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Contributions to the improvement and understanding of new diagnostics for HIV infection and syphilis

A dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy in Epidemiology

by

Claire Catherine Bristow

2015
ABSTRACT OF THE DISSERTATION

Contributions to the improvement and understanding of new diagnostics for
HIV infection and syphilis

by

Claire Catherine Bristow

Doctor of Philosophy in Epidemiology
University of California, Los Angeles, 2015

Professor Frank J. Sorvillo, Chair

Syphilis is a curable disease, yet 10 million persons worldwide are infected each year, of which 1.4 million are pregnant women.\textsuperscript{1-3} Syphilis is an infection caused by the spirochete \textit{Treponema pallidum} and frequently has atypical presentations that may be difficult to differentiate from other sexually transmitted infections (STIs) making effective diagnostics essential to the identification of infection.\textsuperscript{4} Additionally, the manifestations of the disease vary depending on the duration of infection and time of presentation. There are four stages-primary, secondary, latent (early and late), and tertiary.

Syphilis screening and treatment programs that utilize laboratory-based testing have been hampered by limited laboratory access, long turn-around time for results, and loss to follow-up of syphilis infected individuals.\textsuperscript{5-8} When syphilis diagnostic testing involves multiple tests...
performed off-site, only a proportion of infected individuals receive treatment and continued transmission occurs.\textsuperscript{9,10}

A rapid test is a simple point-of-care test that can be used in a variety of settings and provides a result to guide clinical management during the time of the initial consultation (ideally within 30 minutes or less). The advent of rapid point-of-care tests for syphilis has reduced barriers and allowed for new health systems approaches to syphilis prevention, including same day testing and treatment.\textsuperscript{5,6,11-13} Dual rapid tests that have multiple analytes for the detection of antibodies for both HIV and syphilis infections are now available.\textsuperscript{14}

Our first study addressed preferences for dual HIV/syphilis tests through a conjoint survey analysis. Conjoint analysis is a method for systematically estimating consumer preferences across discrete attributes. Conjoint analysis has been used extensively in marketing research and measures the value that consumers place on each feature of a product.\textsuperscript{15} Conjoint analysis has recently begun to be applied in health research.\textsuperscript{16,17} We recruited 298 men and women 18 years of age and over seeking testing or care at GHESKIO (Haitian Study Group for Kaposi’s sarcoma and Opportunistic Infections) clinics. We created 8 hypothetical dual test profiles varying across six dichotomous attributes: cost (free versus $4), accuracy (no false positive versus false positive), time-to-result (20 minutes versus 1 week), blood draw method (finger prick versus venipuncture), number of draws (1 versus 2), and test type (rapid versus laboratory). Cost (free vs. $4; impact score=27.2, SD=36.6, p<.0001) had the highest impact on likelihood of testing, followed by number of blood draws (1 versus 2; impact score=17.5, SD=29.8, p<.0001), blood draw method (fingerprick versus venipuncture; impact score=9.7, SD=26.5, p<.0001), test type
(rapid versus laboratory; impact score= -4.5, SD=21.9, P=.0005), and time-to-result (20 minutes versus 1 week; impact score=3.6, SD=25.6, p=.0139). This analysis showed that implementation of a low cost dual rapid test in the laboratory for HIV and syphilis could be used to improve screening uptake and accessibility to accelerate time to treatment.

Our second study was a field study of a dual HIV and syphilis rapid test in Port-au-Prince, Haiti. Field studies using whole blood fingerprick specimens are essential to understand how the test will perform in real-world settings. We found that for the HIV test component, sensitivity and specificity were 99.2% (95% CI: 95.8%, 100%) and 97.0% (95% CI: 93.2%, 99.0%), respectively; and for the syphilis component were 96.5% (95% CI: 91.2%, 99.0%) and 90.8% (95% CI: 85.7%, 94.6%), respectively. This test performed well and could be considered for wider use to increase rates of HIV and syphilis screening.

Our final analysis was an epidemiological bias analysis using estimates of potential misclassification by use of an imperfect gold standard test. We used regression calibration methods to assess the effect of reference standard misclassification bias using the syphilis component of the dual HIV and syphilis rapid test as an example. We concluded by making recommendations for reporting diagnostic performance ranges instead of point estimates when using an imperfect reference test.

In conclusion, dual HIV and syphilis testing is a preferred screening modality that can be used to streamline HIV and syphilis prevention and case finding. These tests perform well in both laboratory and point-of-care settings and should be considered for wider implementation. Use of
regression calibration methodology can provide accurate information on the potential performance characteristics of these tests when a perfect gold standard test is unavailable.
The dissertation of Claire Catherine Bristow is approved.

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Jeffrey David Klausner

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2015
Table of Contents

LIST OF FIGURES ix
ABBREVIATIONS x
ACKNOWLEDGEMENTS xi
VITA xiii

Chapter I. Introduction and Background 1
  1.1 HIV and syphilis dual prevention 2
  1.2 HIV and syphilis infections 3

Chapter II. Study 1: Attributes of Diagnostic Tests to Increase Uptake of Dual Testing for Syphilis and HIV in Port-au-Prince, Haiti. 5
  2.1 Abstract 5
  2.2 Background 6
  2.3 Study methods 8
  2.4 Results 9
  2.5 Discussion 10
  2.6 Conclusion 13
  2.7 Tables and figures 14

Chapter III. Study 2: Field Evaluation of a Dual Rapid Diagnostic Test for HIV Infection and Syphilis in Port-au-Prince, Haiti 19
  3.1 Abstract 19
  3.2 Background 20
  3.3 Study methods 22
  3.4 Results 24
  3.5 Discussion 25
  3.6 Conclusion 28
  3.7 Tables 29
  3.8 Figures 36

Chapter IV. Study 3: Regression Calibration to Assess the Effect of Reference Standard Misclassification on New Rapid Tests for Detection of Syphilis 38
  4.1 Summary 38
  4.2 Background 38
  4.3 Notation and Model Setup 40
  4.4 Regression calibration 40
  4.5 Results 45
  4.6 Discussion 46
  4.7 Tables and Figures 48

Chapter V. Public Health Importance 51

References 53
LIST OF TABLES

Table 2-1: Acceptability (mean) of hypothetical HIV and Syphilis tests with different attributes in Port-au-Prince, Haiti. (n=298).................................................................14

Table 2-2. Impact of HIV and syphilis test attributes on hypothetical test acceptability among the total sample in Port-au-Prince, Haiti. (N=298).................................................................15

Table 2-3. Impact of HIV and syphilis test attributes on hypothetical test acceptability among pregnant women in Port-au-Prince, Haiti. (n=49).................................................................16

Table 2-4. Impact of HIV and syphilis test attributes on hypothetical test acceptability among Non-pregnant women in Port-au-Prince, Haiti. (n=188).................................................................17

Table 2-5. Impact of HIV and syphilis test attributes on hypothetical test acceptability among men in Port-au-Prince, Haiti. (n=61).................................................................18

Table 3-1. Field performance in Haiti for detection of HIV antibodies using a dual HIV/syphilis test.................................................................29

Table 3-2. Field performance in Haiti for detection of *Treponema pallidum* antibodies using a dual HIV/syphilis test.................................................................30

Table 3-3. Field performance among HIV positive participants in Haiti for detection of *Treponema pallidum* antibodies using a dual HIV/syphilis test.................................................................31

Table 3-4. Field performance among HIV negative participants in Haiti for detection of *Treponema pallidum* antibodies using a dual HIV/syphilis test.................................................................32

Table 3-5. Field performance in Haiti for detection of HIV antibodies using a dual HIV/syphilis test among pregnant women (n=49).................................................................33

Table 3-6. Field performance in Haiti for detection of *Treponema pallidum* antibodies using a dual HIV/syphilis test among pregnant women (n=49).................................................................34

Table 3-7. Visual intensity of the color of the bands indicating positive results for the dual test HIV/syphilis test band intensity.................................................................35

Table 4-1. Field performance for detection of *Treponema pallidum* antibodies using a new dual HIV/syphilis test assuming that the reference test is a true representation of infection status...49

Table 4-2. Sensitivity and specificity of the new test for syphilis using regression calibration to correct for potential misclassification bias caused by use of an imperfect reference standard...50
LIST OF FIGURES

Figure 3-1. Standard for band intensity for SD Bioline HIV/Syphilis Duo test………………36

Figure 3-2. Boxplot of color band intensity among HIV and Treponema pallidum true positive and false positive test results from a dual HIV and syphilis rapid test……………………….37

Figure 4-1. Directed Acyclic graph of the relationship between an imperfect reference test for syphilis (X), an unknown true infection status (T) and a new diagnostic test D……………..48
ABBRVIATIONS

Confidence intervals (CI)
Enzyme linked immunosorbent assay (ELISA)
Haitian Study Group for Kaposi’s sarcoma and Opportunistic Infections (GHESKIO)
Human immunodeficiency virus (HIV)
Prevention of mother-to-child transmission (PMTCT)
Rapid Plasma Reagin (RPR)
Standard Deviation (SD)
*Treponema pallidum* particle agglutination assay (TPPA)
*Treponema pallidum* particle hemagglutination assay (TPHA)
ACKNOWLEDGEMENTS

I would like to express my deep appreciation and gratitude to my advisor, Dr. Frank Sorvillo, for his encouragement and support throughout this program. From the day I started to consider the PhD at UCLA, Dr. Sorvillo provided his honest opinions and advice.

