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Revalidation of the positive serological panel for syphilis – a tool in the quality control of *kits* for syphilis diagnosis

Revalidação do painel sorológico positivo para sífilis – uma ferramenta no controle da qualidade de *kits* para diagnóstico da sífilis

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ABSTRACT

Introduction: Syphilis is a slowly evolving infectious disease caused by a gram-negative bacterium in the group of spirochetes, exclusive to humans, called Treponema pallidum. In the fight against syphilis, an important tool is the laboratory analysis through products for in vitro diagnosis that allows the diagnosis of the disease. The effectiveness of these products is assessed against serological panels composed of true positive and negative samples. **Objective:** To reassess the reactivity of the 153 samples that make up the positive serological panel for syphilis at the Laboratory of Blood and Blood Products (LSH) of the National Institute for Quality Control in Health (INCQS). **Method:** Revalidation of the serological panel for syphilis through retrospective analysis of LSH laboratory data from 2011 to 2015, meeting the positivity criteria in 5 different methodologies and sample volume ≥ 10 mL. **Results:** Of the 172 initial samples, 153 had stock volume ≥ 10 mL. In the proposed period, 46 syphilis diagnosis products were identified with a satisfactory report, comprising 5 different methodologies. The analytical results of the 153 samples belonging to the positive panel were analyzed in the protocols for recording the results of these products. After re-evaluating the reactivity of the 153 samples from the positive panel 130, they were revalidated as positive while 23 were considered to be indeterminate. **Conclusions:** The positive panel for revalidated syphilis will remain an essential instrument in the previous analysis for the purpose of regularizing kits for diagnosing the disease.

KEYWORDS: Syphilis; Serological Panel; Quality Control

RESUMO

Introdução: A sífilis é uma doença infecciosa de evolução lenta causada por uma bactéria Gram-negativa do grupo das espiroquetas, exclusiva do ser humano, chamada *Treponema pallidum*. No combate da sífilis, uma importante ferramenta é a análise laboratorial através de produtos para diagnóstico *in vitro* que permite o diagnóstico da doença. A eficácia destes produtos é avaliada frente a painéis sorológicos compostos por amostras verdadeiro positivas e negativas. **Objetivo:** Reavaliar a reatividade das 153 amostras que constituem o painel sorológico positivo para sífilis do Laboratório de Sangue e Hemoderivados (LSH) do Instituto Nacional de Controle de Qualidade em Saúde (INCQS). **Método:** Revalidação do painel sorológico para sífilis através de análise retrospectiva de dados laboratoriais do LSH do período de 2011 a 2015 atendendo aos critérios de positividade em cinco metodologias diferentes e volume de amostra ≥ 10 mL. **Resultados:** Das 172 amostras iniciais, 153 apresentaram volume de estoque ≥ 10 mL. No período proposto foram identificados 46 produtos para diagnóstico da sífilis com laudo satisfatório compreendendo cinco metodologias diferentes. Os resultados analíticos das 153 amostras pertencentes ao painel positivo foram analisados nos protocolos de registro de resultados destes produtos. Após a reavaliação da reatividade das 153 amostras do painel positivo, 130 foram revalidadas como positivas enquanto 23 foram consideradas como indeterminadas. **Conclusões:** O painel positivo para sífilis revalidado permanecerá sendo instrumento essencial na análise prévia para fins de regularização dos *kits* para diagnóstico da doença.

PALAVRAS-CHAVE: Sífilis; Painel Sorológico; Controle de Qualidade

INTRODUCTION

Syphilis is a slowly evolving infectious disease caused by a Gram-negative bacterium in the group of spirochetes, *Treponema pallidum*, which is exclusive to humans. It is a systemic disease, as it affects practically all organs and systems. This disease has challenged humanity for centuries and, despite being curable through effective and low-cost treatment, it remains a serious public health problem until today[1]. According to data from the World Health Organization (WHO), syphilis affects approximately 5.6 million people worldwide annually, with 90% of these cases in developing countries[2,3]. The disease epidemiological data in Brazil shown in the Epidemiological Bulletin Syphilis 2018 by the Ministry of Health, between the years 2010 and 2017, reveal a significant increase in congenital syphilis rates of detection in pregnant women as well as acquired syphilis[4].

