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Evaluation of rapid diagnostic tests for dengue in Brazil

Avaliação dos testes rápidos para diagnóstico da dengue no Brasil

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ABSTRACT

Introduction: The increasing number of dengue cases worldwide has stimulated the interest to develop products for the diagnosis of this disease in national and international markets. **Objective:** To evaluate the sensitivity and diagnostic specificity of immunochromatographic Rapid Tests (RT) for the detection of NS1 antigen (Ag), antibodies (Ab) IgG and IgM of dengue virus (DENV), and for the detection of combined AgNS1/AbIgG/IgM received from May 2016 to December 2018 at the National Institute for Quality Control in Health from Oswaldo Cruz Foundation for previous analysis and registration by the National Health Surveillance Agency (Anvisa) from Brazil. **Method:** The evaluation was performed using true positive and true negative samples for Ag NS1, Ab IgM and IgG to DENV, commercial performance panels and international standard of the National Institute for Biological Standards and Control/ World Health Organization (NIBSC/WHO). RT that presented sensitivity and/or specificity values higher than or equal to those stated by the manufacturers were considered satisfactory and those with lower values were unsatisfactory. **Results:** A total of 32 RT were evaluated, 23 (71.9%) were satisfactory for sensitivity and specificity, 9 (39.1%) for NS1, 11 (47.8%) for IgG/IgM and 3 (13.0%) for the combined detection NS1/IgG/IgM. From 9 RT considered unsatisfactory, 4 (44.4%) were for the detection of NS1; 2 (22.2%) for IgG/IgM and 3 (33.3%) for NS1/IgG/IgM. Unsatisfactory RT were not registered by Anvisa in Brazil. **Conclusions:** The previous analysis as foreseen in Brazilian regulation is important for the maintenance of RT quality offered to the national market.

KEYWORDS: Quality Control; Rapid Tests; Diagnostic; Dengue

RESUMO

Introdução: O aumento do número de casos de dengue no mundo estimulou o desenvolvimento e a disponibilização no mercado nacional e internacional de testes de execução rápida e simples para o diagnóstico da doença. **Objetivo:** Avaliar a sensibilidade e especificidade diagnóstica de Testes Rápidos (TR) imunocromatográficos para detecção de antígeno (Ag) NS1 e de anticorpos (Ac) das classes G (IgG) e M (IgM) e detecção combinada de Ag e Ac (NS1/ IgG/IgM) do vírus da dengue (DENV), encaminhados para análise prévia no INCQS/Fiocruz, no período de maio de 2016 a dezembro de 2018 para obtenção de registro junto à Anvisa do Brasil. **Método:** A sensibilidade e a especificidade foram avaliadas frente a painéis de amostras verdadeiramente positivas e verdadeiramente negativas para Ag NS1, Ac IgM e IgG do DENV, painéis de desempenho e padrão internacional do *National Institute for Biological Standards and Control*/Organização Mundial da Saúde (NIBSC/OMS). Os TR que apresentaram valores de sensibilidade e especificidade superiores ou iguais aos declarados pelos fabricantes foram considerados satisfatórios e os com valores inferiores, insatisfatórios. **Resultados:** Do total de 32 TR avaliados, 23 (71,9%) foram satisfatórios para sensibilidade e especificidade, destes, nove (39,1%), para NS1, 11 (47,8%) para IgG/IgM e três (13,0%), para os testes combinados NS1/IgG/IgM. Dos nove TR insatisfatórios, quatro (44,4%) foram para detecção de NS1; dois (22,2%), para IgG/IgM e três (33,3%), para NS1/IgG/IgM. Os TR considerados insatisfatórios não foram registrados no Brasil. **Conclusões:** A análise prévia como prevista na legislação brasileira é de grande importância para a manutenção da qualidade dos TR ofertados ao mercado nacional.

