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A field evaluation of a rapid dual immunoassay for human immunodeficiency virus and syphilis antibodies, Hanoi, Vietnam

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Abstract

The SD BIOLINE HIV/Syphilis Duo (SD BIOLINE DUO) rapid test is a dual rapid lateral flow immunoassay that detects antibodies to both human immunodeficiency virus (HIV) and *Treponema pallidum* (TP) 'syphilis' via fingerprick whole blood. We evaluated the field performance of the SD BIOLINE HIV/Syphilis Duo test among two populations in Hanoi, Vietnam – men who have sex with men (MSM) and pregnant women. We also surveyed factors that influence participants' willingness to test for HIV and syphilis. This test has the potential to increase HIV and syphilis screening in low-resource settings. Patients who received healthcare services at a sexual health clinic for MSM and a district antenatal care center in Hanoi, Vietnam were recruited for the study. Participants with HIV and syphilis were intentionally recruited for adequate test performance evaluation via convenience sampling. At each facility, venipuncture blood specimens were obtained for reference testing for HIV and TP using SD BIOLINE HIV 1/2 3.0 and TP particle agglutination, respectively. SD BIOLINE DUO was compared to the standard reference tests and sensitivity and specificity were calculated. We calculated 95% confidence interval (CI) using the exact binomial method. We used conjoint analysis to identify test attributes that are associated with participant likelihood to seek HIV and syphilis testing. Of 280 participants, 100 (35.7%) were MSM and 180 (64.3%) were pregnant women. Of MSM, 17 (17.0%) were HIV positive and 49 (49.0%) were TP seropositive. All women were negative for both HIV and syphilis antibodies. For HIV antibody testing, sensitivity and specificity were 100.0% (95% CI: 80.5–100.0%) and 100.0% (95% CI: 98.6–100.0%), respectively. For the syphilis antibody testing, sensitivity and specificity were 83.1% (95% CI: 71.0–91.6%) and 100.0% (95% CI: 98.3–100.0%), respectively. Potential for false positives, preference for one blood draw over two, and shorter wait time for testing results were the highest ranked attributes by participants according to their willingness to test. The SD BIOLINE HIV/Syphilis Duo rapid test demonstrated very good performance in this field setting and participants preferred attributes that aligned well with this test.

Keywords

Point of care, HIV, syphilis, *Treponema pallidum*, Vietnam

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Background

Human immunodeficiency virus (HIV) and *Treponema pallidum* (TP), the organism that causes syphilis, are a serious public health problem in low-middle income settings (LMICs) such as Vietnam. In Vietnam more than 240,000 adults aged 15 years and over are living with HIV infection with an estimated prevalence of

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0.4% in the general population.[1] However, there is a concentrated HIV subepidemic among special populations in Vietnam including men who have sex with men (MSM), people who inject drugs, and female sex workers.[2] Additionally, many Vietnamese MSM may be less likely to access HIV testing services and unaware of their HIV serostatus.[2] In 2008, the World Health Organization (WHO) reported three million cases of syphilis in the Southeast Asia region.[3] An observational cohort study involving 657 MSM from Taiwan, Hong Kong, and South Korea reported a syphilis incidence of 7.64 per 100 person-years among those HIV positive.[4] Increased screening and treatment programs are needed.

Mother-to-child transmission (MTCT) of HIV and syphilis coinfection is also a significant global public health issue. In 2015, The Global Fund to Fight AIDS, Tuberculosis and Malaria estimated that there were 2760 pregnant women and 455 newly diagnosed infants living with HIV infection in Vietnam.[5] Untreated syphilis infection in pregnant women leads to adverse outcomes including an estimated 212,327 stillbirths, 91,764 newborn deaths, 65,267 premature births, and 151,547 congenital cases globally.[6] The WHO has indicated that targeted efforts to conduct HIV and syphilis testing among pregnant women are needed in order to reach elimination of MTCT.[7] HIV testing services are routinely implemented in antenatal settings in Vietnam; however, screening for syphilis is often not performed.[8,9] A study involving 2872 Vietnamese pregnant women found that simultaneous screening for HIV and syphilis in antenatal settings is possible.[9]

Rapid point-of-care (POC) testing for HIV and syphilis provides an immediate antibody result and can be performed outside a standard laboratory setting. POC tests may allow for increases in screening for both HIV and syphilis infection and have been found to be particularly useful in resource-limited settings.[10] The advent of dual POC testing, which allows for detection of both infections using one specimen and one test, may have additional advantages. Those tests have been found to be cost effective, feasible, easy to use, and have demonstrated high performance in various settings.[10–12]

The SD BIOLINE HIV/Syphilis Duo (SD BIOLINE DUO) rapid test is a qualitative POC, rapid immunoassay that detects both HIV and TP antibodies in whole blood and serum. The SD BIOLINE DUO has shown high performance in both laboratory and field settings.[12–15] In addition to evaluating its performance, it is also important to gain a further understanding of patient preferences regarding HIV and syphilis testing.

