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Revalidation of the serological panel report on the evaluation of Chagas disease diagnostic kits

Revalidação do painel sorológico empregado na avaliação dos kits de diagnóstico da doença de Chagas

Gabriella Pires da Silva Macedo*

Álvaro da Silva Ribeiro

Marisa Coelho Adati

Helena Cristina Balthazar Guedes Borges

Roberto Machado Passo

Valéria Furtado de Mendonça

Yasmin Rosa Ribeiro

José Roberto Niemeyer de Castro

Instituto Nacional de Controle de Qualidade em Saúde, Fundação Oswaldo Cruz (INCQS/Fiocruz), Rio de Janeiro, RJ, Brasil

* **E-mail:** gps.macedo@gmail.com

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ABSTRACT

Introduction: Acute phase of Chagas disease is characterised by the presence of blood parasites while in the chronic phase, parasite titres decrease and antibodies increase. According to RDC nº 36, of August 26, 2015, diagnostic tests for the disease belong to risk class IV, with mandatory registration with the National Health Surveillance Agency. The performance of these products is assessed in the laboratory analysis prior to registration, against serological panels composed of true positive and negative samples. **Objective:** Revalidate the serological panel composed of true positive samples for Chagas disease used in the analysis of in vitro diagnostic kits for the detection of specific antibodies against Trypanosoma cruzi. **Method:** Revalidation of the Chagas serological panel by retrospective analysis of results obtained in the methodologies: ELISA, Rapid Test, Immunofluorescence, Agglutination, Hemagglutination and Chemiluminescence, meeting the criteria of: positivity in 02 Rapid Tests; 03 Immunofluorescences; 01 Agglutination Test; 05 ELISAS, 02 Hemagglutination Tests, 03 Chemiluminescences and volume ≥ 10 mL. **Results:** 45 kits with a satisfactory report were selected, being 60.0% ELISA, 16.0% immunofluorescence, 11.0% chemiluminescence, 7.0% hemagglutination, 4.0% immunochromatographic test and 2.0% agglutination. 160 records were evaluated, 56.2% of which were destined for ELISA, 14.4% of chemiluminescence, 13.1% of immunoflurescence, 8.1% of hemagglutination, 5.6% of rapid tests and 2.5% of agglutination. A standardized spreadsheet was prepared to insert the data in Excel® and evaluate the samples against the methodologies. A total of 64 samples were revalidated. **Conclusions:** The revalidated Panel, composed of 64 samples, was characterized and its use guarantees reliable results, expanding the analytical capacity of the Laboratory of Blood and Blood Products in the quality control of diagnostic kits.

KEYWORDS: Chagas Disease; Revalidation; Serological Panel; *Trypanosoma cruzi*; Diagnosis

RESUMO

Introdução: A doença de Chagas apresenta infecção aguda com alta parasitemia e crônica com queda da parasitemia e aumento de anticorpos. Segundo a RDC nº 36, de 26 de agosto de 2015, os testes de diagnóstico da doença pertencem à classe de risco IV, com obrigatoriedade de registro junto a Agência Nacional de Vigilância Sanitária. O desempenho desses produtos é avaliado na análise laboratorial prévia ao registro, frente a painéis sorológicos compostos por amostras verdadeiro-positivas e negativas. **Objetivo:** Revalidar o painel sorológico composto de amostras verdadeiro-positivas para doença de Chagas utilizado na análise de *kits* de diagnóstico *in vitro* destinados à detecção de anticorpos específicos contra *Trypanosoma cruzi*. **Método:** Revalidação do painel sorológico de Chagas por análise retrospectiva de resultados obtidos nas metodologias: ELISA, teste imunocromatográfico, imunofluorescência, aglutinação, hemaglutinação e quimioluminescência, atendendo aos critérios de: positividade em dois testes rápidos; três imunofluorescências; um teste de aglutinação; cinco ELISA, dois testes de hemaglutinação; três de quimioluminescências e volume ≥ 10 mL. **Resultados:** Foram selecionados 45 *kits* com laudo satisfatório, sendo 60,0% ELISA, 16,0% imunofluorescência, 11,0% quimioluminescência, 7,0% hemaglutinação,