The main route of transmission of the disease is sexual contact (acquired syphilis), followed by vertical transmission from mother to child during pregnancy (congenital syphilis)[5,6,7]. Syphilis transmission by blood transfusion, although possible, has become rare due to mandatory serological screening of donors for the presence of infectious agents such as human immunodeficiency virus (HIV) 1/2, human T-cell lymphotropic virus (HTLV) - 1/2, hepatitis B virus (HBV), hepatitis C virus (HCV), *Trypanosoma cruzi* e *T. pallidum*[8,9]. Allied to this, the short survival time of the bacteria outside the human organism, especially at low temperatures such as those used for blood bags conservation, contributes to decreasing the disease transmission by blood transfusion[10].

Laboratory analysis of biological samples using products for *in vitro* diagnosis is an important tool in the battle against syphilis, allowing diagnosis and, consequently, appropriate treatment and monitoring of treatment response. In addition, the use of these products in blood bags serological screening provides control of disease transmission. The quality control of these products is carried out through prior analysis, an activity implemented to check product characteristics for registration, alteration (when applicable), or revalidation purposes, being a mandatory requirement recommended by the Brazilian National Health Surveillance Agency (Anvisa) for marketing and use of these products in Brazil[11]. This prior analysis is carried out by the Laboratory of Blood and Blood Products (LSH) of the Immunology Department of the National Institute for Quality Control in Health (INCQS). In the quality control of the diagnostic kits, the conformity of the product in relation to two distinct parameters is verified: sensitivity and clinical or diagnostic specificity. Clinical sensitivity is assessed by the incidence of truly positive results obtained when the test is applied to individuals known to have the disease in question. Clinical specificity is assessed by the incidence of truly negative results obtained when the test is applied to individuals known not to have the disease in question[11]. One of the instruments used to assess these two parameters is the serological panels defined as a set of samples produced from processed human plasma. Samples that contain antigenic determinants of a particular marker is marked as the positive serological panel and samples that do not contain it, as the negative serological panel[12]. Thus, the panels created and used in the analysis of products are extremely relevant and their revalidation, with clearly defined criteria, ensures the necessary robustness and consistency for this instrument.

Therefore, the objective of this study was to revalidate the positive serological panel for LSH syphilis, an instrument used to assess the sensitivity of kits for the diagnosis of syphilis.

METHOD

The serological panel is made up of 172 truly positive syphilis samples was made from plasma units from hemotherapy services in different regions of the country. Samples with a volume greater than 200 mL, received by LSH/INCQS between 1996 and 2006, were characterized strictly complying with current legislation with the use of treponemal tests that detect specific antibodies against *T. pallidum* such as: *Enzyme Linked Immunosorbent Assay* (ELISA), rapid immunochromatographic (RT) test, indirect immunofluorescence (IF) and non-treponemic tests such as *Venereal Diseases Research Laboratory* (VDRL) and *Rapid Plasm Reagin* (RPR)[9,13].

The procedure used for positive serological panel sample revalidation for syphilis consisted of a retrospective analysis of the laboratory results of LSH/INCQS obtained in the kits for diagnosis of syphilis, evaluated in the period from January 2011 to December 2015, whose analysis reports (AR) were satisfactory. The protocols for recording the results of all the panel's samples for each of the analyzed products were identified. For the analysis of the results, an Excel® table was prepared, containing the kit, the sample, and the analytical results identification in the different analyzed methodologies. The results corresponding to the ELISA methodology were recorded as a ratio, which corresponds to the ratio between the values obtained from the optical density (OD) of each sample and the cut-off value (CO) or reactivity threshold (OD/CO) for each ELISA test. Samples with ratio values equal to or greater than 1.0 were considered positive and less than 1.0, negative. For the VDRL and IF methodologies, the reaction intensity values of each sample were inserted in "+" crosses, ranging from 1+ to 4+, with 4+ being the maximum intensity response. The results of the samples compared to the RT and RPR methodologies were described as positive (POS) and negative (NEG).

For revalidation of each panel sample, the following criteria were adopted: minimum stock volume of 10 mL; reactivity in three ELISA tests, a VRDL test and/or an RPR test, an RT test, and an IFI confirmatory test. Therefore, samples that met the adopted criteria were considered positive. Samples with non-reactive results in all methodologies were considered negative. Samples not included in the previous parameters were considered indeterminate.

