Performance of a Dual Human Immunodeficiency Virus/Syphilis Rapid Test Compared With Conventional Serological Testing for Syphilis and Human Immunodeficiency Virus in a Laboratory Setting: Results From the Zimbabwe STI Etiology Study

Cornelis A. Rietmeijer, MD, PhD, MSPH,† More Mungati, MBChB, MPH,‡ Peter H. Kilmarx, MD,§ Beth Tippett Barr, DrPH,§ Elizabeth Gonese, MPH,§ Ranmini S. Kularatne, MBChB, MSc,¶|| David A. Lewis, FRCP (UK), PhD,**†† Jeffrey D. Klausner, MD, MPH,‡‡ Luanne Rodgers, BS,§§ and H. Hunter Handsfield, MD¶¶*

Background: Dual human immunodeficiency virus (HIV)/syphilis rapid, point-of-care testing may enhance syphilis screening among high-risk populations, increase case finding, reduce time to treatment, and prevent complications. We assessed the laboratory-based performance of a rapid dual HIV/syphilis test using serum collected from patients enrolled in the Zimbabwe Sexually Transmitted Infections (STI) Etiology study.

Methods: Blood specimens were collected from patients presenting with STI syndromes in 6, predominantly urban STI clinics in different regions of Zimbabwe. All specimens were tested at a central research laboratory using the Standard Diagnostics Bioline HIV/Syphilis Duo test. The treponemal syphilis component of the dual rapid test was compared with the *Treponema pallidum* hemagglutination assay (TPHA) as a gold standard comparator, both alone or in combination with a nontreponemal test, the rapid plasma reagin test. The HIV component of the dual test was compared with a combination of HIV rapid tests conducted at the research laboratory following the Zimbabwe national HIV testing algorithm.

Results: Of 600 men and women enrolled in the study, 436 consented to serological syphilis and HIV testing and had specimens successfully tested by all assays. The treponemal component of the dual test had a sensitivity of 66.2% (95% confidence interval [CI], 55.2%–77.2%) and a specificity of 96.4% (95% CI, 94.5%–98.3%) when compared with TPHA; the sensitivity increased to 91.7% (95% CI, 82.6%–99.9%) when both TPHA and rapid plasma reagin were positive. The HIV component of the dual test had a sensitivity of 99.4% (95% CI, 98.4%–99.9%) and a specificity of 100% (95% CI, 99.9%–100%) when compared with the HIV testing algorithm.

Conclusions: Laboratory performance of the SD Bioline HIV/Syphilis Duo test was high for the HIV component of the test. Sensitivity of the treponemal component was lower than reported from most laboratory-based evaluations in the literature. However, sensitivity of the test increased substantially among patients more likely to have active syphilis for which results of both standard treponemal and nontreponemal tests were positive.

Rapid point-of-care syphilis tests hold promise to enhance syphilis screening and initiate timely treatment among populations at highest risk for this infection and its consequences, including women in antenatal care settings where rapid tests may prove to be an important tool in the prevention of congenital syphilis. Combining syphilis and human immunodeficiency virus (HIV) testing

From the *Rietmeijer Consulting, LLC, Denver, CO; †Colorado School of Public Health, University of Colorado Denver, Denver, CO; ‡Elizabeth Glazer Pediatric AIDS Foundation, Lesotho; §Centers for Disease Control and Prevention, Division of Global Health and Tuberculosis, Harare, Zimbabwe; ¶Centre for HIV and STIs, National Institute for Communicable Diseases, Johannesburg, South Africa; ||Department of Clinical Microbiology & Infectious Diseases, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; **Western Sydney Sexual Health Centre, Western Sydney Local Health District, Parramatta, New South Wales, Australia; ††Marie Bashir Institute for Infectious Diseases and Biosecurity & Westmead Clinical School, University of Sydney, Sydney, New South Wales, Australia; ‡‡Division of Infectious Diseases and Center for World Health, Department of Medicine, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA; §§Biomedical Research and Training Institute, Harare, Zimbabwe; and ¶¶University of Washington School of Medicine, Seattle, WA

Acknowledgments: ZiCHIRe Study Team: Luanne Rodgers, Laboratory Scientist, Vitalis Kupara, State Registered Nurse (SRN), Mebbina Muswera, State Registered Nurse and State Registered Midwife (SRN, SRM), Sarah Vundhla, State Registered Nurse and State Registered Midwife (SRN, SRM), Shirley Tshimanga, State Registered Nurse and State Registered Midwife (SRN, SRM). This study could not have been conducted without the gracious support and collaboration of the staff and patients of the following clinics: Harare: Mbare and Budiriro Clinics, Bulawayo: Nkulumane and Khami Road Clinics, Beitbridge: Dulibadzimu Clinic, Gutu: Gutu Rural Hospital.