4,0% teste imunocromatográfico e 2,0% aglutinação. Foram avaliados 160 registros nos quais, 56,2% destinados a ELISA, 14,4% de quimioluminescência, 13,1% de imunoflurescência, 8,1% de hemaglutinação, 5,6% de testes rápidos e 2,5% de aglutinação. Foi elaborada uma planilha padronizada para inserção dos dados em Excel® e avaliação das amostras frente às metodologias. Um total de 64 amostras foi revalidado. **Conclusões:** O painel revalidado, composto por 64 amostras, foi caracterizado e seu uso garante resultados confiáveis, ampliando a capacidade analítica do Laboratório de Sangue e Hemoderivados no controle de qualidade de *kits* para diagnóstico.

PALAVRAS-CHAVE: Doença de Chagas; Revalidação; Painel Sorológico; *Trypanosoma cruzi*; Diagnóstico

INTRODUCTION

Chagas disease is currently classified by the World Health Organization (WHO) as neglected and endemic among poor populations in Africa, Asia, and Latin America.[1] This situation is related to the social and economic conditions of the countries affected by the disease, which contribute to the spread of neglected diseases with a major impact on Public Health.[2]

The transmission of the etiological agent to men can occur through the bite of a triatomine bug infected by *Trypanosoma cruzi*,[3] by blood transfusion,[4] and vertically.[5] Alternative routes are oral infection,[6] organ transplantation, and accidents.

The WHO estimates that there are 6 to 7 million people with the disease worldwide, and 21 Latin American countries are home to 5,742,167 people infected by *T. cruzi*.[7]

From 2000 to 2013, the Brazilian Ministry of Health reported 1,570 cases of the disease in its acute form, mainly in the North, with 91.1% of cases, followed by the Northeast, with 4.7%. It is important to note that the state of Pará accounted for 75% of all cases in the country.[8]

Laboratory tests for the diagnosis of the disease must take into account the stage of infection. The acute phase is characterized by high parasitemia, and parasitological exams like fresh examination for trypanosomatids, concentration method (Strout), and thick drop are recommended. However, in the chronic phase, there is low parasitemia and presence of specific antibodies (IgG). At this stage, serological diagnosis is made by searching for specific antibodies to the *T. cruzi* parasite antigen with enzyme linked immunosorbent assay (ELISA), immunochromatographic tests, agglutination, hemagglutination, chemiluminescence, and indirect immunofluorescence (IIF).[9]

According to article 12 of Federal Law n. 6.360, of September 23, 1976,[10] no local or imported product covered by this Law may be manufactured, marketed or delivered for consumption without authorization from the Ministry of Health.

In addition, Joint Board Resolution (RDC) n. 36, of August 26, 2015,[11] used as a reference in the evaluation of these products, provides for risk classification, registration, and authorization control, labeling and instructions for use of *in vitro* diagnostic products. According to RDC n. 36/2015,[11] products for *in vitro* diagnosis classified as high risk are subject to prior analysis as part of the marketing authorization process of Brazil's Health Surveillance Agency (Anvisa). This RDC defines prior analysis as "analysis to verify product characteristics for the purpose of marketing authorization, amendment (when applicable) or revalidation".

The Laboratory of Blood and Blood Products (LSH) of the National Institute for Quality Control in Health (INCQS) evaluates the sensitivity and specificity of tests for *in vitro* diagnosis (IVD) e for the purpose of marketing authorization at Anvisa. One of the evaluated parameters is the sensitivity of the products to a serological panel made up of true positive samples. Thus, the true positive serological panel for Chagas disease is an important tool to evaluate the sensitivity of these products.

After a period of 10 years of use, the revalidation of the panel became necessary and the reactivity of the samples was evaluated in light of new methodologies available in the domestic market. The objective of this project was to revalidate the serological panel composed of true positive samples for Chagas disease used in the analysis of *in vitro* diagnostic kits for the detection of specific antibodies against *T. cruzi*, currently used in clinical analysis laboratories and hemotherapy services.

