ORIGINAL RESEARCH ARTICLE

Field performance of a rapid point-of-care diagnostic test for antenatal syphilis screening in the Amazon region, Brazil

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Summary: We evaluated an immunochromatographic point-of-care (POC) syphilis test in 712 pregnant women under field conditions in remote communities of the Amazon region (Brazil), and identified risk factors for syphilis. Women were screened by POC test using whole blood obtained by fingerprick, the fluorescent treponemal antibody absorption (FTA-Abs) test as the gold standard and the Venereal Diseases Research Laboratory (VDRL) test to determine test performance in active syphilis. Multivariate analysis was conducted to identify factors associated with syphilis infection. Among women, 2.2% had syphilis (positive FTA-Abs) and 0.8% active syphilis (FTA-Abs and VDRL positive). In all, 2.2% of samples were positive by the POC test. The sensitivity, specificity, positive and negative predictive values were 62.5% (95% confidence interval [CI]: 38.6–81.5), 99.1% (95% CI: 98.1–99.6), 62.5% (95% CI: 38.6–81.5) and 99.1% (95% CI: 98.1–99.6), respectively. The POC test identified 62.5% (10/16) of syphilis cases, 66.7% (4/6) of active syphilis cases and all high-titre syphilis cases (VDRL > 1:8). Older age was associated with syphilis infection. The rapid test performed moderately well as a screening tool for low-risk populations. This combined with on-site testing and same day treatment could expand antenatal syphilis screening programmes in distant communities characterized by difficult access to antenatal services and infrequent clinical follow-up visits.

Keywords: syphilis, diagnosis, point-of-care technology, pregnant women, evaluation studies, risk factors, prevention and control, Brazil, VisiTect syphilis test

INTRODUCTION

Syphilis remains a major public health problem among pregnant women in Brazil, with a prevalence rate of 1.7%.[1] Congenital syphilis has experienced a resurgence in the country, with an incidence of 1.8 cases per 1000 live births and a mortality rate of 2.4 deaths per 100,000 individuals in 2007.[2]

Given the frequent asymptomatic nature of the infection and the importance of early treatment to prevent complications,[3] antenatal screening has become the main focus for syphilis control.[4] In Brazil, despite high rates of antenatal care, most congenital syphilis cases reported (52.9%) had no syphilis testing during pregnancy.[2] The risk of syphilis in pregnant women is increased among those who are older, have lower income and education level and with multiple sexual partners.[5,6]

The greatest value of rapid point-of-care (POC) syphilis tests is that they can be performed at the clinical setting with results available at the time of the initial patient visit. Therefore, the use of POC tests can prevent the delay between the diagnosis and treatment and the associated risk of persistent infection, failure to follow-up and further sexually transmitted infection (STI) transmission.[7] Further, rapid POC tests present an opportunity for detecting syphilis in non-clinical settings.[8] On-site rapid syphilis screening and same-day treatment of maternal syphilis has been shown to be clinically effective in detecting and treating syphilis among pregnant women,[9] especially in settings where high rates of loss to follow-up might occur.[10]

Such minimally invasive tests can potentially be incorporated into screening programmes at community-based venues and clinics that are remotely located and lack trained staff, laboratory facilities or access to electricity, thereby helping to expand syphilis screening, improve case detection and facilitate treatment delivery in rural areas of developing countries. A recent systematic review identified that immunochromatographic strip rapid syphilis tests have high sensibility and specificity in antenatal clinics.[11]

Our aims were: one, to evaluate the field performance of a rapid POC treponemal test using whole-blood fingerprick specimens against the fluorescent treponemal antibody absorption test (FTA-Abs) assay as the gold standard; two, to assess the usefulness of the rapid POC test for detecting active syphilis cases; and, three, to identify risk factors for syphilis among antenatal clinic attendees in remote communities of Brazil.

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METHODS

Study setting and population

The region of Alto Solimões (214,217 km^2, 83,349 inhabitants) in the Brazilian State of Amazonas includes the tri-border area between Colombia, Brazil and Peru, which is only accessible by a plane or by a boat. The area is highly militarized and has considerable flow of internal migration moving around the Brazilian border.

The study was carried out in 12 antenatal clinics of the municipalities of Tabatinga, Benjamin Constant, Atalaia do Norte (Brazil), Santa Rosa (Peru) and Puerto Nariño (Colombia) of the tri-border area. Between August and December 2006, we recruited consecutive pregnant women accessing the antenatal clinics, with no age restrictions, who agreed to sign the consent form. Ethical approval was obtained from the Ethics Board of Fundação Alfredo da Matta (FUAM) (Manaus, Brazil).

