ORIGINAL ARTICLE

Field evaluation of Standard Diagnostics' Bioline HIV/Syphilis Duo test among female sex workers in Johannesburg, South Africa

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ABSTRACT

Background Point-of-care tests provide immediate results with the opportunity for same-day interventions with improved public health outcomes. A dual HIV/syphilis test enables early treatment of both diseases.

Methods We conducted a field evaluation of the Standard Diagnostics' SD Bioline HIV/Syphilis Duo test (SD Bioline) among female sex workers. SD Bioline was conducted on finger-prick blood according to manufacturer's instructions and compared with (i) Genscreen HIV1/2 (third generation) and Vironostika Ag/Ab (fourth generation) assays for HIV, and (ii) *Treponema pallidum* particle agglutination (TPPA) and rapid plasma reagin (RPR) assays for syphilis. A negative TPPA test was considered negative, a TPPA-confirmed RPR titre ≤1:4 as past infection and a TPPA-confirmed RPR titre ≥1:8 as active syphilis. Sensitivity, specificity, positive and negative predictive values were calculated.

Results Of 263 women recruited, 14 (5.3%) declined an HIV test. Among the remaining 249 women, 187 (75.1%) were HIV positive, 51 (20.5%) had syphilis antibodies with seven (2.8%) active infections. For HIV, the sensitivity and specificity were 98.9% (95% CI 95.8% to 99.8%) and 100% (95% CI 92.7% to 100%). For syphilis, the sensitivity and specificity were 66.7% (95% CI 52.0% to 78.9%) and 98.0% (95% CI 94.5% to 99.3%). Sera with high TPPA titres were more likely to test positive.

Conclusions In field conditions, while the SD Bioline test has high sensitivity and specificity for HIV and high specificity for syphilis, the test has lower sensitivity for syphilis than reported from laboratory evaluations. As the dual test detects only two thirds of syphilis cases, it should only be used in areas with weak screening programmes.

INTRODUCTION

The WHO has developed plans to eliminate both congenital syphilis and paediatric HIV.[1 2] To do this, all pregnant women infected with either HIV or syphilis should be identified early in pregnancy to start them on antiretroviral therapy (ART) and treat them for syphilis, and again later in pregnancy to detect new infections. Syphilis and HIV control measures also need to be implemented among sex workers and other key populations who may be an ongoing source of new infections and may undermine elimination strategies. Point-of-care tests (POCT), using finger-prick blood, provide immediate results so that patients can be counselled on their results, treated for syphilis and started on ART without delay. They also provide an opportunity to screen partners of infected patients who may also be in need of treatment. Providing immediate results and counselling reduces the risk of loss to follow-up as patients do not need to return at a subsequent visit for laboratory results. Combining rapid tests into a single platform is both cost and time saving and reduces the time delays associated with obtaining laboratory results. Earlier treatment and higher treatment coverage for HIV and syphilis have important advantages for public health, improving the health of infected individuals and reducing transmission from mother to child and within communities.

The Standard Diagnostics' Bioline HIV/Syphilis Duo (SD Bioline) test is a POCT which simultaneously tests for HIV-1, HIV-2 and syphilis by detecting antibodies to gp41 for HIV-1 and gp36 for HIV-2 on one band and for antibodies to recombinant *Treponema pallidum* antigens on the second band of a cassette. As such, it is a treponemal-specific test for syphilis and measures previous exposure to syphilis, irrespective of past treatment. Confirmation of recent infections relies on the rapid plasma reagin (RPR) test. Laboratory evaluations of the SD Bioline test, performed by experienced laboratory technologists, using stored or fresh sera, have shown excellent results with most having over 99% sensitivity and specificity for both HIV and syphilis.[3] Before such tests are used, however, they need to be validated, in the laboratory and the field where performance may not be as good as in the laboratory. Here we report on a field evaluation of the SD Bioline assay used to test for HIV-1/2 and syphilis among female sex workers within the inner city of Johannesburg, South Africa.

