

# PILOT EVALUATION OF A RAPID IMMUNOCHROMATOGRAPHIC TEST FOR THE DIAGNOSIS OF GESTATIONAL SYPHILIS

## ESTUDO PILOTO DE AVALIAÇÃO DE UM TESTE RÁPIDO IMUNOCROMATOGRAFICO PARA O DIAGNÓSTICO DE SÍFILIS GESTACIONAL

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### ABSTRACT

**Introduction:** Gestational syphilis is a global public health problem and one of the most common causes of adverse effects during pregnancy due to absence or inadequacy of treatment. Establishing a diagnosis of syphilis during prenatal care prevents the transmission of *Treponema pallidum* to the child. **Objective:** The objective of this study was to evaluate the performance of OL Syphilis (OrangeLife, Brazil), a rapid immunochromatographic test for gestational syphilis diagnosis. **Methods:** A total of 185 pregnant women in prenatal care were evaluated by OL Syphilis. The results were compared by traditional methods: Venereal Disease Research Laboratory and Rapid Plasma Reagin (VDRL and RPR) for screening and fluorescent treponemal antibody absorption test (FTA-Abs) for confirmation. **Results:** The prevalence of syphilis in this population was 6.49% (95%CI 3.40 to 11.06%). Rapid Test (RT) sensitivity was 91.67% (95%CI 61.52 to 99.79%) and specificity was 100% (95%CI 97.89 to 100%). Positive predictive value was 100% (95%CI 71.51 to 100%) and Negative predictive value was 99.43% (95%CI 96.84 to 100%). The agreement measured by Kappa coefficient was 0.954 (95%CI 0.863 to 1.000). **Conclusion:** The OL Syphilis test could be used for screening pregnant women, thus providing rapid diagnosis, increasing the probability of diagnosis and timely treatment, and preventing the devastating consequences of congenital syphilis.

**Keywords:** syphilis; diagnosis; pregnancy; congenital syphilis.

### RESUMO

**Introdução:** A sífilis gestacional é um problema global de saúde pública e é uma das mais comuns causas de efeitos adversos durante a gravidez devido à ausência ou inadequação do tratamento. Estabelecer um diagnóstico de sífilis durante o pré-natal, evita a transmissão de *Treponema pallidum* para a criança. **Objetivo:** o objetivo deste estudo foi avaliar o desempenho de OL Syphilis (OrangeLife, Brasil), um teste imunocromatográfico rápido, para o diagnóstico de sífilis gestacional. **Métodos:** Um total de 185 mulheres grávidas no pré-natal foram avaliadas por sífilis OL. Os resultados foram comparados com os métodos tradicionais: Laboratório de Pesquisa de Doenças Venéreas e Reagens de Plasma Rápido (VDRL e RPR) como ensaios de seleção e FTA-ABS como teste confirmatório. **Resultados:** A prevalência de sífilis nessa população foi de 6,49% (IC 3,40 a 11,06%). A sensibilidade do teste rápido (TR) foi de 91,67% (IC95% 61,52 a 99,79%) e a especificidade foi de 100% (95%IC 97,89 a 100%). O PPV foi 100% (95%CI 71,51 a 100%) e o VPL foi de 99,43 (95%CI 96,84 a 100%). O acordo medido pelo coeficiente Kappa foi de 0,954 (IC95% 0,863 a 1,000). **Conclusão:** O teste OL Syphilis poderia ser usado no rastreio de mulheres grávidas, fornecendo diagnóstico rápido, aumentando a probabilidade de ter a doença diagnosticada e oportuna, evitando as consequências devastadoras da sífilis congênita.

**Palavras-chave:** sífilis; diagnóstico; gravidez; sífilis congênita.

## INTRODUCTION

Syphilis is a serious resurgent disease<sup>(1)</sup>, in which the absence or inadequacy of treatment is still common. Through data collected in 97 countries, it is estimated that 1.36 million pregnant women have active syphilis. Approximately half of them had one or more adverse obstetric outcomes (215,000 abortions or stillbirths, 90,000 neonatal deaths, 65,000 premature births or low birth weight, and 150,000 newborns infected).

