

Rapid diagnostic testing to improve access to screening for syphilis in prison

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ABSTRACT

Objectives: To assess the accuracy of on-site rapid treponemal test for syphilis diagnosis in women deprived of liberty in Bolivia.

Material and methods: Serological tests for syphilis were performed on 219 women deprived of liberty from the San Sebastián prison in Cochabamba, Bolivia. Syphilis was diagnosed using RPR (bioMérieux) and TPPA (Fujirebio) serological tests, and the results were compared to on-site rapid treponemal test (Alere Determine™ Syphilis TP) in whole blood. Diagnostic performance of two FTA tests were also compared (bioMérieux and Biocientífica).

Results: All participants (28) with RPR+/TPPA+ had the rapid syphilis test positive (sensitivity 100%). Eleven participants had rapid syphilis test positive without RPR and TPPA both positive; nevertheless 7 of them had RPR or TPPA positive. Of 33 participants with FTA-bioMérieux positive, 22 (66.6%) had FTA-Biocientífica positive.

Discussion: The rapid syphilis test Determine shows excellent performance as a screening tool among women deprived of liberty affected by high prevalence of syphilis. This test is particularly indicated when there are barriers for access to conventional serological tests. It is inexpensive, easy to use and does not require electricity and laboratory infrastructure. The FTA test performed with reagents from Biocientífica had a suboptimal sensitivity.

Keywords: syphilis; serologic tests; reagent strips; diagnosis; sensitivity and specificity; prisons; vulnerable populations; women.

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INTRODUCTION

Syphilis morbidity and epidemiology within prisons

The World Health Organization (WHO) recommends the screening of syphilis in high-risk populations to reduce associated morbidity, mortality and its transmission. The incarcerated population, and more specifically, female prisoners (FP) represent a high-risk population for syphilis in several countries worldwide¹. In Bolivia, an epidemiological study published in this Spanish Journal of Prison Health (RESP in Spanish), showed a prevalence rate of active syphilis of 12.3% for FP- a three fold increase with regard to the general population²⁻³.

Prisons are a key environment for the control of syphilis, not only for the prevention of serious complications in already infected patients but also for the prevention of mother-to-child transmission in future pregnancies and for the prevention of transmission in the community. Between 25 and 40% of untreated infected patients will develop serious complications⁴.

The risk of mother-to-child transmission is high for primary and secondary syphilis (between 60 and 90% of cases) and it falls for latent syphilis (between 2 and 10%). 40% of pregnancies among women with syphilis entail miscarriage or congenital syphilis which has a mortality rate of 50%⁵⁻⁶. The treatment for syphilis is affordable, efficient and easy to implement.

Syphilis diagnosis

Syphilis control programs in low-income countries are impaired by the lack of laboratories, delayed diagnosis and doubts regarding the interpretation of conventional serological tests⁷. The most widely available non-treponemal tests are the microscopic Venereal Diseases Research Laboratory (VDRL) and the macroscopic rapid plasma reagin (RPR) tests which should be further confirmed by treponemal tests such as the *Treponema pallidum* particle agglutination assay (TPPA) and the fluorescent treponemal antibody absorbed (FTA-ABS) tests.

The essential requirements for these tests are available electricity for the equipment (centrifuge and stirrer) and refrigeration for the storage of reagents, as well as appropriately trained personnel⁸.

For FTA test to be carried out, the fluorescence microscope should have its lamp changed every 200 hours. In low-income countries it is challenging to ensure the maintenance of laboratory equipment to

ensure an appropriate level of quality. The purchase of low-cost reagents and the lack of quality control measures for serological tests impair the reliability of results^{6,9}.

Barriers to diagnosis in prisons

Prisons in low-income countries frequently lack the necessary laboratory infrastructure to process conventional serological tests. When dealing with external laboratories, funding, sample transportation and result follow-up can entail complicated logistics.

Since this process implies significant human resources and laboratory costs, usually syphilis screening is not routinely performed. In some cases, weeks are needed to get the results, notify and initiate treatment in patients deprived of their liberty, if still in custody at all⁸⁻⁹.

Another limiting aspect of non-treponemal and treponemal serological tests is their suboptimal performance in some circumstances, when not processed in laboratories with strict quality control measures. When processed in healthcare facilities, some studies reported a sensitivity rate of only 71% for the RPR test⁷⁻¹⁰.

Advantages of Rapid Syphilis Tests

To diagnose the disease, rapid tests often provide solutions for the previously mentioned barriers. They are stored at room temperature, do not need electricity nor sophisticated lab material and since they are easy to use, healthcare providers need less training to carry them out³⁻⁶.

Acceptance to screening can be encouraged by avoiding drawing a blood sample. The analysis can be carried out from a blood drop collected from a finger-stick test. And since the result can be obtained in a 15-to-20 minute period, treatment can be initiated immediately. The rapid test (immunoassay strip Determine Syphilis TP-PRADS) is marketed in Bolivia at an estimated cost of \$1 per test.

The World Health Organization clearly recommends rapid syphilis tests for people at risk of sexually transmitted infections⁸.