The objective of our study was twofold: (1) to conduct a field evaluation of the SD BIOLINE DUO and (2) identify factors associated with willingness to test for HIV and syphilis infection in MSM and pregnant women in Hanoi, Vietnam. We hypothesized that this lateral flow immunoassay would indicate a high sensitivity and specificity in our participant population. We also hypothesized that factors such as cost and test accuracy would influence participant willingness to test for HIV and syphilis.

Methods

Participant study site and study population

The study was conducted at two clinical sites in Hanoi, Vietnam: Sexual Health Promotions Clinic at Hanoi Medical University and Ha Dong Antenatal Women's Clinic between May 2017 and July 2017. These sites provide services to MSM and pregnant women, respectively. Inclusion criteria consisted of the following: (1) 18 years and older and (2) willingness to be counseled, tested, and treated (if necessary) for HIV and/or syphilis. In order to evaluate the performance of this lateral flow immunoassay, sample size was determined by the sample size formulation. A minimum sample size of 246 participants were required for our study, assuming 80% sensitivity of the device for detection of HIV and syphilis, precision of $\pm 5\%$, and confidence level 95%. For the purpose of our study, we implemented a convenience sampling series. Participants with HIV and TP were intentionally recruited to ensure adequate sample size for calculation of test performance characteristics. The study was approved by the Institutional Review Boards at both University of California Los Angeles (IRB no: 17-000630 and Hanoi Medical University (IRB no: 00003121).

The SD BIOLINE HIV/Syphilis Duo rapid test

The SD BIOLINE HIV/Syphilis Duo rapid test (Alere Inc., United States) is a qualitative rapid immunoassay that detects antibodies (IgG, IgM, IgA) to HIV-specific antigens (HIV-1 gp41, sub O, HIV-2 gp36) and recombinant TP antigen (17 kDa) via fingerprick/whole blood, serum, and plasma.[16] Testing was conducted according to manufacturer's instructions. The SD BIOLINE DUO test device is composed of a HIV, TP, and control line. Whole blood specimens were collected using fingerpick and applied in the application well of the panel. Three drops of buffer solution were added. The test works through lateral flow of the specimen and buffer solution from the application well to the test window. The SD BIOLINE DUO yields results

after 15 min. A positive control line indicates an adequately working test. If HIV and/or TP antibodies are present, then a red indicator will appear next to each respective line which indicates a positive result. Results of this dual rapid antibody test under evaluation were not reported to participants.

Reference testing

Venous whole blood was obtained for reference testing using SD BIOLINE HIV-1/2 3.0 (Standard Diagnostics Inc., Gyeonggi-do, Republic of Korea) and TP particle agglutination (SERODIA-TPPA, Fujirebio Diagnostics, Japan) for HIV and TP antibody detection at both clinical sites. Those tests are used in those settings for standard-of-care testing to assist in clinical diagnosis. Reference testing was performed in a laboratory setting affiliated with each healthcare facility. Standard reference test results were reported to participants. During participant testing, test performers, reader, and evaluators were completely blinded to clinical information and reference standard results. Laboratory reference test evaluators were also completely blinded to the clinical information and SD BIOLINE DUO test results of participants. No clinical interventions were performed as venous blood specimens were obtained simultaneously with the lateral flow immunoassay.

Conjoint survey

To assess attributes associated with willingness to test for HIV and syphilis, all subjects participated in a conjoint survey before fingerprick sample collection. A trained research assistant conducted a one-on-one interview with each participant at both clinical sites. Participants were presented eight hypothetical testing profiles consisting of seven attributes that focused on a person's likelihood to test. Each testing profile was presented on a notecard for the participant to view. The seven attributes included (1) ***cost*** (free versus $4.39 USD), (2) ***accuracy*** (no potential for false positive result versus potential for false positive result), (3) ***testing result time*** (20 min versus one week), (4) ***blood draw method*** (finger prick versus venipuncture), (5) ***number of draws*** (1 versus 2), (6) ***test location*** (POC versus laboratory based), and (7) ***provider initiated testing*** (test was recommended by provider versus test was not routinely ordered by the provider).

