Feasibility and acceptability of HIV/Syphilis Duo assay testing among female sex workers at drop-in centers in **YAOUNDÉ, CAMEROON**

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Aim

The aim of this study was to characterize the feasibility and acceptability of a combined dual HIV and treponemal-based syphilis test for female sex workers for diagnosing both HIV and syphilis in Cameroon.

Rationale

In low and middle-income countries, female sex workers (FSW) are disproportionately affected by HIV [1], and Cameroon is no exception. An estimated 620,000 people have been diagnosed and are living with HIV in Cameroon, corresponding to about 4.5% of the adult population 15-49 years [2].The 2016 Integrated Biological and Behavioral Surveillance (IBBS) survey of FSW estimated HIV prevalence among this population at 24.5% nationally [3]. Regional disparities exist, with FSW reached in Bamenda and Douala experiencing the highest prevalence estimates, 33.8% and 30.7% respectively. In the same study, FSW experienced a high prevalence of syphilis (8.2%). Syphilis is a sexually transmitted infection, which facilitates the acquisition and transmission of HIV [4]. Therefore, the WHO recommends routine screening for syphilis among individuals living with HIV [5]. A recent study of patient data at an HIV clinic in Cameroon estimated that the prevalence of syphilis among patients living with HIV was 11.6%, with the prevalence of 15.6% among men and 9.1% among women [6]. FSW, who are at heightened risk of HIV and who also face structural barriers to health care, may be especially at risk for primary syphilis and their children may be at particular risk for congenital syphilis [7]. Syphilis can be responsible for serious consequences including stillbirth, prematurity, and neonatal death.

In Cameroon, and other low and middle-income countries, the standard for syphilis testing has been to use a rapid plasma reagin (RPR) non-treponemal test which is cheap, requires minimal infrastructure, and can be used to assess treatment needs in real time. There are several issues in using RPR alone: lower specificity given cross-reactivity, lower sensitivity in warm temperatures, and lower sensitivity in detecting late syphilis or latent syphilis [8]. Moreover, some people remain in a serofast condition where titers of non-treponemal antibodies do not decrease by at least four times after treatment—and titering of these tests is time intensive and not often done [8]. Because of these issues, there is significant interest in moving to a rapid treponemal enzyme immunoassay (EIA) followed by using a non-treponemal as a confirmatory test for active syphilis and treatment indication.

Concurrently, in Cameroon, there are an increasing number of facilities that do rapid HIV testing in the context of "one-stop shops" or drop-in centers (DIC). However, syphilis testing is not routinely conducted in these DICs because of the need for separate supply chain management systems to order these tests and appropriate reagents and separate staff training to read and interpret the results. STI care is generally limited to syndromic screening and management. Many STIs are commonly asymptomatic [9-11], and syndromic screening is likely to result in untreated STIs among beneficiaries. Further, although screening pregnant

women for syphilis is recommended in many countries and antenatal care can thus serve as an important setting for diagnosis [12], syphilis screening is not routinely conducted during antenatal care in Cameroon.

In response to such needs, there has been the development of a rapid kit HIV/Syphilis Duo Test (SD BIOLINE) that uses a single drop of plasma, serum, or whole blood and can rapidly assess the presence of both HIV and syphilis infections. In countries around the world, the use of a single test has been shown to reduce training time, reduce workload, and allow for a single procedure for HIV and syphilis. Given the high pregnancy rates among FSW in Cameroon and the high burden of syphilis observed in larger urban centers, increased yield and treatment of active syphilis infection has the potential to mitigate HIV and congenital syphilis.

Methods

A total of 400 FSW who are at risk for HIV and are indicated to receive HIV testing were tested simultaneously for HIV and syphilis to assess number of syphilis infections detected, that would otherwise have been undiagnosed. SD BIOLINE HIV/Syphilis tests (described below) were administered to those women recruited at drop-in centers (DIC) supported by the Continuum of Prevention, Care and Treatment (CoPCT) of HIV/AIDS with Most At-Risk Populations (CHAMP) Program and implemented in partnership with Horizons Femmes, an organization based in Cameroon who provide HIV testing services and are dedicated to improving the health and lives of particularly marginalized women. The DIC have experience with both treponemal and non-treponemal syphilis testing in the context of the cross-sectional and prospective biobehavioral surveys but does not provide this as a standard service.

At each DIC, the population recruited to participate was FSW indicated to receive an HIV test.

Inclusion criteria

- Assigned female biological sex at birth
- 18 years of age or older
- Reported having exchanged sex for money as a main source of income for the last 12 months
- Indicated to receive an HIV test

Exclusion criteria

- Demonstrated mental incapacity or any other illness preventing comprehension of the study procedures or informed consent
- Confirmed HIV-positive result at DIC

All study interactions, including the informed consent process and brief questionnaire, were offered to participants in either English or French. At the DIC, individuals were provided the option to enroll in the program and participate in HIV/Syphilis testing. After consent to participate in the program, the participants were assigned a unique identification number which was pasted on the consent document, the questionnaire, the sample tube, and the results of tests of HIV and syphilis. All documents in the program for each participant had the same unique ID code as well as the date of collection.

