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ORIGINAL ARTICLE

WHAT IS THE BEST PROTOCOL FOR DIAGNOSING SYPHILIS IN PREGNANT WOMEN AND REWBORNS?

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ABSTRACT

Syphilis is a systemic disease caused by *Treponema pallidum* and may be diagnosed using treponemal and non-treponemal tests. In order to verify the concordance of the rapid test for syphilis in pregnant women and newborns, when compared to the venereal disease research laboratory test (VDRL) and the need for confirmatory tests, a study was carried out in a public hospital in the north of Espírito Santo. To analyze the pregnant women, rapid tests and the VDRL test were applied. If one or both tests were positive, the confirmatory *T. pallidum* hemagglutination test (TPHA) was applied. The results indicated that 146 (52.9%) out of the 276 samples tested positive for both methods (VDRL and rapid test). In total (276), 91 (33.0%) samples were positive for the rapid test, and 55 (19.9%) samples were positive for VDRL. Out of the 91 reactive samples for the rapid test, 53 (58.2%) were positive for both tests, and 38 (41.7%) were positive only for the rapid test. Of the 55 VDRL positive samples, 53 (96.4%) were positive for both tests, and only two samples (3.6%) were positive solely for VDRL. Additionally, three rapid test samples were classified as inconclusive by the testing team, with one (1.1%) of them being positive in the VDRL examination. On the other hand, 89 samples (32.2%) remained positive when the confirmatory test was carried out, and 35 (39.3%) of the pregnant women whose syphilis diagnosis was confirmed by the TPHA were positive only for the rapid test. The VDRL and TPHA tests were applied to newborns, and 61.0% and 97.6% (25 and 40 individuals, respectively) were positive. The results indicate that there is a need to adjust the diagnostic procedure for newborns and that the implementation of the rapid test in prenatal care can provide early access to the diagnosis and treatment of syphilis in pregnant women and their sexual partners.

KEY WORDS: Congenital syphilis; comparison test; prenatal; rapid test; VDRL; indirect hemagglutination-*T. pallidum* hemagglutination test

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INTRODUCTION

Syphilis is a systemic disease that has been known since the 15th century, although its origin remains a subject of controversy. The most widely accepted hypothesis suggests that treponemes, the causative agents of the disease, already existed in European territories and underwent mutations over time, allowing for sexual transmission (Rothschild, 2005; Neto et al., 2009).

The spread of the disease has been associated with the military campaign of the French king, who, upon entering Rome in December 1494 with an army of 12,000 men, stayed for about a month, engaging in orgies and celebrations. Similar events occurred in Naples the following year, further facilitating the spread of the disease (Neto et al., 2009). On the other hand, according to the Colombian theory, the disease originated in the New World and was brought to the Old World by sailors during Christopher Columbus' expeditions (Talhari & Talhari 2005).

The name syphilis became commonly used in the late 18th century, and physicians began studying the harmful effects of the disease on the children of infected individuals. Years later, in 1880, Master Fournier presented data showing that two out of three children born to infected parents died (Carrara, 1996).

In 1905, Schaudinn and Hoffman identified the etiological agent of syphilis as *Treponema pallidum* (Talhari & Talhari, 2005), and in 1918, Rabelo attributed 80% of cases of congenital debility to syphilis (Carrara, 1996). Since then, numerous studies have been conducted, and much is now known about syphilis, a systemic disease with a chronic course, subject to acute crises and periods of latency (Vaz, 2007; Freitas et al., 2021).

In Brazil, congenital syphilis became a notifiable disease through Ordinance No. 542 of December 22, 1986. In 1993, Latin American countries identified congenital syphilis as a public health problem. However, despite the availability of clinical guidelines, diagnostic tests, and therapy for pregnant women, the goal of achieving an incidence of one case per 1,000 live births has not been met (Brasil, 1986). From 1998 to 2017, a total of 159,890 cases of congenital syphilis in children under one year old were reported in the Notifiable Diseases Information System (SINAN) in Brazil, with 70,558 (44.12%) cases in the Southeast region, 49,585 (31.01%) in the Northeast, 17,257 (10.79%) in the South, 13,625 (8.52%) in the North, and 8,865 (5.55%) in the Midwest (Brasil, 2017). Starting in 2010, there has been a progressive increase in the incidence of the disease, with a national average of 8.6 cases per thousand live births reported in 2017 (Brasil, 2019).