METHODS

The Wits Reproductive Health and HIV Institute has worked with female sex workers in the inner city of Johannesburg since 1998. Current services include HIV counselling and testing (HCT), tuberculosis diagnosis and referral, syndromic management of sexually transmitted infections (STI), family planning, pregnancy testing, ART initiation and management, male and female condom distribution and management of minor common ailments. These services are provided in a dedicated area within a government primary healthcare clinic

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Table 1 Performance of the SD Bioline HIV/Syphilis Duo test among female sex workers in Johannesburg, South Africa

Laboratory-confirmed cases	Prevalence % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
HIV N=187	75.1 (69.0 to 80.1)	98.8 (95.8 to 99.8)	100 (92.7 to 100)	100 (97.5 to 100)	96.9 (88.2 to 99.5)
Syphilis N=51	20.5 (15.8 to 26.1)	66.7 (52.0 to 78.9)	98.0 (94.5 to 99.3)	89.5 (74.3 to 96.6)	91.9 (87.2 to 95.1)

NPV, negative predictive values; PPV, positive predictive values.

and as outreach services to women working in brothels. As part of a study of surveillance of STIs among these female sex workers, we evaluated the SD Bioline assay for HIV and syphilis. Female sex workers attending the above services were invited to participate in the study. Those consenting to participate completed an informed consent form. Additional consent was provided prior to HIV testing as women consenting to be tested for HIV receive standard pretest and posttest HIV counselling as per the South African HCT policy.[4]

A research nurse was trained on conducting the test, according to the manufacturer's instructions, at the start of the study. Finger-prick blood was used for the evaluation of the SD Bioline test. Participants who tested positive for either HIV or syphilis on the SD Bioline test were managed accordingly.

The SD Bioline test was compared with laboratory tests for HIV and syphilis which we used as the gold standard. Blood was collected by venipuncture and sent to the National Institute of Communicable Diseases' (NICD) Centre for HIV and STIs for separation of serum prior to further testing. Laboratory technologists were blinded to the SD Bioline result. For HIV, the third generation Genscreen HIV 1/2 EIA V2 (BioRad, France) and the fourth generation Vironostika Ag/Ab EIA (bioMérieux, France) assays were used to confirm serostatus. For syphilis, a combination of the *T. pallidum* particle agglutination (TPPA) assay (Serodia, Fujirebio, Japan) and the RPR assay (Immutrep RPR, Omega Diagnostics, UK) were used to determine syphilis status. A negative TPPA test, regardless of RPR titre, was interpreted as no prior syphilis infection; an RPR titre ≤1:4 with a positive TPPA test was interpreted as a past infection without current syphilis; a TPPA-confirmed RPR titre ≥1:8 was interpreted as active syphilis. For syphilis, the SD Bioline result was compared with the TPPA result and to active syphilis (TPPA positive, RPR titre ≥1:8).

To confirm the sample size estimates, we use a Monte Carlo simulation. We set the true prevalence of the condition under investigation to be 25%, 50% or 75% with the true sensitivity and specificity set to either 95% or 75% and the sample size set to 250. This can measure the sensitivity and specificity to within ±5% for true values of the sensitivity and specificity set to 95% and to within ±10% with true values of the sensitivity and specificity set to 75%. A sample of 250 gives us an acceptable level of precision in estimates of the true prevalence, sensitivity and specificity.

Sensitivity, specificity, positive predictive values and negative predictive values with 95% CIs were calculated for HIV and syphilis assuming binomial errors. To test an association between TPPA titre and sensitivity for detection of syphilis, we compared the mean TPPA titre values, given as the $\log_{10}$(number of dilutions), between false negative and true positives, using Student's t test.

The study was approved by the Human Research Ethics Committee University of Witwatersrand (Protocol M130907).