Syphilis diagnostics

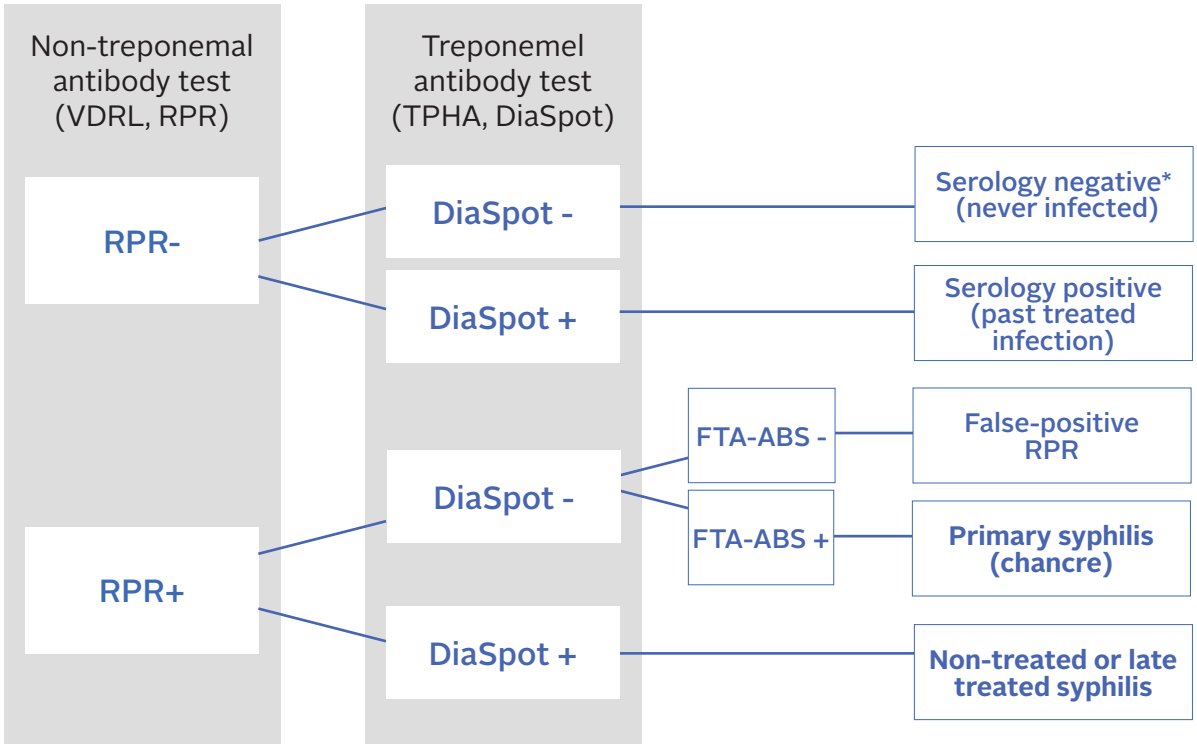
Syphilis diagnosis is made through detection of antibodies. There are two groups of serological tests for syphilis:

1. Non-specific, non-treponemal tests detect antibodies associated with syphilis infection but not specific to *Treponema pallidum*. These antibodies are present during active infection but subside after treatment, thus non-treponemal tests can distinguish between past treated and active infection. Examples include the Rapid Plasma Reagin (RPR) test and Venereal Disease Reference Laboratory (VDRL) test.
2. Specific, treponemal tests detect antibodies to *T. pallidum* antigens. As these are retained for years, even after infection is treated, treponemal tests cannot distinguish between past and active infection. Examples include Treponema pallidum Hemagglutination Assay (TPHA), DiaSpot, FTA-ABS, and the SD BIOLINE HIV/Syphilis Duo Test. Most rapid syphilis tests are treponemal tests.

Although results from an RPR test can be read in less than 10 minutes, unlike rapid treponemal tests, the non-treponemal tests require more resources such as correct storage and preparation (e.g. refrigerated reagent, a rotator and centrifuge to prepare plasma).

For this study, we utilized the SD BIOLINE HIV/Syphilis Duo Test for dual detection of HIV and treponemal antibodies. This was combined with an RPR test to distinguish active syphilis. Given that SD Bioline HIV/Syphilis Duo is not validated for non-study use in Cameroon, to conform with national guidelines for syphilis diagnosis (**Figure 1**), a second treponemal test, DiaSpot, was used for all participants, and syphilis treatment decisions were based on RPA and DiaSpot results (**Figure 1**). Notably, while the RPR non-treponemal and DiaSpot treponemal test combination represents a standard in the country, most centers do not do syphilis testing.

Figure 1. National algorithm for syphilis diagnosis



** Does not exclude early syphilis in the case of recent infection*

SD BIOLINE HIV/Syphilis Duo Test

SD BIOLINE HIV/Syphilis Duo is a solid phase immunochromatographic assay for the qualitative detection of all isotypes (IgG, IgM, IgA) specific to HIV-1/2 and/or Treponema pallidum. SD BIOLINE HIV/Syphilis Duo tests are manufactured by Standard Diagnostics, Incorporated and Alere. Test kits provide results within 15-20 minutes and require one drop of serum, plasma, or whole blood. The kits are stable for up to 24 months if kept at temperatures between 1 and 30°C. SD BIOLINE HIV/Syphilis Duo was approved for prequalification of in vitro diagnostics by World Health Organization (WHO) in 2015 [13]. Based on laboratory performance, the sensitivity for HIV is 99.91% (95% CI 99.51-100.00%) and specificity of 99.67% (99.16%, 99.91%). The sensitivity for Treponema Pallidum antibodies is 99.67% (98.82%, 99.96%) with a specificity of 99.72% (99.29%, 99.92%) [13].