METHOD

The positive serological panel for Chagas disease at the LSH consists of 76 plasma samples characterized as true positive. It was revalidated using the criterion of reactivity in the different methodologies applicable to the *in vitro* diagnosis of Chagas disease. The kits that received a satisfactory analysis report (LA) after evaluation at the INCQS, from January 2010 to December 2015, were selected and used as a parameter to evaluate the reactivity and behavior of the samples that make up the panel. Analytical protocols were identified with the recording of sample results in different methodologies, and an Excel® spreadsheet was created. The results corresponding to the ELISA and chemiluminescence methodology were recorded as a ratio between the optical density (OD) and the cut-off value (CO). For agglutination, hemagglutination and IIF, the intensity of the response of each sample was observed and is given by "+" crosses, ranging from 1+ to 4+, with 4+ as the maximum intensity response. The results of the samples in the rapid tests were described as reactive (R) and non-reactive (NR).

Based on the methodology used in the validation of the panel at the LSH in 2008,[12] which included positivity in three ELISA tests, in a hemagglutination test, in an agglutination test, in an

IIF test and in a Western Blot, the following criteria for sample revalidation were established: reactivity in at least five ELISA with a ratio above 1.5; reactivity in two rapid tests; reactivity in at least three chemiluminescence tests; positivity in at least three IIF tests, in one agglutination test, and in two hemagglutination tests. In addition to reactivity in the tests of choice, the samples should have a volume greater than or equal to 10 ml, since the loss of volume is common in panel samples over the years. This is due to the great demand of the laboratory, especially in automated analysis, where larger volumes are required. In addition, the received samples undergo a filtration process to reduce fibrin, which results in a decrease in their total volume and constant need for renewal.

RESULTS AND DISCUSSION

From January 2010 to December 2015, 54 kits for *in vitro* diagnosis of Chagas disease were received for analysis at the LSH of the INCQS. They were distributed as follows: nine kits (16.7%) in 2010, 15 (27.8%) in 2011, eight (14.8%) in 2012, one (1.8%) in 2013, 11 (20.4%) in 2014 and ten (18.5%) in 2015. The number of kits received annually is related to the demand for marketing authorizations from Anvisa, which, in 2013 was lower than in the other years. Of the total 54 analyzed products, 45 (83.0%) received a satisfactory report and nine (17.0%) were unsatisfactory. Thus, the number of kits used in the revalidation of the samples was 45 products, distributed in the following methodologies: 27 (60.0%) ELISA, three (7.0%) hemagglutination tests, five (11.0%) chemiluminescence tests, seven (16.0%) IIF, two (4.0%) immunochromatographic tests, and only one (2.0%) agglutination test (Figure).

The ELISA test was the most demanded serological method for analysis (60.0%). According to Fitarelli,[13] since 2000, ELISA has been the methodology of choice for the determination of Chagas disease in the diagnostic market, and this situation has remained constant since then.[13,14]

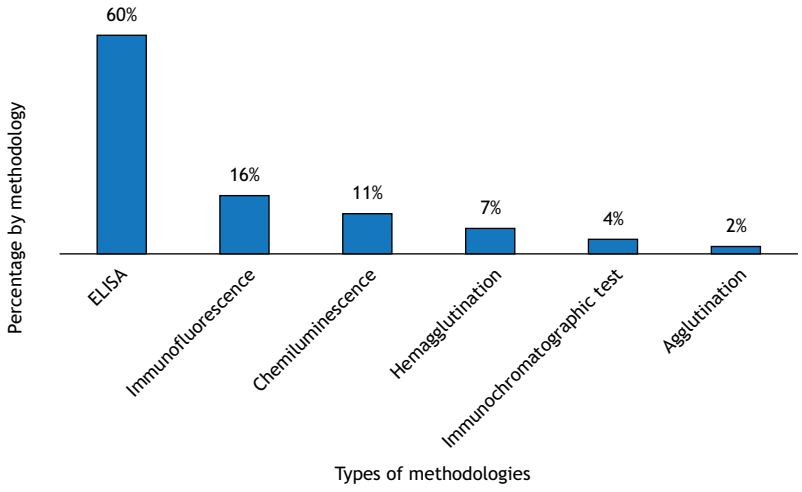
After the selection of 45 satisfactory kits, 160 analysis protocols were identified, of which 90 (56.25%) were destined for ELISA, 23 (14.4%) for chemiluminescence, 21 (13.1%) for IIF, 13 (8.1%) for hemagglutination, nine (5.6%) for rapid tests and four (2.5%) for agglutination.