PALAVRAS-CHAVE: Controle de Qualidade; Testes Rápidos; Diagnóstico; Dengue

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INTRODUCTION

Dengue is a major public health concern in all tropical and subtropical regions of the world. The disease is endemic in more than 125 countries and has expanded globally driven by climate change, increased circulation of people, and urbanization, coupled with the lack of vector control programs[1,2,3].

In Brazil, according to data from the Ministry of Health, there were 873,093 probable cases of the disease from 12/29/2019 to 6/27/2020, which are transmitted through the bite of the infected *Aedes* mosquito[4]. *Aedes aegypti* is the main vector, with wide distribution in intertropical regions in the world and Brazil[5,6]. Other forms of disease transmission have been described and include tissue and organ transplantation, blood transfusion, and breast milk[7,8]. The dengue virus (DENV) belongs to the genus *Flavivirus* and to the family Flaviviridae and has four distinct serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) that can circulate concurrently in regions where the disease is hyperendemic[9]. Infection in humans by a serotype confers permanent immunity against subsequent reinfections by the same serotype but only partial and temporary protection against the other serotypes[10,11].

The disease has a spectrum that varies from asymptomatic to the occurrence of severe hemorrhage and shock, which can progress to death. Treatment is based mainly on symptoms, as there is no specific antiviral medication, and is therefore limited to the use of analgesics and/or fluid replacement[12,13].

The diagnosis, when based exclusively on clinical symptoms, can be compromised by the presence of subclinical or asymptomatic infections, which vary from 0.7% to 87% depending on the population studied[14,15,16]. In addition, dengue can be confused with other diseases, both in the febrile phase and in the critical phase, among them, febrile diseases accompanied or not by rash, such as those caused by the Epstein-Barr virus, herpes virus type 6, parvovirus B19, rubella, measles, and bacterial infections[17] or by seasonal diseases similar to dengue, such as leptospirosis[18]. There is a description of phylogenetic cross-reactions in serological tests, especially in endemic areas where it can be confused with malaria, yellow fever, mayaro, Oropouche fever, zika, and chikungunya[19,20,21].

The diagnosis of DENV infection involves one or more methodologies that may include viral isolation, molecular, and serological tests. These allow the detection of viruses, viral RNA, NS1 viral antigen (Ag), and antibodies (Ab) to DENV, immunoglobulins (Ig) of classes G (IgG) and M (IgM), each with applicability associated with different stages of the disease[22,23].

Currently, tests for the detection of Ag NS1 are widely used in the initial phase of the disease, which occurs before the appearance of specific Ab. The NS1 protein is present in the serum of infected individuals from the first day of the onset of symptoms and remains detectable until the fifth or sixth day of the evolution of the infection[24,25,26].

A small percentage of individuals have detectable levels of IgM on the first day of the disease (8%), which increase rapidly, reaching their peak around two weeks, remaining detectable for two to three months, which makes these Ab indicators of recent infection. In the secondary response, IgM levels are considerably lower than in the primary response[27].

In primary infection, IgG Ab begins to appear a few days after IgM Ab, being detectable from the fifth day of infection. IgG titers increase slowly from the first week of infection and remain detectable for life. Individuals with immunity prior to DENV or even another *Flavivirus* develop a secondary response characterized by a rapid increase in IgG titer almost immediately after the onset of symptoms[19,28].

The detection of IgA Ab present in the saliva of individuals with DENV infection has already been evaluated, however, they are not markers frequently used in the diagnosis of the disease. The combined diagnostic tests that detect Ag and Ab, (NS1/IgG/IgM and IgA) facilitate the diagnosis of individuals with DENV infection in any period of the clinical course of the disease[28,29].