RPR test

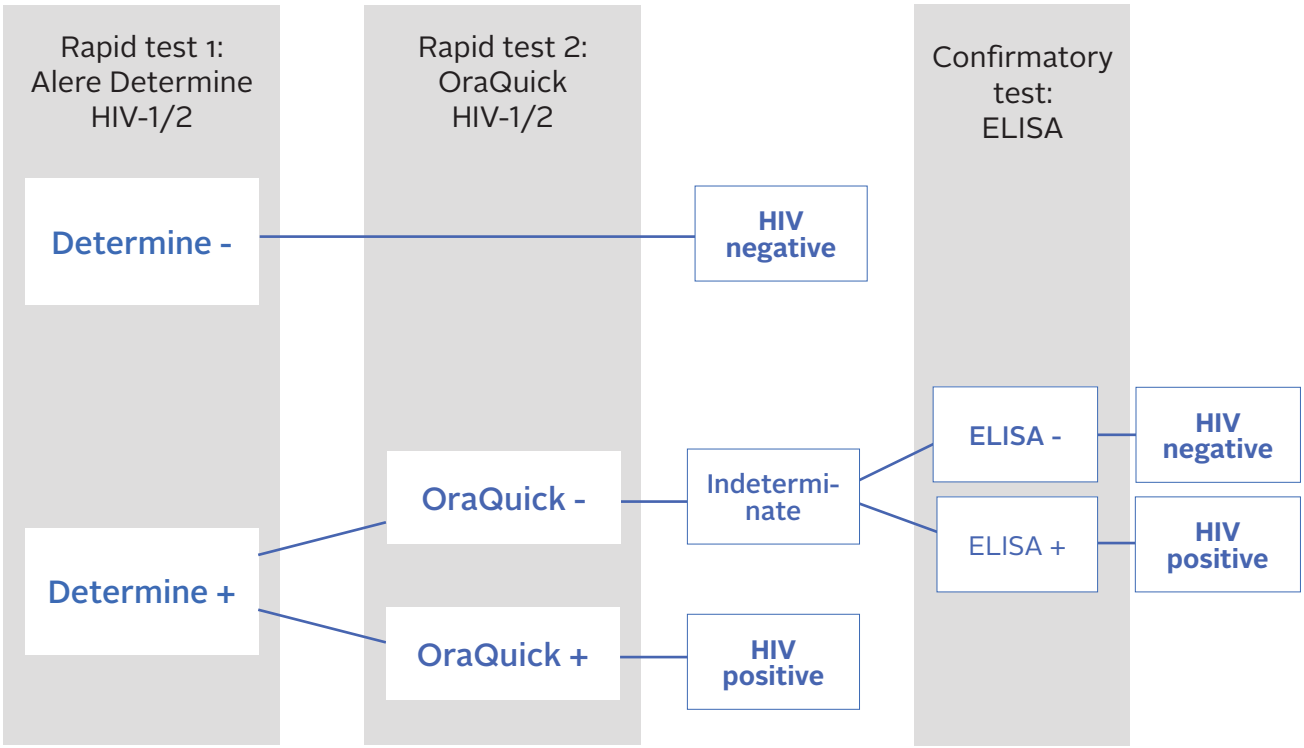
RPR test is non-specific treponemal test. RPR test measures IgM and IgG antibodies to lipoidal material released from damaged host cells as well as to lipoprotein-like material, and possibly cardiolipin released from the treponemes.

DiaSpot

DiaSpot™ Rapid Diagnostic Test kit is a rapid treponemal test which detect antibodies (IgG ad IgM) to T. pallidum in serum, plasma and whole blood. Its rapid test strip is based on lateral flow immunoassay. It has a relative sensitivity of 99.7% and specificity of 99.6% [14].
HIV diagnostics

As for syphilis diagnosis, for HIV diagnosis SD Bioline HIV/Syphilis Duo was combined with additional rapid HIV tests to conform with national guidelines. Serial rapid tests for HIV using Alere DetermineTM HIV – ½ and OraQuick HIV – ½ were completed based on venous blood draw, according to the national testing protocol (**Figure 2**).

Figure 2. National algorithm for clinical HIV screening



** Does not exclude early syphilis in the case of recent infection*

Determine HIV-1/2

The Cameroonian HIV screening algorithm utilizes Alere DetermineTM HIV-1/2 is used as the initial screening test based on high sensitivity. Determine detects antibodies to HIV types 1 and 2 in serum, plasma or whole blood sampled. An evaluation by WHO demonstrated sensitivity of 100% and specificity of 99.4% [15], but issues with lower specificity (e.g. 96.8%, 95% CI: 95.9%-97.6% in whole blood) have been highlighted in some studies [16, 17].

Based on the national algorithm, individuals with a non-reactive Determine test are declared HIV negative. Individuals with a reactive Determine test receive a second discriminatory rapid test.

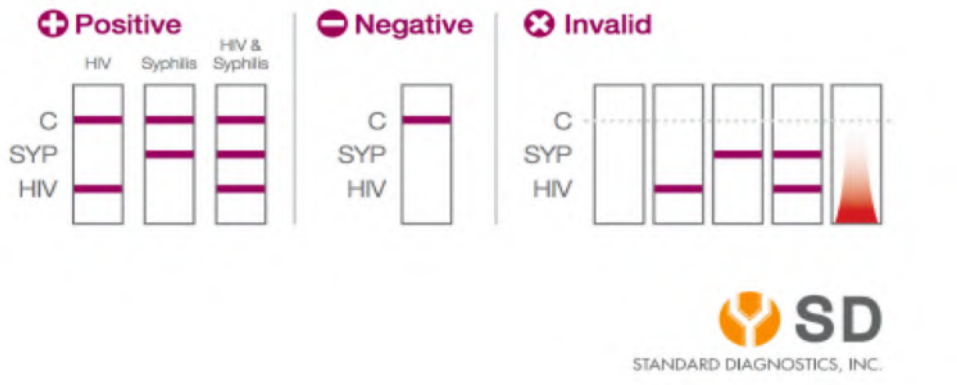
OraQuick HIV-1/2

OraQuick rapid HIV test detects antibodies to HIV types 1 and 2 in human oral fluid, whole blood, serum or plasma. A United States-based study demonstrated sensitivity of 99.2% (97.8%–99.8%) and specificity of 100% (97.2%–100%) on whole blood samples [18].

Study procedures

Upon provision of informed consent, pre-test counseling was conducted followed by the taking of a sample of whole blood. This was conducted by a qualified study nurse and/or phlebotomist. Each sample test was marked with the participants UIC. A venous blood sample was taken, after the area had been cleaned using an alcohol swab. A capillary pipette was used to add 20 µL of blood into a round sample well. Provided assay diluent was added into the same round well. Results were available 15-20 minutes later, and read immediately to reduce a false interpretation. A schematic diagram of interpretation of SD Bioline HIV/Syphilis Duo results is shown in **Figure 3**.