Study procedures

Participants were interviewed on sociodemographics, sexual behaviour, and syphilis testing and treatment history by trained health professionals who attended to the services. Subsequently, the same health professional collected a 20-μL fingerprick capillary blood sample for on-site testing with a POC syphilis test based on an immunochromatographic technique (VisiTect Syphilis test; Omega Diagnostics, Alloa, Scotland). Results were ready within 30 minutes and participants received immediate treatment based on the POC test result.

To perform syphilis serology, an 8-mL venous blood sample was drawn and serum was stored at −20°C until shipment to the FUAM reference laboratory. We considered the FTA-Abs test (WAMA Diagnostica, São Paulo, Brazil) as the most appropriate reference standard test comparator since the rapid POC test was also *Treponema*-specific.[12] The Venereal Diseases Research Laboratory Test (VDRL; Winer Laboratorios, Rosario, Argentina) was performed on all samples and titres were determined. Syphilis infection was defined as a positive FTA-Abs test result. Active syphilis was defined as sera reactive with both FTA-Abs and VDRL, with high (HTS) and low-titre active syphilis defined based on VDRL titres ≥ or, <1:8, respectively.

Statistical analysis

Data were analysed using STATA version 9.0 (StataCorp, College Station, TX, USA). The performance characteristics (sensitivity, specificity, and predictive values) of the POC test against the gold standard (FTA-Abs) were calculated according to standard methods. Bivariate analysis compared infected and non-infected women on baseline characteristics using the χ^2 test, and crude odds ratios (OR), with 95% confidence interval (CI) were calculated. To identify factors independently related to syphilis infection, all factors were included in a multivariate logistic regression model. To build the final model, a backward stepwise procedure was used and a variable was retained in the model if the likelihood ratio test P value was less than 0.05.

RESULTS

A total of 712 women participated in the study, with a mean age of 24.2 years (SD: 6.7). Most subjects were Brazilian (90.5%), of which almost three quarters (72.7%) were from the municipality of Tabatinga, which is the largest in the region. Approximately 10% of the women were illiterate, 61.8% completed the first two years of primary school and none of them reached secondary school. Among women, the mean gestational age was 22.4 weeks (SD 9.3). A total of 192 (27.0%) women had ever been tested previously for syphilis, of which 122 (63.5%) had tested in the past year and four (2.1%) reported having syphilis in the past. However, 51 subjects (7.5%) reported having received treatment for syphilis, which might have included penicillin injections. With regard to sexual behaviour, mean age at first sex was 15.1 years (SD 1.9). Most of the women (92.4%) had a regular partner and 22.1% had an occasional partner during the last year.

Table 1 presents the characteristics of the 16 women with a positive FTA-Abs test result. A comparison of demographic, syphilis testing history and sexual behaviour using bivariate analysis showed women with a positive FTA-Abs test result were more likely to be at least 20 years old (Table 1). In the multivariate logistic analysis, age was the only factor that remained marginally associated with syphilis infection, the adjusted OR of infection in the age group 20–29 years being 7.62 (95% CI: 0.96–60.60, $P = 0.05$), and among those at least 30 years old 7.56 (95% CI: 0.84–68.32, $P = 0.07$), compared with those aged less than 20.

Table 1 **Correlates of syphilis infection among women attending antenatal clinics in Alto Solimões, Brazil**

Variable	Number of women*	Number (%) of infected women†	Crude OR (95% CI)‡	*P* value
Age (years)				
<20	247	1 (0.40)	1	
20–29	312	10 (3.21)	8.15 (1.02–64.86)	0.02
≥30	149	5 (3.36)	8.54 (0.97–75.15)	0.02
Country of origin				
Brazil	644	14 (2.17)	1	
Peru/Colombia	68	2 (2.94)	1.36 (0.30–6.14)	0.69
Education				
Illiterate	73	1 (1.37)	1	
<3 years	435	11 (2.53)	1.87 (0.24–14.73)	0.55
≥3 years	195	3 (1.54)	1.12 (0.11–11.04)	0.92
Weeks of pregnancy				
1–12	119	3 (2.52)	1	
13–26	306	7 (2.29)	0.90 (0.23–3.57)	0.89
≥27	260	6 (2.31)	0.91 (0.22–3.72)	0.90
Prior syphilis test				
No	518	10 (1.93)	1	
Yes	192	6 (3.13)	1.64 (0.59–4.58)	0.34
Previous treatment for syphilis				
No	628	12 (1.91)	1	
Yes	51	2 (3.92)	2.10 (0.45–9.65)	0.33
Age at first sex (years)				
<15	251	7 (2.79)	1	
≥15	424	8 (1.89)	0.67 (0.24–1.87)	0.44
Regular partner				
No	54	2 (3.70)	1	
Yes	652	12 (1.84)	0.49 (0.11–2.41)	0.35
Occasional partner				
No	550	10 (1.82)	1	
Yes	156	5 (3.21)	1.79 (0.60–5.32)	0.29

