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Revalidation of the positive serological panel intended for the quality control of kits for HIV serological diagnosis

Revalidação do painel sorológico destinado a avaliação de kits para o diagnóstico sorológico do HIV

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ABSTRACT

Introduction: Kits used in the diagnosis of the human immunodeficiency virus (HIV) must meet the requirements of RDC No. 36, of August 26, 2015 for registration with the National Health Surveillance Agency and Law No. 6.360, of September 23 1976 for commercialization in the country. One of the registration steps corresponds to the previous laboratory analysis of the products with the highest risk class (class IV), carried out by the Laboratory of Blood and Blood Products (LSH). In the analysis of the products, serological panels consisting of true positive samples are used as the main tool in the sensitivity assessment. **Objective:** To revalidate a true HIV positive serological panel for the evaluation of in vitro HIV diagnostic kits. **Method:** A retrospective evaluation and selection of the panel results was performed against the kits that obtained satisfactory results and were received for prior analysis from January 2010 to December 2011. The reactivity of the panel samples in three immunoenzymatic assays (ELISA), in three chemiluminescence assays (CLIA), in three immunochromatographic assays (rapid tests) and in three western blots was used as revalidation criterion; and reactivity in an enzyme-linked fluorescent enzyme assay (ELFA), in addition to a volume equal to or greater than 1 mL. **Results:** During the period, 73 kits for in vitro diagnosis of HIV infection were received for analysis at the LSH, 47 (64.4%) of which were satisfactory, distributed as follows: 43.0% (20/47) ELISA, 34.0% (16/47) immunochromatographic assays, 13.0% (06/47) western blot, 2.0% (01/47) ELFA, 8.0% (04/47) chemiluminescence assays. After the evaluation, 77.0% (34/44) of the units were revalidated, and 23.0% (10/44) were excluded from the panel, as they did not meet the established criteria. **Conclusions:** The revalidated panel currently consists of 34 units of true positive samples, with consistent results, thus increasing the reliability and safety of the analyses carried out and of the tests marketed in the country.

KEYWORDS: HIV; Serological Panel; AIDS

RESUMO

Introdução: Os *kits* empregados no diagnóstico do vírus da imunodeficiência humana (HIV) devem cumprir requisitos da RDC n° 36, de 26 de agosto de 2015, para registro junto à Agência Nacional de Vigilância Sanitária e da Lei n° 6.360, de 23 de setembro de 1976, para comercialização no país. Uma das etapas do registro é a análise prévia laboratorial dos produtos de maior classe de risco (classe IV), realizada pelo Laboratório de Sangue e Hemoderivados (LSH). Na análise dos produtos são utilizados painéis sorológicos constituídos de amostras verdadeiro positivas como principal ferramenta na avaliação de sensibilidade. **Objetivo:** Revalidar painel sorológico verdadeiro positivo para HIV, destinado à avaliação de *kits* de diagnóstico *in vitro* do HIV. **Método:** Foram realizadas a avaliação retrospectiva e a seleção dos resultados do painel frente aos *kits* recebidos para análise prévia de janeiro de 2010 a dezembro de 2011 que obtiveram resultado satisfatório. Foi utilizado como critério de revalidação a reatividade das amostras do

painel em três ensaios imunoenzimáticos (ELISA), em três ensaios de quimiluminescência (CLIA), em três ensaios imunocromatográficos (testes rápidos) e em três *western blot*; e reatividade em um ensaio enzimático fluorescente ligado à enzima (ELFA), além do volume igual ou superior a 1 mL. **Resultados:** No período foram recebidos para análise no LSH 73 *kits* para diagnóstico *in vitro* da infecção pelo HIV, sendo 47 (64,4%) satisfatórios, assim distribuídos: 43,0% (20/47) ELISA, 34,0% (16/47) ensaios imunocromatográficos, 13,0% (06/47) *western blot*, 2,0% (01/47) ELFA e 8,0% (04/47) ensaios de quimiluminescência. Após a avaliação, 77,0% (34/44) das unidades foram revalidadas, sendo excluídas do painel 23,0% (10/44), pois não alcançaram os critérios estabelecidos. **Conclusões:** O painel revalidado atualmente é composto por 34 unidades de amostras verdadeiro positivas, com resultados consistentes, aumentando, assim, a confiabilidade e a segurança das análises realizadas e dos testes comercializados no país.

PALAVRAS-CHAVE: HIV; Painel Sorológico; AIDS

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS), identified in 1981, became a milestone in history[1]. In search of answers about the disease, a race against time began, which continues to this day, in the search for a cure for this syndrome. In October 1983, the Pasteur Institute carried out the first isolation of the human immunodeficiency virus (HIV) from the lymph nodes of patients with lymphadenopathy, characteristic of an early stage of AIDS[2].