Data analysis

This lateral flow dual immunoassay was compared to the standard clinic reference tests. We calculated sensitivity and specificity. The exact binomial method was used to calculate 95% confidence intervals (CI). We measured the agreement between the dual HIV/TP antibody and reference tests by using Cohen's kappa statistic.

For the metric conjoint analysis, participants responded on a Likert scale of 1–5. The Likert scale consisted of five levels: (1) very unlikely, (2) somewhat unlikely, (3) neutral/do not know, (4) somewhat likely, (5) very likely. The Likert score was converted to a 100-point preference score using multiplication. Higher scores indicate increased willingness to use the test. A part-worth utility value was calculated for each level of each attribute.[17] A part-worth utility is the preference or utility associated with each level of each attribute used to define the test. Attributes with the highest part-worth utility range are considered the most important in determining preference. We fit a conjoint analysis model for each participant using a main effects ANOVA.[17] We calculated mean importance values for each attribute using the percentages from relative ranges in the attribute's utility values. We summarized results across participants. We conducted all data analyses using SAS v9.4 (Cary, NC, USA). Attributes that were incorrectly ranked were not calculated in the analysis.

There were no indeterminate results for the index and standard tests. However, regarding the conjoint analysis survey, some participants elected not to answer certain components. Unanswered choices were not calculated in the overall score for that particular participant. In addition, any data recording error by the research assistant during collection was not calculated in the overall participant score.

Results

Of 280 participants, 100 (35.7%) were MSM and 180 (64.3%) were pregnant women. The median age was 26 years with a range of 18–49 years. Table 1 shows the detailed demographics of the sample. Of the 100 MSM, 17 (17.0%) were HIV infected and 49 (49.0%) tested positive for antibody to TP using reference tests. All women were negative for both HIV and TP antibodies. For HIV, sensitivity and specificity were 100.0% (95% CI: 80.5–100.0%) and 100.0% (95% CI: 98.6–100.0%), respectively (kappa coefficient: 1.00 [95% CI, 1.00–1.00]). For TP antibody testing, sensitivity and specificity were 83.1% (95% CI: 71.0–91.6%) and 100.0% (95% CI: 98.3–100.0%), respectively (kappa coefficient: 0.89 [95% CI: 0.82–0.95]) (Table 2) (see Tables 2 to 4). Please refer to Figure 1 for a total participant flow chart diagram of the dual rapid immunoassay for HIV and TP.

Figure 2 shows the average part-worth utility values and average importance values. Among the attributes that impacted likelihood to test, accuracy ranked the highest and on average accounted for 45.30 of test type

preference (no potential for false positive versus some potential for false positive result; SD =14.38), followed by number of blood draws (1 versus 2; average importance value= 11.96, SD = 8.23), testing result time (20 min versus one week; average importance value = 11.66, SD= 8.21), cost (free versus not free; average importance value = 10.48, SD= 7.94), blood draw method (fingerprick versus venipuncture; average importance value = 8.12, SD = 6.39), provider-initiated testing (test was recommended by provider versus test is not a routinely recommended test; average importance value = 6.49, SD = 5.88), and test type (POC versus laboratory-based; average importance value = 5.95, SD = 5.5).

Table 1. Participant demographics.

Demographic characteristics	N (%)
Gender	
Men	100 (35.7%)
Women	180 (64.3%)
Age	
Median age: 26 years	
Range of 18–49 years	
Education	
High school education	90 (32%)
Currently in or finished college	164 (58.7%)
Monthly household income	
Less than $219.50 USD	9 (3.21%)
$219.50–$439.00 USD	111 (39.6%)
$439.00–$658.50 USD	101 (36.1%)
More than $658.50 USD	48 (17.14)

Discussion

Our study evaluated the field performance of a lateral flow dual immunoassay for HIV and syphilis and testing attributes that impact a patient's willingness to test. This test was found to have excellent specificity for HIV (100.0%) and TP (100.0%) antibodies. This test also revealed excellent sensitivity for HIV (100.0%) and good sensitivity for TP (83.1%) antibodies.