OR = odds ratio; CI = confidence interval

*Total numbers do not always add up to 712 because of missing data

†Women with a positive fluorescent treponemal antibody absorption (FTA-Abs) test result

‡Estimated by bivariate logistic regression analysis

Performance of the POC syphilis test

The sensitivity of the POC syphilis test compared with FTA-Abs was 62.5% (95% CI: 38.6–81.5), specificity 99.1% (95% CI: 98.1–99.6), positive predictive value 62.5% (95% CI: 38.6–81.5) and negative predictive value 99.1% (95% CI: 98.1–99.6).

We categorized syphilis serology according to the combined results of FTA-Abs and VDRL testing (Table 2). Overall, 2.2% (16/712) of women had evidence of syphilis (FTA-Abs positive), of whom 0.8% (6/712) had evidence of active syphilis (dually positive for FTA-Abs test and VDRL) and 0.1% (1/712) showed HTS. Only 16.6% (1/6) of the women with active syphilis reported having received treatment for syphilis, which might have included penicillin injections.

Using the POC syphilis test, 2.2% (16/712) of the samples were positive and there were no indeterminate results. Five VDRL tests results were lost because results were not recorded after the test had been performed. However, we did not exclude these participants and stratified Table 2 to show the potential impact of these missing samples on the performance of the POC test if the results had been positive or had been negative. The POC test detected 62.5% (10/16) of syphilis cases, 62.5–66.7% of active syphilis cases and all the HTS. Among samples, two out of six 'false-negatives', defined as positive samples by the FTA-Abs but yet negative by the rapid POC test, were VDRL positive, at a titre of 1:1 and 1:2.

DISCUSSION

To our knowledge, this is the first study that evaluates a new rapid POC treponemal test using fingerprick under real field conditions in antenatal populations from remote communities of the Amazon region. The rapid POC test performed moderately well in detecting syphilis infection compared with FTA-Abs among pregnant women in Alto Solimões. The sensitivity of the POC syphilis test was within the range (45.8–66.7%) obtained in a previous field validation of this assay conducted at a reference STI clinic in Manaus, Brazil.[13]

However, in a previous evaluation of VisiTect Syphilis against FTA-Abs in the same STI clinic using whole blood, we found that the POC test had high sensitivity (96.1%).[14] This might be explained by the higher proportion of active syphilis cases among the study population, which included sex workers and their potential clients. The test performance might have been affected by more adverse field conditions such as greater variability in temperature and humidity. Given the high turnover of health professionals that characterizes the interior of the Amazon region, lack of training might also have affected the test performance. Similar tests evaluated using fingerprick specimens in prenatal populations of developing countries have shown a high sensitivity (84.1–91.8%), when compared with results of a combined gold standard rapid plasma reagin test and *Treponema pallidum* haemagglutination assay.[9,15,16] The specificity of the rapid POC test was excellent and in the range found in previous studies.[13–16]

Access to screening was a major barrier as suggested by the high proportion of participants who had never tested for syphilis. In addition, in some remote areas in Brazil, results are available 30–60 days later. In this context, the use of rapid POC tests of moderate sensitivity combined with immediate on-site treatment may lead to the treatment of more infected women than the use of a more sensitive test that requires a return visit.[17] In this low-risk population, the POC test identified a high proportion of active syphilis cases, and all the HTS, which is strongly associated with poor birth outcome.[10] In resource-constrained settings, immediate treatment and reduction of losses to follow-up[18] provide considerable advantages since the benefits of preventing serious sequelae and onward transmission of syphilis clearly outweigh the risks and costs of overtreatment.[19]

The prevalence of syphilis in pregnant women was 2.2%, which is consistent with a previous study.[20] High syphilis rates and lack of access to services may increase the risk of acquisition of HIV among pregnant women[19] and fuel the expansion of the HIV epidemic.

In our study population, almost a quarter had an occasional partner during the last year, which suggests that STI-related risk behaviours were substantial in a region characterized by intense migration and temporary residence. Migrant populations are more vulnerable to acquiring STIs while mobile and face greater obstacles in accessing health services.[21] In multivariate analysis, the only factor that showed some evidence for an association to have syphilis was age and this is related to the use of a treponemal test, which is a marker of cumulative risk and longer exposure period.