Figure 3. Reading the results of SD BIOLINE HIV/Syphilis Duo, adapted from http://www.standardia.com/en/home/product/Rapid_Diagnostic_Test/HIVSyphilisDuo.html



Using the same blood sample, an alternative treponemal test–DiaSpot–and RPR non-treponemal test were simultaneously run. Whole blood was centrifuged to obtain plasma and ready to use in room temperature (20–30°C). RPR antigen was then mixed with plasma on a plastic-coated card. If antibodies were present, they combine with the lipid particles of the antigen, causing them to agglutinate. The charcoal particles co-agglutinate with the antibodies and show up as black clumps against the white card. If antibodies are not present in the test serum, the test mixture is uniformly gray.

While waiting for their results, the study participants took part in a 15-20-minute socio-behavioral questionnaire, which was administered by a study team interviewer. Questionnaires covered participant demographics, sexual practices, STI symptoms, and STI and HIV testing and treatment history. The data collected from questionnaires was entered electronically using tablets, and laboratory forms will be filled on paper and subsequently entered electronically.

Following the socio-behavioral questionnaire, a qualified counselor or the study nurse/phlebotomist conducted post-test counseling with the participant and made the rapid test results available to study participants at the time of study participation. Participants may have chosen to decline to receive their test results.

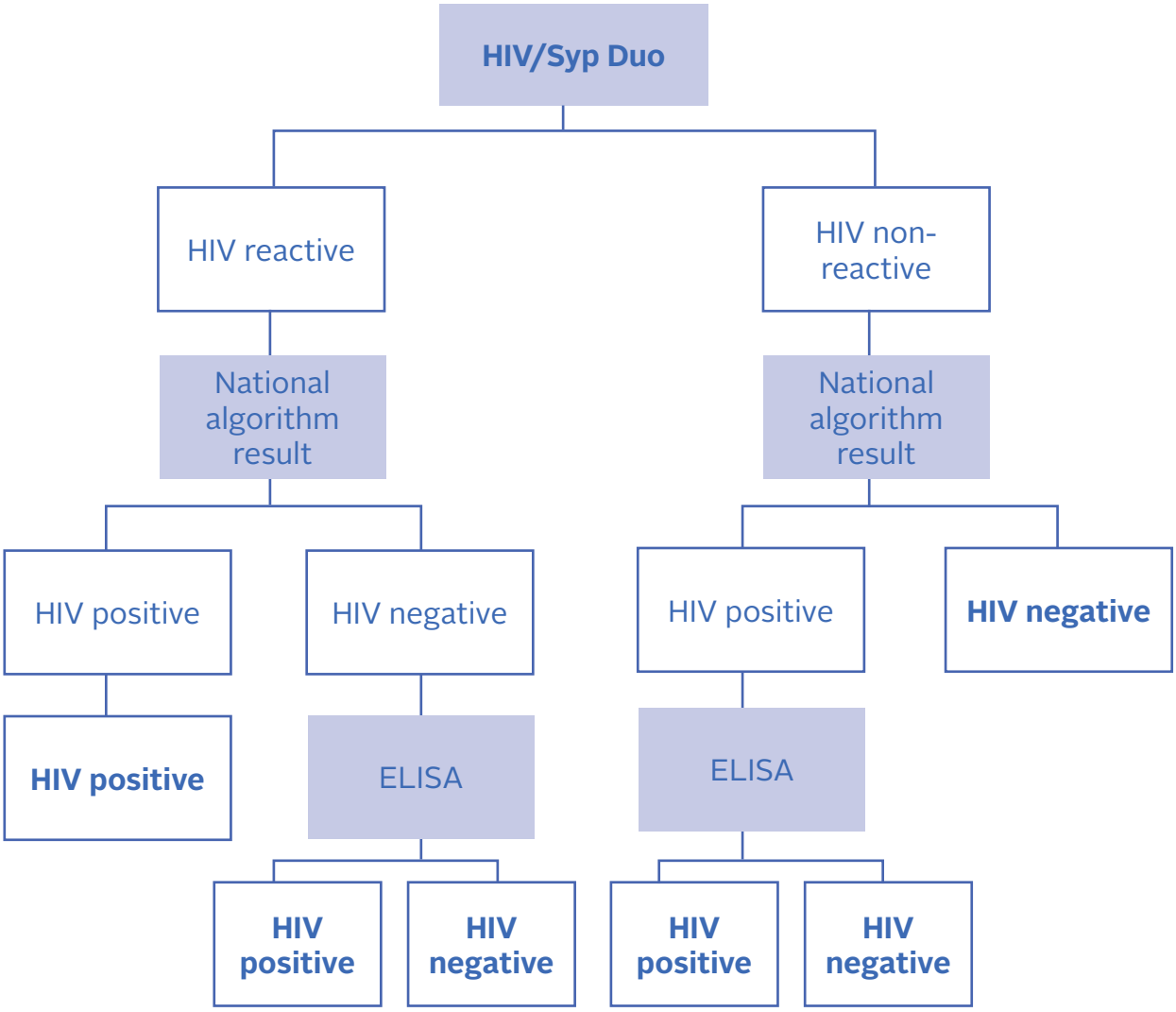
Treatment decision

Treatment was provided based on the results of RPR and DiaSpot, which are approved nationally, and consisted of 14 days (early syphilis) or 30 days (late syphilis) of oral doxycycline. If there was a suspicion of pregnancy, then treatment was 4 weekly injections of benzathine penicillin. Preferred treatment for syphilis in the DIC was doxycycline packs rather than Pen-G injections to reduce loss to follow-up for treatment, as the full oral course could be provided at once.