After retrospective analysis of the data and preparation of an Excel® spreadsheet, the reactivity of the samples was evaluated in relation to the different methodologies for the diagnosis of Chagas disease. According to the established revalidation criteria, 100.0% of the samples were reactive in at least five ELISA tests, with values of the ratio greater than 1.5, as well as in the three IIF tests. As for the other methodologies, the panel's reactivity corresponded to 92.1% in the immunochromatographic test, 97.4% in the agglutination test, 93.4% for hemagglutination, and 98.7% for chemiluminescence.

A total of 12 (15.8%) samples have shown non-reactive results in one or more methodologies. Four samples were non-reactive in rapid tests, five were negative in the hemagglutination test, one in the agglutination test, one sample did not fulfill the immunochromatographic and agglutination test simultaneously, and another sample had non-reactive results for both the immunochromatographic test and the chemiluminescence test (Table).

The 12 samples that have shown discordant results in the rapid tests, hemagglutination, agglutination, and chemiluminescence tests were not revalidated and became part of a new indeterminate serological panel.

The six samples that failed the immunochromatographic test represented 43.0% of the discordant results, whereas five samples with negative results in hemagglutination were responsible for 36.0%. The revalidation criterion for agglutination represented 14.0% of the discordant results. In the chemiluminescence methodology, we found 7.0% of discordant results, from a sample with a negative result.



Source: LSH, 2018.

Figure. Distribution of the methodologies used in the revalidation of samples from the positive panel of Chagas disease.

Table. Quantitative of samples that did not meet the revalidation criteria compared to the established tests.

Tests	Not revalidated (%)
Immunochromatographic test	4 (5.3%)
Immunochromatographic test and agglutination	1 (1.3%)
Immunochromatographic test and chemiluminescence	1 (1.3%)
Hemagglutination	5 (6.6%)
Agglutination	1 (1.3%)

Source: LSH, 2018.

The loss of reactivity of the 12 samples when compared to the results of the panel validation[12] may be related to the drop in the antibody titer, the time of use and the detection limit of the kit. In addition, some samples have different levels of reactivity.

The freezing and thawing of the panel does not interfere in the reduction of the antibody titer. The University of São Paulo (USP) did an assessment of the stability of positive samples for the human immunodeficiency virus (HIV), which were subjected to ELISA, Western Blot and IIF assays. In that study, 11 cycles of freezing and thawing were performed and no effect on the reactivity of specific antibodies was observed.[15] This was also proven in the present study, as the samples were thawed at least 45 times during the revalidation process.

The last criterion established for revalidation is related to the volume of the samples, which should be at least 10 mL, according to the laboratory's activity protocol, in order to guarantee sufficient volume for the next LSH demands. Only one sample did not meet this criterion, with 9 mL. However, it had already been excluded by the criteria of positivity of the analyzed methodologies.

After the revalidation process, the panel then had 64 (84.0%) samples duly revalidated. A total of 16.0% of the samples (12/76) that did not meet the criteria for revalidation became a panel of indeterminate samples, which will be analyzed with other markers.

CONCLUSIONS

After the analysis of the 76 samples that formed the serological panel of Chagas disease, compared to the 45 kits with a satisfactory analysis report, with six different methodologies, it was possible to revalidate the panel, which now consists of 64 true positive samples.

The revalidated panel is intended for the prior analysis for marketing authorization purposes, a mandatory step for selling the kits for serological diagnosis of Chagas disease in the country. It is a tool used in product analysis to ensure reliable results and expand the analytical capacity of LSH.

It is worth mentioning that the kits that obtained unsatisfactory results were not used in this retrospective revalidation process, ensuring the reliability of the panel's results.

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Authors' Contribution

Macedo GPS, Ribeiro YR - Data acquisition, analysis and interpretation and review of the manuscript. Adati MC - Conception and planning (study design). Borges HCBG, Ribeiro AS, Passo RM, Mendonça VF, Castro JRN - Writing of the manuscript. All authors approved the final draft of the manuscript.

Conflict of Interest

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