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Evaluation of positive and false-positive results in syphilis screening of blood donors in Rio de Janeiro, Brazil

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ABSTRACT

Objectives: We propose to analyse the positive and false-positive results of treponemal and nontreponemal tests in blood donors from Brazil and to evaluate possible factors associated with the results of treponemal tests.

Background: Treponemal tests have been used widely for syphilis screening in blood banks. The introduction of these tests in donor screening has caused an impact and a loss of donors who need to be assessed.

Methods: This was a retrospective cross-sectional study of syphilis screening and confirmatory test results of blood donors that were obtained before and after adopting a chemiluminescent immunoassay (CLIA). A comparative analysis was performed using a second sample drawn from positive donors. The possible factors associated with CLIA-positive or CLIA-false-positive results were investigated in a subgroup. Statistical tests were used to compare the proportions and adjusted estimates of association.

Results: The reactivity rate increased from 1·01% ($N = 28\,158$) to 2·66% ($N = 25\,577$) after introducing the new test. Among Venereal Disease Research Laboratory (VDRL)- and CLIA-confirmed results, the false-positive rates were 40·5% ($N = 180$) and 37·4% ($N = 359$), respectively ($P = 0{\cdot}5266$). Older donors (OR = 1·04; $P = 0{\cdot}0010$) and donors with lower education levels (OR = 6·59; $P = 0{\cdot}0029$) were associated with a higher risk of positivity for syphilis.

Conclusions: CLIA represents an improvement in blood bank serological screening. However, its use in a healthy population appears to result in high rates of false positives. Identifying which characteristics can predict false positives, however, remains a challenge.

Key words: blood donors, serological tests, syphilis.

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Syphilis is a disease that has been studied for centuries, and serological tests represent an important tool for diagnosis. Since the discovery of the first syphilis serological test 110 years ago (Bialynicki-Birula, 2008), many testing options with varied methodologies have become available. However, no test alone can be considered definitive.

Treponemal tests such as enzyme-linked immunosorbent assay (ELISA) and chemiluminescent immunoassay (CLIA) have been widely used as alternatives to the traditional nontreponemal tests, i.e. the Venereal Disease Research Laboratory (VDRL) and Rapid Plasma Reagin (RPR), due to the formers' advantages, such as objectivity and the possibility of automation. However, the treponemal assays used for syphilis screening, in contrast to the confirmatory testing of the initial nontreponemal positive results, have a low positive predictive value for infectious syphilis among low-prevalence populations. Thus, these methods require reflex testing and possibly a second treponemal test with an alternative platform for confirmation (Young, 2000). This reverse algorithm has resulted in increased reactivity rates for syphilis (Mishra *et al.*, 2011), and false-positive cases represent a significant proportion of the results (van Dommelen *et al.*, 2015). Unconfirmed treponemal screening tests require close monitoring. This is a clinical dilemma for follow-up and treatment and can represent a loss of healthy donors and the increased disposal of blood products in blood banks.

The use of high-sensitivity testing in blood donor screening is essential to ensure transfusion safety. However, it is also important that the tests maintain high specificity to minimise the possible losses of healthy donors, particularly in areas with few donors.

This study analysed the positive and false-positive result profiles among blood donors from the Instituto Nacional de Câncer José Alencar Gomes da Silva (INCA), Rio de Janeiro, Brazil, by comparing the use of a nontreponemal test (VDRL) with a treponemal test (CLIA). Additionally, we investigated possible factors associated with positive and false-positive results of the recently introduced test.