Study endpoints

The primary endpoint was to assess the performance of PRADS in a population at high risk of suffering syphilis: female prisoners in a prison in Bolivia, by using RPR and TPPA as reference tests. The secondary endpoint was to compare the performance of two different brands of FTA treponemal tests.

MATERIAL AND METHODS

A cross-sectional study of 220 women imprisoned in the prison of San Sebastian, in the city of Cochabamba, Bolivia was carried out over the period between September 1st and October 31st 2013.

Ethical considerations

The study was previously approved by the Bioethics committee of the University Mayor of San Simon in Cochabamba, Bolivia (on December 21st 2012). All the imprisoned women were invited to take part in the study throughout this period. Each received an information sheet and provided informed consent. Refusing to take part in the study did not lead to any sanctions whatsoever.

Collection of biological samples and laboratory diagnostic tests

A 5ml blood sample was drawn from each of the 219 voluntary participants. One remaining drop was used to permeate the PRADS strip by the main researcher.

After centrifuging the samples, the serum was stored at -20°C until the analysis was processed by the reference laboratory LABIMED, in the University Mayor of San Simon, Cochabamba, Bolivia. A non-treponemal test was also performed (RPR, NOSTICON TMII bioMérieux Ltd.). The RPR titer was determined by serial dilutions.

For treponemal testing, TPPA (*Treponema pallidum* particle agglutination; Serodia® Fujirebio Inc.) and two different brands of FTA tests were used (Immunofluor FTA-ABS Biocientífica Ltd. and bioMérieux Ltd.). All serological tests were processed according to the producer regulations. The interpretation of FTA tests was done with a fluorescence microscope (Leitz) with a new mercury lamp. BioMérieux FTA results were also compared with those from the same microscope with a four-year-old lamp (approximately with 800 hours of use) and with another fluorescence microscope (Olympus) with halogen lamp. Biochemists performing these tests were not aware of the results provided by the other serological tests (blind test).

Definition of cases

In this study, syphilis cases were considered for those women with both positive RPR and TPPA tests⁴. Only participants with positive RPR and TPPA tests (RPR+ AND TPPA+) were considered true positive for calculating purposes.

Statistical analysis

Sensitivity and specificity rates and both positive and negative predictive values for the Determine rapid test were calculated for the diagnostic criteria of syphilis (both RPR and TPPA positive tests). Confidence intervals were calculated by the exact binomial method. Both the sensitivity and specificity of the FTA treponemal test (Immunofluor FTA-ABS Biocientífica Ltd.) were compared to those of FTA (BioMérieux Ltd.).

The sensitivity and specificity rates of FTA (bioMérieux Ltd) were also calculated when used with the fluorescence microscope both with the mercury and the halogen lamps.

RESULTS

Table 1 describes the prevalence of each of the serological markers assessed in female prisoners in the study population.

Performance of PRADS

The twenty-eight FP with both RPR+/TPPA+ had a positive PRADS (sensitivity rate of 100%). Eleven participants had a positive PRADS without positive results for both reference tests: five were TPPA+, two RPR+ and four both RPR and TPPA negative.

Out of the two participants with TPPA-/RPR+ one had a reactive FTA test (borderline reaction). Out of the 28 RPR+/TPPA+ participants, 26 had a positive FTA-bioMérieux test and two a negative result.

Performance of the FTA-Biocientífica test

The FTA-Biocientífica test has a sensitivity rate of 66.7%: 22/33, 95% confidence interval (CI) 49.6-80.2 when taking the FTA-bioMérieux test as a reference. The FTA-Biocientífica test has a sensitivity rate of 75% (21/28; 95%CI: 56.7-87.3) for the detection of active syphilis (RPR+/TPPA+).

Performance of the FTA-bioMérieux test with non-recommended laboratory material

The interpretation of all FTA samples was repeated with a fluorescence microscope with a four-year-old lamp (approximately 800 hours of use). Two patients who were found FTA positive with the new mercury lamp were negative with the used lamp. No false-positive results were found.

All samples were subject to a fluorescence microscope with halogen lamp. Four patients with

positive FTA according to the mercury lamp were interpreted as FTA negative. No false-positive results were obtained.

DISCUSSION

Performance of PRADS

The study shows how PRADS in whole blood sample presents a high sensitivity (100% 95% CI: 87.7-100) and specificity rates (94.2% 95% CI: 89.9-97.1) when compared with the reference test for active syphilis (RPR+/TPPA+). Moreover, it has a maximal negative predictive value, with a minimum confidence interval (98-100%), which is also an essential feature for an optimal screening test.

Our results support four studies which compared the Determine rapid test with the reference test defined by both a treponemal and non-treponemal positive tests, with a sensitivity rate between 91 and 100%^{9,11-13}. Therefore, PRADS ensures the requirements of the Bolivian Ministry of Health, which recommends diagnostic tests for syphilis with a sensitivity rate over 80%⁹.