With the growth in demand for more sensitive and specific tests for early detection, diagnosis, and monitoring of the clinical status of dengue, a wide variety of tests for in vitro diagnostic are sold and used on the national and international market[30,23]. Among the diagnostic methods offered, the use of lateral flow immunochromatographic assays known as rapid tests (RT) is worth mentioning[31]. These tests are widely used in the detection of Ag NS1 and IgM and IgG Ab in many public and private health services. They are characterized by being simple to perform and require, in the great majority, 15 to 20 min to obtain the result. They have a low comparative cost when used in large populations, in addition to being convenient for distribution in the most distant places from the main health centers. However, the sensitivity and specificity of RT can vary considerably and depend on the stage and type of infection (primary or secondary) and the infecting serotype[32,33].

Performance evaluations of these products have shown sensitivity and specificity values that differ from those declared by the manufacturers[5,34], which, in some cases, are carried out against inappropriate reference standards or reduced sample quantity[36,36]. This fact implies the possibility of the occurrence of false-negative (FN) results. In samples from asymptomatic or affected individuals with the most severe form of the disease, FN results can lead to the absence of adequate treatment and health risk. On the other hand, false-positive (FP) results imply incorrect treatment, possibly due to infection by another *Flavivirus*[37]. Sensitive and specific diagnostic tests are necessary for infected individuals to receive appropriate care and for epidemiological data to be reliable in the efficient implementation of public health policies[38].

Registration of diagnostic kits in Brazil

The registration of diagnostic kits in Brazil with the Ministry of Health is a legal act that recognizes the adequacy of a product to

the health legislation. Trade in these products is subject to registration with the Brazilian National Health Surveillance Agency (Anvisa), and is regulated by Law No. 5,991, of December 17, 1973[39], and its Decree No. 74,170, of June 10, 1974[40]. Currently, the Resolution of the Collegiate Board (RDC) No. 36, of August 26, 2015[41], which establishes the risk classification, the registration, and registration control regimes, in addition to labeling requirements and instructions for use of diagnostic kits produced in the country and those imported. The diagnostic kits belonging to risk class I (low risk to the individual and public health) are subject to notification and class II (medium risk to the individual and low risk to public health) to be registered. The dengue diagnostic kits (class III) and those for the detection of pathologies transmitted by transfused blood (sexually transmitted diseases by the human immunodeficiency virus (HIV) and by the human T-cell lymphotropic virus (HTLV), Chagas disease, syphilis, and hepatitis B and C belonging to risk class IV are subject to registration with Anvisa[41,42].

In the application for the granting of registration, information related to manufacturing, composition, performance, functionality, sensitivity, and clinical or diagnostic specificity is evaluated, in addition to compliance with the regulatory requirements of RDC No. 36/2015, in order to minimize any risks associated with products[41].

In this context, the National Institute for Quality Control in Health (INCQS), belonging to the Oswaldo Cruz Foundation (Fiocruz) and technically subordinated to Anvisa, acts as a reference for scientific and technological issues related to the quality control of products, environments, and services linked to health surveillance. INCQS routinely assesses the diagnostic kits belonging to risk class IV used to detect pathologies transmitted by transfused blood, in the different available methodologies, with a view to registering with Anvisa as provided for in RDC No. 36/2015[41].

From May 2016, as part of the process of obtaining or revalidating the registration, products intended for the diagnosis of dengue started to be subjected to prior analysis by INCQS, as provided in item IV of Art. 16 of Law 6,360, of September 23, 1976[43], and in item VII of Art. 19 of RDC No. 36/2015[41]. The approval of the registration petition depends on the satisfactory analysis report of the INCQS and on the fulfillment of the requirements determined in the current legislation.

The objective of this study was to evaluate the sensitivity and clinical or diagnostic specificity of the immunochromatographic RT used in the serological diagnosis of dengue, as part of the process of obtaining registration with Anvisa. The results obtained were compared to those declared in the instructions for use of the products by the manufacturers.