Due to national guidelines and the lack of a reference standard test or FTA-ABS tests, treatment decisions for syphilis were conservatively made, as over-treatment is preferred given the consequences of missed treatment. The WHO recommend that in high risk or vulnerable populations, including FSW, all individuals with a positive rapid treponemal test be treated [19]. Women were treated for syphilis if a positive RPR or positive DiaSpot test were detected.

HIV treatment decision was based on national treatment guidelines, but individuals with discordant results by national algorithm and SD Bioline HIV/Syphilis HIV result received confirmatory testing via ELISA (**Figure 4**).

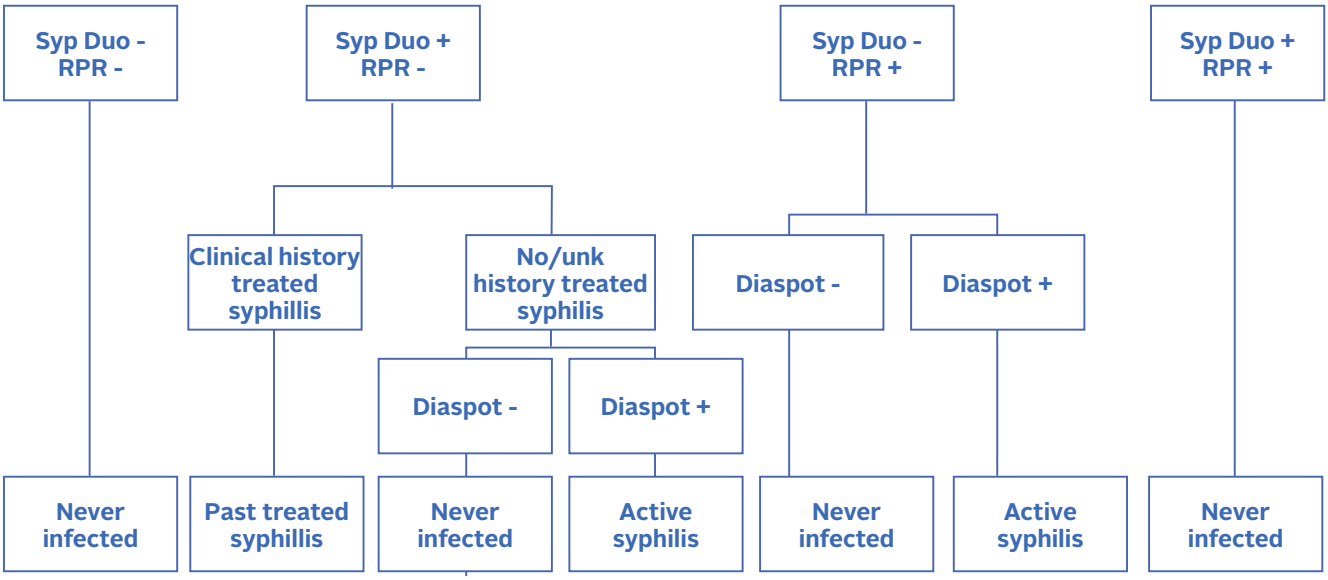
Figure 4. Derivation of final HIV diagnosis and treatment decision. Final result shown in boldface.



Study result interpretation

To classify active syphilis infection, an algorithm was developed based on SD Bioline HIV/Syphilis Duo syphilis result, RPR, and clinical history (**Figure 5**). Interpretation of HIV result for study purposes was the same as for treatment purposes (**Figure 4**).

Figure 5. Algorithm used for classification of active syphilis



Ethical considerations

Ethical approval was obtained from the National Research Ethics Committee in Cameroon (No. 2017/10/942/CE/CNERSH/SP) and Johns Hopkins University Institutional Review Board (IRB#00008011). Administrative clearance was received through the Ministry of Public Health No. 631-21-17.

Results

In total, 400 FSW were recruited from 7 February to 9 April 2018. The median age of participants was 27 years (IQR: 23-34 years), and most women had some secondary school education (74%), including 51 (13%) women with university education.

Table 1. Sexual partners and consistent condom use (CCU) with given partner type

	N	n	%
SOCIODEMOGRAPHIC			
AGE GROUP			
18-19	400	36	9.0
20-24	400	110	27.5
25-29	400	92	23.0
30-34	400	63	15.8
35-39	400	32	8.0
40-49	400	43	10.8
50+	400	24	6.0
Any secondary schooling or higher	400	297	74.3
Monthly income			
<=100,000 FCFA	386	277	71.8
>100,000 FCFA	386	109	28.2

In addition to regular (94%) and casual (99%) clients, most participants also reported a regular non-paying partner (NPP; 80%) in the past month, and 27% of participants reported a casual NPP in the past month (Table 2).

Consistent condom use (CCU) was higher with casual clients (77%) than regular clients (54%), and lowest with regular NPP (6%, **Figure 6**).

Report of experiencing any STI symptoms in the past year was high (83%). Although some symptoms are not specific and may not represent an STI, more specific symptoms remained high (**Figure 7**), and 25% of all women specifically reported genital or anal blisters and sores. Approximately two-thirds of participants reported even being tested for STIs, and nearly half (49%) reported ever being told they had an STI. Overall, 44% of women reported a prior syphilis test, while 30 (8%) women reported ever being diagnosed with syphilis, of whom nearly all (93%) reported being treated for syphilis. The most commonly diagnosed STIs were chlamydia (39%) and gonorrhea (12%).