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Chapter I. Introduction and Background

Syphilis is a curable disease, yet 10 million persons worldwide are infected each year, of which 1.4 million are pregnant women.\textsuperscript{1-3} Syphilis is an infection caused by the spirochete Treponema pallidum and frequently has atypical presentations that may be difficult to differentiate from other sexually transmitted infections (STIs) making effective diagnostics essential to the identification of infection.\textsuperscript{4}

Syphilis screening and treatment programs that utilize laboratory-based testing have been hampered by limited laboratory access, long turn-around time for results, and loss to follow-up of syphilis infected individuals.\textsuperscript{5-8} When syphilis diagnostic testing involves multiple tests performed off-site, only a proportion of infected individuals receive treatment and continued transmission occurs.\textsuperscript{9,10}

A rapid test is a simple point-of-care test that can be used in a variety of settings and provides a result to guide clinical management during the time of the initial consultation (ideally within 30 minutes or less). The advent of rapid point-of-care tests for syphilis has reduced barriers and allowed for new health systems approaches to syphilis prevention, including same day testing and treatment.\textsuperscript{5,6,11-13} Rapid syphilis tests are primarily solid-phase immunochromatographic assays that use lateral flow technology, in which antibodies move by capillary action over antigen immobilized on a nitrocellulose membrane strip.\textsuperscript{18} Antibodies in the specimen bind to the antigen site in the test region, resulting in the appearance of a visible, colored line.
1.1 HIV and syphilis dual prevention

The World Health Organization is calling for a harmonized approach to the elimination of human immunodeficiency virus (HIV) and syphilis. Combined prevention is warranted because both syphilis and HIV infections have evidence-based, scalable interventions using current health care mechanisms. The introduction of point-of-care rapid tests into health services can improve syphilis screening and results in increased rates of treatment for those infected with syphilis. However, in practice we must determine what are the preferred diagnostic modalities that are important to specific target populations. Chapter 2 of this dissertation is an analysis of preferences around testing for HIV and syphilis.

This dissertation also includes, in chapter 3, an evaluation of a new dual HIV and syphilis rapid test compared to Treponema pallidum hemagglutination assay (TPHA) as a reference standard. However, point-of-care tests are subject to false positive and false negative results when compared to a treponemal reference test. The TPHA and other reference tests are imperfect, also subject to false positive and false negative test results. Using an imperfect reference test can lead to misclassification of disease status and therefore could lead to biased estimates of sensitivity and specificity of the new rapid tests. Therefore, chapter 4 describes an epidemiological bias-corrected analysis simulation using estimates of potential misclassification by the TPHA using regression calibration.
1.2 HIV and syphilis infections

The manifestations of syphilis disease vary depending on the duration of infections and time of presentation. There are four stages—primary, secondary, latent (early and late), and tertiary. The primary stage classically presents with a single chancre—a firm, painless, non-itchy skin ulceration at the site of exposure, secondary syphilis with a diffuse rash which frequently involves the palms of the hands and soles of the feet, latent syphilis with little to no symptoms, and tertiary syphilis with gummas, neurological, or cardiac symptoms. However, syphilis frequently has atypical presentations that may be difficult to differentiate from other systemic medical conditions (e.g., autoimmune diseases, infections, malignancies, collagen-vascular diseases). This makes effective diagnostics essential to the identification of infection.4

The primary route for transmission of syphilis is through sexual contact. However, it can also be transmitted from mother to fetus during pregnancy or at birth, resulting in congenital syphilis. If syphilis remains untreated during pregnancy, it can lead to fetal loss or stillbirth or, in a live-born infant, neonatal death, prematurity, low birth weight or syphilis infection in the infant.3,26 In syphilis-infected pregnant women, adverse birth outcomes are common and have been shown to be 4.5 times higher in those with untreated syphilis than those without syphilis.26 Congenital syphilis can be prevented by screening early in pregnancy, treating seropositive pregnant women, and preventing re-infection.3,26

HIV and syphilis co-infection is a dangerous combination.27 Syphilis infection, like other genital ulcer diseases, may facilitate HIV acquisition and transmission.28-30 Anogenital ulcers interfere with the natural mucosal and epithelial barriers and cause inflammation providing a portal of
entry for HIV.\textsuperscript{29} In co-infected patients, syphilis can increase transmission of HIV by increasing viral shedding\textsuperscript{31,32} and viral load\textsuperscript{33,34}. Secondary syphilis may cause a potent immune activation that decreases the percentage of CD4+ T-cells, which may further enhance the progression of HIV.\textsuperscript{30} Additionally, persons with HIV infection are at increased risk for neurosyphilis.\textsuperscript{4,35} It has been demonstrated that \textit{Treponema pallidum pallidum} and its pro-inflammatory components can cause expression of CCR5 (the major co-receptor for HIV entry) on human monocytes within syphilitic chancre, which enhances the susceptibility of the cells to HIV infection.\textsuperscript{30} Syphilis and HIV co-infection is increasingly common; approximately 25% of cases of primary and secondary syphilis reported in the United States occur in HIV-infected individuals.\textsuperscript{36}

Integrating the screening of syphilis into HIV prevention programs such as prevention of mother-to-child transmission (PMTCT) of HIV and targeted screening for high-risk populations would add little to the cost of screening but would have a major effect on case finding of syphilis and the prevention of transmission.\textsuperscript{1,37} The large sum of donor funds available for HIV prevention and the global advocacy for HIV presents an opportunity for diseases like syphilis with less-advocacy to be twinned into the same platform of diagnosis and treatment during HIV-related prevention or care visits.\textsuperscript{38} Implementing a simple and affordable dual testing strategy for HIV and syphilis could improve screening uptake, the accessibility of testing and ultimately accelerate the time to treatment.
Chapter II. Study 1: Attributes of Diagnostic Tests to Increase Uptake of Dual Testing for Syphilis and HIV in Port-au-Prince, Haiti.

2.1 Abstract

Introduction: Syphilis and HIV screening is highly recommended for pregnant women and those at risk for infection. Enhanced control and prevention can be accomplished through integrated dual testing. We used conjoint analysis, an innovative method for systematically estimating consumer preferences across discrete attributes, to identify factors associated with testing preferences for HIV and syphilis infection.

Methods: We recruited 298 men and women 18 years and over seeking testing or care at GHESKIO (Haitian Study Group for Kaposi’s sarcoma and Opportunistic Infections) clinics. We created 8 hypothetical dual test profiles varying across six dichotomous attributes: cost (free versus $4), accuracy (no false positive versus false positive), time-to-result (20 minutes versus 1 week), blood draw method (finger prick versus venipuncture), number of draws (1 versus 2), and test type (rapid versus laboratory). Participants were asked to rate each profile using Likert preference scales. Ratings were converted to 100-point preference scores; higher scores suggest increased preference. An impact score was generated for each attribute by taking the difference between the preference scores for the preferred and non-preferred level of each attribute. Two-sided one-sample t-test was used to generate p-values.
**Results:** Of 298 study participants, 61 (20.5%) were male. Of 237 females, 49 (20.7%) were pregnant. Cost (free vs. $4; impact score=27.2, SD=36.6, p<.0001) had the highest impact on likelihood of testing, followed by number of blood draws (1 versus 2; impact score=17.5, SD=29.8, p<.0001), blood draw method (fingerprick versus venipuncture; impact score=9.7, SD=26.5, p<.0001), test type (rapid versus laboratory; impact score= -4.5, SD=21.9 , P=.0005), and time-to-result (20 minutes versus 1 week; impact score=3.6, SD=25.6, p=.0139).

**Conclusion:** HIV and syphilis testing preferences for this study sample in Port-au-Prince prioritized cost, single fingerprick and timeliness. Implementing a low cost dual rapid test in the laboratory for HIV and syphilis could improve screening uptake and accessibility to accelerate time to treatment.

**2.2 Background**

Screening for syphilis and HIV is highly recommended for pregnant women and those at risk for infection.\(^39\)-\(^42\) Syphilis is caused by the spirochete *Treponema pallidum*, which, like HIV, can be transmitted through sex, blood and from mother-to-child during pregnancy or at birth. The similarities in screening recommendations for HIV and syphilis offer an important opportunity to strengthen prevention programs for the elimination of congenital syphilis along with preventing mother-to-child transmission of HIV infection by means of integrated screening.\(^1\) Peeling and colleagues commented on the tragedy of babies avoiding HIV through effective prevention of mother-to-child transmission of HIV programs but dying of syphilis because of the lack of screening for syphilis available to the women.\(^37\) Integrated screening could profoundly change medical and public health practice.\(^1,43\) With a shortened time to diagnosis, patients may be
treated and rendered less infectious or non-infectious much quicker resulting in reduced complications from untreated infection as well as the decreased spread of infection to others.

In Haiti, a demographic health survey conducted in 2005–2006 showed an HIV prevalence of 2.3% among women aged 15 to 49 and a 2.0% prevalence among men. Additionally, in parts of Haiti a significant proportion (7.6%) of pregnant women have serologic evidence of syphilis. Screening is the main strategy for identifying HIV and syphilis infections. Enhanced control and prevention can be accomplished through increased uptake of testing and subsequent treatment for those infected.