HIV transmission can occur mainly through sexual intercourse, blood contamination or vertical transmission[3]. HIV infection can be characterized in three phases: acute phase (in the first weeks of infection, in which there is intense viral replication), persistent phase (characterized by the maintenance of TCD4+ levels and low plasma HIV concentration) and AIDS (when there is a significant reduction in TCD4+ cells, high plasma HIV levels and the appearance of characteristic clinical manifestations and opportunistic infections)[4]. There are two retroviruses capable of causing AIDS, HIV-1 and HIV-2, with type 2 being found in individuals who have had contact with people from the African continent[5].

In Brazil, the diagnosis of the disease in individuals over the age of two is based on the detection of antibodies, in accordance with Minister's Office/Ministry of Health (GM/MS) Ordinance No. 59 of January 28, 2003. To identify HIV infection in children under the age of two, tests are used to quantify the HIV-1 viral load, due to the passive transfer of antibodies from mother to baby, which can lead to false positive results in antibody detection tests[6].

Tests to detect HIV antibodies can be classified as screening or confirmatory. Screening tests are the first tests carried out to identify possible HIV-infected individuals, which are: the enzyme-linked immunosorbent assay (ELISA), the enzyme-linked fluorescent assay (ELFA), the chemiluminescence assay (CLIA) and the immunochromatography (rapid test), as they have a high degree of sensitivity. The confirmatory tests are tests or sets of tests that can define the diagnosis of a blood unit after an initial reactive result. These tests have a high degree of specificity, i.e. they correspond to the percentage of negative results obtained when there is no certain marker in the sample, these are: the *western blot*, the indirect immunofluorescence reaction (IFI) and the detection of the HIV virus genome[6].

According to Collegiate Board Resolution (RDC) No. 36, of August 26, 2015, diagnostic products for *in vitro* use are classified by epidemiological relevance and for regularization purposes with the National Health Surveillance Agency (Anvisa) in four risk classes (I, II, II and IV). HIV diagnostic kits belong to the highest risk class (IV) and, according to current legislation, must be registered. According to Law No. 6,360 of September 23, 1976, only products registered with Anvisa can be marketed. One of the stages of registration is the prior laboratory analysis to verify the product's performance, in which the parameters of sensitivity and specificity must be met[7,8].

Since 1995, prior evaluation of these products has been carried out by the Blood and Blood Products Laboratory (LSH) belonging to the National Institute for Quality Control in Health (INCQS) of the Oswaldo Cruz Foundation (Fiocruz)[8].

Serological panels made up of true positive samples are used in the preliminary analysis of products intended for HIV diagnosis as the main tool for assessing clinical or diagnostic sensitivity, in addition to the use of international standards and commercially available international panels. The panel is a set of plasma units considered unsuitable for therapeutic use, from hemotherapy services in different regions of the country. Plasma units obtained from the fractionation of whole blood from donors who showed HIV reactivity and met the LSH validation criteria, carried out from 1996 onwards, form the true positive serological panel (PSVP) for HIV, currently made up of 44 samples.

Thus, the aim of this study was to revalidate the HIV panel, which is extremely important for maintaining the reliability and safety of the tests used in serological diagnosis, since new kits are made available every year on the national and international market, in order to meet the population's demand for increasingly sensitive tests[9].

METHOD

In order to revalidate the HIV-positive serological panel, the results of the units that make up the HIV panel used to assess the quality of the diagnostic kits for this pathology were evaluated and sent to LSH for prior analysis in compliance with RDC No. 36/2015 for registration with Anvisa.

This study only considered kits that obtained a satisfactory report for the parameters of sensitivity (100.0%) and specificity (≥ 99.5%) between January 2010 and December 2011; these parameters were adopted from Anvisa. A survey was carried out of the results of the samples that make up the serological panel using ELISA, immunochromatographic assay, western blot, ELFA and CLIA methodologies, carried out in the laboratory during the selected period. The data obtained was compiled in an Excel® spreadsheet. In the ELISA, ELFA and CLIA test spreadsheets, the ratio values were added, which are obtained by calculating the optical density (OD) and cut-off point (CO) - (OD/CO) of the rapid tests and in the *western blot* test, the reactivity (R) or non-reactivity (NR) presented in the test was added.

For the revalidation of the HIV panel, the criteria established were that the samples were positive in three ELISA tests, three immunochromatographic assays, three western blot tests, one ELFA test and three CLIA tests, in addition to a volume equal to or greater than 1 mL of the samples in stock or in the laboratory routine. The revalidation criteria established were based on the validation criteria carried out when the panel was created, in order to guarantee reliable results[10].

In the ELISA and CLIA methodologies, only samples with a (DO/CO) ratio ≥ 1.5 were considered positive, in order to ensure greater reactivity and more uniform results[10].