MATERIALS AND METHODS

This was a retrospective cross-sectional analysis of syphilis screening and confirmatory test results from INCA blood donors

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obtained before and after the adoption of a CLIA. Data were extracted from the database, and a comparative analysis was performed using the results from a second sample that was drawn from positive donors to retest and confirm the results from the initially reactive samples. This second sample was collected when the donor could return, which occurred between 1 week and 1 year after donation. The periods analysed were from January 2010 to December 2011 for VDRL (Weiner Lab, Rosario, Argentina) and from January 2012 to December 2013 for CLIA (Architect® Syphilis TP, Abbott, Wiesbaden, Germany). The CLIA used the recombinant antigens TpN15, TpN17 and TpN47, and it was performed using the Architect® system Module i2000 (Abbott, North Chicago, IL, USA).

The titre cut-off for positive VDRL was 2 (dilution 1/2). Confirmed positives were defined as samples that showed reactivity in both the screening test and the confirmatory second test [Fluorescent Treponemal Antibody-Absorption (FTA-ABS), WAMA Diagnóstica, São Carlos/SP, Brazil, or Scimedx, Denville, NJ, USA]. Additionally, CLIA-reactive samples were subjected to VDRL to distinguish between active syphilis (CLIA+/VDRL+/FTA−ABS+) and past syphilis (CLIA+/VDRL−/FTA-ABS+). After retesting the second samples from positive donors, false-positive results were classified as transitory (if the retest result was CLIA− or VDRL−) and permanent (if the retest result was CLIA+/FTA-ABS− or VDRL+/FTA-ABS−). Cases of disagreement or borderline results in the FTA-ABS test were resolved by *Treponema pallidum* hemagglutination test (TPHA, WAMA Diagnóstica, São Carlos/SP, Brazil).

During the CLIA period, variables that were possibly associated with positive (age, gender, marital status and education level) and/or false-positive results (age, gender, use of medicines in the 15 days prior to donation and concomitant reactivity to other infectious agents) were investigated in a subgroup of donors, with a second sample collected and confirmed between July and December 2013, and in a contrasting group, with initially CLIA-negative samples frozen at −20 °C. Negative samples were randomly selected from the same period and confirmed with FTA-ABS. The number of negative samples was estimated to be 2000 in accordance with the proportion of reactivity for syphilis in this population. Data were collected from the electronic system and from the manual form filled out by donors at the time of donation.

Samples for which it was not possible to perform any test or retrieve the necessary data were excluded.

Statistical analysis

Data were recorded in a spreadsheet. χ^2 and Fisher's exact tests were used to compare the means, verify the possible associations between variables and positive/false-positive results and compare categorical variables. Multiple logistic regression was applied when there were significant associations between variables. The level of significance was set at 0·05. All analyses were carried out using the S-Plus version 8·0 software (TIBCO Software Inc., Palo Alto, CA, USA).

Ethical issues

This study was approved by the Ethics and Research Committee of the Instituto Nacional de Câncer José Alencar Gomes da Silva (Approval number: 933,706).

RESULTS

The INCA blood bank received 53 735 donations between 2010 and 2013. Donors were aged between 17 and 69 years, and most were male (66·90%). The reactivity rate for syphilis increased from 1·01% to 2·66% after the introduction of the treponemal test (Figs 1 and 2).

It was possible to retest a second sample from 180 and 359 returning donors among the samples tested with VDRL and CLIA, respectively. From these, negative results were obtained in 33 (18·33%) samples from the VDRL group and 25 (6·96%) from the CLIA group, representing transitory false positives in the first sample. VDRL presented more transitory false positives than CLIA did ($P < 0{\cdot}05$). Positive results persisted in 147 (81·67%) samples from the VDRL group and 334 (93·04%) from the CLIA group. FTA-ABS was performed on samples that remained reactive, and this result was confirmed in 107 (59·45%) samples from the VDRL group and 225 (62·68%) from the CLIA group. Positivity was not confirmed in 40 (22·22%) and 109 (30·36%) samples from the VDRL and CLIA groups, respectively, thus representing permanent false positives. The permanent false-positive rates were not significantly different between tests ($P = 0{\cdot}5$).