It is imperative to understand the variable determinants of test uptake in order to reduce barriers. Screening and treatment programs that utilize laboratory-based testing have been hampered by limited laboratory access, long turn-around time for results, and loss to follow-up of syphilis infected individuals. When diagnostic testing involves multiple tests performed off-site, only a proportion of infected individuals receive treatment and continued transmission occurs. There have been several advances in point-of-care diagnostic tests; however, it is unknown what factors are associated with increased preference for testing.

Conjoint analysis is a technique that has been used successfully in health care and is gaining widespread use. Conjoint analysis is a method for systematically estimating consumer preferences across discrete attributes. It allows for estimation of the relative importance of different aspects of a product or healthcare, the trade-offs between these attributes, and the total satisfaction or preference that participants associate with the product or care. This analytic
method is perfect for use in determining preferences of respondents in a low-resource setting like Haiti where informed decisions must be made about how to prioritize limited resources.

In order to understand preferences for the integration of HIV and syphilis testing, we used conjoint analysis to identify factors associated with willingness to test for HIV and syphilis infection.

### 2.3 Study methods

We recruited 298 men and women 18 years and over seeking testing or care at GHESKIO (Haitian Study Group for Kaposi’s sarcoma and Opportunistic Infections) Health Centers between March and July of 2014. Currently, GHESKIO receives about 100,000 patient visits annually. Central to the GHESKIO model is the concept that an individual at risk or already infected with HIV should be quickly identified and provided access to a package of services including voluntary counseling and testing, management of sexually transmitted infections, tuberculosis screening and treatment, reproductive health services, HIV care including antiretroviral therapy, and services to prevent mother to child transmission of HIV.

We utilized conjoint analysis methods to assess likelihood of testing (willingness to test).\(^\text{17}\) The testing attributes were identified using characteristics of existing HIV and syphilis testing strategies.\(^\text{5,43,47}\) Testing attributes included cost (free versus US$4), accuracy (no potential for false positive syphilis result versus potential for false positive syphilis result), time-to-result (20 minutes versus 1 week), blood draw method (finger prick versus venipuncture), number of draws (1 versus 2), and test type (rapid versus laboratory). We created scenarios that describe all
possible combinations of attributes to create a hypothetical test profile. Because each attribute has two levels and we have six attributes, there will be 64 ($2^6$) different combinations that can be made using these attributes. Using the fractional factorial design, we reduced the number of scenarios to 8 hypothetical test scenarios across the six dichotomous attributes to measure the main effect of each attribute. This design method assumes no interactions between attributes.

Preferences for the hypothetical test scenarios were determined using an interview conducted by a trained counselor in Haitian Creole. We assessed willingness to test by asking participants to rate how likely they were to test using each individual test profile on 5-level Likert preference scales: (1) Very unlikely, (2) Somewhat unlikely, (3) Neutral/Don’t know, (4) Somewhat likely, (5) Very likely.

Ratings were converted to 100-point preference scores; higher scores suggest increased willingness to test. The mean of each hypothetical test scenario was determined. An impact score was generated for each attribute by taking the difference between the average preference scores between the preferred scenarios and non-preferred scenarios of each attribute. Two-sided one-sample t-test was used to generate p-values for the comparisons between the preferred and non-preferred levels for each attribute. Data analysis was conducted using SAS software v9.3 (Cary, NC, USA).

### 2.4 Results

Of 298 study participants, 61 (20.5%) were male. Of 237 females, 49 (20.7%) were pregnant.
For the overall population, cost (free vs. $4; impact score=27.2, SD=36.6, p<.0001) had the highest impact on willingness to test, followed by number of blood draws (1 vs. 2; impact score=17.5, SD=29.8, p<.0001), blood draw method (fingerprick vs. venipuncture; impact score=9.7, SD=26.5, p<.0001), test type (rapid vs. laboratory; impact score= -4.5, SD=21.9 , P=.0005), and time-to-result (20 minutes vs. 1 week; impact score=3.6, SD=25.6, p=.0139) [Tables 2-1 and 2-2].

Additionally, we looked at differences among 3 groups included in our sample: pregnant women, non-pregnant women and men [Tables 2-3 through 2-5]. Each of the groups had similar prioritization of attributes. Cost was the most important driving factor for all groups, followed by number of blood draws and sample collection method. However, among the 3 groups, only pregnant women prioritized time to result (impact score=17.22, SD=30.15, p=0.0002). Additionally, males did not prioritize test type (impact score=-2.77, SD=20.4, p=0.2937), while females did.

2.5 Discussion

We used conjoint analysis to determine factors associated with willingness to test simultaneously for HIV and syphilis in Port-au-Prince, Haiti. The study participants in all three groups, males, pregnant and non-pregnant females, prioritized cost and a single blood draw using a fingerprick. It was only pregnant women that prioritized timeliness from specimen collection to result for HIV and syphilis tests. In addition, females prioritized laboratory-based testing while males did not.
We found that the most impactful attribute of HIV and syphilis tests was the cost. GHESKIO Health Centers offers HIV and syphilis screening free of charge; however, we recommend that screening tests be offered free of charge in other settings around Haiti that are aiming to increase test uptake. The results of this study can influence the way that people are getting tested for syphilis and HIV. Additionally, groups prioritized one blood draw over two when testing for the two infections and preferred fingerprick specimen collection to venipuncture. Dual rapid tests could be used to meet these preferred methods of testing. Dual rapid tests that have multiple analytes for the detection of antibodies for both HIV and syphilis infections are now available. Those tests use one drop of fingerprick wholeblood and one device to test for two infections in minutes at the point-of-care. Dual tests enable testing for both HIV and syphilis at the same time. There are several advantages of rapid point-of-care tests that include rapid time to result, low cost, minimal equipment, minimal training needed (easy to perform), and suitable for use in non-clinical settings.

In contrast with our hypothesis, women prioritized laboratory testing over rapid testing that can be performed at the point-of-care. We hypothesized that rapid testing would be preferred however we had a negative, but statistically significant among females, impact score. One explanation for this is the setting in which the study was performed may have driven this preference. At GHESKIO, most testing for syphilis and HIV is performed in a laboratory/phlebotomy setting even when rapid tests are used. Therefore, the participants could be more comfortable in this setting away from the waiting room and patient rooms. In a study in a U.S. urban hospital, patients reported that they believed the rapid test was less accurate than a laboratory-based test. This is perhaps also the case in Haiti, which would highlight the need for
appropriate pre-screening education to explain to the patient the utility and high performance of rapid testing.

We also found attributes of HIV and syphilis tests that are less important to consumers. Participants’ willingness to test for HIV and syphilis was not as affected by a potential for false positive syphilis result. This result has implications on roll out of dual testing for HIV and syphilis, which can include screening tests that may require further confirmatory testing for positive results.

This study was subject to some limitations. Some of the attributes were not necessarily 100% mutually exclusive. Sample collection method could be related to test type, laboratory based versus rapid point-of-care. Therefore, there might be some overlap in attributes even though our analysis assumed independence. However, creating separate attributes for potentially related factors allowed us to parse out the specific impact of a characteristic related to willingness to test. An additional limitation is that participants were presenting to GHESKIO, a site where they had access to free HIV and syphilis testing, making these results less generalizable to other places where the patient population may be unaccustomed to diagnostic access.

Based on this first study using conjoint analysis in Haiti to detect preferences around attributes of tests for HIV and syphilis, we have found several attributes that affect people’s decision about how to test. Future research could look at interactions across the most impactful attributes as well as additional levels of each attribute. For example we used only 2 levels of cost, free and $4.
Additional levels of cost could be explored to identify a threshold of cost that would be prohibitory.

2.6 Conclusion

Our study provides important information on preferences for HIV and syphilis testing which in combination with studies on test efficacy, cost, and feasibility can help identify best practices for prevention, screening, and treatment to reduce the continued burden of sexual and reproductive health-related diseases, like HIV and syphilis, in low-resource settings. Other studies have also found that the implementation of an accurate and low cost integrated rapid testing strategy for HIV and syphilis has been deemed acceptable, often preferred by patients and providers, and has the capacity to improve the rates of screening.\textsuperscript{52-54} Implementation of a low-cost dual rapid test in the laboratory for HIV and syphilis could improve screening uptake and accessibility to accelerate time to treatment.
2.7 Tables and figures

Table 2-1. Acceptability (mean) of hypothetical HIV and Syphilis tests with different attributes in Port-au-Prince, Haiti. (n=298)