RESULTS

We recruited 263 women of whom 14 (5.3%) declined to have an HIV test. Among the remaining 249 women, the NICD laboratory results showed that 187 (75.1%, 95% CI 69.2% to 80.3%) were HIV positive with no cases of acute HIV infection and 51 (20.5%) women had evidence of past exposure to syphilis while 7 of these had active syphilis. There were no false-positive RPR tests. Table 1 gives the sensitivity and specificity of the SD Bioline HIV and syphilis results. For HIV, the sensitivity and specificity were 98.9% (95% CI 95.8% to 99.8%) and 100% (95% CI 92.7% to 100%), respectively. For syphilis, the sensitivity and specificity were 66.7% (95% CI 52.0% to 78.9%) and 98.0% (95% CI 94.5% to 99.3%), respectively, but when considering only those with active syphilis (higher TPPA titres), the sensitivity increases to 85.8% (95% CI 42.0% to 99.2%). The mean TPPA titre for active syphilis was 4.3 (3.6 −4.8) and for past infection it was 3.0 (2.4−3.5) (p=0.004) (figure 1).

Figure 2 shows that at the lowest titre all test results were false negatives, whereas at the highest titre all correctly tested positive. This association between higher titre and sensitivity was confirmed by the finding of a significantly higher mean titre (3.51) in the true positives than the false negatives (2.78), p=0.007 by t test. HIV status did not affect the sensitivity of the syphilis result (data not shown).

DISCUSSION

Our field evaluation of the SD Bioline test demonstrates high sensitivity and specificity for HIV, with results comparable with well-established rapid HIV diagnostic tests.[5 6] HIV POCTs have been the mainstay of HIV diagnosis in HIV programmes around the world for decades but many different tests have been used including some with lower field validation sensitivity and specificity compared with laboratory evaluations.[7 8] WHO recommends a sensitivity and specificity of 99% and 98%, respectively, for HIV POCTs.[9] The SD Bioline test fulfils these criteria, both in laboratory and in field evaluations and would be a suitable option to use clinically and in screening programmes in the diagnosis of HIV infection.

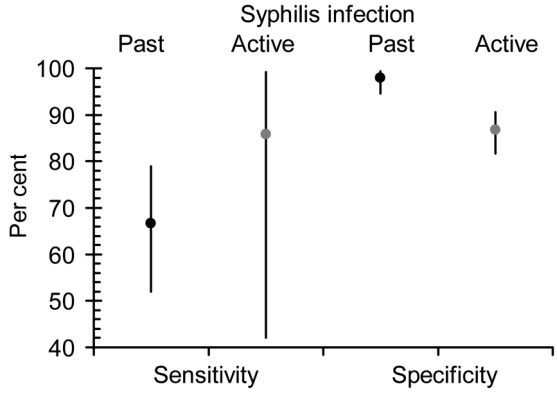

Figure 1 Plot of sensitivity and specificity of past and active syphilis infection. Black: past syphilis infection; grey: active syphilis infection.

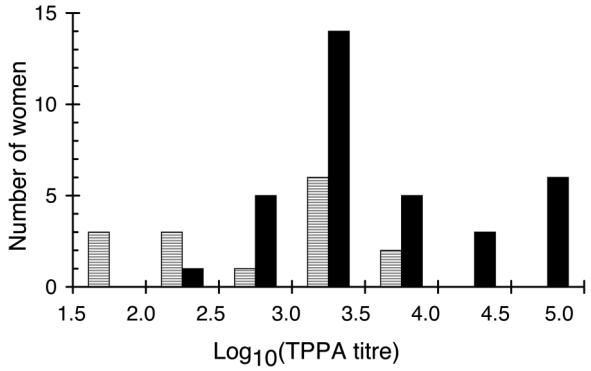

Figure 2 Plot of $\log_{10}$(*Treponema pallidum* particle agglutination (TPPA) assay titre) for women with positive TPPA assay results comparing false negative with true positive syphilis tests as detected by SD Bioline HIV/Syphilis Duo test. Black: true positive; horizontal grey: false negatives.