The States with syphilis incidence rates higher than the national average were Rio Grande do Sul, Rio de Janeiro, Espírito Santo, and Pernambuco, all with 10.4 cases per thousand live births, followed by Tocantins and Sergipe with rates of 9.9 and 8.8 cases per thousand live births,

respectively (Brasil, 2017). Currently, looking at data from the last decade, there has been a considerable increase in the number of cases detected, from 18,243 cases in 2011 to 167,523 cases in 2021, still presenting itself as a major challenge in Brazil (Ramos Jr, 2022; Brasil, 2023; Silva et al., 2023).

Given that syphilis can cause serious sequelae and disabilities in affected individuals, studies to determine the extent and origin of the problem become important. Although it can spontaneously cure, the sores caused by syphilis can serve as entry points for various microorganisms, including HIV and Hepatitis B virus, which can lead to co-infections with other sexually transmitted infections (STIs) (Hinrichsen, 2009; Pinto Neto et al., 2021).

The treponemal methods of direct detection of the etiological agent are based on the detection of the etiological agent itself, whereas indirect detection methods are based on the detection of antibodies against the components of *T. pallidum* (Fiumara, 1980). Among the treponemal tests are fluorescent treponemal antibody absorption (FTA-abs), indirect hemagglutination tests (TPHA), immunoenzymatic assays (ELISA - Enzyme-Linked Immunosorbent Assay), including chemiluminescent assays and rapid tests (Brasil, 2010; Peçanha Jr & Brasil, 2022).

Non-treponemal tests are methods that detect antibodies that are not specific to *T. pallidum* but are present in syphilis. Non-treponemal tests can be qualitative or quantitative, and their titers are indicated by the last dilution of the sample that still shows reactivity or visible flocculation (Brasil, 2010).

The most commonly used non-specific serological test is the Venereal Disease Research Laboratory (VDRL) test. This microagglutination technique utilizes cardiolipin, yielding good responses as early as the secondary phase with a sensitivity ranging from 78% to 86%. Being a quantitative test, it serves both for diagnosis and monitoring (Brasil, 2016).

In this sense, the present study aimed to verify the concordance of the rapid test for syphilis in pregnant women compared to VDRL and to compare the performance of treponemal and non-treponemal serological tests in pregnant women and newborns at a public hospital in a municipality in northern Espírito Santo.

MATERIAL AND METHODS

Study Site

The study was conducted between January and December 2018, utilizing data from pregnant women admitted to a reference hospital in northern Espírito Santo. According to the State Government, the hospital makes approximately 1,764 deliveries per year and has 37 beds from the Unified Health System (SUS), including 26 obstetric beds. The hospital only handles routine risk deliveries, follows the Ministry of Health's protocol

(Brasil, 2015), and routinely performs rapid tests for syphilis screening in pregnant women. After the rapid test screening, pregnant women with positive results undergo the VDRL test as a quantitative examination to determine the mother's infection load.

For neonates born to infected mothers, the VDRL test is performed shortly after birth. Neonates with titers equal to or lower than the mother's are evaluated for the degree of infection and the need for maternal treatment. Neonates with titers higher than the mother's and/ or abnormalities in cerebrospinal fluid (CSF) receive treatment according to the guidelines and therapeutic protocols of the Ministry of Health (Brasil, 2015).

Sampling

Since it was a descriptive study that included the entire eligible population, no sample size calculation was used, all pregnant women admitted to the hospital in the northern region of Espírito Santo (HRNES) between January and December 2018, who met the inclusion criteria and signed the informed consent form, were enrolled in the present study.

The results of rapid tests for syphilis were evaluated in 2,437 pregnant women admitted to the hospital for delivery or curettage from January to December 2018. Out of the total number of pregnant women, 106 (4.35%) tested positive for syphilis. Among the pregnant women admitted for delivery or curettage during the study period, 276 women agreed to participate in the screening evaluation phase.

Inclusion criteria: Pregnant women with results from rapid tests and VDRL, and who remained in the hospital for delivery or curettage.

Exclusion criteria: Pregnant women admitted for clinical treatment.

For the analysis of newborns, a total of 41 samples were collected from neonates born to mothers infected with syphilis who agreed to participate in the study, from January to December 2018.

Study Design

This is a cross-sectional, descriptive study of quantitative and qualitative nature that aimed to compare the results of rapid tests and VDRL in pregnant women, as well as TPHA in pregnant women and newborns.