The main weakness of the study is the rather small number of women with syphilis, which widens the CI for sensitivity of the

Table 2 **Accuracy of the point-of-care (POC) syphilis test by syphilis serological status among low-risk population in Alto Solimões, Brazil**

Serological syphilis category and interpretation	Five missing values in VDRL	Sensitivity (95% CI) (number positive onsite/number positive at reference laboratory)	Specificity (95% CI) (number positive onsite/number positive at reference laboratory)	Positive predictive value (95% CI) (number true positive onsite/number positive onsite)	Negative predictive value (95% CI) (number true negative onsite/number negative onsite)
FTA-Abs+ and VDRL+ active syphilis	+ ($n = 8$)	62.5 (24.5–91.5) (5/8)	98.4 (97.2–99.2) (693/704)	31.3 (11.0–58.7) (5/16)	99.6 (98.7–99.9) (693/696)
	− ($n = 6$)	66.7 (22.3–95.7) (4/6)	98.3 (97–99.1) (694/706)	25.0 (7.3–52.4) (4/16)	99.7 (99.0–100.0) (694/696)
FTA-Abs+ and VDRL− old or treated syphilis	+ ($n = 8$)	62.5 (24.5–91.5) (5/8)	98.4 (97.2–99.2) (693/704)	31.3 (11.0–58.7) (5/16)	99.6 (98.7–99.9) (693/696)
	− ($n = 10$)	60 (26.2–87.8) (6/10)	98.6 (97.4–99.3) (692/702)	37.5 (15.2–64.6) (6/16)	99.4 (98.5–99.8) (692/694)
FTA-Abs− and VDRL+ biological false-positive	+ ($n = 8$)	12.5 (0.3–52.7) (1/8)	97.9 (96.5–98.8) (689/704)	6.25 (0.2–30.2) (1/16)	99.0 (97.9–99.6) (689/696)
	− ($n = 5$)	20 (0.5–71.6) (1/5)			

FTA-Abs = fluorescent treponemal antibody absorption test; VDRL = Venereal Diseases Research Laboratory test; + = positive; − = negative; CI = confidence interval

POC test. The sample size might have been insufficient to find association between other known risk factors and syphilis.[5,6] In order to guide the implementation of syphilis screening policies in this area, recruitment is ongoing among indigenous populations which will allow the study to reach generalizable conclusions about the findings. Secondly, one of the major drawbacks of all *Treponema*-specific tests, including current POC tests, is the persistency of test positivity over time, which limits the utility of such tests in high-risk populations that might have been previously tested and treated.[22] POC tests are better suited for prenatal populations who are only seen infrequently or who have difficult access to screening. Nevertheless, a new generation of rapid POC tests will enable clinicians to differentiate active versus past syphilis infection. Finally, the definition of active syphilis (both FTA-Abs and VDRL positive with any titre) includes those with infectious (primary and secondary) syphilis, and also cases of untreated latent syphilis, as well as some who have been successfully treated in whom the VDRL can remain positive for a long period.

Under field conditions, the rapid test performed moderately well as a screening tool in remote communities characterized by difficult access to antenatal services and infrequent clinical follow-up visits. These combined with the test operational suitability,[23] its ability to identify HTS cases, and the advantages of on-site testing and same day treatment could increase the uptake of syphilis screening in resource-constrained settings[10] and reduce syphilis-related adverse outcomes of pregnancy. This is especially important if we aim to scale-up syphilis screening programmes in distant settings of high maternal syphilis prevalence like the tri-border area of the Amazon region which is characterized by population migration, the need for river transport, and lack of well equipped laboratories, electricity, refrigeration, adequate training and continuity of supplies.