Figure 6. Reports always using a condom in the past month with given partner type, where applicable

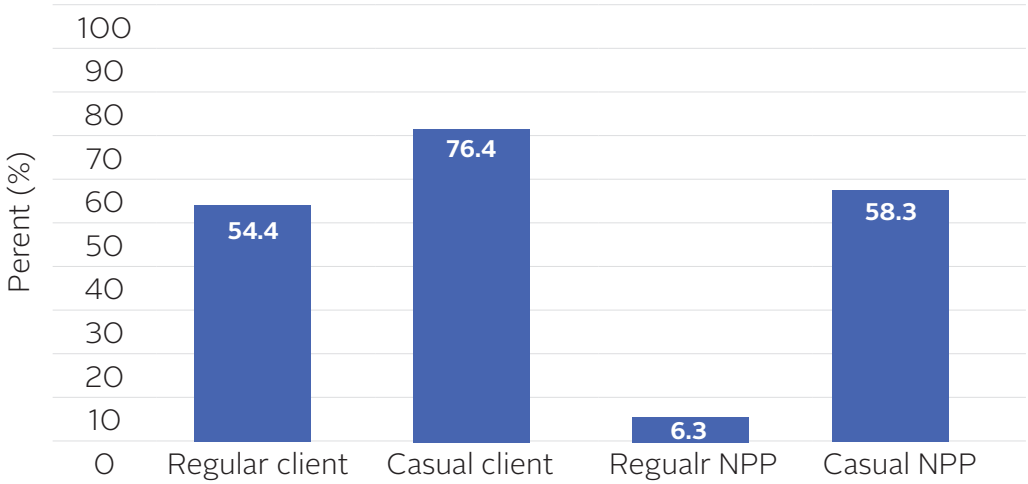
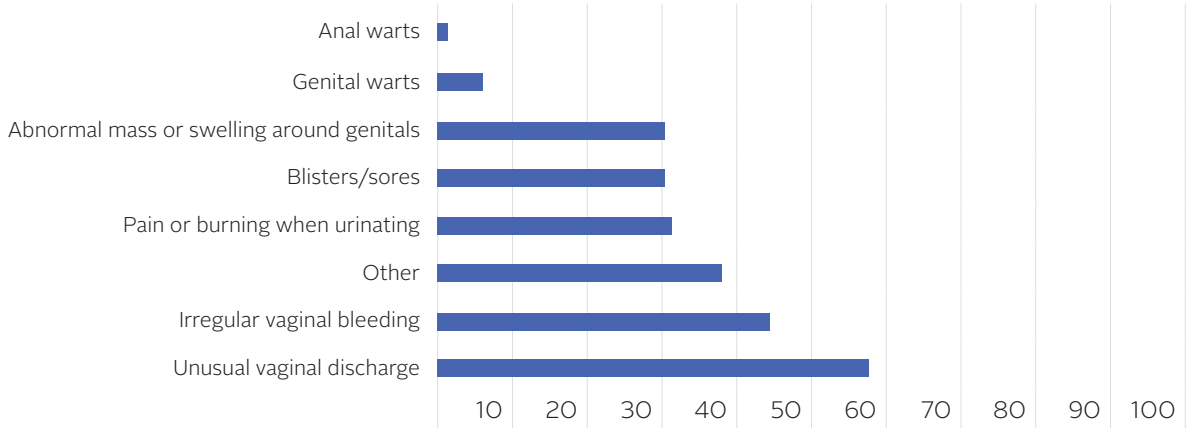


Figure 7. Types of genital/anal symptoms experienced by participants reporting any STI symptoms in the past year



Most participants reported prior HIV testing (Table 3); 376 (94%) women reported ever being tested for HIV, and 333 (84%) women reported an HIV test in the past 12 months. Among those ever tested, nine (2.4%) women reported a previous positive result and were still eligible for study participation if result was not confirmed/ registered within CHAMP.

Most women had ever been pregnant (85%), of which 298 (88%) currently had living children. Among women with a history of pregnancy, 26% had ever had a miscarriage or still birth and 75% had sought antenatal care at last pregnancy. Overall 24 (6%) women reported being currently pregnant, and 15 (4%) women did not know if they were pregnant. While 261 (71%) of women reported that it was important to avoid getting pregnant right now, only 122 (31%) women reported using any hormonal or long-acting contraception. Most women (77%) reported intentions to have children in the future (Table 3).

The most commonly reported reasons for taking an HIV/Syphilis test at the time of enrollment were part of regular testing pattern (44%), engaged in risky behavior (20%) and someone suggested they get tested (15%, Table 4). Of participants reporting the latter reason, they were most commonly recommended for testing from a peer educator (45%) or friend (32%).

Table 3. HIV and STI testing and reproductive health and history

	N	n	%
STI AND TESTING HISTORY			
Any symptoms of STIs in past year	398	331	83.2
Blisters or sores in the genital region or in the anus	400	102	25.5
Ever been tested for STI	400	272	68.0
Ever been tested for syphilis	398	174	43.7
Ever told have:			
Any STI	400	196	49.0
Syphilis	383	30	7.8
Treated syphilis	29	27	93.1
Gonorrhoea	394	49	12.4
Chlamydia	390	150	38.5
Herpes	399	21	5.3
Human papillomavirus	394	0	0.0
Hepatitis B	386	1	0.3
Hepatitis C	388	0	0.0
Unknown STI	399	3	0.8
Other STI	399	4	1.0
Ever tested for HIV	400	376	94.0
Self-reported HIV positive result	369	9	2.4
HIV test in past 12 months	399	333	83.5

Table 3. HIV and STI testing and reproductive health and history *cont.*

	N	n	%
REPRODUCTIVE HEALTH AND HISTORY			
Ever pregnant	399	338	84.5
Ever miscarriage or still birth	338	88	26.0
Any living children	338	298	88.2
Visited clinic for ANC at last pregnancy	338	253	74.9
Using any hormonal or long-acting contraception	400	122	30.5

Table 4. Reason for test at enrollment

	N	n	%
REASON FOR HIV/SYPHILIS TEST			
Reason for HIV/Syphilis test			
Engaged in risky behaviour	398	80	20.1
Sex partner engaged in risky behaviour	398	19	4.8
Had sex with someone living with HIV	398	3	0.8
Condom slipped or broke	398	11	2.8
Someone suggested I get tested	398	60	15.1
Part of my regular testing pattern	398	173	43.5
To confirm another positive test result#	398	19	4.8
Know status/curiosity	398	22	5.5
Other	398	11	2.8
Who suggested to get an HIV test			
Sexual partner	60	3	5.0
Peer Educator	60	27	45.0
Doctor	60	2	3.3
Family member	60	9	15.0
Friend	60	19	31.7

Although specifically phrased as to confirm a positive result, only 9/19 of these individuals self-reported a previous HIV-positive result, and question may have been interpreted as to confirm a prior test result (regardless of result).