RESULTS

Out of the 172 samples identified in the positive syphilis panel, 19 (11%) were initially excluded because they had a volume of less than 10 mL, so 153 samples went on to the subsequent revalidation steps.

The survey of laboratory data referring to the analyzes carried out from January 2011 to December 2015 allowed the identification of 46 *in vitro* diagnostic products for syphilis analyzed by LSH/INCQS with satisfactory AR, distributed as follows: 13 kits in the year 2011, six kits in 2012, eight kits in 2013, 11 kits in 2014 and eight kits in 2015. It is worth mentioning that the number of kits received annually is related to the demand for products to obtain registration by Anvisa. The distribution of this total of kits by methodology analyzed was as follows: ELISA (12 products), VDRL (eight products), RPR (five products), RT (13 products), and IF (eight products) (Table 1).

From the selection of 46 satisfactory kits, 145 protocols for recording the results of all 153 samples belonging to the positive panel for syphilis were identified. Of the total of 145 work protocols analyzed, 31 (21%) involved the ELISA methodology, 29 (20%) from VDRL, 19 (13%) from RPR, 26 (18%) from RT, and 40 (28%) from IFs distributed between 2011 and 2015 (Table 2).

The results of the samples from the positive syphilis panel obtained in the 145 protocols analyzed in relation to the selected methodologies were inserted in an Excel® spreadsheet. According to the reactivity criteria established for the revalidation of the samples, it was observed that, among the 153 samples of the positive panel for syphilis, none presented a negative result in the analyzed methodologies. A total of 118 samples (77%) tested positive for three ELISA tests with a ratio ≥ 1, a VDRL test, an RPR test, and an IF test, fulfilling all reactivity criteria being revalidated as positive samples; 12 samples (8%) tested positive for three ELISA tests with a ratio ≥ 1, a VDRL test or an RPR test, an RT test, and an IF test, and were also validated as positive samples, totaling 130 samples (85%) with proven reactivity. Twenty-three samples (15%) showed negative results for two or more methodologies and were classified as indeterminate samples (Figure).

Among the 23 samples classified as indeterminate, three (13%) presented positive results for the three methodologies of treponemic tests, which detect specific antibodies against *T. pallidum* (ELISA, RT, IF), and negative or undetermined result for two methodologies of non-treponemal tests, those that detect antibodies against the lipid material released by cells damaged due to syphilis (VDRL, RPR); 13 showed a positive result for the IFI and a negative result for the ELISA and RT methodologies; five showed negative results for ELISA, RT, VDRL and/or RPR and two samples did not show results for IF (Table 3).

In summary, after the positive panel revalidation for syphilis composed of 153 samples, 130 (85%) were confirmed as positive, while 23 (15%) samples started to be classified as indeterminate, thus requiring new analyzes in the different methodologies.

Table 1. Distribution of the number of *in vitro* syphilis diagnostic products analyzed by year and by methodology (period 2011 - 2015).

Methodology	2011	2012	2013	2014	2015	Total
ELISA	5	1	3	1	2	12
VDRL	2	2	2	1	1	8
RPR	0	0	1	2	2	5
RT	5	2	1	4	1	13
IF	1	1	1	3	2	8
TOTAL	13	6	8	11	8	46

Source: Elaborated by the authors, 2020.

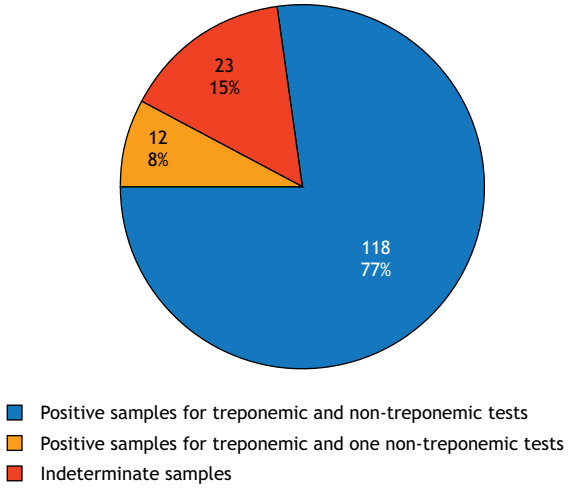
ELISA: immunoassay test; VDRL: *venereal disease research laboratory*; RPR: *rapid plasm reagin*; RT: immunochromatographic rapid test; IF: indirect immunofluorescence.