<table>
<thead>
<tr>
<th>Hypothetical test profile</th>
<th>Test acceptability among total sample mean (SD)</th>
<th>Test acceptability among pregnant females (n=49) (SD)</th>
<th>Test acceptability among non-pregnant females (n=188) (SD)</th>
<th>Test acceptability among males (n=61) (SD)</th>
<th>Test Attributes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cost</td>
<td>Potential for Syphilis False Positive</td>
<td>Time to Result</td>
<td>Number of Blood Draws</td>
<td>Blood Draw Method</td>
</tr>
<tr>
<td>One</td>
<td>45.05 (43.41)</td>
<td>33.16 (37.98)</td>
<td>46.94 (44.19)</td>
<td>48.77 (44.12)</td>
<td>Free</td>
</tr>
<tr>
<td>Two</td>
<td>85.91 (30.20)</td>
<td>91.84 (20.66)</td>
<td>84.97 (31.19)</td>
<td>84.02 (33.24)</td>
<td>Free</td>
</tr>
<tr>
<td>Three</td>
<td>66.70 (43.86)</td>
<td>51.02 (47.04)</td>
<td>69.41 (42.98)</td>
<td>70.90 (41.88)</td>
<td>Free</td>
</tr>
<tr>
<td>Four</td>
<td>42.11 (45.74)</td>
<td>48.98 (46.20)</td>
<td>39.23 (45.83)</td>
<td>45.49 (45.07)</td>
<td>$4</td>
</tr>
<tr>
<td>Five</td>
<td>35.40 (45.10)</td>
<td>36.22 (45.66)</td>
<td>32.98 (44.36)</td>
<td>42.21 (46.89)</td>
<td>$4</td>
</tr>
<tr>
<td>Six</td>
<td>57.97 (46.89)</td>
<td>48.46 (47.16)</td>
<td>58.64 (47.34)</td>
<td>63.52 (44.85)</td>
<td>Free</td>
</tr>
<tr>
<td>Seven</td>
<td>48.74 (47.99)</td>
<td>44.39 (46.57)</td>
<td>48.80 (46.66)</td>
<td>52.05 (47.50)</td>
<td>$4</td>
</tr>
<tr>
<td>Eight</td>
<td>27.85 (42.89)</td>
<td>19.90 (35.35)</td>
<td>28.59 (44.15)</td>
<td>31.97 (44.28)</td>
<td>$4</td>
</tr>
</tbody>
</table>

Abbreviation: SD, standard deviation

Test acceptability score is based on a 5-point Likert scale converted to 0-100 point scale; higher results indicate higher preference

Overall test acceptability: 51.22 (SD: 25.05)
Overall test acceptability among pregnant women: 46.75 (SD: 19.68)
Overall test acceptability among non-pregnant women: 51.20 (SD: 26.39)
Overall test acceptability among men: 54.87 (SD: 24.38)
Table 2-2. Impact of HIV and syphilis test attributes on hypothetical test acceptability among the total sample in Port-au-Prince, Haiti. (N=298)

<table>
<thead>
<tr>
<th>Test Attributes</th>
<th>Attribute values</th>
<th>Acceptability of testing with preferred attribute (mean)</th>
<th>Acceptability of testing with non-preferred attribute (mean)</th>
<th>Impact on testing acceptability Mean (SD)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost</td>
<td>Free vs. $4</td>
<td>64.83</td>
<td>37.60</td>
<td>27.22 (36.62)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of Blood Draws</td>
<td>1 vs. 2</td>
<td>59.94</td>
<td>42.49</td>
<td>17.45 (29.80)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sample Collection Method</td>
<td>Fingerprick vs. Venipuncture</td>
<td>56.08</td>
<td>46.35</td>
<td>9.73 (26.52)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Test Type</td>
<td>Rapid vs. Laboratory</td>
<td>48.97</td>
<td>53.46</td>
<td>-4.49 (21.85)</td>
<td>0.0005</td>
</tr>
<tr>
<td>Time to Result</td>
<td>20 minutes vs. 1 week</td>
<td>53.04</td>
<td>49.39</td>
<td>3.64 (25.46)</td>
<td>0.0139</td>
</tr>
<tr>
<td>Potential for Syphilis False</td>
<td>No vs. Yes</td>
<td>51.89</td>
<td>50.55</td>
<td>1.34 (23.69)</td>
<td>0.3288</td>
</tr>
</tbody>
</table>

Abbreviation: SD, standard deviation
Scores were converted from preferences described using a 5-scale Likert to scores on a 100-point scale.
Table 2-3. Impact of HIV and syphilis test attributes on hypothetical test acceptability among pregnant women in Port-au-Prince, Haiti. (n=49)

<table>
<thead>
<tr>
<th>Test Attributes</th>
<th>Attribute values</th>
<th>Acceptability of testing with preferred attribute (mean)</th>
<th>Acceptability of testing with non-preferred attribute mean)</th>
<th>Impact on testing acceptability Mean (SD)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost</td>
<td>Free vs. $4</td>
<td>58.93</td>
<td>34.57</td>
<td>24.36 (37.02)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of Blood Draws</td>
<td>1 vs. 2</td>
<td>56.25</td>
<td>37.24</td>
<td>19.01 (32.67)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Sample Collection Method</td>
<td>Fingerprick vs. Venipuncture</td>
<td>52.43</td>
<td>41.07</td>
<td>11.35 (25.00)</td>
<td>0.0026</td>
</tr>
<tr>
<td>Test Type</td>
<td>Rapid vs. Laboratory</td>
<td>41.20</td>
<td>52.30</td>
<td>-11.10 (21.17)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Time to Result</td>
<td>20 minutes vs. 1 week</td>
<td>55.35</td>
<td>38.14</td>
<td>17.22 (30.15)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Potential for Syphilis False Positive</td>
<td>No vs. Yes</td>
<td>47.32</td>
<td>46.17</td>
<td>1.14 (23.23)</td>
<td>0.731</td>
</tr>
</tbody>
</table>

Abbreviation: SD, standard deviation

Scores were converted from preferences described using a 5-scale Likert to scores on a 100-point scale.
Table 2-4. Impact of HIV and syphilis test attributes on hypothetical test acceptability among non-pregnant women in Port-au-Prince, Haiti. (n=188)

<table>
<thead>
<tr>
<th>Test Attributes</th>
<th>Attribute values</th>
<th>Acceptability of testing with preferred attribute (mean)</th>
<th>Acceptability of testing with non-preferred attribute (mean)</th>
<th>Impact on testing acceptability Mean (SD)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost</td>
<td>Free vs. $4</td>
<td>65.46</td>
<td>36.93</td>
<td>28.52 (36.56)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of Blood Draws</td>
<td>1 vs. 2</td>
<td>60.14</td>
<td>42.25</td>
<td>17.89 (27.95)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sample Collection Method</td>
<td>Fingerprick vs.</td>
<td>55.88</td>
<td>46.51</td>
<td>9.38 (26.72)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Venipuncture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test Type</td>
<td>Rapid vs. Laboratory</td>
<td>49.53</td>
<td>52.86</td>
<td>-3.32 (22.27)</td>
<td>0.0421</td>
</tr>
<tr>
<td>Time to Result</td>
<td>20 minutes vs. 1 week</td>
<td>51.50</td>
<td>50.90</td>
<td>0.60 (23.17)</td>
<td>0.7237</td>
</tr>
<tr>
<td>Potential for Syphilis</td>
<td>No vs. Yes</td>
<td>52.33</td>
<td>50.07</td>
<td>2.26 (24.22)</td>
<td>0.2021</td>
</tr>
</tbody>
</table>

Abbreviation: SD, standard deviation

Scores were converted from preferences described using a 5-scale Likert to scores on a 100-point scale.
Table 2-5. Impact of HIV and syphilis test attributes on hypothetical test acceptability among men in Port-au-Prince, Haiti. (n=61)

<table>
<thead>
<tr>
<th>Test Attributes</th>
<th>Attribute values</th>
<th>Acceptability of testing with preferred attribute (mean)</th>
<th>Acceptability of testing with non-preferred attribute (mean)</th>
<th>Impact on testing acceptability Mean (SD)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost</td>
<td>Free vs. $4</td>
<td>67.62</td>
<td>42.11</td>
<td>25.51 (36.89)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of Blood Draws</td>
<td>1 vs. 2</td>
<td>62.30</td>
<td>47.44</td>
<td>14.86 (33.13)</td>
<td>0.0009</td>
</tr>
<tr>
<td>Sample Collection Method</td>
<td>Fingerprick vs.</td>
<td>59.63</td>
<td>50.10</td>
<td>9.53 (27.50)</td>
<td>0.0088</td>
</tr>
<tr>
<td></td>
<td>Venipuncture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test Type</td>
<td>Rapid vs. Laboratory</td>
<td>53.48</td>
<td>56.25</td>
<td>-2.77 (20.40)</td>
<td>0.2937</td>
</tr>
<tr>
<td>Time to Result</td>
<td>20 minutes vs. 1 week</td>
<td>55.94</td>
<td>53.79</td>
<td>2.15 (25.05)</td>
<td>0.5049</td>
</tr>
<tr>
<td>Potential for Syphilis False</td>
<td>No vs. Yes</td>
<td>54.20</td>
<td>55.53</td>
<td>-1.33 (22.54)</td>
<td>0.6461</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: SD, standard deviation

Scores were converted from preferences described using a 5-scale Likert to scores on a 100-point scale.
Chapter III. Study 2: Field Evaluation of a Dual Rapid Diagnostic Test for HIV Infection and Syphilis in Port-au-Prince, Haiti

3.1 Abstract

Introduction: Congenital syphilis is responsible for over 500,000 adverse pregnancy outcomes globally every year, while 240,000 mother-to-child transmissions of HIV infection occur every year. We evaluated the field performance of the SD BIOLINE HIV/Syphilis Duo test in Port-au-Prince, Haiti using whole blood fingerprick specimens.