Biological results

Overall 37 (9%) women tested HIV positive by SD Bioline. They were eight results discordant with the national algorithm for HIV testing, highlighted in Table 5. All discordant results were sent for confirmatory ELISA testing.

Table 5. HIV diagnosis table for SD Bioline and national algorithm for HIV testing

		NATIONAL GUIDELINES HIV			
		Negative	**Positive**	**Indeterminate**	**Total**
HIV SD Bioline	Negative	362	0	1	363
	Positive	6	30	1	37
	Total	368	30	2	400

Among the eight individuals with discordant HIV results, two women were confirmed positive based on ELISA. This includes one women who tested HIV negative and one women who had an indeterminate status based on the national algorithm. The final HIV prevalence among the included sample was 8.0% (Table 6).

Table 6. Final biological results for HIV and syphilis

BIOLOGICAL RESULTS	n	%
Total participants	400	100.0
HIV positive	32	8.0
Treponemal-based syphilis results		
SD Bioline Syphilis Duo	36	9.0
DiaSpot	30	7.5
Non-treponemal-based syphilis results		
RPR	0	0.0
Interpreted syphilis result		
Active syphilis	14	3.5
Past treated syphilis	12	3.0
Never infected	374	93.5
HIV/syphilis coinfected	1	0.3

Of the 32 women with a confirmed HIV positive result, 24 (75%) were newly diagnosed with HIV and eight (25%) women self-reported a prior HIV positive result. The women who self-reported a prior HIV positive result remained eligible for the study because there was no formal record of HIV status at CHAMP.

Overall, 36 (9%) women had a reactive SD Bioline result for syphilis, and no woman had a positive RPR test. When interpreted alongside Syphilis DiaSpot and history of syphilis, 14 (3.5%) were classified as having active syphilis, 12 (3.0%) were classified as having past treated syphilis (Table 6). Due to national guidelines and the lack of a reference standard test, treatment decisions for syphilis were conservatively made, and in total 30 women were treated for syphilis based on having a positive Syphilis DiaSpot (n=30). All were treated as per guidelines.

Of 32 women with confirmed HIV infection, one (3.1%) was coinfected with syphilis. Two (6%) women newly diagnosed with HIV were currently pregnant, but no women with active syphilis were currently pregnant.

The median age of women diagnosed with HIV was 32 years (IQR 25.5–40.5) whereas the median age of women classified with active syphilis was 48 years (IQR 45–51). Infection by age group is shown in Table 7.

Table 7. HIV and active syphilis infections by age group

Age group (years)	Total	HIV		Active syphilis	
	n	n	%	n	%
18-19	36	1	2.8	0	0.0
20-24	110	6	5.5	0	0.0
25-29	92	5	5.4	1	1.1
30-34	63	8	12.7	1	1.6
35-39	32	3	9.4	1	3.1
40-49	43	7	16.3	6	14.0
50+	24	2	8.3	5	20.8

Discussion

Providing dual HIV/Syphilis testing to FSW was a functional approach to increasing access to syphilis testing services in a community-based HIV service. Overall, the detected prevalence of HIV was 8.0%, and 3.5% of women were classified as having active syphilis, and 6.5% were classified as having past or current syphilis. Women reached were at high risk of HIV and other STIs, with a substantial proportion of women testing due to potential risk exposure, many women reporting inconsistent condom use, and very high self-report of STI symptoms. Most women had ever been pregnant and intended to have children in the future. This highlights the importance of access to syphilis testing among these women, with the potential to mitigate both vertical transmission of HIV and congenital syphilis.

Prevalence of active syphilis was higher among older FSW. Given no women tested RPR positive, these infections may represent early primary infection, misclassification of active infection due to unknown treatment history or alternatively late latent infection [20], in which case the observed trend could represent greater likelihood of previous exposure to syphilis with increasing age. Despite higher observed prevalence among older women, syphilis screening remains particularly important for young FSW who are more likely to have pregnancy intentions, with implications for congenital syphilis.

History of diagnosed syphilis was relatively high, at 8%, and adds further complexity to diagnosing active syphilis and determining treatment need. There is no single rapid treponemal test that can differentiate between past treated and active infection due to the retention of antibodies to treponemal antigens. In this study, SD Bioline HIV/Syphilis duo test was combined with a second rapid diagnostic test, the non-treponemal based RPR, to differentiate active syphilis. Although all participants tested negative for RPR, due to FSW being at high risk of syphilis and unavailability of a reference standard test, a conservative approach was taken to treatment. The WHO recommend over-treating syphilis when RPR or reference standard testing is not available, due to the harms of untreated infection outweighing those of over-treatment [19]. In a context with relatively high prevalence of syphilis and ongoing engagement with women at increased risk through testing and treatment, more precise detection and differentiation of infection and corresponding treatment decisions may be appropriate, and further studies are needed to resolve these issues.

In Cameroon, FSW are marginalized and stigmatized, and community programs are often the preferred source of HIV services due to greater accessibility and acceptability. Currently syphilis and other etiological STI testing are not available through these programs. HIV/Syphilis Duo tests are simple point-of-care tests, can be easily integrated into existing community HIV services, and be offered outside of a clinical or laboratory setting. Our findings indicate the utility of dual rapid testing for detecting new HIV and syphilis infections among FSW to promote prompt linkage to treatment and reduce ongoing risk of transmission.