Findings from our study are consistent with a systematic review that summarized SD BIOLINE performance in a number of laboratory and field settings.[14] It found that the SD BIOLINE for HIV had a sensitivity

Table 2. Field performance of the SD BIOLINE HIV/Syphilis Duo rapid test in Vietnam for detection of human immunodeficiency virus and *Treponema pallidum* antibodies using a dual HIV/syphilis test, 2017.

Antibody detection	N	True Pos	False Pos	False Neg	True Neg	Sensitivity estimate (exact 95% CI)	Specificity estimate (exact 95% CI)	Kappa coefficient (95% CI)
HIV	280	17	0	0	263	100.0% (80.5–100.0%)	100% (98.6–100.0%)	1.00 (1.00, 1.00)
T. pallidum	280	49	0	10	221	83.1% (71.0–91.6%)	100.0% (98.3–100.0%)	0.89 (0.82–0.95)

SD BIOLINE DUO: SD BIOLINE HIV/Syphilis Duo.
HIV reference test was SD BIOLINE DUO HIV 1/2 3.0 (Standard Diagnostics Inc., Gyeonggi-do, Republic of Korea).
Treponemal reference test was *T. pallidum* particle agglutination (SERODIA-TPPA, Fujirebio Diagnostics, Japan).

Table 3. Field performance of the SD BIOLINE HIV/Syphilis Duo rapid test in Vietnam for detection of human immunodeficiency virus and *T. pallidum* antibodies using a dual HIV/syphilis test, 2017 by MSM.

Antibody detection	N	True Pos	False Pos	False Neg	True Neg	Sensitivity estimate (exact 95% CI)	Specificity estimate (exact 95% CI)	Kappa coefficient (95% CI)
HIV	100	17	0	0	83	100.0% (80.5%, 100.0%)	100.0% (95.7%, 100.0%)	1.00 (1.00, 1.00)
T. pallidum	100	49	0	10	41	83.1% (71.0%, 91.6%)	100.0% (91.4%, 100.0%)	0.80 (0.69, 0.92)

MSM: men who have sex with men; SD BIOLINE DUO: SD BIOLINE HIV/Syphilis Duo.

Table 4. Field performance of the SD BIOLINE HIV/Syphilis Duo rapid test in Vietnam for detection of human immunodeficiency virus and *T. pallidum* antibodies using a dual HIV/syphilis test, 2017 by pregnant women.

Antibody detection	N	True Pos	False Pos	False Neg	True Neg	Sensitivity estimate (exact 95% CI)	Specificity estimate (exact 95% CI)	Kappa coefficient (95% CI)
HIV	180	0	0	0	180	NA	100.0% (98.0%, 100.0%)	NA
T. pallidum	180	0	0	0	180	NA	100.0% (98.0%, 100.0%)	NA

SD BIOLINE DUO: SD BIOLINE HIV/Syphilis Duo.