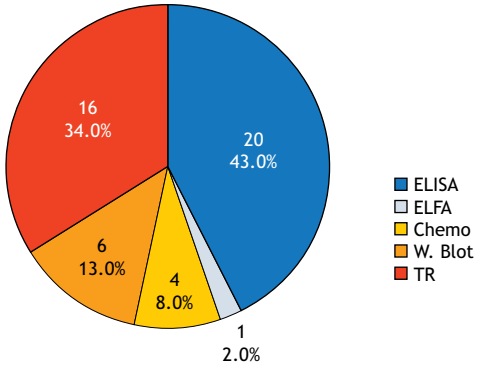
Only the sample units that met all the previously established criteria were revalidated. Samples that did not fully meet the criteria were excluded from the HIV panel and segregated, becoming part of a panel of undetermined samples.

RESULTS AND DISCUSSION

From January 2010 to December 2011, 73 HIV *in vitro* diagnostic kits were submitted for prior analysis at INCQS as a requirement for product registration with Anvisa, in the ELISA, rapid test, western *blot*, ELFA and CLIA methodologies. After laboratory evaluation, 47 (64.0%) products were considered satisfactory in terms of sensitivity (100.0%) and specificity (> 99.5%) and 26 (36.0%) were unsatisfactory. With regard to the methodologies evaluated, the satisfactory tests corresponded to 20 (43.0%) ELISA tests, 16 (34.0%) rapid tests, six (13.0%) *western blot*, one (2.0%) ELFA, and four (8.0%) CLIA (Figure 1).

The evaluations were carried out in order to guarantee the performance of the products available on the national market. The kits with unsatisfactory reports were not registered with Anvisa and were therefore not included in the study. In Brazil, the use of unregistered *in vitro* diagnostic products is not only illegal, but can also have an impact on the quality and reliability of the results, which cannot happen when it comes to the health safety of the user[10].

A retrospective evaluation of the results, with the aim of revalidating the HIV serological panel, initially made up of 44 true positive samples, was carried out on the 47 kits that were evaluated and obtained satisfactory reports. The values of the ratio



Source: Blood and Blood Products Laboratory, 2020.

Figure 1. Distribution by methodology of the number of kits for the HIV diagnosis with a satisfactory report.

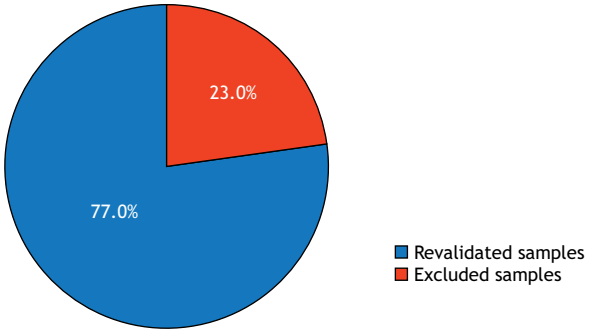
(OD/CO) of the samples in the ELISA, ELFA and CLIA tests and the reactivity or not in the immunochromatographic and *western blot* tests were compiled and compared in an Excel® spreadsheet. Based on the revalidation criteria established, we found that eight (18.0%) did not meet one or more of the criteria. A total of seven one (12,0%) was non-reactive in the ELFA test, and two (25.0%) were negative in the western blot. All the samples analyzed were positive in the three immunochromatographic tests and showed ratio values (OD/CO) greater than 1.5 in the three ELISA tests evaluated.

It should be emphasized that HIV-positive panel samples have been used in the laboratory routine for more than 20 years, and the long period of use could explain the loss or decrease in reactivity, especially in units with low antibody titers, thus making it difficult to identify them[10].

Another hypothesis evaluated in relation to reactivity was the serum freezing and thawing process, however, according to a study by Castejón et al.[11] , the impact of multiple freezing and thawing cycles of serum samples stored at -20°C has no significant effect on the reactivity of specific antibodies, thus not interfering with their reactivity[11].

For revalidation, in addition to meeting the criteria related to the methodology, the samples had to have a minimum volume of 1 mL in stock or in use at LSH[10]. We found that 26 (59.0%) samples had a volume of 5 mL or less. A total of 16 (36.0%) had a volume greater than 5 mL and 5.0% (2/44) were not available in stock. Therefore, two sample units were excluded because they did not have sufficient volume for use.

Of the total of positive samples from the HIV panel, initially composed of 44 validated samples, eight (18.0%) did not show reactivity in three (7.0%) CLIA tests, one (2.0%) in ELFA and two (4.0%) in the western blot and two (4%) did not have sufficient volume in stock. After excluding samples with discordant results or insufficient volume, 34 (77.0%) were revalidated and ten (23.0%) were excluded because they did not meet the criteria established for revalidation in terms of methodology and volume (Figure 2).



Source: Blood and Blood Products Laboratory, 2020.