METHOD

In compliance with the requirement to carry out prior product analysis issued by Anvisa, from May 2016 to December 2018, 500 units of each product (RT) were sent to INCQS by the respective applicants (manufacturers, importers/distributors) accompanied by the production dossier and quality control, in addition to the batch release certificate. Thirty-two RT from different manufacturers were evaluated for a total of 16,000 analyzes. In order to eliminate the risks to guaranteeing the confidentiality of information and results and to the impartiality of the processes that culminate in the institutional conclusions, all information regarding the RT such as trade name, manufacturer, distributor/importer, origin, as well as individual sensitivity values and diagnostic specificity contained in the packaging and instructions for use of the products, have been omitted.

Serological panels

The sensitivity of RT was evaluated against serological panels made up of true positive (TP) serum/plasma samples for Ag NS1 (n = 100), for IgM Ab (n = 100) and for IgG Ab (n = 100). The samples were obtained during dengue epidemic outbreaks in the state of Rio de Janeiro (Brazil), from March 2010 to May 2013 and characterized by two or more methodologies. In the detection of Ag NS1, IgM, and IgG Ab the enzyme linked immunosorbent assay (ELISA) and RT were used. The reverse transcription polymerase chain reaction (RT-PCR) was used to determine the serotype of samples that had a detectable viral load. Specificity was determined against a serological panel consisting of 200 true negative (TN) serum/plasma samples collected in non-endemic dengue regions (states in the southern region of the country), in the period from 2013 to 2014, characterized by two or more methodologies (ELISA, RT, and RT-PCR). In addition, were included in the evaluation, serological performance panels (PVD 201- *Anti-Dengue Mixed Titer Performance Panel* (n = 21) and 0845-0051 *Anti-Dengue Mixed Titer Accuset Performance Panel Modified* (n = 16), *Sera Care Life Science*®) and the international standard of the World Health Organization (WHO), *Anti-Dengue Virus Types* 1+2+3+4 (*Reference Reagent* 02/186)[44].

Evaluation of the sensitivity and specificity of the rapid tests

The RT were evaluated strictly following the procedure described in the instructions for use for each product. In RTs that included the use of whole blood samples, a minimum number of 25 positive whole blood samples (spikes) and 25 negative whole blood samples were evaluated. To make the spikes, venous blood samples collected with anticoagulant were centrifuged for 10 min / 4,200 g, the plasma was removed and the red blood cell concentrate obtained diluted 1: 2 in plasma/TP serum for the evaluated dengue serological markers (NS1, IgM and IgG). An approximate amount of 50 tests per batch was reserved for repetition in case of conflicting results. Two specific interfering reagent samples for each of the following etiologic agents were included in the specificity assessment: HIV-1/2, hepatitis C virus (HCV), hepatitis B virus (HBV), and HTLV-1/2.

The test results were interpreted according to the manufacturers' instructions and the percentage values of sensitivity and specificity were calculated[45]. The sensitivity value of each product was obtained based on the number of TP samples analyzed and correctly identified by the evaluated test, by the ratio of TP added to the number of FN samples. Specificity was determined

by the number of correctly identified TN samples, by the ratio of the TN added to the number of FP samples for DENV infection. The values of sensitivity and diagnostic specificity obtained were compared with those declared in the instructions for use of the evaluated RT. The RT that obtained values of sensitivity and specificity equal to or higher than those declared were considered satisfactory. RTs with sensitivity and/or specificity values lower than those declared by the manufacturer were considered unsatisfactory. After the analysis was completed, as part of the product registration process, the analysis reports were forwarded to Anvisa, which is responsible for completing the processes and granting the records.

This study was authorized by the Human Research Ethics Committee (CEP) of the Oswaldo Cruz Institute (IOC)/Fiocruz (CAAE: 55365316.7.0000.5248) under opinion No. 1590251.

RESULTS

Of the total of 32 RT sent for prior analysis at INCQS, 31.3% (10/32) was received in 2016, 53.1% (17/32) in 2017, and 15.6% (5/32) in 2018. As for the origin of the products received, 65.6% (21/32) were national and 34.4% (11/32) imported from Asia and Europe.