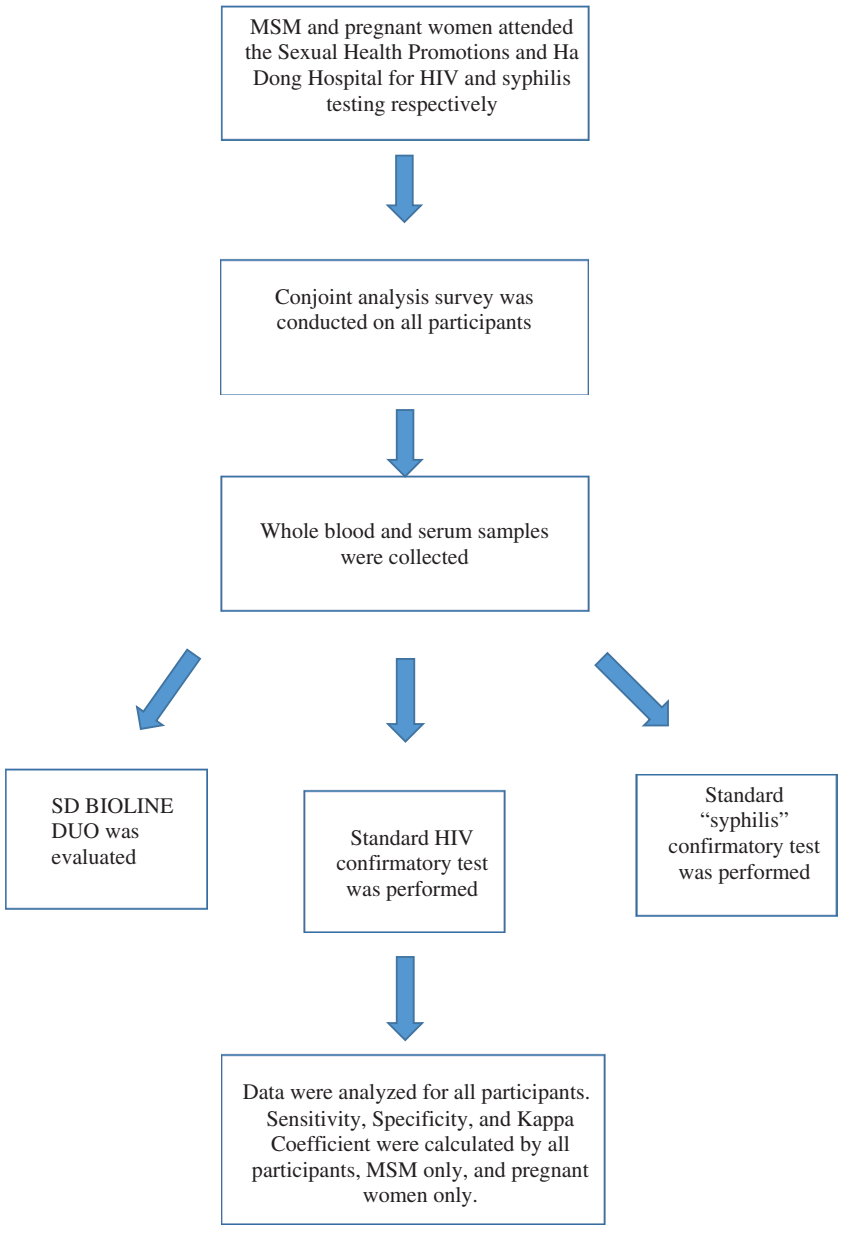

Figure 1. Procedural flow chart. MSM: men who have sex with men; SD BIOLINE DUO: SD BIOLINE HIV/Syphilis Duo.

and specificity ranging from 98 to 100% and 97 to 100%, respectively.[14] For treponemal antibody detection, the reported sensitivity and specificity ranged from 67 to 100% and 91 to 100%, respectively.[14] Therefore, our findings of the sensitivity for TP antibody are within the range found in other field studies.[10,14]

We also identified several factors that impacted participant preferences on willingness to test. High accuracy, shorter wait time, and one blood draw were ranked as the highest preferences by participants according to their willingness to test. Our findings were similar to another study, who also found that patients in antenatal care settings preferred rapid testing due to their shorter wait times and faster result times.[8] In a study conducted by Nguyen et al.[18] it was also suggested that testing factors such as access to HIV testing services, stigma, and patient acceptability may also influence women in Vietnams' preference to utilize HIV testing services. We observed that cost (free testing versus paying $4.39 USD) was not a highly-ranked attribute among participants. These findings were consistent with Hoang et al.[19] who found that Vietnamese MSM were willing to pay up to $8.78 USD for HIV services (including testing) as long as services provided less waiting time and

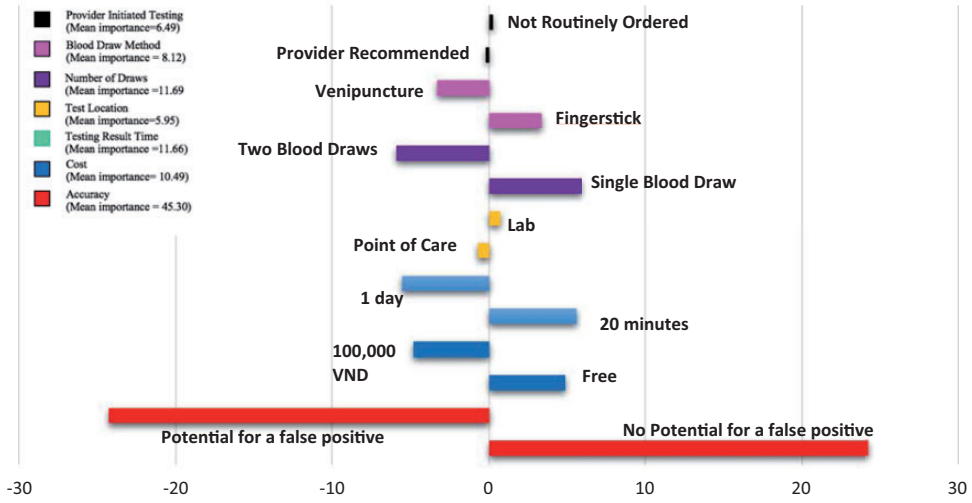

Figure 2. Average part-worth utility values on ranked attributes impacting participant willingness to engage in HIV and syphilis testing services. VND: Vietnamese Dong.