Methods: GHESKIO (Haitian Study Group for Kaposi’s sarcoma and Opportunistic Infections) clinic attendees 18 years of age or older were invited to participate. Venipuncture blood specimens were used for reference testing using standard commercially available tests in Haiti. Sensitivity and specificity were calculated and the exact binomial method was used to determine 95% confidence intervals (CI).

Results: Of 298 study participants, 237 (79.5%) were female, of which 49 (20.7%) were pregnant. For the HIV test component, sensitivity and specificity were 99.2% (95% CI:95.8%,100%) and 97.0% (95% CI:93.2%,99.0%), respectively; and for the syphilis component were 96.5% (95% CI:91.2%,99.0%) and 90.8% (95% CI:85.7%,94.6%), respectively. In pregnant women, the sensitivity and specificity of the HIV test component were 93.3% (95%
CI: 68.0%, 99.8%) and 94.1% (95% CI: 80.3%, 99.3%), respectively; and for the syphilis component were 100% (95% CI: 81.5%, 100%) and 96.8% (83.3%, 99.9%), respectively.

**Conclusion:** The Duo dual test performed well in a field setting in Haiti and should be considered for wider use.

### 3.2 Background

HIV and syphilis infections cause substantial burden of disease.\(^{55-57}\) Those with syphilis infection may be asymptomatic but if left untreated syphilis can lead to neurological complications and in pregnant women can result in severe adverse outcomes of pregnancy. The primary route for the transmission of syphilis is through sexual contact. However, it can also be transmitted from mother to fetus during pregnancy or at birth, resulting in congenital syphilis. Transmission and complications that occur as a result of infection can be prevented through more testing and subsequent treatment for those who test positive.

Up to 80% of syphilis infections in pregnancy cause adverse outcomes including as stillbirths or fetal deaths, neonatal deaths, preterm or low birth weight infants, and infants born with congenital disease.\(^3\) In addition, syphilis infection during pregnancy also leads to a 2.7 fold increase in the risk of mother-to-child transmission of HIV infection.\(^{35}\) In order to address the risk of adverse outcomes of pregnancy and mother-to-child transmission of HIV and syphilis, the World Health Organization has called for the dual elimination of HIV and syphilis.\(^{19}\)
Integrating the screening of syphilis into HIV prevention programs would add little to the cost of screening but would have a major effect on case finding of syphilis and the prevention of transmission.\textsuperscript{1,37} Syphilis and HIV dual testing provides the opportunity to test for both infections using one fingerprick of blood with one device in minutes, allowing for same-day testing and treatment. Combined testing is warranted because both syphilis and HIV infections have evidence-based, scalable interventions using the antenatal care platform for pregnant women.\textsuperscript{20-22}

Mother-to-child transmission of HIV and syphilis can be prevented through early access to antenatal care, testing and treatment. With the advent of dual testing, HIV and syphilis have affordable and accurate point-of-care integrated tests making it feasible for use in any setting – not just those with laboratory capacity. A dual point-of-care test has the potential to reduce missed opportunities to return results and can improve efficiency along the treatment cascade.\textsuperscript{22,58}

In Haiti 90\% of pregnant women report at least one antenatal visit.\textsuperscript{59} Therefore, by including rapid syphilis screening in the first antenatal visit, 90\% of pregnant Haitian women could have access to testing and treatment. The recent development of a new dual rapid HIV and syphilis test is ideal for Haiti, a country with limited resources.

The SD BIOLINE HIV/Syphilis Duo test is a qualitative solid phase immunochromatographic assay. It is easy to perform and interpret and does not require special storage or transport conditions with results available in 20 minutes. This test has been shown to be highly sensitive and specific in settings using plasma and serum.\textsuperscript{60-64} However, rapid tests are intended for use at the point-of-care using a fingerprick whole blood specimen. We evaluated the performance of
the SD BIOLINE HIV/Syphilis Duo test in Port-au-Prince, Haiti using whole blood fingerprick specimens.

### 3.3 Study methods

Participants included men and women, at least 18 years of age from GHESKIO (Haitian Study Group for Kaposi’s sarcoma and Opportunistic Infections) clinics in Port-au-Prince, Haiti enrolled from March through July 2014. Working in partnership with the Haitian Government, GHESKIO is a nonprofit that provides integrated primary care services, including HIV counseling, AIDS care, antenatal care, and management of tuberculosis and sexually transmitted infections. GHESKIO receives about 100,000 patient visits annually and all of the health care provided by GHESKIO is free of charge, including services and medications. Known pregnant women were actively recruited for participation at the GHESKIO antenatal clinic. Additionally, known HIV and syphilis-infected participants were actively recruited to supplement the study population.

After participants gave their informed consent, a trained health worker collected a single drop of blood from the participant using a fingerprick. The SD BIOLINE HIV/Syphilis Duo test (Standard Diagnostics, Giheung-gu, Korea) was conducted using the whole blood fingerprick specimen according to the manufacturers instructions. In short, the participant’s finger was pricked with a lancet, a capillary pipette was used to collect one drop of blood, the drop of blood was added into the test ‘sample well’ followed by 3 drops of buffer solution. After 20 minutes the health worker read the test results. The presence of a color band for the control, marked as ‘C’, indicated a valid test. At two other regions marked with ‘SYP’ and ‘HIV’ color bands
appeared to indicate positive results. Additionally, visual intensity of the color band indicating a positive result was recorded by the health worker using the intensity standard [Figure 3-1]. The visual intensity was recorded on a 100-point scale by two separate health workers. We analyzed the average intensity result between the two.

Venipuncture blood specimens were collected by phlebotomists and transported to the reference laboratory for serum separation and reference testing. HIV reference testing varied depending on which clinic at GHESKIO participants presented. The reference tests for comparison to the HIV antibody component of the dual rapid test was Murex HIV-1.2.0 (DiaSorin S.p.A., Saluggia, Italy) or Alere Determine HIV (Alere Inc., Waltham, MA) rapid tests. Positive results were confirmed with the KHB rapid test (Shanghai Kehua Bio-Engineering Co., LTD, China). For the rapid *Treponema pallidum* antibody comparison, the *Treponema Pallidum* Hemagglutination Assay (TPHA) (Human Gesellschaft fur Biochemica und Diagnostica mbH, Wiesbaden, Germany) was used. For TPHA indeterminate results, specimens were retested using a *Treponema pallidum* enzyme-linked immunosorbent assay test (ELISA) (Architect Syphilis TP; Abbott, Wiesbaden, Germany). Rapid plasma reagin (RPR) (Syphilis RPR test, Human Gesellschaft fur Biochemica und Diagnostica mbH, Wiesbaden, Germany) results were also available for all participants to assist with clinical diagnosis but were not used as a reference test. RPR titer levels were determined using serial dilutions.

Sensitivity and specificity were calculated for the whole study population and for a subset of pregnant women. The exact binomial method was used to determine 95% confidence intervals (CI). The Kappa statistic was used to determine concordance between the rapid Duo test and
reference test results. Visual color intensity for positive results was summarized using descriptive statistics. Additionally, we used simple linear regression to explore the association between the RPR titer level and syphilis color band intensity. All analyses were conducted using SAS v9.3 (Cary, NC, USA).

3.4 Results

Of 298 study participants, 61 (20.5%) were male. Of 237 females, 49 (20.7%) were pregnant. The median age of participants was 34 years (interquartile range: 26, 42). All participants had a Duo rapid test and gold standard reference tests for antibodies to HIV and syphilis. Of the 298 participants, 21 had inconclusive TPHA results. Of those 21 inconclusive results, all (100%) were *Treponema pallidum* ELISA positive and of note 15 (71.4%) were RPR positive.

The test performance results can be seen in Tables 3-1 and 3-2. For the HIV component, sensitivity and specificity were 99.2% (95% CI: 95.8%, 100%) and 97.0% (95% CI: 93.2%, 99.0%), respectively. For the *Treponema pallidum* component, sensitivity and specificity were 96.5% (95% CI: 91.2%, 99.0%) and 90.8% (95% CI: 85.7%, 94.6%), respectively. Two of the 17 false positive results (Duo *Treponema pallidum* positive, TPHA negative) were reactive on the RPR test with titers of 1:4 and 1:64. Additionally, among HIV positive specimens the performance for the *Treponema pallidum* component of the Duo test had a sensitivity of 94.4% (72.7%, 99.9%) and a specificity of 92.8% (86.3%, 96.8%) [Table 3-3] and among HIV negatives the sensitivity was 96.8% (91.1%, 99.3%) and the specificity was 87.8% (78.2%, 94.3%) [Table 3-4].
The test performance results for pregnant women are shown in Table 3-5 and 3-6. In pregnant women, the HIV component sensitivity and specificity of the HIV component were 93.3% (95% CI: 68.0%, 99.8%) and 94.1% (95% CI: 80.3%, 99.3%), respectively. For the *Treponema pallidum* component, the sensitivity and specificity were 100% (95% CI: 81.5%, 100%) and 96.8% (83.3%, 99.9%), respectively.

The color line indicating a positive result on the Duo test tended to be more intense (darker) for the HIV antibody component (median intensity = 100%) of the test versus the *Treponema pallidum* antibody component (median intensity = 20%) [Table 3-7, Figure 3-2]. Additionally, the color band intensity was lower among false-positive test results for both HIV and syphilis results than among true positive results. There was a weak correlation between syphilis color band intensity and RPR titer (R=0.226, p=0.0118).