The analysis of possible variables associated with positive or false-positive results included 79 second samples that were initially CLIA-reactive. Five samples were excluded because it was not possible to retrieve some of the results. The contrasting group included 2032 samples with negative screening. After excluding 38 replicated donations from the same donors, 1994 samples were analysed. We identified seven (9·46%) transitory false-positive results and 25 (33·78%) permanent false-positive results from the group of reactive samples ($N = 74$) compared to the FTA-ABS test. Of the 1994 non-reactive samples. 5 were borderline results in the FTA-ABS test, but after analysing the repetition in the FTA-ABS and TPHA results, these samples were considered negative. Thus, there were no cases of false-negative tests for CLIA compared with FTA-ABS.

When interviewing donors who were considered suitable in clinical screening, the drugs that were reported as being used in the study period were included. In this subgroup ($N = 2068$), 515 (25%) of the donors had used at least one drug within the last 15 days before donation, including herbal and homoeopathic medicines. The most frequent therapeutic classes found were antihypertensive drugs (136), contraceptives (110), analgesics (80) and anti-inflammatories (65). Brazilian legislation recommends that the use of medicines be evaluated on a case-by-case

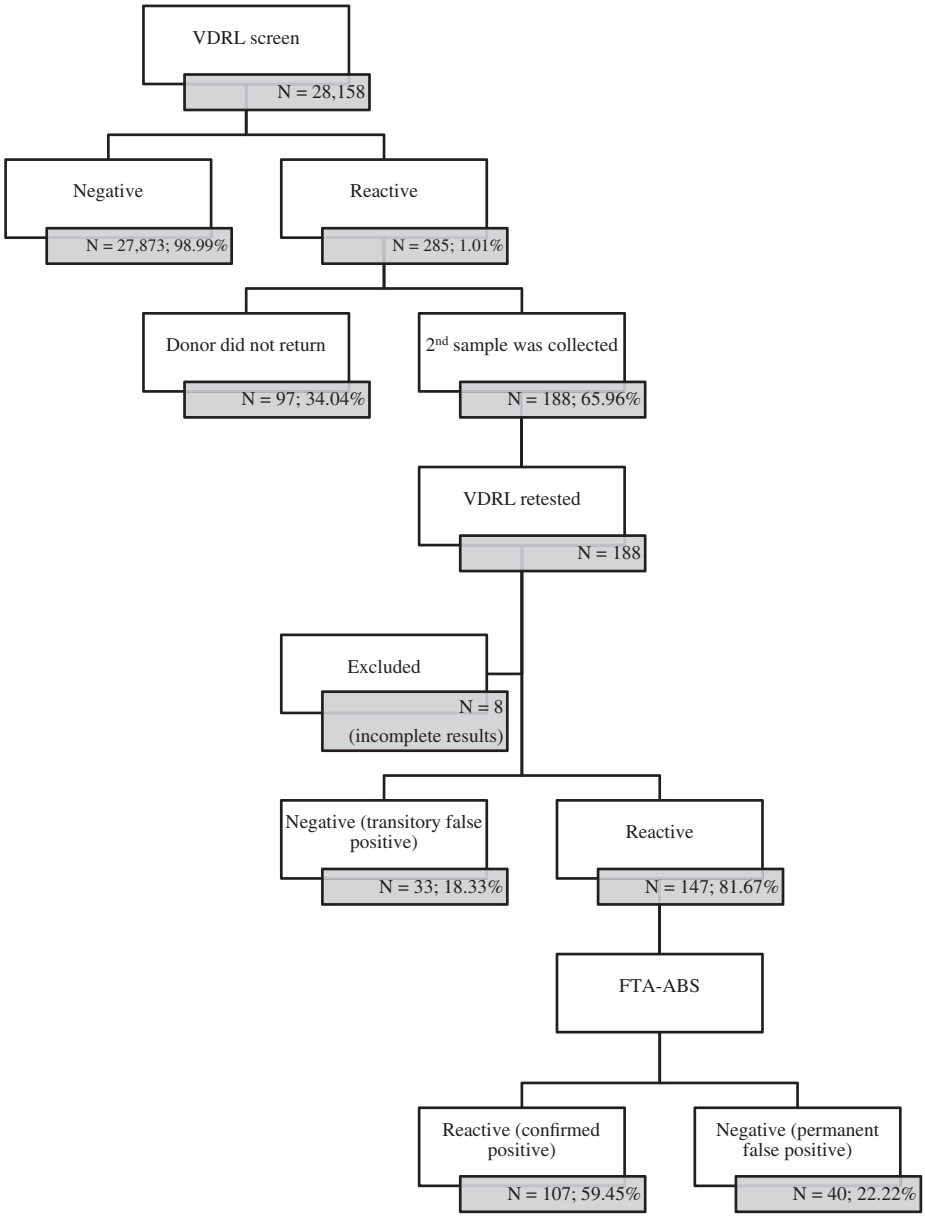

Fig. 1. Results of syphilis screening during the VDRL (venereal disease research laboratory) test period (2010–2011).

basis. Some classes of drugs cause temporary non-eligibility of donors. These include antibiotics, anticoagulants, anticonvulsants, anorectics, vasodilators, antiarrhythmic drugs, some types of antihypertensive drugs such as beta blockers, some types of hormones and teratogenic drugs (Brazil, 2016).

Additionally, reactive results for the following serological markers were found: Chagas (4), Anti-HBc (16), Anti-HBc/HBsAg (1) HBsAg (1), HCV (5) and HTLV (1).

There was a significant association of both age and education level with positive results (Table 1). There was no significant association between age, gender, drug use or positivity to other infectious agents and transitory or permanent false-positive results, as shown in Table 2.

DISCUSSION

CLIA was introduced in 2012 for the syphilis screening of INCA blood donors. Traditional donor screening uses nontreponemal tests as the first line of investigation and has the advantage of having a low cost, quick execution and ease of implementation, particularly in small laboratories. Traditional tests also make it possible to follow the evolution of the treatment. Treponemal tests were previously used only as a confirmatory step to exclude false positives from traditional screening; screening with them first ensured the advantages of objective results and a possibility of automation. This optimised the routine of laboratories with large volumes of samples and ensured higher quality. Furthermore,

