.*
114 *pallidum* and HIV, 24 (16%) were positive for *T. pallidum* but negative for HIV, 35
115 (23.3%) were positive for HIV but negative for *T. pallidum,* and 62 (41.3%) were positive
116 for both HIV and *T. pallidum*,, by the reference methods. All HIV EIA positive specimens
117 were confirmed by a positive HIV-1 western blot (not shown). RPR titers for the 86

specimens positive by the TP-PA assay ranged from not reactive (n=28) to 1:512 (mean titer of reactive specimens, 1:8).

Performance of the RDTs is listed in Table 2. Sensitivity for HIV antibody detection by the RDTs was 98-99% and specificity was 94-100%, as compared to the Siemens Advia HIV EIA. Similarly, detection of *T. pallidum* antibodies was excellent for all three methods, ranging from 93 – 95% sensitivity, and 97-100% specificity, as compared to the TP-PA assay (Table 2). The Kappa coefficient between the three RDTs was 0.95 (95% CI, 0.80-1.0) for the HIV component and 0.93 for the *T. pallidum* component (95% CI, 0.78-1.0).

Repeat testing did not resolve any false negative or false positive results observed by the RDTs. All false negative *T. pallidum* antibody results (n=7) were from HIV-positive specimens (Table 3). Two of these were from specimens with high (≥1:8) RPR results. Two false positive *T. pallidum* results were observed, both only with the MedMira assay and again in HIV-positive specimens (Table 3). Four false positive HIV results were observed; three of these by the MedMira assay (Table 4), two of which had repeatedly faint reactions for HIV. Two specimens yielded false-negative HIV reactions (Table 4). One of these specimens was negative by all three RDTs, whereas specimen SF5 was negative by the MedMira and SD RDTs, but positive by the Chembio RDT (Table 4). Faint HIV reactions were observed in 2 MedMira (1.3%, both false positive), 3 SD (2%, all true positives) and 2 ChemBio tests (1.3%, one false positive). Faint *T. pallidum* reactions were noted for 16 MedMira (10.7%, including 2 false positives), 10 SD (6.7%, all true positives) and 6 ChemBio (4%, all true positives) tests. Repeat testing yielded similarly difficult-to-interpret results. Overall, our evaluation showed

comparable performance by the RDTs to reference methods, with excellent sensitivity and specificity. Ease of use was qualitatively comparable.

In the U.S., several HIV RDTs have been approved for clinical use by the U.S. Food and Drug Administration (FDA) since 2002. The CDC recommends a second specimen be collected and tested by an HIV 1/2 immuno-differential test, for those patients with a positive HIV RDT. If positive, the diagnosis is confirmed, and if negative, additional testing with an appropriate HIV RNA NAT is recommended (11). In contrast, the clinical experience with syphilis RDTs is limited in the U.S., as only one manufacturer has recently achieved FDA clearance for their syphilis RDT. The management and follow-up testing for patients that test positive with a syphilis RDT remains to be defined, but will likely include confirmation with laboratory-based treponemal tests, and non-treponemal testing for disease staging and monitoring of treatment. Nonetheless, global experience with syphilis RDTs has shown excellent results. A systematic review of multiple syphilis RDTs used in 15 studies from over 22,000 whole, plasma, or fingerstick specimens show a sensitivity (median 86%, interquartile range 75%–94%) and specificity (99%, 98%–99%), comparable with non-treponemal screening tests characteristics (18). A more recent meta-analysis further reports performance that is estimated to be comparable to laboratory-based treponemal tests for these rapid treponemal tests (16).

Dual RDTs for HIV and syphilis infection have been less well evaluated in either laboratory or clinical settings. Recently, a multi-site laboratory evaluation from 6 countries demonstrated excellent sensitivity and specificity of the SD BIOLINE HIV/Syphilis Duo RDT. The sensitivity and specificity of the HIV antibody test

component (n = 2336 specimens tested) was reported to be 99.9% and 99.7%, respectively. For the *T. pallidum* antibody component (n = 2059 specimens tested), the sensitivity and specificity was 99.7% and 99.7%, respectively (19). These values are comparable to those obtained in the present study.