**3.5 Discussion**

We evaluated a dual rapid point-of-care test for detection of HIV and syphilis infection. The HIV antibody component of the dual test showed excellent performance with a sensitivity of 99.2% and specificity of 97%. That high performance using whole blood is similar to what was observed in laboratory evaluations using serum or plasma.\(^{61,63-65}\) That high performance suggests the dual test could be used at the point-of-care with whole blood fingerprick specimens as a screening test. The few false positive HIV results that were found reinforces the need for HIV confirmatory testing for those who screen positive.
The *Treponema pallidum* antibody component was highly sensitive however the specificity somewhat lower. False positives could have resulted because the reference test may use different antibody targets than the Duo test, which utilizes 17 kDA recombinant *Treponema pallidum* antigen as the target. Additionally, other reactive antibodies could be responsible for the false positive results observed with the Duo test. We also calculated performance of the *Treponema pallidum* component of the Duo test stratified by HIV status and found that differences were small with overlapping confidence intervals and therefore are unlikely to be clinically significant.

The Duo gave 17 *Treponema pallidum* false positive results. Of which, Two were RPR reactive indicating that these were most likely falsely negative on the reference test. If we reclassified those as true positives then there would be a slight increase in specificity. This finding highlights the limitations of using an imperfect reference test to determine validity of a new test.

A limitation of all syphilis tests is that they can produce false negative results when the infection has been acquired recently. Additional limitations occur with treponemal tests because treponemal antibodies can persist for life, even following curative treatment therefore rapid tests for syphilis which detect treponemal antibodies will give a positive result if there is a history of syphilis infection. In some settings confirmatory tests may be necessary while in others – such as antenatal clinics – the benefits of providing same day syphilis treatment might outweigh the risks of unnecessary treatment.
Among pregnant women, we found that the *Treponema pallidum* component of the dual test actually had higher sensitivity and specificity compared with the total study population. The risks of untreated syphilis infection in pregnancy are very high and an accurate screening test to detect syphilis is paramount to reduced adverse pregnancy outcomes.\(^{3,19}\) The HIV component of the test in pregnant women showed somewhat lower performance than among the total study population, however the sample size was small and the lower performance could be due to random error.

The Duo test is ideal for use in resource-limited settings where laboratory services are often unavailable and follow-up is difficult. By combining testing for HIV and syphilis into one device using one drop of blood from a fingerprick, the Duo test has the potential to reduce testing barriers and increase uptake of testing for both HIV and syphilis.\(^{22,68}\)

We determined a mild correlation between RPR titer level and the color band intensity of the treponemal antibody result. However, the color band intensity was subjective. Using an electronic rapid test reader would provide an objective reading of the color band intensity to further explore this relationship.\(^{69}\) The manufacturer instructions indicate that any color band should be interpreted as a positive result. In our study, false positive results provided a much lower intensity reading, however due to the substantial overlap in intensity between false and true positive results, we could not determine an accurate cutoff value.
3.6 Conclusion

The SD Bioline Duo test should be used in settings to increase the uptake of dual screening for HIV infection and syphilis.
### 3.7 Tables

**Table 3-1.** Field performance in Haiti for detection of HIV antibodies using a dual HIV/syphilis test.

<table>
<thead>
<tr>
<th>HIV reference test</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Kappa Coefficient (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pos</td>
<td>Neg</td>
<td></td>
</tr>
<tr>
<td>SD BIOLINE HIV/Syphilis Duo test</td>
<td>128 5 133</td>
<td>99.2% (95.8%, 100%)</td>
<td>97.0% (93.2%, 99.0%)</td>
</tr>
<tr>
<td></td>
<td>Pos</td>
<td>Neg</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>129 169 298</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Reference tests were Murex HIV-1.2.0 (DiaSorin S.p.A., Saluggia, Italy) or Alere Determine HIV (Alere) rapid testing. Positive results were confirmed with a colloidal gold test from KHB (Shanghai Kehua Bio-Engineering Co., LTD, China).*
Table 3-2. Field performance in Haiti for detection of *Treponema pallidum* antibodies using a dual HIV/syphilis test.

<table>
<thead>
<tr>
<th>Treponema pallidum reference test</th>
<th>Total</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Kappa Coefficient (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos</td>
<td>109</td>
<td>17</td>
<td>126</td>
<td>96.5%</td>
</tr>
<tr>
<td>Neg</td>
<td>4</td>
<td>168</td>
<td>172</td>
<td>(91.2%, 99.0%)</td>
</tr>
</tbody>
</table>

*Reference test was conducted using *Treponema Pallidum* Hemaglutination Assay (TPHA) (Human Gesellschaft fur Biochemica und Diagnostica mbH, Wiesbaden, Germany). TPHA indeterminate results were retested using a *Treponema pallidum* enzyme-linked immunosorbent assay test (ELISA)(Architect Syphilis TP; Abbott, Wiesbaden, Germany).
Table 3-3. Field performance among HIV positive participants in Haiti for detection of *Treponema pallidum* antibodies using a dual HIV/syphilis test.

<table>
<thead>
<tr>
<th>Treponema pallidum reference test</th>
<th>Total</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Kappa Coefficient (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD BIOLINE HIV/Syphilis Duo test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pos</td>
<td>17</td>
<td>8</td>
<td>25</td>
<td>94.4%</td>
</tr>
<tr>
<td>Neg</td>
<td>1</td>
<td>103</td>
<td>104</td>
<td>92.8%</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>111</td>
<td>129</td>
<td></td>
</tr>
</tbody>
</table>

*Reference test was conducted using Treponema Pallidum Hemaglutination Assay (TPHA) (Human Gesellschaft fur Biochemica und Diagnostica mbH, Wiesbaden, Germany). TPHA indeterminate results were retested using a Treponema pallidum enzyme-linked immunosorbent assay test (ELISA)(Architect Syphilis TP; Abbott, Wiesbaden, Germany).
Table 3-4. Field performance among HIV negative participants in Haiti for detection of *Treponema pallidum* antibodies using a dual HIV/syphilis test.

<table>
<thead>
<tr>
<th>SD BIOLINE HIV/Syphilis Duo test</th>
<th>Treponema pallidum reference test</th>
<th>Total</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Kappa Coefficient (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos</td>
<td>92</td>
<td>9</td>
<td>101</td>
<td>96.8%</td>
<td>87.8%</td>
</tr>
<tr>
<td>Neg</td>
<td>3</td>
<td>65</td>
<td>68</td>
<td>(91.1%, 99.3%)</td>
<td>(78.2%, 94.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>95</td>
<td>74</td>
<td>169</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Reference test was conducted using *Treponema Pallidum* Hemaglutination Assay (TPHA) (Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany). TPHA indeterminate results were retested using a *Treponema pallidum* enzyme-linked immunosorbent assay test (ELISA)(Architect Syphilis TP; Abbott, Wiesbaden, Germany).
Table 3-5. Field performance in Haiti for detection of HIV antibodies using a dual HIV/syphilis test among pregnant women (n=49).

<table>
<thead>
<tr>
<th>HIV reference test</th>
<th>Total</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Kappa Coefficient (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pos</td>
<td>Neg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD BIOLINE HIV/Syphilis Duo test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pos</td>
<td>14</td>
<td>2</td>
<td>16</td>
<td>93.3%</td>
</tr>
<tr>
<td>Neg</td>
<td>1</td>
<td>32</td>
<td>33</td>
<td>(68.1%, 99.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>34</td>
<td>49</td>
<td></td>
</tr>
</tbody>
</table>

*Reference tests were Murex HIV-1.2.0 (DiaSorin S.p.A., Saluggia, Italy) or Alere Determine HIV (Alere) rapid testing. Positive results were confirmed with a colloidal gold test from KHB (Shanghai Kehua Bio-Engineering Co., LTD, China).
Table 3-6. Field performance in Haiti for detection of *Treponema pallidum* antibodies using a dual HIV/syphilis test among pregnant women (n=49).

<table>
<thead>
<tr>
<th>SD BIOLINE HIV/Syphilis Duo test</th>
<th>T. pallidum reference test</th>
<th>Total</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Kappa Coefficient (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pos</td>
<td>Neg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pos</td>
<td>18</td>
<td>1</td>
<td>19</td>
<td>100%</td>
<td>96.8% (81.5%, 100%)</td>
</tr>
<tr>
<td>Neg</td>
<td>0</td>
<td>30</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>31</td>
<td>49</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Reference test was conducted using *Treponema Pallidum* Hemaglutination Assay (TPHA) (Human Gesellschaft fur Biochemica und Diagnostica mbH, Wiesbaden, Germany). TPHA indeterminate results were retested using a *Treponema pallidum* enzyme-linked immunosorbent assay test (ELISA)(Architect Syphilis TP; Abbott, Wiesbaden, Germany).
Table 3-7. Visual intensity of the color of the bands indicating positive results for the dual test HIV/syphilis test band intensity

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Median</th>
<th>Quartile Range</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIV band color intensity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV true positives</td>
<td>133</td>
<td>88.3</td>
<td>28.0</td>
<td>100</td>
<td>0</td>
<td>0.3</td>
<td>100</td>
</tr>
<tr>
<td>HIV false positives</td>
<td>128</td>
<td>91.7</td>
<td>22.4</td>
<td>100</td>
<td>0</td>
<td>0.3</td>
<td>100</td>
</tr>
<tr>
<td><strong>T. pallidum band color intensity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. pallidum true positives</td>
<td>126</td>
<td>30.3</td>
<td>31.9</td>
<td>20</td>
<td>37</td>
<td>0.3</td>
<td>100</td>
</tr>
<tr>
<td>T. pallidum false positives</td>
<td>109</td>
<td>34.3</td>
<td>32.4</td>
<td>23</td>
<td>40</td>
<td>0.3</td>
<td>100</td>
</tr>
<tr>
<td><strong>T. pallidum false positives</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: Intensity was not recorded for 3 of the tests that were syphilis positive on the Duo test.
3.8 Figures

Figure 3-1. Standard for band intensity for SD Bioline HIV/Syphilis Duo test.