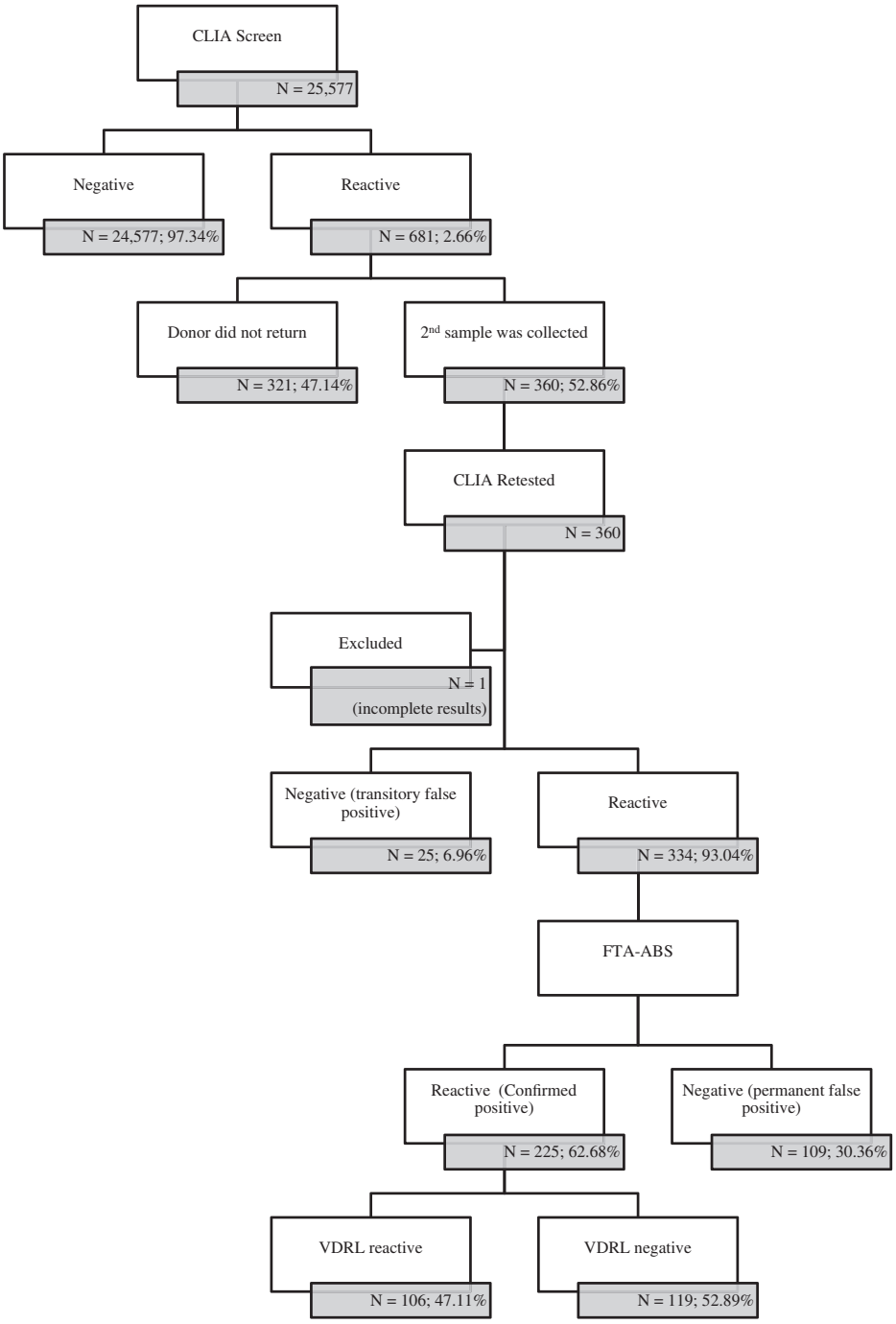

Fig. 2. Results of syphilis screening during the CLIA (chemiluminescent immunoassay) test period (2011 – 2012).

the test is more sensitive to cases of recent and latent syphilis (Kaur & Kaur, 2015).

Although the CLIA used specific *Treponema pallidum* antigens, no significant difference was observed in the false-positive profile between the CLIA and the previously used VDRL tests. A significant difference was found when transitory false positives were compared with the highest proportion in the VDRL test. VDRL is a manual technique and is thus more susceptible to interference, which could explain this result. Moreover, some patient transitory states can affect the test results (Seña *et al.*, 2010).

A study conducted in another Brazilian blood bank showed a VDRL reactivity rate of 0·75%, which increased to 0·93% with a treponemal test (Baião *et al.*, 2014). Moreover, the false-positive rate with VDRL was 60·7%, but it decreased to 25·6% with CLIA. These authors found that 18·4% of the VDRL-reactive tests and

Table 1. Association of variables with positive or negative results from retested donors of the subgroup from the chemiluminescent immunoassay period (from July to December 2013, $N = 2068$)