The performance of the syphilis component of the SD Bioline test is less satisfactory. When compared with the laboratory *T. pallidum* agglutination assay, the sensitivity is 67% (table 1), meaning that a third of those cases with proven exposure to syphilis are missed with this POCT. The SD Bioline measures exposure to syphilis (TPPA) rather than active disease. The TPPA titre values for true positive tests, as determined by laboratory-confirmed positive SD Bioline treponemal antibody results, were significantly higher than for false-negative syphilis tests; women whose sera had lower TPPA assay titres were more likely to test false negative for syphilis with the SD Bioline assay (figure 2). These data suggest that the test needs to be sufficiently sensitive to a TPPA titre of 2.5 or a dilution of 1:320. In our study, the seven women who had active disease based on those cases with high RPR results (titre ≥1:8) shows a higher sensitivity, although the small number limits the interpretation of this result. However, cases with active syphilis should have higher TPPA titres potentially making this test more sensitive in identifying people who need to be treated. As the SD Bioline measures exposure to syphilis rather than active disease, it will result in overtreatment for syphilis in countries where syphilis management is based on RPR testing alone and where patients' recollection of prior treatment is poor.

The performance of the SD Bioline test in our study is lower than that found in a previous evaluation which used laboratory personal and techniques to conduct the tests and reported sensitivities and specificities for both HIV and syphilis of 100%.[9 10] Our results for detection of treponemal antibodies by the SD Bioline test are similar to other POCT evaluations where syphilis tests show a lower sensitivity compared with laboratory standards when evaluated in the field rather than the laboratory.[11 12] This highlights the importance of quality assurance (QA) for POCT testing to ensure tests conducted in the field approach existing laboratory standards. Robust QA programmes require laboratory support and programme funding to operate effectively but will ensure optimal POCT performance and minimise patient harm from erroneous results.

To increase sensitivity for the detection of syphilis, repeat testing at a later time point should be done for patients with negative SD Bioline test. To avoid overtreatment of people with previously treated syphilis, RPR serological testing could be done, although in resource-poor environments it may not be possible to meet the laboratory and personnel demands required for an effective syphilis diagnostic programme.

Interventions to screen for STIs among key populations have been poorly implemented, particularly in the African region, which relies predominantly on symptomatic screening for case identification. The prevalence of HIV and syphilis has recently been increasing in a number of key populations around the world.[13–20] As both infections are predominantly asymptomatic for long periods of time, there is a need to diagnose these infections through use of cheap screening tests to prevent ongoing spread within the community. Introduction of syphilis POCTs into the Avahan programmes in India more than doubled testing rates among clinic attendees.[21] The introduction of a combined POCT into screening and treatment programmes for pregnant women and key populations in countries with poor or no screening programmes, such as sex workers and men who have sex with men, will strengthen prevention efforts and is required if countries are to achieve elimination of congenital syphilis and paediatric HIV.

Limitations of this study include that the tests were evaluated by a trained research nurse whose performance may not reflect the technical capacity of clinical staff in busy clinics, particularly in resource-poor settings. Studies with larger sample sizes are needed to more precisely define the relationship between titre and the probability of false-negative results. As the original sample size was calculated to evaluate syphilis overall, the sample of active syphilis cases was too small to accurately define the performance of the SD Bioline test in those enrolled women with active syphilis.

CONCLUSION

When evaluating a new POCT, it is important to undertake both laboratory and field evaluations to determine its performance in both settings. Although the SD Bioline performs well for HIV diagnosis, the assay has lower sensitivity for syphilis detection in our field setting compared with published laboratory evaluations. Using the SD Bioline test, in screening programmes will detect both HIV and two thirds of individuals exposed to syphilis. However, the test's performance is suboptimal at low titres of treponemal antibody and so its use should only be considered in settings where routine laboratory testing is weak.

Key messages

- Field evaluation of Standard Diagnostics' HIV/Syphilis Duo test performs well for HIV but is less sensitive and specific for syphilis.
- Sera with high *Treponema pallidum* particle agglutination titres were more likely to test positive.
- The test should only be implemented where existing syphilis screening programmes are absent or weak.

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Contributors VB, DAL, FR and VM conceptualised the study and wrote the protocol and conducted the work for the study; VB and BGW analysed the data; VB, BGW and DAL wrote the manuscript; VB, BGW, DAL and HVR edited the manuscript.

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Competing interests None declared.

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