The color bands on this reference standard were compared to positive results on the Duo test and the percent intensity was recorded by two trained phlebotomists.
Figure 3-2. Boxplot of color band intensity among HIV and Treponema pallidum true positive and false positive test results from a dual HIV and syphilis rapid test.

◇ Mean value
The ends of the whisker are set at 1.5*IQR above the third quartile and 1.5*IQR below the first quartile
Chapter IV. Study 3: Regression Calibration to Assess the Effect of Reference Standard Misclassification on New Rapid Tests for Detection of Syphilis

4.1 Summary
Diagnostic validation studies are essential to determine how a test performs. If an imperfect reference standard is used, the calculated diagnostic accuracy of the new test may be biased because of the misclassification of true disease status. We conducted an epidemiological bias-corrected analysis simulation using estimates of potential reference standard misclassification using regression calibration methodology. We concluded by making recommendations for reporting diagnostic performance ranges instead of point estimates when using an imperfect reference test.

4.2 Background
Diagnostic validation studies are essential to determine how a test performs. However, when calculating performance of new tests, one must use a “gold standard”. Gold standards are rarely 100% sensitive and specific, leading to reference standard misclassification bias; therefore, there has been a move to using the term reference standard instead of gold standard. If an imperfect reference standard is used, the calculated diagnostic accuracy of the new test may be biased because of the misclassification of true disease status. If the reference test has lower than perfect sensitivity then there will be true cases of disease that are measured as negative (i.e. false
negative on the reference test). If those are also negative on the new test being validated then the sensitivity estimate that calculated for that new test is an over-estimate and we will conclude that the test performs better than it actually does. However, if those specimens that are positive on the reference test, then they will appear to be false negative results when they are actually true positives. Additionally, this will reduce the precision of the new test sensitivity and decrease the specificity estimate.

The introduction of point-of-care rapid tests into health services can improve syphilis screening and results in increased rates of treatment for those infected with syphilis. Rapid tests provide advantages over laboratory-based reference test methods including rapid time to result, ease of use, use of fingerprick blood specimens and no need for special transport or storage conditions. The aim of an evaluation of a new rapid test is usually to find that it is similarly performing to the existing laboratory methods, but since it has those advantageous attributes it is better suited for use. Those advantageous rapid tests are subject to false positive and false negative results when compared to a laboratory-based reference test, such as *Treponema pallidum* particle agglutination assay (TPPA). However, the TPPA is an imperfect reference test, also subject to false positive and false negative test results.

Methods to correct for misclassification error have been developed and include regression calibration. The basis for this methodology is to use information gained from an imperfectly measured variable for disease status, X, for the unknown true variable, which we will call T [Figure 4-1]. We must estimate the missing information for T that eliminates the misclassification bias caused by X that influences our final results.
We conducted an epidemiological bias-corrected analysis simulation using estimates of potential misclassification by the TPPA. We aimed to quantify the potential ranges of sensitivity and specificity for a new test when using an imperfect TPPA as a reference standard. We will conclude by making recommendations for reporting diagnostic performance ranges instead of point estimates when using an imperfect reference test.

Our article contains the following sections. In Section 4.3, we describe the data and models for the data, which include a misclassified discrete covariate, and in Section 4.4, we describe methods to correct bias induced from the misclassified covariate using regression calibration. In section 4.5, we describe the new results once data have been calibrated. The discussion and concluding remarks are given in Section 4.6.

### 4.3 Notation and Model Setup

**Data**

The data for this analysis were from an evaluation study of a dual HIV and syphilis rapid test that was conducted in Lima, Peru among men who have sex with men and transgender women. We only used the syphilis component of the dual test for this analysis.

### 4.4 Regression calibration

**Overview of regression calibration**
Regression calibration is a method to develop and fit the calibration model for the regression of our unknown variable $T$ on $D$.$^{71,72}$ For the variable of true syphilis infection status, $T$, we have some measurement error (misclassification) due to the use of an imperfect reference standard test. This imperfect standard is subject to bias because of false positive and false negative rates that are greater than zero. Our analysis began by estimating the misclassification of the true infection status by the TPPA. We quantified the potential misclassification and corrected for it using estimates of sensitivity and specificity for the TPPA. We then recalculated sensitivity and specificity of the syphilis component of the new dual rapid test. The true infection status variable ($T$) is mismeasured by the TPPA test result ($X$). The TPPA result ($X$) and the syphilis dual test ($D$) result are independently functions of the true infection status ($T$) [Figure 4-1].

If we had information on the true infection status, we would regress that variable, $T$, on the predictor variables $X$ and $D$ to get estimates of how closely $X$ and $D$ independently predict $T$ from which we could get true estimates of sensitivity and specificity of $X$ (the imperfect reference test, TPPA) and $D$ (the new rapid test for syphilis).

Define $T=1$ when the true infection status is syphilis infected, and $T=0$ when truly syphilis uninfected. $T$ is unknown.

Additionally, define $X=1$ if classified as syphilis infected, i.e. TPPA gives a positive result, and $X=0$ if TPPA gives a negative result.

Lastly, define $D=1$ if the new rapid test gives a positive result and $D=0$ if the rapid test gives a negative result.
We assume that each relationship in the directed acyclic graph (Figure 4-1) follow a generalized linear model and used the identify link where

\[ P(D=1|T=t) = \beta_0 + \beta_1 t \]

is the true association we are interested in measuring but T is unknown. Instead we have

\[ P(D=1|X=x) = \theta_0 + \theta_1 x, \]

which is biased because X is a misclassified proxy of T. We use prior information to inform the relationship between X and T where \( P(X=1|T=t) = \gamma_0 + \gamma_1 t \)

and we use Bayes rule to find \( P(T=1|X=x) = \lambda_0 + \lambda_1 x. \)

To use those above relationships in regression calibration, we plugged in \( P(T=1|X=x) \) for the unknown T by replacing X in the model \( P(D=1|X=x) \) to yield \( P(D=1|T=t) \). In other words, we replaced X with an expression for T, in terms of X, in the model \( P(D=1|X=x) \) to get \( P(D=1|T=t) \).

This left us with

\[ \beta_0 + \beta_1 (\lambda_0 + \lambda_1 x) \]

which simplifies to

\[ \beta_0 + \beta_1 \lambda_0 + \beta_1 \lambda_1 x \]

and is equal to

\[ \theta_0 + \theta_1 x. \]

Therefore \( \beta_0 = \theta_0 - (\theta_1 / \lambda_1) \times \lambda_0 \)

and \( \beta_1 = \theta_1 / \lambda_1. \)

**Regression of X on T**

We have the following four probabilities for the validity of X as a proxy of T (assuming non-differential misclassification):

1. Sensitivity (Se_{XT}) = probability TPPA test is positive given truly infected = \( P(X = 1 \mid T = 1) \)
2. False-negative probability (\( F_{NX} \)) = Probability TPPA test is negative given truly infected 
   \[ = P(X = 0 \mid T = 1) = 1 - S_{XT} \]
3. Specificity (\( S_{pX} \)), Probability TPPA test is negative given being truly uninfected 
   \[ = P(X = 0 \mid T = 0) \]
4. False-positive probability (\( F_{PX} \)) = Probability TPPA test is positive given truly uninfected 
   \[ = P(X = 1 \mid T = 0) = (1 - S_{pX}) \]
5. Positive predictive value (\( P_{PX} \)) = Probability that there is true infection given that the TPPA test is positive 
   \[ = P(T = 1 \mid X = 1) \]
6. Negative predictive value (\( N_{PX} \)) = Probability that there is no infection given that the TPPA test is negative 
   \[ = P(T = 0 \mid X = 0) \]

Note that given \( P(X = 1 \mid T = t) = \gamma_0 + \gamma_1 t, \)
\[ \gamma_0 = P(X=1\mid T=0) = F_{PX} \]
and \( \gamma_1 = P(X=1\mid T=1) - P(X=1\mid T=0) = S_{XT} - F_{PX} = S_{XT} - (1 - S_{pX}). \)

Additionally, given \( P(T=1\mid X=x) = \lambda_0 + \lambda_1 x, \)
\[ \lambda_0 = P(T=1\mid X=0) = 1 - N_{PX} \]
and \( \lambda_1 = P(T=1\mid X=1) - P(T=1\mid X=0) = P_{PX} - (1 - N_{PX}). \)

Because \( T \) is unknown, we used a range of estimates to conduct a sensitivity analysis around this effect. We used a range from 94% to 98% to reflect the possible uncertainty in performance of the TPPA. Additionally, used a range of 95% to 99% for specificity.