Conflicts of Interest: None declared.

Sources of Funding: The Zimbabwe STI Etiology Study was supported by funds from the President's Emergency Plan for AIDS Relief (PEPFAR) through a cooperative agreement between the U.S. Centers for Disease Control and Prevention and the University of Zimbabwe Department of Community Medicine SEAM Project under the terms of Cooperative Agreement Number IU2GGH000315-01.

SD HIV/Syphilis BIOLINE Duo test kits were provided as an in-kind contribution to the study by the test manufacturer.

Disclaimer: The findings and conclusions of this report are those of the authors and do not necessarily represent the official position of the funding agencies.

Correspondence: Cornelis A. Rietmeijer, MD, PhD, MSPH, Rietmeijer Consulting LLC, 533 Marion Street, Denver, CO 20218. E-mail: kees@rietmeijer.us.

Received for publication April 15, 2019, and accepted May 29, 2019.

DOI: 10.1097/OLQ.0000000000001022

in a single rapid test device can expand syphilis testing coverage in areas where HIV testing is ubiquitous but syphilis testing lags behind.[1] Furthermore, combined testing offers the potential to leverage existing HIV testing resources to enhance syphilis screening among pregnant women to achieve the ultimate goal of elimination of mother to child transmission of both diseases.[2] A recently published systematic review shows that dual HIV/syphilis rapid tests have very high sensitivity and specificity for HIV and a lower but generally adequate sensitivity and specificity for syphilis.[3] The systematic review also highlights a higher sensitivity for the treponemal component of dual rapid tests when they are used to test serum in a laboratory setting compared with testing finger-prick blood in a clinic setting.[3]

Finally, dual tests are more cost-effective than separate single rapid tests for HIV and syphilis and prevent more adverse pregnancy outcomes.[4]

On such assay is the SD Bioline HIV/Syphilis Duo test, a solid phase immunochromatographic assay for the qualitative detection of antibodies to all isotypes (IgG, IgM, and IgA) specific to HIV-1/2 and *Treponema pallidum* simultaneously in human serum, plasma, or whole blood. The syphilis component of this lateral-flow assay tests for the presence of treponemal antibodies. According to the manufacturer, test sensitivity/specificity are, respectively, 99.9% and 99.67% for the HIV component of the test and 99.67% and 99.72% for the syphilis component.[5] In 2015, the SD Bioline HIV/Syphilis Duo Test (Abbott Laboratories, Lake Bluff, IL—hereafter "Bioline") was accepted for the World Health Organization list of prequalified in vitro diagnostics.[3] We evaluated the Bioline test as part of the Zimbabwe Sexually Transmitted Infections (STI) Etiology Study.

METHODS

The Zimbabwe STI Etiology Study, conducted in 2014 to 2016, examined the etiology of 3 common STI syndromes: vaginal discharge in women, urethral discharge in men, and genital ulcer disease in both men and women. Details of the study are available online,[6] and the main outcomes of the study have been published elsewhere.[7–10] An analysis of serological syphilis markers among patients in this study is published as a companion article in this issue of the Journal.[11] The following methodological details are pertinent to this report. Among 600 patients with STI syndromes recruited for this study at 6 mostly urban clinics in Zimbabwe, 494 accepted optional venipuncture for syphilis testing, all but 5 of whom also accepted HIV testing. All specimens were kept refrigerated after collection and shipped in a cooler box with cooling packs by overnight courier to the study laboratory at Wilkins Hospital in Harare, where they were kept refrigerated until further processing. All syphilis and HIV tests were conducted at the study laboratory. Serum specimens were tested with the Bioline test according to the manufacturer's instructions. Additional syphilis serology included both treponemal and nontreponemal tests, the *Treponema pallidum* hemagglutination assay (TPHA) and rapid plasma reagin (RPR) test respectively (both SPINREACT, Girona, Spain). All TPHA test runs included positive and negative controls. Rapid plasma reagin reactive samples were subsequently titrated up to 1:32 dilution for quantitative analysis.