Initially, a rapid test was performed, and if positive, a VDRL test was conducted. In case of positivity for at least one of the tests, a TPHA test was performed (Figure 1A). For pregnant women with a non-reactive rapid test result, a serum sample was collected by venipuncture at the study hospital for VDRL testing. If positive, a confirmatory TPHA test was also performed for that sample (Figure 1B).

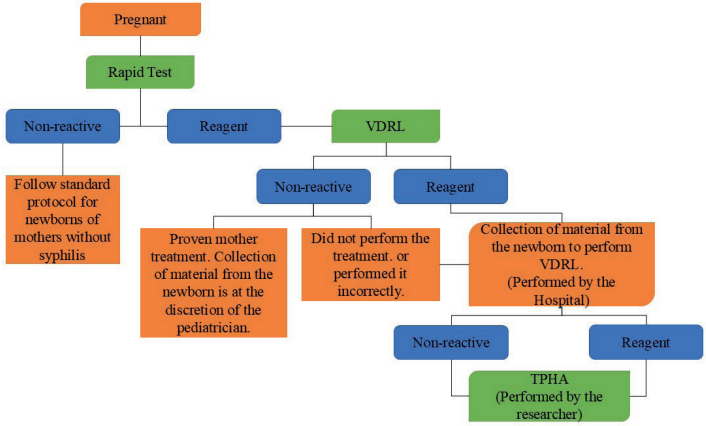

Figure 1. (A) Flowchart of the tests performed in pregnant women with a reactive rapid test result at a hospital in the northern region of Espírito Santo, from January to December 2018. (B) Flowchart of the tests performed in pregnant women with a non-reactive rapid test result at a hospital in the northern region of Espírito Santo, from January to December 2018.

In neonates, serum samples were collected by the hospital for VDRL testing, and the TPHA test was performed on serum samples provided by the hospital's laboratory.

The clinical repercussions and treatment of the newborn depend directly on the serological status and whether treatment is carried out for the pregnant woman. To understand the dynamics of testing in neonates, the most common situations in which these newborns are exposed are represented in Figure 2.

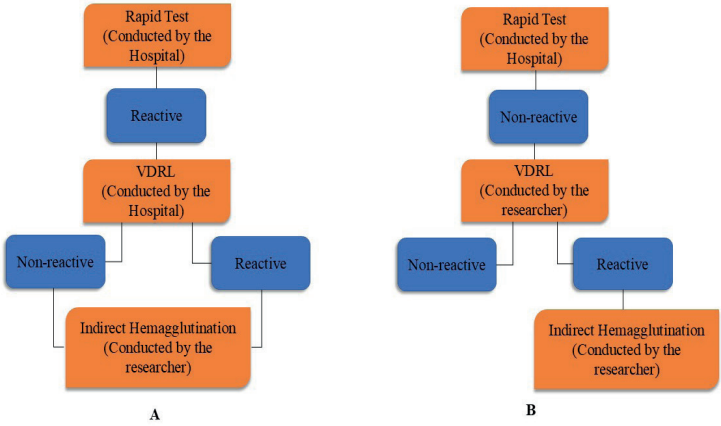

Figure 2. Flowchart of the tests performed in newborns at a hospital in the northern region of Espírito Santo, from January to December 2018.

All analyses in this research were conducted in the Clinical Laboratory of the hospital under study. The results of the syphilis tests were provided to the physician responsible for the care of the pregnant women and newborns.

Pregnant women under 18 years old were only able to participate in the project with the consent of their parents or guardians and after signing the assent form, which was mandatory for all study participants.

Ethical aspects

The study obtained authorization from the hospital's administrative board through a letter of agreement for its execution and was submitted to and approved by the Research Ethics Committee with Human Subjects (CEP) of the Centro Universitário Norte do Espírito Santo (CEUNES) of the Universidade Federal do Espírito Santo (UFES) under the opinion number 77247317.1.000.5063.

Statistical Analysis

Statistical analysis was performed using Stata 14.0 software, and the tests used were Pearson's chi-square and Fisher's exact tests. Descriptive statistics were applied to obtain the mean, standard deviation, and percentage of collected data. The Kappa coefficient (IC95%) was used for test agreement analysis, generated through Bioestat 5.0. It is widely used in inter-rater reliability studies, where the aim is to understand the level of agreement

between different raters or measuring instruments (Miot, 2016). Cohen suggested the Kappa result be interpreted as follows: values ≤ 0 as indicating no agreement and 0.01-0.20 as none to slight, 0.21-0.40 as fair, 0.41-0.60 as moderate, 0.61-0.80 as substantial, and 0.81-1.00 as almost perfect agreement (Cohen, 1960; McHugh, 2012).