Limitations to our study include a relatively small number of patient specimens evaluated. Another limitation includes the use of patient serum as opposed to fingerstick whole blood specimens, which would be used for point-of care testing. Furthermore, the performance of these tests may be higher in our study than what would be observed in the real world, as testing was performed in a controlled laboratory setting by a small number of highly skilled technicians. Future research should include field evaluations of dual HIV/syphilis rapid tests.

While RDTs are not intended to replace standard reference methods, the development of quality RDTs can have an enormous impact on public health initiatives, by providing earlier identification of patients infected with HIV and/or *T. pallidum.* The fact that syphilis is a co-factor in HIV transmission and HIV infection affects the clinical presentation of syphilis (9-10), coupled with the high rate of HIV and syphilis co-infection among MSM in the U.S., underscores the need for both HIV and syphilis testing at the point of care. Since no HIV/Syphilis dual RDTs remain research use only in the U.S., such testing requires use of two RDTs. The recent FDA clearance of the Syphilis Health Check RDT, makes such testing now feasible. In regions of the U.S. where co-infection rates are high, or if targeted to high-risk patient populations, the availability of such testing may not only improve detection of syphilis infection, in addition to HIV, but also prevent further transmission by immediate treatment (18).

In summary, our study is the first to evaluate the sensitivity and specificity of three commercial combination HIV and syphilis RDTs in parallel, and demonstrates comparable performance to reference methods for all three RDTs. Further use of these RDTs in the clinical setting may more adequately determine their performance as point-of-care tests. However, until the manufacturers submit data to the FDA for clearance of these products, this testing will not be available in the U.S.

Acknowledgments: No financial support was received for this study. We would like to thank Dr. Mark Pandori for contribution of remnant, de-identified serum specimens, and the UCLA Clinical Microbiology Laboratory for their assistance. All RDTs were donated by the manufacturers.

References

1. **Blocker ME, Levine WC, St Louis ME.** 2000. HIV prevalence in patients with syphilis, United States. Sexually transmitted diseases **27:**53-59.
2. **Centers for Disease Control and Prevention.** 2001. Outbreak of syphilis among men who have sex with men--Southern California, 2000. MMWR. Morbidity and mortality weekly report **50:**117-120.
3. **Centers for Disease Control and Prevention.** 2002. Primary and secondary syphilis among men who have sex with men--New York City, 2001. MMWR. Morbidity and mortality weekly report **51:**853-856.
4. **Centers for Disease Control and Prevention.** 1999. Resurgent bacterial sexually transmitted disease among men who have sex with men--King County, Washington, 1997-1999. MMWR. Morbidity and mortality weekly report **48:**773-777.
5. **D'Souza G, Lee JH, Paffel JM.** 2003. Outbreak of syphilis among men who have sex with men in Houston, Texas. Sexually transmitted diseases **30:**872-873.
6. **Chen SY, Gibson S, Katz MH, Klausner JD, Dilley JW, Schwarcz SK, Kellogg TA, McFarland W.** 2002. Continuing increases in sexual risk behavior and sexually transmitted diseases among men who have sex with men: San Francisco, Calif, 1999-2001, USA. American Journal of Public Health **92:**1387-1388.
7. **Mertz KJ, Trees D, Levine WC, Lewis JS, Litchfield B, Pettus KS, Morse SA, St Louis ME, Weiss JB, Schwebke J, Dickes J, Kee R, Reynolds J, Hutcheson D, Green D, Dyer I, Richwald GA, Novotny J, Weisfuse I, Goldberg M, O'Donnell JA, Knaup R.** 1998. Etiology of genital ulcers and prevalence of human immunodeficiency virus coinfection in 10 US cities. The Genital Ulcer Disease Surveillance Group. The Journal of infectious diseases **178:**1795-1798.
8. **Mertz KJ, Weiss JB, Webb RM, Levine WC, Lewis JS, Orle KA, Totten PA, Overbaugh J, Morse SA, Currier MM, Fishbein M, St Louis ME.** 1998. An

investigation of genital ulcers in Jackson, Mississippi, with use of a multiplex polymerase chain reaction assay: high prevalence of chancroid and human immunodeficiency virus infection. The Journal of infectious diseases **178:**1060-1066.