**Regression of \( D \) on \( T \)**

We have the following four probabilities for the validity of test \( D \) compared to the true infection status, \( T \):

1. Sensitivity (\( S_{DT} \)) = \( P(D = 1 \mid T = 1) \)
2. False-negative probability ($\text{FN}_{DT}$) = $P(D = 0 \mid T = 1) = (1 - \text{Se}_{DT})$

3. Specificity ($\text{Sp}_{DT}$) = $P(D = 0 \mid T = 0)$

4. False-positive probability ($\text{FP}_{DT}$) = $P(D = 1 \mid T = 0) = (1 - \text{Sp}_{DT})$

We assumed that the true values follow a generalized linear model and used the identify link for regression of $T$ on $D$ where

$$P(D=1|T=t) = \beta_0 + \beta_1 t.$$ 

Note that again the regression coefficients can be used to find sensitivity and specificity where

$$\beta_0 = P(D=1|T=0) = \text{FP}_{DT}$$

and

$$\beta_1 = P(D=1|T=1) - P(D=1|T=0) = \text{Se}_{DT} - \text{FP}_{DT} = \text{Se}_{DT} - (1 - \text{Sp}_{DT}).$$

However, the variable $T$ is unknown. Instead we have data that used a proxy for $T$ given by $X$. Misclassification occurs when some of the values of $X$ do not equal the corresponding values of $T$; instead of $T$ we observed $X$. Some measurements of $T$ using the proxy $X$ are correctly classified (measures without error). This means that for some observations $X=T$. Regressing $D$ on $X$ results in biased $\beta$ estimates that here we call $\theta$.

**Regression of $D$ on $X$**

We have the following four probabilities for the validity of test $D$ compared to the biased reference standard, $X$:

1. Sensitivity ($\text{Se}_{DX}$) = $P(D = 1 \mid X = 1)$
2. False-negative probability ($FN_{DX}$) = $P(D = 0 \mid X = 1) = (1 - Se_{DX})$

3. Specificity ($Sp_{DX}$) = $P(D = 0 \mid X = 0)$

4. False-positive probability ($FP_{DX}$) = $P(D = 1 \mid X = 0) = (1 - Sp_{DX})$

We assumed the relationship between $X$ and $D$ follow a logistic model where

$$P(D=1\mid X=x) = \theta_0 + \theta_1 x.$$ 

Note that these regression coefficients can be used to find sensitivity and specificity of the new test $D$ based on the biased gold standard where

$$\theta_0 = P(D=1\mid X=0) = FP_{DX}$$

and

$$\theta_1 = P(D=1\mid X=1) - P(D=1\mid X=0) = Se_{DX} - FP_{DX} = Se_{DX} - (1 - Sp_{DX}).$$

All analyses and simulations were conducted using SAS v9.4 (SAS Institute Inc., Cary, NC). Logistic models were fit in PROC NLMIXED.

4.5 Results

There were three false positive results and 18 false negative results. Under an assumption that the reference test was a true representation of the infection status (i.e. the $X$ to $D$ relationship), the sensitivity and specificity of the new syphilis test were 89.2% (95% CI: 83.5%, 93.5%) and 98.8% (95% CI: 96.5%, 99.8%), respectively [Table 4-1].
The results of the relationship between T and D, found using regression calibration show a wide range of potential values for sensitivity and specificity of the new syphilis test (D) generated to account for uncertainty in the misclassification of the true infection status [Table 4-2].

4.6 Discussion

Perfect knowledge of the magnitude of measurement error is rarely known and unlikely in practice. We used regression calibration for a range of estimates accounting for potential measurement error to correct for disease status misclassification from an imperfect reference standard test. We then recalculated the validity estimates for a new test based on those infection status results. We found that the estimates of sensitivity ranged between 100% to about 67% and the specificity ranged between 100% to about 90%. This is a much wider range than the 95% confidence interval reflected when we used the TPPA reference test as a true gold standard – even when using the conservative exact binomial method to generate confidence intervals.

Even laboratory-based diagnostics for syphilis are subject to infection status misclassification. Diagnostic validation studies for new tests using an accepted reference standard are essential to determine the performance of the new test, however, if an imperfect reference standard test is used, the calculated diagnostic accuracy of the new test will be biased. We recommend that reporting of new test performance information use a range estimates of sensitivity and specificity generated using a calibrated model that corrects for reference standard misclassification using external or internal information on the extent of misclassification.
A limitation of this analysis is that we did not account for time since infection with *Treponema pallidum* (the organism that causes syphilis). Serologic tests for syphilis detect humoral antibodies. Antibodies, however, usually do not appear until 1 - 4 weeks after the initial syphilitic chancre has formed, which usually appears within 3 weeks after infection.\(^{74,75}\)

Therefore the sensitivity of serologic tests tends to be lower during primary stage syphilis.

Antibodies increase in concentration during the second stage of syphilis infection and therefore sensitivity of serologic tests are improved during the secondary stage. We assume that both the rapid test and the reference test, TPPA, are both subject to similar, but potentially slightly different, misclassification by stage of disease because they are both antibody detection methods.

An additional limitation of this analysis is that we did not have internal validation data to use for the regression calibration. In the absence of internal validation data, we used external prior information to inform the calibration model.

In conclusion, when a validation study is subject to reference standard misclassification bias, regression calibration can be a useful tool to detect possible ranges of sensitivity and specificity estimates for the new diagnostic test.
4.7 Tables and Figures

Figure 4-1. Directed Acyclic graph of the relationship between an imperfect reference test for syphilis (X), an unknown true infection status (T) and a new diagnostic test (D).
Table 4-1. Field performance for detection of *Treponema pallidum* antibodies using a new dual HIV/syphilis test assuming that the reference test is a true representation of infection status.

<table>
<thead>
<tr>
<th>T. pallidum Component</th>
<th>Number of samples</th>
<th>total</th>
<th>Sensitivity (95% CI)*</th>
<th>Specificity (95% CI)*</th>
<th>Kappa Coefficient (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref test +</td>
<td>149</td>
<td>153</td>
<td>(83.5%, 93.5%)</td>
<td>(96.5%, 99.8%)</td>
<td>(.96, 1.00)</td>
</tr>
<tr>
<td>Ref test -</td>
<td>3</td>
<td>261</td>
<td>89.2%</td>
<td>98.8%</td>
<td>.98</td>
</tr>
<tr>
<td>Dual Test +</td>
<td>18</td>
<td>243</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dual Test -</td>
<td>246</td>
<td>413</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>167</td>
<td>246</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The exact binomial method was used to calculate confidence intervals for sensitivity and specificity*
Table 4-2. Average value of sensitivity and specificity generated using a simulated range of misclassified reference standard results.

<table>
<thead>
<tr>
<th></th>
<th>Mean value</th>
<th>Minimum value</th>
<th>Maximum Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>90.3%</td>
<td>67.5%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100.0%</td>
<td>90.8%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>
Chapter V. Public Health Importance

Over 10 million persons worldwide have new syphilis infections each year.² Syphilis case finding and subsequent treatment can help prevent transmission to sex partners and unborn infants. The World Health Organization recommends syphilis screening for pregnant women as part of antenatal care and for key populations like men who have sex with men and transgender women.¹⁹,⁷⁶,⁷⁷ Because syphilis screening has been shown to be cost-effective in a number of settings to prevent adverse outcomes of pregnancy and continued transmission, most countries have adopted policies for antenatal syphilis screening.⁷⁷,⁷⁸ However, despite these policies, syphilis screening is not routinely conducted in many settings.⁷⁹-⁸¹

The advent of point-of-care rapid tests could result in early detection and treatment of syphilis infections, expand case finding outside of clinical settings, and has the potential for enhancing the global control of syphilis to save lives. The potential impact of point-of-care HIV and syphilis testing using rapid tests is not just from the knowledge of infection status but from the implementation of effective treatment.⁸²,⁸³ Rapid tests may require lower levels of infrastructure for testing when compared to laboratory-based diagnostics, however this does not mean that it requires lower levels of programming, commitment or support. Implementing point-of-care testing along is not enough, a focus of point-of-care testing needs to be on clinical action. Rapid tests are a tool in the spectrum of care to reduce adverse outcomes related to infection and to reduce further transmission. The introduction of a new test requires program support, training, systems for communication of results to both patients and providers, patient data storage and adequate follow up. Additionally, new technology requires quality assurance, which means that
the tests must remain at a sustainable price to allow for continued quality assurance programs and systems.

The World Health organization published *Guidance on global processes and criteria for validation of elimination of mother-to-child transmission of HIV and syphilis* in 2014, which provides a framework for elimination with key targets.\(^\text{19}\) So far, Cuba has been the first and only country to receive validation from the WHO that it has eliminated mother-to-child transmission of HIV and syphilis by meeting certain targets including less than 50 cases of mother-to-child transmission of HIV per 100 000 live births; and less than 5% in breastfeeding populations or less than 2% in non-breastfeeding populations.\(^\text{84,85}\) Additionally, the rate of mother-to-child transmission of syphilis must be less than 50 cases per 100 000 live births. We look forward to other countries following Cuba’s lead and receiving confirmation of the elimination of mother-to-child transmission of HIV and syphilis as a public health threat.
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