Limitations

There are a few limitations in this study. Speed of recruitment and uptake of HIV/Syphilis Duo testing was slower than expected given study participation was contingent on program recruitment, which focused on targeted verses number of tests completed. In addition, given that no women tested positive for RPR, and there was a lack of a reference standard test or FTA-ABS tests, there may be an over or misclassification of active syphilis. Finally, history of syphilis was based on self-report rather than clinical record, which may be subject to social desirability and recall bias.

Next steps

Further studies should consider the value of HIV/syphilis Duo testing for improving STI diagnoses in other populations, such as men who have sex with men (MSM). In some cities of Cameroon, men who have sex with men (MSM) also have high prevalence of syphilis – for example the estimated prevalence of active syphilis was 5.6% among MSM in Yaoundé in 2016 [3]. Further, dual testing may support programmatic implementation of HIV pre-exposure prophylaxis (PrEP), which requires periodic HIV and STI testing.

The SD Bioline HIV/Syphilis Duo test is not yet approved in Cameroon for general clinical use. Further collaboration and discussion with the Cameroonian Ministry of Health is required to advocate for local validation and scale up of HIV/Syphilis duo testing among FSW and other populations with increased risk of syphilis and related harms. Broader studies including the general adult population should also be considered to evaluate the SD Bioline HIV/Syphilis Duo test against national algorithms for HIV and syphilis testing.

References

1. Baral S, Beyrer C, Muessig K, Poteat T, Wirtz AL, Decker MR, et al. Burden of HIV among female sex workers in low-income and middle-income countries: a systematic review and meta-analysis. Lancet Infect Dis. 2012;12(7):538-49.

2. UNAIDS. HIV and AIDS estimates (2015) [Available from: http://www.unaids.org/en/regionscountries/countries/cameroon.

3. Johns Hopkins School of Public Health, CARE, Metabiota. 2016 Integrated Biological and Behavioral Surveillance (IBBS) survey among female sex workers and men who have sex with men in Cameroon: Final Report. Yaounde: Johns Hopkins Cameroon Program; 2018.

4. WHO. Antiretroviral therapy for HIV infection in adults and adolescents. Recommendations for a public health approach: 2010 revision. 2010.

5. Zetola NM, Klausner JD. Syphilis and HIV infection: an update. . Clinical infectious diseases: an official publication of the infectious diseases society of America. 2007;44(9):1222-8.

6. Zoufaly A, Onyoh EF, Tih PM, Awasom CN, Feldt T. High prevalence of hepatitis B and syphilis co-infections among HIV patients initiating antiretroviral therapy in the north-west region of Cameroon. 2012. 2012;International Journal of STD & AIDS(23):6.

7. Willis B, Welch K, Onda S. Health of female sex workers and their children: a call for action. Lancet Global Health. 2016;4(7):e438-9.

8. CDC. 2015 Sexually Transmitted Diseases Treatment Guidelines: Syphilis 2015 [Available from: https://www.cdc.gov/std/tg2015/syphilis.htm.

9. Peipert JF. Genital Chlamydial Infections. N Engl J Med. 2003;349(25):2424-30.

10. Detels R, Green AM, Klausner JD, Katzenstein D, Gaydos C, Handsfield H, et al. The incidence and correlates of symptomatic and asymptomatic Chlamydia trachomatis and Neisseria gonorrhoeae infections in selected populations in five countries. Sex Transm Dis. 2011;38(6):503-9.

11. Nessa K, Waris SA, Alam A, Huq M, Nahar S, Chawdhury FA, et al. Sexually transmitted infections among brothel-based sex workers in bangladesh: high prevalence of asymptomatic infection. Sex Transm Dis. 2005;32(1):13-9.

12. World Health Organisation. WHO guideline on syphilis screening and treatment for pregnant women. Geneva: World Health Organisation.

13. World Health Organization. Prequalification of In Vitro Diagnostics Programme, Public Report: SD BIOLINE HIV/Syphilis Duo 2015 [cited 2018 May 15]. Available from: http://www.who.int/diagnostics_laboratory/evaluations/151028_final_report_0179-012-00_sd_bioline_hiv_syphilis2.pdf?ua=1.

14. DiaSpot Diagnostic. DiaSpot: Syphilis rapid test (dipstick) 2018 [cited 2018 May 15]. Available from: https://www.diaspot.com/index.php/support/download-inserts/file/syphilis-strip-test-insert?id=14.

15. WHO Department of Essential Health Technologies. HIV Assays: Operational Characteristics - (Phase I) - Report 12 - Simple/Rapid Tests Whole Blood Specimens. Geneva: World Health Organisation; 2002 [cited 2018 Jun 4]. Available from: http://apps.who.int/medicinedocs/en/d/Js15203e/.

16. Gray RH, Makumbi F, Serwadda D, Lutalo T, Nalugoda F, Opendi P, et al. Limitations of rapid HIV-1 tests during screening for trials in Uganda: diagnostic test accuracy study. BMJ. 2007;335(7612):188.

17. Kroidl I, Clowes P, Mwalongo W, Maganga L, Maboko L, Kroidl AL, et al. Low Specificity of Determine HIV1/2 RDT Using Whole Blood in South West Tanzania. PLoS ONE. 2012;7(6):e39529.

18. Delaney KP, Branson BM, Uniyal A, Phillips S, Candal D, Owen SM, et al. Evaluation of the performance characteristics of 6 rapid HIV antibody tests. Clin Infect Dis. 2011;52(2):257-63.

19. WHO, TDR. The use of rapid syphilis tests. Geneva: World Health Organisation (WHO)/ Special Programme for Resarch & Training in Tropical Diseases (TDR); 2006 [cited 2018 May 14]. Available from: http://www.who.int/reproductivehealth/publications/rtis/TDR_SDI_06_1/en/.

20. Unemo M. Laboratory diagnosis of sexually transmitted infections, including human immunodeficiency virus. Geneva: World Health Organization; 2013 [cited 2018 June 4]. Available from: apps.who.int/iris/bitstream/10665/85343/1/9789241505840_eng.pdf.