HIV testing followed the algorithm promoted by the Zimbabwe Ministry of Health and Child Care standard national HIV testing algorithm: an initial test by First Response HIV1–2-O (Premier Medical Corporation, Daman, India); if positive, a confirmatory Alere Determine HIV1/2 test (Alere Inc. Waltham, MA); and, in case of discrepancy between these results, an INSTI HIV1/HIV2 test (bioLytical Laboratories Inc. Richmond, BC, Canada) as tie breaker.

All tests were performed according to their package inserts.

For this report, we compared the results of the treponemal component part of the Bioline test to TPHA as the gold standard and also to a combined TPHA/RPR outcome. We also applied our study data to 2 alternative syphilis screening algorithms: one based on a nontreponemal screening test (in our case RPR) followed, if reactive, by a treponemal (in our case TPHA) confirmatory test (also known as the "traditional" algorithm); and one based on a treponemal screening test (in our case the Bioline test) followed, if positive, by a second but different treponemal (TPHA) confirmatory test (a.k.a. the "reverse" algorithm).[12]

The HIV component of the Bioline test was compared with results of the standard HIV rapid test algorithm. Performance measures of the dual test included sensitivity, specificity, and predictive values of positive and negative results, and 95% confidence intervals (95% CI) were calculated. Yates' corrected chi square was used to test for statistical significance. Statistical analyses were conducted with the SAS software package (Cary, NC).

All patients were treated syndromically according to their presenting STI syndrome using the Zimbabwe STI treatment guidelines,[13] as summarized in the accompanying article in this issue of the journal.[11] Study participants were informed that they would be notified should test results come back positive, and study nurses called all patients for further management based on laboratory results.[6]

The study protocol was reviewed and approved by the Joint Research and Ethics Committee of Parirenyatwa Central Hospital, the Zimbabwe Medical Research Council, and the US Centers for Disease Control and Prevention.

RESULTS

Samples of 436 participants were successfully tested for syphilis and HIV by all assays. The TPHA was positive in 71 (16.3%) and the RPR was positive in 50 (11.5%). Both TPHA and RPR were positive in 36 (8.2%). Isolated RPR positivity was seen in 14 (3.2%) and both TPHA and RPR were negative in 351 (80.5%).

When compared with a positive TPHA alone as the gold standard, the syphilis component of the Bioline test had a sensitivity of 66.2% (95% CI, 55.2%–77.2%) and a specificity of 96.4% (95% CI, 94.5%–98.3%, Table 1).

Among samples positive by both TPHA and RPR, the Bioline test had a statistically significant higher sensitivity (91.7%, Table 1) compared with samples positive by TPHA alone (40.0%, $P < 0.0001$). The predictive value of a positive Bioline test was 78.3% (95% CI, 67.9%–88.8%) in samples testing positive for TPHA and 71.7% (95% CI, 58.7%–84.8%) in samples testing positive for both TPHA and RPR, whereas predictive values of a negative result were 93.6% (95% CI, 91.1%–96.1%) and 99.2 (95% CI, 98.2%–99.9%), respectively (Table 1).

Of the 71 TPHA-positive syphilis cases in our study, 36 (50.7%) would have been detected using the RPR-based "traditional" screening algorithm and 47 (66.2%) by the Bioline-based "reverse" algorithm ($P < 0.0001$). Of 50 RPR-positive cases, 36 (72.0%) were confirmed by TPHA, and 33 (91.7%) of these were also positive by Bioline. Of the 14 RPR-positive/TPHA-negative cases, 5 (35.7%) were positive by Bioline. Conversely, of 60 Bioline-positive cases, 47 (78.3%) were confirmed by TPHA, and of these 33 (70.2%) were positive by RPR. Of 13 Bioline-positive/TPHA-negative cases, 5 (28.4%) had a positive RPR test. Of the 36 RPR/TPHA dually positive cases, 3 (8.3%) were negative by Bioline, and 2 of these had RPR titers of 1:8 or greater.

TABLE 1. SD BIOLINE HIV/Syphilis Duo Test Performance

	Comparator								
	Syphilis Component						*HIV Component*		
	TPHA Positive			TPHA Positive and RPR Positive*			Standard HIV Testing Algorithm (see text)		
	n/N	%	95% CI	n/N	%	95% CI	n/N	%	95% CI
Sensitivity	47/71	66.2	55.2–77.2	33/36	91.7	82.6–99.9	180/181	99.4	98.4–99.9
Specificity	352/365	96.4	94.5–98.3	352/365	96.4	94.5–98.3	250/250	100	99.9–100
Positive predictive value	47/60	78.3	67.9–88.8	33/46	71.7	58.7–84.8	180/180	100	99.9–100
Negative predictive value	352/376	93.6	91.1–96.1	352/355	99.2	98.2–99.9	250/251	99.6	98.8–99.9

*Excluding samples positive on TPHA alone (n = 35).