The disease caused the loss of 3.5 million disability-adjusted life years (DALYs) and represents a direct medical cost of US\$ 309 million<sup>(2)</sup>. High-income countries were also shown to have an increasing incidence. In the United States, for example, there was an annual

increase after the year 2000, reaching 5 cases per 100,000 inhabitants in 2012<sup>(3)</sup>. In Canada, in the same year, the incidence of acquired syphilis was the highest in 30 years<sup>(4)</sup>.

Morbidity and mortality rates, however, are not homogeneously distributed, as low and medium-income countries suffer the greatest impacts<sup>(5)</sup>. In Brazil, a nation-wide representative cross-sectional study, conducted between 2011 and 2012, pointed out syphilis prevalence of 1.02% (95%CI 0.84–1.25) among 23,894 postpartum women<sup>(6)</sup>.

According to the Epidemiological Bulletin of the Ministry of Health, in 2013, the detection rate was 7.4 cases of maternal syphilis per 1,000 live births<sup>(7)</sup>. In spite of increasing prenatal care coverage, the quality of care did not accompany this increase<sup>(8)</sup>. As a result, the country is far from eradicating syphilis as a public health problem, since the incidence proposed as a goal by the World Health Organization (WHO) would be of 0.5 cases per 1,000 live births<sup>(9)</sup>.

*Treponema pallidum* infection is a polymorphic disease that alternates symptomatic and asymptomatic periods in which the only finding is positive serology<sup>(10)</sup>. Vertical transmission of syphilis can occur at any time during pregnancy, being more frequent in women with recent infection. Miscarriages, stillbirths, low birth weight, and even neonatal deaths are common when transmission to the conceptus occurs<sup>(11)</sup>.

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In clinical practice, the diagnosis of syphilis depends on serology by treponemal and/or non-treponemal tests. Among non-treponemal, VDRL (venereal disease research laboratory), RPR test card (rapid plasma reagin), and TRUST (toluidine red unheated serum test) are generally used for screening. These tests can be titrated, a procedure that is essential for post-treatment follow-up<sup>(12)</sup>. Since 2012, the Brazilian Ministry of Health has required that a positive non-treponemal test be confirmed by a treponemal test<sup>(7)</sup>. The most commonly used treponemal tests are FTA-Abs (fluorescent treponemal antibody absorption test), TPHA (*T. pallidum* haemagglutination test), TPPA (*T. pallidum* passive particle agglutination test), EIA (treponemal enzyme immunoassay), and CIA (chemiluminescence immunoassay)<sup>(12)</sup>. Laboratories with large volume of samples have started to perform screenings by automated treponemal tests (e.g., CMIA tests)<sup>(13)</sup>, a system named “reverse flowchart”<sup>(14)</sup>. When using reverse flowchart, non-treponemal quantitative tests remain mandatory for both confirmation and post-treatment follow-up.

More recently, rapid tests (RT) started being used for syphilis screening, particularly gestational syphilis. These are treponemal tests which do not depend on laboratory infrastructure, sophisticated equipment, refrigeration or electricity. They can be used by trained professionals even in resource-scarcity settings<sup>(15,16)</sup>. They are also considered point-of-care tests whose main advantage is to provide diagnosis at the time of consultation, with results in 15 to 30 minutes. Treatment can supposedly be started immediately, which is crucial for obstetrical prognosis. Rapid tests were evaluated in a laboratory environment, showing satisfactory sensitivity and specificity when compared to conventional treponemal tests<sup>(17)</sup>.

They also presented adequate performance in field situations, including hard to reach locations<sup>(18)</sup>. The WHO defined ideal characteristics for the choice of a point-of-care test: sensitivity and specificity, quick results, simplicity, low cost, robustness, independence of equipment, and availability to those in need<sup>(15)</sup>. They are part of the public health recommendations for primary care and mother-and-child hospitals across Brazil, being performed free of charge by the public health system (*Sistema Único de Saúde — SUS*)<sup>(19)</sup>.