9. **Buchacz K, Patel P, Taylor M, Kerndt PR, Byers RH, Holmberg SD, Klausner JD.** 2004. Syphilis increases HIV viral load and decreases CD4 cell counts in HIV-infected patients with new syphilis infections. Aids **18:**2075-2079.

10. **Kofoed K, Gerstoft J, Mathiesen LR, Benfield T.** 2006. Syphilis and human immunodeficiency virus (HIV)-1 coinfection: influence on CD4 T-cell count, HIV-1 viral load, and treatment response. Sexually transmitted diseases **33:**143-148.

11. **Centers for Disease Control and Prevention and the Association of Public Health Laboratories** 2014, posting date. Laboratory testing for the diagnosis of HIV infection: Updated Recommendations. http://stacks.cdc.gov/view/cdc/23447. Accessed August 16, 2014.

12. **Wong EH, Klausner JD, Caguin-Grygiel G, Madayag C, Barber KO, Qiu JS, Liska S, Pandori MW.** 2011. Evaluation of an IgM/IgG sensitive enzyme immunoassay and the utility of index values for the screening of syphilis infection in a high-risk population. Sexually transmitted diseases **38:**528-532.

13. **Shrestha RK, Sansom SL, Schulden JD, Song B, Smith LC, Ramirez R, Mares-DelGrasso A, Heffelfinger JD.** 2011. Costs and effectiveness of finding new HIV diagnoses by using rapid testing in transgender communities. AIDS Education and revention **23:**49-57.

14. **Schulden JD, Song B, Barros A, Mares-DelGrasso A, Martin CW, Ramirez R, Smith LC, Wheeler DP, Oster AM, Sullivan PS, Heffelfinger JD.** 2008. Rapid HIV testing in transgender communities by community-based organizations in three cities. Public Health Reports **123 Suppl 3:**101-114.

15. **Centers for Disease Control and Prevention.** 2010. Expanded HIV testing and trends in diagnoses of HIV infection - District of Columbia, 2004-2008. MMWR. Morbidity and Mortality Weekly Report **59:**737-741.

16. **Jafari Y, Peeling RW, Shivkumar S, Claessens C, Joseph L, Pai NP.** 2013. Are Treponema pallidum specific rapid and point-of-care tests for syphilis accurate enough for screening in resource limited settings? Evidence from a meta-analysis. PloS one **8:**e54695.

17. **Centers for Disease Control and Prevention.** 2014. Primary and secondary syphilis – United States, 2005-2013. Morbidity and Mortality Weekly Report. **63:** 402-406.

18. **Tucker JD, Bu J, Brown LB, Yin YP, Chen XS, Cohen MS.** 2010. Accelerating worldwide syphilis screening through rapid testing: a systematic review. The Lancet. Infectious diseases **10:**381-386.

19. **Bristow CC, Adu-Sarkodie Y, Ondondo RO, Bukusi EA, Dagnra CA, Oo KY, Pe EH, C. K, Huong LT, Campuzano RV, J. E, Klausner JD.** 2014. Multisite Laboratory Evaluation of a Dual Human Immunodeficiency Virus (HIV)/Syphilis point-of-care Rapid test for Simulataneous Detection of HIV and Syphilis Infection. Open Forum Infectious Diseases **1**.