Figure 2. Number of samples revalidated and excluded from the HIV panel from the Blood and Blood Products Laboratory.

CONCLUSIONS

The study evaluated the LSH HIV serological panel, made up of 44 sample units, against 47 kits from different methodologies and manufacturers received at INCQS between January 2010 and December 2011, with the aim of revalidating the panel.

The human plasma used to make the serological panel has a limited and exhaustible volume, so there is a great need to insert new samples into the panel, and the process is dynamic and random. As new plasmas meet the required specifications, they will be characterized and injected into the HIV-positive panel.

The study showed that a large part of the positive panel has a low volume, with 26 (59.0%) samples with a volume ≤ 5 mL, 16 (36.0%) samples with a volume > 5 mL and two (5.0%) with no volume, thus confirming the need to constantly receive plasmas for registration, characterization and consequent expansion of the total number of true positive samples for HIV.

The true-positive HIV panel, initially made up of 44 samples, now consists of 34 units after revalidation. A total of ten units did not meet the revalidation criteria and were reallocated to the panel of undetermined samples.

The revalidation of the HIV panel is extremely important, as it guarantees the reliability and safety of the sample results compared to the kits used in the *in vitro* diagnosis of HIV and, consequently, the safety of the products sold *in* the country.

REFERENCES

1. Goldani LZ. Descoberta do HIV: o reconhecimento. Rev HCPA. 2008;28:205-6.
2. Brito AM, Castilho EA, Szwarcwald CL. AIDS e infecção pelo HIV no Brasil: uma epidemia multifacetada. Rev Soc Bras Med Trop. 2001;34(2):207-17. https://doi.org/10.1590/S0037-86822001000200010
3. Santos NSO, Romanos MTV, Wigg MD. Virologia humana. 3a ed. Rio de Janeiro: Guanabara Koogan; 2015.
4. Lopes AC. Tratado de clínica médica. 3a ed. São Paulo: Roca; 2006.
5. Ministério da Saúde (BR). Recomendações para terapia antiretroviral em adultos e adolescentes infectados pelo HIV 2007/2008: documento preliminar. Brasília: Ministério da Saúde; 2008[acesso 30 ago 2018]. Disponível em: www.aids.gov.br
6. Ministério da Saúde (BR). Portaria Nº 59, de 28 de janeiro de 2003. A sub-rede de laboratórios do programa nacional de DST e AIDS, no que concerne ao diagnóstico laboratorial da infecção pelo HIV, será composta por todos os laboratórios, públicos e conveniados ao SUS, que realizam testes sorológicos para a detecção de anticorpos anti-HIV e de antígenos do HIV, organizados hierarquicamente, de acordo com a esfera de gestão do SUS à qual pertencem. Diário Oficia União. 29 jan 2003.
7. Agência Nacional de Vigilância Sanitária - Anvisa. Resolução RDC Nº 36, de 26 de agosto de 2015. Dispõe sobre a classificação de risco, os regimes de controle de cadastro e registro e os requisitos de rotulagem e instruções de uso de produtos para diagnóstico in vitro, inclusive seus instrumentos e dá outras providências. Diário Oficial União. 27 ago 2015.
8. Brasil. Lei N° 6.360, de 23 de setembro de 1976. Dispõe sobre a vigilância sanitária a que ficam sujeitos os medicamentos, as drogas, os insumos farmacêuticos e correlatos, cosméticos saneantes e outros produtos, e dá outras providências. Diário Oficial União. 24 set 1976.
9. Chiattone CS, Pereira JPM, Langhi Junior DM, Rugani MA, Souza CA, Saraiva JCP et al. Urgência na introdução do NAT: é fundamental não cometer os erros do passado. Rev Bras Hematol Hemoter. 2008;31(2):113-4. https://doi.org/10.1590/S1516-84842009000200015
10. Ribeiro AS. Confecção de painel sorológico para controle da qualidade de conjuntos de diagnósticos para detecção do anti-HIV [monografia]. Rio de Janeiro: Fundação Oswaldo Cruz; 2006.
11. Castejón MJ, Yamashiro R, Oliveira CC, Olivieri JC, Oliveira CAF, Ueda M. Avaliação dos múltiplos ciclos de congelamento e descongelamento na estabilidade dos soros para a detecção de anticorpos anti-HIV. Rev Inst Adolfo Lutz. 2012;71(3)573-81.

Authors' Contribution

Ribeiro YR, Macedo GPS - Acquisition, analysis, data interpretation, and writing of the work. Borges HCBG - Conception, planning (study design). Vigo DC, Passo DCD, Silva MM, Castro JRN - Analysis and interpretation of results. All the authors approved the final version of the work.

Conflict of Interest

The authors inform that there is no potential conflict of interest with peers and institutions, political or financial, in this study.

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