RESULTS

Of 2,437 pregnant women admitted for delivery or curettage, 106 (4.35%) had a positive rapid test result for syphilis, and of the total number of pregnant women admitted, 276 (Table 1) agreed to participate in the screening evaluation phase.

The results indicated that 146 (52.9%) out of the 276 samples tested positive for both methods (VDRL and rapid test). In total (276), 91 (33.0%) samples were positive for the rapid test, and 55 (19.9%) samples were positive for VDRL. Out of the 91 reactive samples for the rapid test, 53 (58.2%) were positive for both tests and 38 (41.7%) were positive only for the rapid test. Of the 55 VDRL positive samples, 53 (96.36%) were positive for both tests, and only two samples (3.63%) were positive solely for VDRL. Additionally, three rapid test samples were classified as inconclusive by the testing team, with one (1.1%) of them being positive in the VDRL examination.

Table 1. Results of the samples analyzed by the rapid test and VDRL screening methods, in pregnant women seen at a hospital in the northern region of Espírito Santo, between January and December 2018.

		VDRL			
		Reactive	Non-reactive	Uncertain	Total
Rapid Test	Reactive	53	38	0	91
	Non-reactive	1	181	0	182
	Uncertain	1	2	0	3
	Total	55	221	0	276

Kappa coefficient (IC95% 0,41-0,60, p < 0,0001)

The confirmatory TPHA test was used as a reference test. The test was applied to 94 samples, and the results indicated that 89 samples remained positive when compared to the rapid test, as well as the same number when compared to the VDRL (Table 2).

Table 2. Result of TPHA assay of positive samples for the screening methods, rapid test, and VDRL, in pregnant women attended at a hospital in the northern region of Espírito Santo, between January and December 2018.

		Rapid Test			
		Reactive	Non-reactive	Uncertain	Total
TPHA	Reactive	88	0	1	89
	Non-reactive	3	0	2	5
	Total	91	0	3	94
		VDRL			
		Reactive	Non-reactive	Uncertain	Total
TPHA	Reactive	54	35	0	89
	Non-reactive	1	4	0	5
	Total	55	39	0	94

For the analysis of agreement between the tests, the concordance index analysis method was used (Concordance index= Agreements/ Agreements + Disagreements × 100), which consists of comparing identical results applied to the same individual using different methods or instruments, or the same method at two different time points (Miot 2016).

The results indicated a concordance of 98.9% when analyzing the positivity of the rapid tests compared to the TPHA assay, while using VDRL as a screening method yielded only 60.7% concordance.

Application of Confirmatory Test for Neonates

As part of the care protocol for neonates born to mothers infected with syphilis, the hospital performs VDRL testing on these newborns.

A total of 41 samples were collected from neonates born to mothers infected with syphilis who agreed to participate in the study, from January to December 2018. The VDRL tests indicated that only 24 (58.5%) of the neonates showed positivity for syphilis. However, when evaluated with the confirmatory method of TPHA, 40 (97.6%) of the newborns tested positive for syphilis. Thus, 39.0% of the neonates had a non-reactive VDRL test, while only 2.4% of the newborns had a negative confirmatory test (Figure 3).

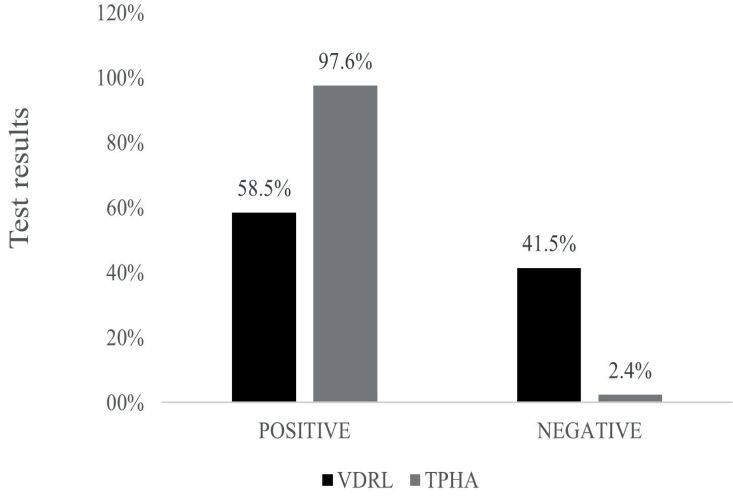

Figure 3. Comparison between VDRL and indirect hemagglutination test in neonates born to mothers with positive results for syphilis at a hospital in the northern region of Espírito Santo, between January and December 2018.