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REFERENCES

1 Rodrigues CS, Guimaraes MD. Syphilis positivity in puerperal women: still a challenge in Brazil. *Rev Panam Salud Publ* 2004;**16**:168–75
2 Ministério da Saúde. Secretaria de Vigilância em Saúde. Programa Nacional de DST e Aids. Vigilância epidemiológica da sífilis congênita no Brasil: Definição de casos. *Boletim Epidemiol – AIDS* (serial online) (last accessed 15 July 2007) Jan–Jun 2007;**1**:12–7. See http://www.aids.gov.br/data/Pages/LUMIS9A49113DPTBRIE.htm (last accessed 25 January 2010)
3 Mullick S, Watson-Jones D, Beksinska M, Mabey D. Sexually transmitted infections in pregnancy: prevalence, impact on pregnancy outcomes, and approach to treatment in developing countries. *Sex Transm Infect* 2005;**81**:294–302
4 Peeling RW, Ye H. Diagnostic tools for preventing and managing maternal and congenital syphilis: an overview. *Bull World Health Organ* 2004;**82**:439–46
5 Miranda AE, Figueiredo NC, Schmidt R, Page-Shafer K. A population-based survey of the prevalence of HIV, syphilis, hepatitis B and hepatitis C infections, and associated risk factors among young women in Vitoria, Brazil. *AIDS Behav* 2008;**12**:S25–31
6 Zhou H, Chen XS, Hong FC, *et al.* Risk factors for syphilis infection among pregnant women: results of a case-control study in Shenzhen, China. *Sex Transm Infect* 2007;**83**:476–80
7 Peeling RW. Testing for sexually transmitted infections: a brave new world? *Sex Transm Infect* 2006;**82**:425–30
8 Peeling RW, Holmes KK, Mabey D, Ronald A. Rapid tests for sexually transmitted infections (STIs): the way forward. *Sex Transm Infect* 2006;**82**:S1–6
9 Bronzan RN, Mwesigwa-Kayongo DC, Narkunas D, *et al.* On-site rapid antenatal syphilis screening with an immunochromatographic strip improves case detection and treatment in rural South African clinics. *Sex Transm Dis* 2007;**34**:S55–60
10 Watson-Jones D, Oliff M, Terris-Prestholt F, *et al.* Antenatal syphilis screening in sub-Saharan Africa: lessons learned from Tanzania. *Trop Med Int Health* 2005;**10**:934–43
11 Tucker JD, Bu J, Brown LB, Yin YP, Chen XS, Cohen MS. Accelerating worldwide syphilis screening through rapid testing: a systematic review. *Lancet Infect Dis* 2010;**10**:381–6
12 Singh AE, Romanowski B. Syphilis: review with emphasis on clinical, epidemiologic, and some biologic features. *Clin Microbiol Rev* 1999;**12**:187–209
13 Benzaken AS, Sabido M, Galban EG, *et al.* Field evaluation of the performance and testing costs of a rapid point-of-care test for syphilis in a red-light district of Manaus, Brazil. *Sex Transm Infect* 2008;**84**:297–302
14 Mabey D, Peeling RW, Ballard R, *et al.* Prospective, multi-centre clinic-based evaluation of four rapid diagnostic tests for syphilis. *Sex Transm Infect* 2006;**82**:S13–6
15 Montoya PJ, Lukehart SA, Brentlinger PE, *et al.* Comparison of the diagnostic accuracy of a rapid immunochromatographic test and the rapid plasma reagin test for antenatal syphilis screening in Mozambique. *Bull World Health Organ* 2006;**84**:97–104
16 Tinajeros F, Grossman D, Richmond K, *et al.* Diagnostic accuracy of a point-of-care syphilis test when used among pregnant women in Bolivia. *Sex Transm Infect* 2006;**82**:S17–21
17 Gift TL, Pate MS, Hook EW III, Kassler WJ. The rapid test paradox: when fewer cases detected lead to more cases treated: a decision analysis of tests for *Chlamydia trachomatis*. *Sex Transm Dis* 1999;**26**:232–40
18 Vickerman P, Watts C, Peeling RW, Mabey D, Alary M. Modelling the cost effectiveness of rapid point of care diagnostic tests for the control of HIV and other sexually transmitted infections among female sex workers. *Sex Transm Infect* 2006;**82**:403–12
19 Reynolds SJ, Risbud AR, Shepherd ME, *et al.* High rates of syphilis among STI patients are contributing to the spread of HIV-1 in India. *Sex Transm Infect* 2006;**82**:121–6
20 Benzaken AS, Galbán EG, Rodrigues AO, Mora C, Sanchez MS. Madre Fronteriza: estudo conjunto de prevalencia de sífilis, trichomoníase e HIV em gestantes da tríplice fronteira do Alto Solimoes. *J Bras Doenças Sex Transm* 2004;**16**:15–23
21 Lydie N, Robinson NJ, Ferry B, Akam E, De Loenzien M, Abega S. Mobility, sexual behavior, and HIV infection in an urban population in Cameroon. *J Acquir Immun Defic Syndr* 2004;**35**:67–74
22 Campos PE, Buffardi AL, Chiappe M, *et al.* Utility of the determine syphilis TP rapid test in commercial sex venues in Peru. *Sex Transm Infect* 2006;**82**:S22–5
23 Sabido M, Benzaken AS, de-Andrade-Rodrigues EJ, Mayaud P. Rapid point-of-care diagnostic test for syphilis in high-risk populations, Manaus, Brazil. *Emerg Infect Dis* 2009;**15**:647–9

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