Performance of a conventional FTA treponemal test

The study shows how serological tests conventionally considered as reference tests do not necessarily have a good performance. We found a high percentage of RPR+/TPPA+ participants with a negative FTA-Biocientífica test, leading to a quality control by means of a thoroughly validated FTA test (bioMérieux).

Reagents of FTA-Biocientífica routinely used by reference labs during the study period are cheaper than those of bioMérieux. However, these reagents can not be recommended due to a lack of sensitivity.

In the study population almost one third of the patients with positive FTA-bioMérieux tests were false negatives according to FTA-Biocientífica. In addition, if willing to use the FTA treponemal test, our study confirms that keeping up with high quality standards is imperative to ensure the optimal performance of the test, which entails regularly replacing mercury lamps (which are quite expensive) and not using fluorescence microscopes with halogen lamps, among others.

Consequences of the lack of a gold standard for the diagnosis of syphilis

The rate of positive PRADS with a negative reference test is 5% (11/219) in our study population. Despite this, reference tests are not considered a gold

Table 1. Prevalence of serological markers for syphilis in female prisoners (FP), Cochabamba, Bolivia.

	Rective test % (n=219)
PRADS+*	17.8% (39)
RPR+†	13.7% (30)
TPPA+‡	18.3% (40)
RPR+ & TPPA+	12.3% (28)
FTA-bioMérieux+§	15.1% (33)
FTA-Biocientífica+	10.0% (22)

Note. *PRADS: rapid test Alere Determine™ Syphilis TP. †RPR: rapid plasma reagin (bioMérieux S.A.). ‡TPPA: *Treponema pallidum* particle assay (Serodia® Fujirebio Inc.). §fluorescent treponemal antibody-absorption: FTA-bioMérieux reactive, 4/33 with borderline reaction ||fluorescent treponemal antibody-absorption: FTA-Biocientífica reactive, 4/22 with borderline reaction.

standard for the diagnosis of syphilis since their sensitivity and specificity rates are not 100%. Therefore, we can not rule out that some positive PRADS considered false positives in the study are actually cases of active syphilis. Three out of the five participants with PRADS+/RPR-/TPPA+ have a reactive FTA test and so does one of the two patients with PRADS+/RPR+/TPPA-. Therefore, false negatives for RPR and/or TPPA tests can not be excluded.

The risks of misdiagnosis can lead to an increase of allergy (anaphylaxis) and stigmatization⁹. The reported risk for anaphylaxis after the administration of parenteral penicillin is 32/100000. Only 1.8% (4/219) of PRADS+ participants had a negative result in either RPR or TPPA tests.

When considering the benefit-risk equation of the use of PRADS without a confirmatory test, we can assume that the benefits for the study population outweigh the risks of overtreatment^{8,9,14}.

Strengths and Limitations

Despite the size of the sample (n=219), the results of sensitivity rate, positive predictive value (PPV) and negative predictive value (NPV) are accurate enough, thanks to the high prevalence of active syphilis diagnosed by the reference test (RPR+/TPPA+).

A positive factor of this study is the high participation rate of FP (99.5%, n=219/220) in spite of the nuisance of drawing a blood sample. We can consider that if implementing a screening method which would only entail a finger-stick test the acceptance would inevitably be high.

Table 2. Sensitivity rate, specificity rate, positive predictive value (PPV) and negative predictive value (NPV) of the rapid test Alere Determine™ Syphilis TP (PRADS) when compared with the reference test RPR/TPPA† in the female prison of Cochabamba, Bolivia.

	Reference test TPPA & RPR‡ (N with both positive tests=28)					Reference test TPPA (N positive=40)		
	N positive	Sensitivity rate (95 CI*%)	Specificity rate (95 CI%)	PPV (95 CI%)	NPV (95 CI%)	N† positive	Sensitivity rate (95 CI%)	Specificity rate (95 CI%)
PRADS (N positive=39)	28	100% (87.7-100%)	94.2% (89.9-97.1%)	71.8% (55.1-85%)	100% (98-100%)	33	82.5% (68.4-92%)	96.6% (93.2-98.6%)

Note. PRADS: rapid test Alere Determine™ Syphilis TP. *CI: confidence interval. †N: number of participants. ‡TPPA: *Treponema pallidum particle assay* (Serodia® Fujirebio Inc.). RPR: Rapid Plasma Reagin (bioMérieux SA).

CONCLUSION

The study shows that PRADS presents an excellent performance for the screening of syphilis in the imprisoned population of FP in Bolivia and an alternative to conventional serological tests. This result supports the meta-analysis by Jafari, which proved that PRADS had a superior performance than non-treponemal conventional tests, usually processed in areas with limited resources¹⁴.

It is an affordable test (approximately \$1 per test in Bolivia) whose implementation does not need a laboratory infrastructure, electric light, refrigeration nor a sophisticated training. It is particularly indicated when there are access barriers to conventional serological tests. Performing this test in whole blood without needing centrifugation and providing a result in 15 minutes leads to the possibility of immediately prescribing treatment.

The study also proves that when conventional treponemal and non-treponemal tests are used, we need to buy reliable reagents, implement regular quality controls and use validated materials.

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