Burden of Infection in Puerperal Women and Newborns

The quantitative test used to assess the burden of *T. pallidum* infection was the non-treponemal VDRL test. For this analysis, the VDRL results of pregnant women who were positive for both the rapid test and the confirmatory TPHA test were used. A total of 89 samples from pregnant women and 40 from neonates (confirmed by TPHA) were counted.

The results showed a predominance of low infection rates in both pregnant women and newborns, with 39.3% of pregnant women and 40.0% of newborns having non-reactive serology for the VDRL test. Tests with reactive results accounted for more than half of the cases, 60.7% and 60.0% respectively. The 1:1, 1:2, 1:4, and 1:8 titers, considered to be of low to intermediate infection load, accounted for 49.4% of the results obtained for pregnant women, and 55.0% for neonates. Only 11.3% of pregnant women and 5.0% of newborns had titers above 1:16 (Table 3).

Table 3. Burden of *T. pallidum* infection in pregnant women and newborns treated at a hospital in northern Espírito Santo.

VDRL (Title)	Pregnant women (%)	Neonates (%)
Non-reactive serum	35 (39.3)	16 (40.0)
Reagent 1:1	12 (13.5)	10 (25.0)
Reagent 1:2	13 (14.6)	05 (12.5)
Reagent 1:4	14 (15.7)	04 (10.0)
Reagent 1:8	05 (5.6)	03 (7.5)
Reagent 1:16	05 (5.6)	00 (0.0)
Reagent 1:32	00 (0.0)	00 (0.0)
Reagent 1:64	02 (2.3)	01 (2.5)
Reagent 1:128	03 (3.4)	01 (2.5)
Total	89	40

DISCUSSION

The Ordinance nº 3.242, issued on December 30, 2011, is the first regulation in Brazil that governs the diagnostic research of syphilis, and it indicates the possibility of "reverse screening," a term used when employing a treponemal test to initiate the syphilis investigation (Miot, 2016). However, the most commonly used test for screening the causative agent of the disease in the country is VDRL, a non-treponemal test (Brasil, 2022).

Recently, in the country, the treponemal rapid test was introduced into the routine prenatal protocol to use it in reverse screening of pregnant women (Miot, 2016). The present study aimed to analyze the concordance between the tests for detecting positive results using the treponemal rapid test and VDRL, as well as their concordance with the confirmatory TPHA test. It found a moderate level of agreement between the rapid test and VDRL. In the analyzed sample, 35 (39.3%) pregnant women, with a confirmed diagnosis of syphilis through TPHA, tested positive only for the rapid test. However, VDRL can experience a decrease in titers or become negative when the patient is treated, undergoes spontaneous cure, or is in the primary or latent stages of the disease (Senã et al., 2010; CDC, 2015).

The agreement between the rapid test and the confirmatory TPHA assay, as observed in the present study, was 98.9%, demonstrating that the test can be used as a key tool for the prevention of vertical and congenital transmission of syphilis. This result was expected, as studies on the sensitivity and specificity of the treponemal rapid test for syphilis, such as the study conducted by Benzaken et al. (2007), indicated a sensitivity of 90.2% and a specificity of 99.4%.

The sensitivity and specificity of the rapid test can reach 99.8% and 100%, respectively, using TPHA as the reference (Brasil, 2006). One advantage of using the rapid test in reverse screening of pregnant women is its ease of execution, as the rapid test does not require a laboratory setting for its performance, thereby increasing access for pregnant women residing in rural or remote areas (Benzaken et al., 2007).

Screening using the rapid test as the first-choice examination has been increasingly employed (Brasil, 2011; Brasil, 2015), as seen in the hospital in the northern region of Espírito Santo, which changed its protocol in 2017, adopting the rapid test as the primary screening method.