		FTA-ABS result					Logistic regression analysis	
		Negative ($N = 2205$)		Positive ($N = 43$)		Univariate analysis	Odds ratio	
	Variable	*N*	%	*N*	%	*P* value	(IC 95%)	*P* value
Gender	Female ($N = 758$)	745	98·3	13	1·7	0·5	–	–
	Male ($N = 1310$)	1280	97·7	30	2·3			
Marital status	Married ($N = 866$)	846	97·7	20	2·3	0·6	–	–
	Not married[1] ($N = 1202$)	1179	98·0	23	2·0			
Education level[2]	Incomplete elementary school ($N = 131$)	124	94·7	7	5·3		7·72 (2·22-26·8)	<0·05
	Elementary school ($N = 128$)	122	95·3	6	4·7		6·72 (1·87-24·19)	< 0·05
	Incomplete high school (N = 69)	67	97·1	2	2·9	<0·05	4·08 (0·73-22·71)	0·1
	Complete high school ($N = 927$)	906	97·7	21	2·3		3·17 (1·08-9·28)	< 0·05
	Incomplete superior education ($N = 240$)	237	98·8	3	1·2		1·73 (1·08-9·28)	0·4
	Complete superior education ($N = 551$)	547	99·3	4	0·7		1·0 (Ref.)	–
	Age, mean (SD) ($N = 2068$)	36·96 (11·53)		43·72 (11·79)		< 0·05	1·04 (1·02-1·08)	< 0·05

FTA-ABS, fluorescent treponemal antibody-absorption; ref., reference; SD, standard deviation.
P value – Statistical Significance ($P < 0·05$).
[1]The group includes single, widowed and divorced people.
[2]22 samples were excluded because the education level was not available.

Table 2. Association of variables with false-positive or positive results from retested donors of the subgroup from the chemiluminescent immunoassay period compared to Fluorescent Treponemal Antibody-Absorption (FTA-ABS) (from July to December 2013, $N = 74$)

		CLIA results compared to FTA-ABS						
		Positive ($N = 43$)		Transitory false-positive ($N = 6$)		Permanent false-positive ($N = 25$)		
Variable		*N*	%	*N*	%	*N*	%	*P* value
Gender	Female ($N = 26$)	13	50·0	3	11·5	10	38·5	
	Male ($N = 48$)	30	62·5	3	6·2	15	31·3	0·5
Drug use	Yes ($N = 17$)	10	58·8	3	17·6	4	23·6	
	No ($N = 57$)	33	57·9	3	5·3	21	36·8	0·2
Reactivity to other infectious agents	Yes ($N = 6$)	3	50·0	0	-	3	50·0	
	No ($N = 68$)	40	58·8	6	8·8	22	32·4	0·8
Age, mean (SD) ($N = 74$)		43·72 (11·80)		41·16 (15·32)		42·44 (11·20)		0·6

CLIA, chemiluminescent immunoassays; SD, standard deviation.
P value – statistical significance ($P < 0·05$).

6·1% of the CLIA-reactive tests became negative on follow-up testing. These transitory false-positive rates were consistent with those found in our study.

Marangoni, along with other researchers, checked the CLIA from the same manufacturer in two different studies. In the first case (Marangoni *et al.*, 2009), the researchers found a specificity rate of 98·4% on the analysed samples from patients suffering from syphilis or other biological conditions associated with false-positive results and samples that were previously considered false positive in CLIA. These authors evaluated 129 CLIA-reactive samples that were negative by western blotting and TPHA and found that 25 of these samples had weak responses to the TpN47 antigen, 14 samples were reactive to TmpA, and two samples were reactive to TpN 17 and TpN15. According to the authors, these false positives did not appear to be caused by cross-reactivity with treponemal antigens. Thus, many CLIA false-positive results have unknown causes. In the second study (Marangoni *et al.*, 2013), the researchers analysed samples with similar characteristics to the first case, adding samples from donors and patients who were subjected to the routine lab tests. In this study, a specificity rate of 78·4% was found.

Older subjects were more likely to test positive for syphilis. This finding agrees with another study that raised the possibility that this association is related to an increase in primary and secondary syphilis in this population (Vera *et al.*, 2014). Another explanation may be the presence of immunological memory antibodies of past syphilis in older people (de Almeida Neto *et al.*, 2009). A study conducted by Ferreira *et al.* (2014) in São Paulo showed that a reactive treponemal test and negative nontreponemal test profile were more common among older people.