276 Table 1. Comparison of the three HIV / *T. pallidum* antibody RDTs used in this study

	ChemBio DPP® HIV-Syphilis Assay	SD Bioline HIV/Syphilis Duo	MedMira Muliplo rapid Syphilis / HIV antibody test
Specimen	Whole blood, serum or plasma	Whole blood, serum, or plasma	Whole blood, serum, or plasma
Time to detection	25 minutes	20 minutes	3 minutes
Equipment	Requires a timer	Requires a timer	None
Shelf life	24 months at room temperature	24 months at room temperature	18 months at room temperature
HIV component	Recombinant HIV-1 and HIV-2 antigens (not specified)	Recombinant HIV-1 gp41, sub-O antigens Recombinant HIV-2 gp36 antigen	Synthetic HIV-1 gp41, gp120 and group O peptides Synthetic HIV-2 gp36 peptide IgM and IgG
T. pallidum component	Recombinant antigen (not specified)	Recombinant antigen (17 kDa)	Recombinant antigens (15 kDa, 17kDa, 47kDa)
Method	Solid phase immunochromatographic assay	Solid phase immunochromatographic assay	Vertical flow immunoassay
Antibodies Detected	IgM and IgG antibodies to HIV and *T. pallidum* antigens	IgG, IgM and IgA antibodies to HIV and *T. pallidum* antigens	IgM and IgG antibodies to HIV and *T. pallidum* peptides

277

278

279

Table 2. Laboratory performance of 3 rapid diagnostic tests for the dual detection of HIV and *T. pallidum* antibodies, as compared to reference methods*

RDT	HIV antibody		*T. pallidum* antibody	
	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
SD	97.9 (92.0-99.6)	100 (91.5-100)	93.0 (84.8-97.1)	100 (92.9-100)
Chembio	98.9 (93.6-99.9)	98.1 (88.6-99.9)	95.3 (87.9-98.5)	100 (92.9-100)
MedMira	97.9 (92.0-99.6)	94.2 (83.1-98.5)	94.1 (86.3-97.8)	96.9 (88.2-99.5)

*Reference HIV antibody method was the Siemens Advia Centaur HIV 1/O/2 enzyme immunoassay and the GS HIV-1 Western Blot kit, and *T. pallidum* antibody reference method was the Serodia TP-PA.

285 Table 3. Characteristics of specimens with discordant *T. pallidum* antibody test results

286 by one or more RDT for the dual detection of HIV and syphilis antibodies

Specimen	Reference Result		TP Component of RDT Result			RPR	HIV Western Blot
	TPPA	HIV EIA	MedMira	SD	Chembio		
74	-	+	faint +[1]	-	-	NR[2]	reactive
93	-	+	faint +	-	-	NR	reactive
41	+	+	faint +	-	+	1:2	reactive
64	+	+	-	+	+	NR	reactive
75	+	+	-	-	-	1:4	reactive
81	+	+	-	-	-	1:32	reactive
84	+	+	-	faint+	faint+	NR	reactive
SF11	+	+	faint +	-	-	1:4	reactive
SF14	+	+	faint +	-	-	1:32	reactive
SF3	+	+	-	-	+	1:2	reactive

287 Shaded box indicates discordant result. [1]faint results were considered positive. [2]NR, not

288 reactive

289 Table 4. Characteristics of specimens with discordant HIV antibody results by one or
290 more RDT for the dual detection of HIV and syphilis antibodies
291

Specimen	Reference		HIV Component of RDT			RPR	HIV Western Blot
	TP-PA	HIV EIA	MedMira	SD	Chembio		
96	-	-	faint+[1]	-	-	NR	negative
16	+	-	-	-	faint +	1:1	negative
40	+	-	+	-	-	1:2	negative
SF23	+	-	faint+	-	-	1:4	negative
SF5	+	+	-	-	+	1:8	reactive
SF8	+	+	-	-	-	1:4	reactive

292 Shaded box indicates discordant result. [1]faint reactivity was considered positive. [2]NR,
293 not reactive
294