One point to consider when discussing syphilis screening using the treponemal rapid test is immunological memory. Patients who have been adequately treated generally show reactive results (Workowski & Berman, 2010; Brasil, 2016). However, the presence of antibodies does not confer immunity against a new infection. Therefore, the decision to recommend therapy should be considered by the clinician, taking into account the epidemiological factors and clinical condition of the patient, which may expose the pregnant woman to reinfection (Brasil, 2011).

The traditional approach of using VDRL for screening pregnant women may need to be reconsidered, as the results of the present study indicate that it is a test with reduced capacity to capture pregnant women in certain stages of the disease. Additionally, being a non-treponemal test, it can produce false-positive results in patients with malaria, infectious mononucleosis, viral infections, Virchowian leprosy, and autoimmune diseases such as lupus, among others, as reported by Avelleira & Bottino in 2006.

In 2017, the Government of Espírito Santo launched the State Plan to Address Congenital Syphilis, with the objective of drastically reducing the cases of congenital syphilis in the State to achieve a target of 0.5 cases per thousand live births (SESA, 2017). The program proposed actions to facilitate access for pregnant women in basic health units and immediate implementation of rapid tests for syphilis, HIV, and Hepatitis B, as well as the inclusion of their partners in testing. It aimed to ensure that 100% of pregnant women undergo rapid tests at the first prenatal consultation and also in the third trimester of pregnancy. The plan also proposed decentralizing and distributing strategic inputs to address these diseases, aiming to facilitate logistics and ensure a supply in all municipalities (Matyskina et al., 2017). Similar actions were

documented by Fitzgerald et al. (2003) in Haiti, where they observed a 75% reduction in congenital syphilis rates in the Artibonite region following the decentralization of screening.

Thus, the prevention of vertical transmission is of vital importance for reducing rates of congenital syphilis, but it is necessary to focus on improving early diagnosis guidelines for the disease.

In the present study, a positivity rate of 61.0% was detected in newborns of syphilis-infected mothers using the non-treponemal VDRL test, while the treponemal TPHA test yielded a positivity rate of 97.6%. These results corroborate the study by Matyskina et al. (2017), who found a positivity rate of 65.8% using VDRL and 88.9% using TPHA. The findings in São Mateus can contribute to healthcare professionals' decisions, as they highlight the need for adjustments in diagnostic procedures for neonates.

The IgM test for syphilis can be a useful tool for the early detection of syphilis in newborns. However, a negative result cannot rule out the possibility of this condition due to its low sensitivity (Salazar et al., 2000; Matyskina et al., 2017). Passively transferred IgG antibodies from the mother to the neonate can persist in a baby until 15 months of age, and the results of treponemal tests in newborns may remain positive despite effective treatment (Herremans et al., 2010). Among treponemal tests, the most commonly used in clinical practice for confirmatory diagnosis are FTA-Abs, ELISA, and TPHA after 18 months of age (Rodrigues-Cerdeira et al., 2012; Chiumento & Griep, 2015; Feitosa et al., 2016).

Rawstron et al. (2001), using the FTA-ABS test, showed that only half of a group of neonates with clinical or laboratory evidence of syphilis remained reactive when the test was performed at 12 months of age, while in a group of newborns without evidence of the disease, approximately two-thirds of the children had positive results. The authors suggest that this difference may exist because not all children underwent a complete clinical or laboratory evaluation at birth.

Therefore, the need for clinical and laboratory monitoring of all neonates born to syphilis-infected mothers is evident, with protocols that include specific laboratory tests for the disease. Consequently, children with a non-reactive confirmatory test and those who have already received appropriate treatment would be discharged from further follow-up.

Our findings show that the treponemal rapid test used in the study showed moderate agreement with the VDRL test, indicating that reverse screening for syphilis in pregnant women using the rapid test can be considered for immediate initiation of treatment in positive cases. Considering that the rapid test was positive in 98.9% of pregnant women who had a reactive laboratory report for syphilis, we can say that the test can help expedite and provide greater resolution for pregnant women tested during prenatal consultations, without the need to wait for laboratory diagnosis. When we

observe that more than half of the newborns (78 individuals) in this study (62/79.5%) are asymptomatic for the disease (unpublished data), which makes clinical diagnosis challenging, we understand that early laboratory diagnosis of congenital syphilis in newborns, on the first day of life, necessarily requires specific methods that use treponemal reactions, such as TPHA.

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CONFLICT OF INTEREST

The authors declare they have no conflicts of interest to disclose.

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