CI indicates confidence interval; RPR, rapid plasma reagin; TPHA, *Treponema pallidum* hemagglutination assay.

Compared with the standard HIV testing algorithm, the HIV component of the Bioline test had near perfect performance with only one discrepant (false negative) result (Table 1).

DISCUSSION

The results of our study add to the growing literature on dual HIV/syphilis rapid testing. Obviously, any advantages a dual HIV/syphilis test may bring to enhanced screening access, reduction in time to treatment, and cost-effectiveness should be weighed against test performance. For HIV infection, our study confirms the excellent performance characteristics of the Bioline test as compared with the standard HIV rapid testing algorithm employed in Zimbabwe and many other developing countries.[3] For syphilis, the sensitivity of the Bioline compared with TPHA (66.1%) in our study was very similar to a 69.7% sensitivity found in a recently published study by Holden et al.[14] in a laboratory-based comparison between Bioline and the *Treponemal pallidum* particle agglutination assay from samples collected at a Baltimore STI clinic. These sensitivities are substantially lower than those found in a number of laboratory-based studies conducted elsewhere, but similar to the sensitivity found in clinic-based settings that used whole blood rather than serum samples.[3] However, in our study, sensitivity improved substantially when the Bioline was compared with a combination reference of both TPHA and RPR positivity, as has been shown elsewhere.[15] Compared with those with isolated positive TPHA results, patients testing positive by both treponemal and nontreponemal tests may be more likely to have active syphilis,[16] and the higher sensitivity of the dual test in this group is, therefore, encouraging.

When applying our data to screening algorithms based on either nontreponemal or treponemal testing, the Bioline test detected significantly more syphilis cases as defined by the TPHA gold standard. However, the value of correctly identifying an additional 14 (19.7%) TPHA-positive but RPR-negative cases was off-set by missing 3 dual TPHA/RPR-positive cases (of which 2 with RPR titers ≥1:8), given that these latter cases more likely represent active treponemal infections.

In addition to its potential role as a screening test, the Bioline can also be considered as a rapid confirmatory test in a nontreponemal ("traditional") screening algorithm. Our finding that 33 (91.7%) of 36 of dual RPR/TPHA-positive cases also had a positive Bioline, could have important practical advantages as the confirmatory results would not have to await delays of laboratory-based treponemal confirmatory tests and would allow for faster treatment initiation, as has been suggested elsewhere.[14]

The number of false-positive results for the treponemal component of the Bioline (3.6% in our study) is higher than what has been reported elsewhere for laboratory-based evaluations of the Bioline test.[3] The positive predictive value of 78% for the Bioline test found in our study implies that approximately one out of 5 positive tests in our population may have been false-positive results. Because the positive predictive value is dependent on prevalence, a greater proportion of positive rapid tests may be false-positive in settings where syphilis prevalence is lower than in STI clinics, including antenatal care settings. All positive rapid syphilis tests should, therefore, be followed by additional treponemal and nontreponemal testing and by a thorough medical and STI history and clinical examination to assess stage of infection and to determine appropriate treatment. However, in the absence of follow-up testing and assessment, the World Health Organization recommends treatment on the basis of rapid test results alone because the serious consequences of untreated syphilis are considered to outweigh the harm of overtreatment.[17]

There are a number of limitations to this study. First, the value of rapid testing is diagnosis at the point of care. In our study, specimens were collected in the field but the rapid test was conducted using serum at a central study laboratory by a trained and experienced laboratorian and not on-site by less-experienced, field-based clinicians using capillary finger-prick blood specimens, which may have resulted in potential biases toward better test performance. Second, comparison tests included locally readily available treponemal (TPHA) and nontreponemal (RPR) tests for the syphilis component of the rapid dual test and a standard rapid test algorithm for the HIV component. Potentially more sensitive/specific comparison tests, including the *Treponema pallidum* particle agglutination assay or treponemal antibody enzyme-linked immunosorbent assay for syphilis and a fourth-generation laboratory-based HIV antibody/antigen enzyme-linked immunosorbent assay could have resulted in different outcomes in the evaluation of the dual test performance. Thus, our finding that of the 13 Bioline false-positive syphilis tests (as determined by a negative TPHA), 5 had a positive RPR could be interpreted as a potential lack of sensitivity of the TPHA in our study. However, we were not able to confirm or reject this hypothesis because no other treponemal tests were available.