The odds of having a positive syphilis test were 7·72 times greater among people who did not complete elementary education than in the other groups. The odds dropped to 6·72 times among donors who completed elementary school and 3·17 times for donors who completed high school. Thus, people with higher education levels were more protected. An association of positivity for syphilis and low education levels was also found in China (Liu *et al.*, 2012). This result indicates the need for intensifying the educational prevention campaigns against syphilis and other sexually transmitted diseases among this population.

No association was found between CLIA false-positive results and variables such as age, gender, use of medicines or reactivity to other infectious agents, although the small sample size in this analysis might have been a limitation. A larger sample size might reveal associations not found here.

Tong *et al.* (2014) showed higher sensitivity to syphilis when a treponemal test was used as the first line of testing. This option fits the purpose of blood bank syphilis screening, which is to maximise sensitivity and remove positive specimens. However, low specificity implies significant losses that must be assessed from another angle. In addition to the financial loss associated with unnecessarily discarded specimens, which increases costs and inputs in the production of blood products, there is an intangible loss with respect to donors, particularly repeat donors.

Liu and colleagues (2012) indicated that the standardisation of tests with high specificity and sensitivity and the establishment of algorithms could be cost-effective solutions to overcome the rejection of blood donors. For instance, 134 donations (corresponding to 0·52% of the total donations) would have been saved if an algorithm that used FTA-ABS as the second test had been used in syphilis screening during the period 2012 – 2013.

New technologies such as polymerase chain reaction (PCR)-based tests for syphilis detection or pathogen inactivation have been suggested, but these methods also have drawbacks, such as lower sensitivity to detect syphilis and reduced platelet activity, respectively (Gayet-Ageron *et al.*, 2013; Schlenke, 2014; Simpore *et al.*, 2014; Kaur & Kaur, 2015; Osman *et al.*, 2015).

Kane *et al.* (2015) found a low prevalence of syphilis among blood donors in the United States (162·6/100 000 first-time donors and 17·9/100 000 repeat donors) and concluded that a cost-utility analysis and continuity of serological screening for syphilis should be discussed, although these approaches could benefit public health by identifying cases that are unlikely to be treated. A study in the United States suggested that complications of untreated syphilis can be more frequent than previously estimated (Dombrowski *et al.*, 2015). In Brazil, few studies have assessed the prevalence of confirmed cases.

This study has some limitations. Only the second confirmed samples were analysed, which resulted in a loss of almost 50% of the initially reactive samples. Thus, it was impossible to calculate the prevalence of syphilis in the population. Furthermore, the VDRL and CLIA results were compared in different populations.

Another limitation is the use of FTA-ABS as the confirmatory test. This method has important constraints, including the subjectivity of results and the potential for false positives and negatives. FTA-ABS is widely used as a confirmatory test due to its easy implementation and low cost, which explains the availability of this test in the institution where the work was carried out. The lack of clinical data is also an important limitation.

Finally, CLIA represented an improvement in blood bank serological screening because it leads to the optimisation of large routines and to higher-quality automated tests. This improvement is demonstrated by the significantly lower rate of transient false positives for CLIA than for VDRL. In addition, its high sensitivity contributes to transfusion safety. However, its use in a healthy population appears to result in higher rates of false positives, which in turn imply a loss of donors. To minimise the discomfort in donors caused by false-positive results, it is important that the medical staff be aware of the characteristics of the serological tests used for screening, the confirmatory tests and the patient's clinical history.

Identifying characteristics that could predict false positives and reduce this problem remains a challenge to be overcome when improving efficiency in syphilis screening.

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V. S., S. S. and I. M. performed the research. V. S. and L. G. V. analysed the data. V. S. and S. C. wrote the paper.

CONFLICT OF INTEREST

The authors have no competing interests.

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