Table 2. Distribution of the number of results recording protocols per year and by methodology of *in vitro* syphilis diagnostic products analyzed.

Methodology	2011	2012	2013	2014	2015	Total
ELISA	12	2	6	2	9	31
VDRL	5	7	11	4	2	29
RPR	0	0	5	4	10	19
RT	8	4	3	9	2	26
IF	7	6	10	11	6	40
TOTAL	32	19	35	30	29	145

Source: Elaborated by the authors, 2020.

ELISA: immunoassay test; VDRL: *venereal disease research laboratory*; RPR: *rapid plasm reagin*; RT: immunochromatographic rapid test; IF: indirect immunofluorescence.



Source: Elaborated by the authors, 2020.

Figure. Distribution of sample results from the positive panel for revalidated syphilis.

DISCUSSION

The percentage of 11% (19/172) of the samples excluded due to the volume of less than 10 mL is due to the great demand of the laboratory to analyze the kits for diagnosing syphilis over these years, mainly in automated analyzes that require a larger sample volume for its realization.

It was possible to observe after all the revalidation process of the samples of the positive panel for LSH syphilis that none of the initial 153 samples was discarded because it was considered a negative sample and 23 samples started to be characterized as indeterminate samples.

Among the samples characterized as indeterminate, it is possible to suggest those with a positive result in all treponemic tests and a negative result for non-treponemic tests that demonstrate a serological response pattern characteristic of recent infection. In literature, this response pattern in primary syphilis is well described and ratified [5,6,14].

In the case of samples characterized as indeterminate, which showed a positive result for IFI, but negative for other treponemic tests and negative for one or more non-treponemic tests, it is possible to suggest that they have a serological response pattern characteristic of a serological scar. Serological scarring is considered to be the persistence, after two years of post-treatment follow-up, of the serological response to non-treponemal tests at low titers (up to 1:4) accompanied by positive treponemal tests[14]. In about 85% of patients with successful treatment, this profile of positive response to treponemic tests can be maintained for several years[15,16].

Table 3. Distribution of the results of the indeterminate samples in the revalidation of the positive panel for syphilis.

Methodology	Samples not revalidated
VDRL and RPR	3
ELISA and RT	13
ELISA, RT, VDRL and/or RPR	5
IF	2
Total	**23**

Source: Elaborated by the authors, 2020.
VDRL: *venereal disease research laboratory*; RPR: *rapid plasm reagin*; ELISA: immunoassay test; RT: immunochromatographic rapid test; IF: indirect immunofluorescence.

Every production process has very well-defined stages and, among them, is the validation stage prior to the product's implementation, as well as stages following this one for the control of the product's effectiveness and quality. The positive syphilis panel was constituted as a product to be evaluated through the revalidation stage, its relevance is based on these samples time of use, which is more than 10 years, considering that this panel was constituted by plasma samples received by LSH/INCQS between the years 1996 and 2006. Several studies on the evaluation of products for diagnosing syphilis have demonstrated the use of panels made up of samples from blood banks and the importance of a very well characterization through treponemic and non-treponemic tests of different methodologies[17,18].

CONCLUSIONS

After analyzing the 153 samples that constituted the positive serological panel for syphilis, compared to 46 products with satisfactory AR, from five different methodologies, it was possible to revalidate the panel, which now consists of 130 true positive samples.

The positive serological panel for revalidated syphilis will remain an essential instrument in the previous analysis for the purpose of regularizing the kits for the serological diagnosis of syphilis. It is a tool used in the analysis of products in order to guarantee reliable results and expand the analytical capacity of LSH.

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Author's Contributions

Passo DCD - Conception, planning (study design), acquisition, analysis, data interpretation, and writing of the work. Zamith HPS, Coelho MA - Conception, planning (study design), analysis, data interpretation, and writing of the work. Vigo DC, Ribeiro AS, Passo RM – Data acquisition. All authors approved the final version of the work.

Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.