In conclusion, laboratory performance of the Bioline test was high for the HIV component of the test. The sensitivity of the treponemal component of the rapid test was lower than reported from laboratory-based evaluations in the literature. However, the sensitivity was higher with specimens from patients more likely to have active syphilis for which results of both standard treponemal and nontreponemal tests were positive.

REFERENCES

1. Dinh TH, Kamb ML, Msimang V, et al. Integration of preventing mother-to-child transmission of HIV and syphilis testing and treatment in antenatal care services in the Northern Cape and Gauteng provinces, South Africa. Sex Transm Dis 2013; 40:846–851.

2. Taylor MM, Peeling RW, Toskin I, et al. Role of dual HIV/syphilis test kits in expanding syphilis screening. Sex Transm Infect 2017; 93:458–459.
3. Gliddon HD, Peeling RW, Kamb ML, et al. A systematic review and meta-analysis of studies evaluating the performance and operational characteristics of dual point-of-care tests for HIV and syphilis. Sex Transm Infect 2017; 93(S4):S3–S15.
4. Bristow CC, Larson E, Anderson LJ, et al. Cost-effectiveness of HIV and syphilis antenatal screening: a modelling study. Sex Transm Infect 2016; 92:340–346.
5. Abbott. SD BIOLINE HIV/Syphilis Duo product information. https://www.alere.com/en/home/product-details/sd-bioline-hiv-syphilis-duo.html. Accessed 15 February 2019.
6. Rietmeijer C, Mungati M, Machiha A, et al. The Zimbabwe STI etiology study: design, methods, study population. STD Prevention Online Web site. http://www.stdpreventiononline.org/index.php/resources/detail/2114. Published 2017. Accessed May 29, 2019.
7. Mungati M, Machiha A, Mugurungi O, et al. The etiology of genital ulcer disease and coinfections with *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Zimbabwe: Results from the Zimbabwe STI etiology study. Sex Transm Dis 2018; 45:61–68.
8. Rietmeijer CA, Mungati M, Machiha A, et al. The etiology of male urethral discharge in Zimbabwe: Results from the Zimbabwe STI etiology study. Sex Transm Dis 2018; 45:56–60.
9. Kilmarx PH, Gonese E, Lewis DA, et al. HIV infection in patients with sexually transmitted infections in Zimbabwe—Results from the Zimbabwe STI etiology study. PLoS One 2018; 13:e0198683.
10. Chirenje ZM, Dhibi N, Handsfield HH, et al. The etiology of vaginal discharge syndrome in Zimbabwe: Results from the Zimbabwe STI etiology study. Sex Transm Dis 2018; 45:422–428.
11. Rietmeijer CA, Mungati M, Kilmarx PH, et al. Serological markers for syphilis among persons presenting with syndromes associated with sexually transmitted infections: results from the Zimbabwe STI Etiology Study. Sex Transm Dis 2019; 46:579–583.
12. (CDC) CfDCaP. Syphilis testing algorithms using treponemal tests for initial screening—four laboratories, New York City, 2005–2006. MMWR Morb Mortal Wkly Rep 2008; 57:872–875.
13. Ministry of Health and Child Care—AIDS and TB Unit. Management of Sexually Transmitted Infections and Reproductive Tract Infections in Zimbabwe. Harare, Zimbabwe: Ministry of Health and Child Care, 2013.
14. Holden J, Goheen J, Jett-Goheen M, et al. An evaluation of the SD Bioline HIV/syphilis duo test. Int J STD AIDS 2018; 29:57–62.
15. Black V, Williams BG, Maseko V, et al. Field evaluation of Standard Diagnostics' Bioline HIV/syphilis duo test among female sex workers in Johannesburg, South Africa. Sex Transm Infect 2016; 92:495–498.
16. Snowden JM, Konda KA, Leon SR, et al. Recent syphilis infection prevalence and risk factors among male low-income populations in coastal Peruvian cities. Sex Transm Dis 2010; 37:75–80.
17. World Health Organization. The use of rapid syphilis tests. http://apps.who.int/iris/bitstream/handle/10665/43590/TDR_SDI_06.1_eng.pdf. Published 2006. Accessed September 6, 2016.