## OBJECTIVE

The objective of this study was to evaluate OL Syphilis (OrangeLife, Rio de Janeiro, Brazil), a rapid immunochromatographic test for syphilis diagnosis in pregnant women seen at a prenatal service of a university hospital.

## METHODS

This was a diagnostic test study that included 189 serum samples from pregnant women attending prenatal care, from October 2014 to January 2015, at a university hospital reference for high-risk pregnancies in the city of Francisco Beltrão, Paraná, Brazil. Three samples were excluded due to presence of hemolysis, lipemic appearance, signs of contamination and/or insufficient volume for testing. Another sample was excluded because its result was considered false positive (reactive VDRL, non-reactive RPR, and negative FTA-Abs). Serum samples collected as part of the prenatal care routine were coded and had all personal identification data related eliminated.

A true “case of syphilis” criteria was reactivity found in one or both non-treponemal tests (VDRL and/or RPR), and confirmed by a treponemal test (FTA-Abs). The VDRL test (Winer, Rosario, AR) was comprised of a solution of cardiolipin, lecithin and cholesterol, and a choline-stabilized buffer. Flocculation was identified by optical microscopy. The RPR test (Laborclin, Pines, Brazil) contained an antigenic suspension that, in the presence of serum containing specific antibodies, would present flocculation visible under a light source. Treponemal fluorescent antibodies were identified by indirect immunofluorescence microscopy. The rapid test used was OL Syphilis, an immunochromatographic with lateral flow test using recombinant *T. pallidum* antigens immobilized in the test line region. It is applicable to whole blood, serum or plasma. All tests were performed according to manufacturers’ instructions.

Sensitivity, specificity, positive and negative predictive values were presented as proportions and respective confidence intervals. Concordance was measured using Cohen’s Kappa Coefficient. The study was submitted and approved by the human research ethics committee of Universidade Luterana do Brazil under protocol 1047020/2015. An informed consent form was signed (Resolution National Council of Health 466/12 item IV.8), since there was no additional risk to participants: no changes in care routines and observational character of study, in which aliquots were taken from samples previously obtained and tests were performed without any personal identifiers.

## RESULTS

Out of 185 samples from pregnant women in prenatal care, 12 were considered positive by non-treponemal tests (11 samples were reagent to VDRL and 7 were positive to RPR). When tested by FTA-Abs, all 12 samples were positive and, therefore, defined as “syphilis cases”. All samples that tested negative, defined as “non-syphilis cases”, were also negative when tested by RT (**Table 1**). The prevalence of “syphilis cases” in our sample was 6.49% (95%CI 3.40% to 11.06%). RT sensitivity was 91.67% (95%CI 61.52 to 99.79%) and specificity was 100% (95%CI 97.89 to 100%). Positive predictive value (PPV) was 100% (95%CI 71.51 to 100%) and negative predictive

**Table 1** – Samples with positive rapid tests.

Sample	RT	VDRL	RPR	FTA-Abs*	Case of Syphilis
1	+	-	-	+	No
67	+	+	-	+	Yes
147	+	-	+	+	Yes
151	+	+	+	+	Yes
159	+	+	+	+	Yes
161	+	+	+	+	Yes
168	+	+	+	+	Yes
173	+	+	-	+	Yes
174	+	+	+	+	Yes
175	+	+	-	+	Yes
176	-	+	-	-	

\*Presence of IgG antibodies.

RT: rapid test; VDRL: Venereal Disease Research Laboratory; RPR: Rapid Plasma Reagin; FTA-Abs: fluorescent treponemal antibody absorption test.

value (NPV) was 99.43% (95%CI 96.84 to 100%). The agreement, measured by Kappa coefficient, was 0.954 (95%CI 0.863 to 1.000). A sample that tested positive in RT and non-reactive in VDRL and RPR also resulted positive when FTA-Abs was performed.