healthcare workers displayed friendly attitudes. However, García et al.[20] found that MSM who come from households with incomes less than $219.50 USD per month were less likely to access HIV testing services. Bristow et al.[15] found that Haitian patients ranked cost as a prioritizing factor when deciding on whether to test for HIV and syphilis. Cost may have been ranked as less important than other attributes among our sample, given that a majority of the participants in our study came from household incomes greater than $219.50 USD per month.

Targeted dual HIV and syphilis screening interventions among at-risk populations in Vietnam are critically important for both treatment and prevention. The Integrated Biological and Behavioral Surveillance 2013 projected significantly low uptake of HIV testing among MSM in four major Vietnamese provinces including Hanoi.[2] Furthermore, a previous Internet survey involving 2077 Vietnamese MSM found that 76.5% of participants reported they had never been tested for HIV.[20] A number of factors may be associated with low uptake in HIV testing among this population including stigma and discrimination by healthcare providers.[19] Rapid POC dual tests may have the potential to increase screening coverage by being implemented outside of healthcare settings. WHO reported that expanding HIV testing services could significantly reduce the spread of HIV infection.[7] In addition, dual POCs, such as the SD BIOLINE, have been found to be quick, easy to use, cost effective, and acceptable in a number of low- and middle-resource countries.[10,11] Screening for syphilis, using dual POCs, should be implemented in settings where screening for syphilis is limited.[9] A systematic review found that antenatal settings with limited uptake of both HIV and syphilis in LMICs showed rapid improvement in testing with the introduction of rapid diagnostic testing.[21] Therefore, dual POCs may have the potential to increase detection of both HIV and syphilis infections and improve clinical outcomes in key populations.[9]

There is no perfect test for detection of syphilis and HIV infection and therefore, misclassification of disease status by reference tests may lead to biased evaluation results. However, our study used highly accurate reference tests and the tests used for reference testing are those that are used in clinical practice in our study settings. Participants in our study were concerned about the potential for false positive results. It is important to note that TP antibodies can persist even after curative treatment and qualitative tests, like the SD BIOLINE DUO, may therefore deliver a positive result when treatment is not needed, especially in high prevalence settings. Therefore, there may still be a need for additional testing. Reactive HIV antibodies of dual immunoassays are to be interpreted as a preliminary positive result and should always be followed by a confirmatory test. Selection bias may also play a role in our study given the intentional recruitment of previously diagnosed HIV and syphilis participants. Lastly, patient preference and the calculated importance values depend on the particular attribute levels chosen for the study. For example, with a wider range of prices, cost may have been deemed more important. In addition, when we compute an attribute's importance, it is always relative to the other

attributes being used in the study; therefore our results may not be comparable to other studies that use different attributes. Overall, the SD BIOLINE DUO performed well in this field setting. Furthermore, we were able to access populations who are highly vulnerable to HIV and syphilis infection.

Our study was subject to some limitations. First, all women were negative for both HIV and TP infections, thereby making us unable to evaluate test sensitivity among pregnant women. Second, the moderate sample size in our study, particularly those with HIV infection and syphilis, impacts the precision of our estimates for both HIV and syphilis. Third, due to the intentional recruitment of HIV- and syphilis-infected MSM our findings may not be generalizable to other populations. However, our evaluation findings for the SD BIOLINE DUO have been found to be consistent with other studies performed in other settings.

The SD BIOLINE DUO demonstrates very good performance for screening for HIV and syphilis. Participants preferred tests that were accurate, had fewer blood draws, and reported faster result times, which are all qualities that are consistent with this lateral flow immunoassay. The SD BIOLINE DUO has the potential to help increase screening for both syphilis and HIV infections in resource-limited settings such as Vietnam. This study highlights key populations who have been historically less engaged with HIV testing services both within Vietnam and globally. Future studies should evaluate the rollout of dual rapid POC tests time to treatment and clinical outcomes among high-risk populations in resource-limited settings.

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