## DISCUSSION

The findings of this study indicate that OL Syphilis (OrangeLife), a rapid immunochromatographic test, could be used for diagnosing syphilis in pregnant women, so, it may be an additional option in individual care as well as in public health activities. Like all RT, it can be conveniently performed at the time of consultation, providing timely results that allow starting treatment before patients leave the health-care facilities. This is suitable when it comes to healthcare for pregnant women and their partners, to whom implementation of early treatment prevents severe adverse obstetric outcomes<sup>(20)</sup>. In spite of providing valid results in environments where traditional tests do not work, RT may be misinterpreted, but it can be avoided by training programs with internal and external quality control<sup>(18)</sup>. The high prevalence of positive results in our sample — over 6% — upholds the serious epidemiological situation in Brazil. It is well above the prevalence found in nation-wide studies<sup>(6)</sup>. The service being a reference for high-risk pregnancies could have influenced prevalence upwards, but it does not diminish the importance of our findings. We compared OL Syphilis with the Brazilian flowchart used before the implementation of RT in Brazil. The recommendations then were non-treponemal tests for screening, and treponemal tests for confirmation. It is well known that treponemal tests (such as OL Syphilis RT) have greater sensitivity than non-treponemal ones, especially to detect early infections and post-treatment immunological memory<sup>(17)</sup>. This was found once in our sample.

The sample size can be considered a limitation of this study, however, it allowed us to establish acceptable confidence intervals, as well as positive and negative predictive values. The results were similar to those reported in the literature, where RT was used to diagnose syphilis in pregnant women. The performance of a rapid syphilis diagnostic test known as SD BIOLINE Syphilis 3.0 was evaluated elsewhere (SD Biostandard Diagnostics), and IMMUTREP Treponema pallidum hemagglutination assay (TPHA) was used as control. The standard reference and sensitivity, specificity, and PPV/NPV values of SD BIOLINE Syphilis 3.0 were 92.86% (95%CI: 80.52–98.50%), 98.28% (90.76–99.96%), 97.50% (86.84–99.94%), and 95.00% (86.08–98.96%), respectively, compared to TPHA as the gold standard<sup>(21)</sup>. Further evaluation was performed using Accu-Tell rapid anti-TP tests; Alere Determine Syphilis TPO; Cypress Diagnostics Syphilis Quick test; and SD Biotline Syphilis 3.0 test. Sensitivity ranged from 78 to 93% and specificity from 95 to 98% in a group of 120 patients. All four tests were proven to have good diagnostic specificity for syphilis (95–98%), and healthcare professionals found them easy to use<sup>(22)</sup>. As for Chembio MedMira SD Biotline test, the results in a group of 1,514 patients was 94.2–99.67% for sensitivity and 97.2–99.72% for specificity<sup>(23)</sup>.

Using non-treponemal tests for screening and treponemal tests for confirmation is a procedure used in many settings. As a result, multiple visits increase the costs for services, patients, and, most importantly, it implies lost opportunities: many pregnancies have come

to term without adequate treatment while waiting for test results<sup>(16)</sup>. RT has been recently consolidated as a better option to expand testing for pregnant women, especially in peripheral health settings<sup>(9)</sup>. The results of RT must also be considered in the light of clinical information — they remain positive in spite of previous treatments; but a positive result does not always indicate active syphilis. A non-treponemal test should always be performed to enable adequate follow-up; nonetheless, it is unacceptable that treatment be postponed until results are available.

## CONCLUSION

In conclusion, the OL Syphilis test could be used for screening pregnant women, thus providing rapid diagnosis, increasing the probability of diagnosis and timely treatment, and preventing the devastating consequences of congenital syphilis. It is known that preventing congenital syphilis is one of the most cost-effective measures in public health, as its occurrence in current times is unacceptable and reveals the failure of health systems.

## Conflict of interests

The authors declare no conflict of interests.

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