A total of 40.6% (13/32) corresponded to the RT for the detection of Ag NS1, 53.8% (7/13) of national origin, and 46.2% (6/13), imported. Of the 13 tests (40.6%) for the detection of IgG/IgM, 84.6% (11/13) were manufactured in Brazil and 15.4% (2/13) were imported. The tests for detection of Ag/Ab NS1/IgG/IgM corresponded to 18.8% (6/32) of the evaluated products, with 50.0% manufactured in the country and 50.0% of imported origin.

After evaluating the parameters of sensitivity and specificity against TP and TN samples, 71.9% (23/32) of the products were considered satisfactory and 28.1% (9/32) unsatisfactory for one or both performance parameters. Of the satisfactory RT, 65.2% (15/23) were of national origin and 34.8% (8/23) imported. Of the tests considered unsatisfactory, 66.7% (6/9) were national and 33.3% (3/9) imported. Of the 23 satisfactory products, 39.1% (9/23) corresponded to RT for the detection of Ag NS1, the majority of which 62.5% (5/8) were imported products. A total of 47.8% (11/23) corresponded to the tests for detection of IgG/IgM Ab, of which 66.7% (10/15) were national and 12.5% (1/8) imported. Tests for detection of Ag/Ab (NS1/IgG/IgM) corresponded to 13.1% (3/23) of the analyzed products, of which 6.7% (1/15) were national and 25.0% (2/8), imported.

In the RT for the detection of satisfactory Ag NS1, the sensitivity values ranged from 88.3% to 100.0%, being higher than the 82.0% to 99.1% declared by the manufacturers. RT for the detection of IgG/IgM Ab showed sensitivity values for IgG Ab ranging from 97.5% to 100.0%, equal to or greater than 88.0% to 99.1% declared. For the detection of IgM Ab, the sensitivity varied from 99.0% to 100.0%, obtaining values equal to or greater than the 88.0% to 100.0% declared by the manufacturers (Table 1).

In the RT for the detection of combined Ag/Ab (NS1/IgG/IgM), the sensitivity values for NS1 were 85.7% to 100.0%, and 100.0% for IgG and IgM Ab, being higher or equal to the expected values of 82.0% to 99.1% for NS1 and 88.0% to 99.1% for IgG/IgM considered, therefore, satisfactory (Table 1).

The specificity values presented in the satisfactory RT for the detection of Ag NS1 varied from 99.0% to 100.0% and were greater than or equal to those declared by the manufacturers. In the RT for the detection of IgG/IgM Ab, the specificity for IgG Ab was 96.7% to 100.0%, being equal to or greater than the 96.2% to 100.0% declared. For the detection of IgM Ab, the sensitivity varied from 98.1% to 100.0%, a value greater than or equal to the 96.2% -100.0% declared by the manufacturers. In the combined tests AgNS1/AbIgG/IgM, the sensitivity values obtained for NS1 were 98.5% to 100.0%, higher than those declared for 81.0% to 100.0%. The specificity achieved for IgG Ab was 97.1% to 99.3% and the stated, 96.2% to 100.0%. The specificity obtained for IgM Ab ranged from 98.6% to 100.0% (Table 1).

Table 1. Sensitivity and specificity values (%) obtained through the previous analysis and the values declared by the manufacturers for satisfactory rapid tests for the detection of Ag (NS1), Ab (IgG/IgM), and Ag/Ab NS1/IgG/IgM of dengue.

Rapid Tests	Markers	Satisfactory (n = 23)			
		Sensibility		Specificity	
		Obtained	Declared	Obtained	Declared
Ag NS1					
9/23	NS1	88.3% - 100.0%	82.0% - 99.1%	99.0% - 100.0%	95.0% - 100.0%
(39.1%)					
Ac IgG/IgM	IgG	97.5% -100.0%	88.0% - 99.1%	96.7% - 100.0%	96.2% - 100.0%
11/23	IgM	99.0% -100.0%	88.0% - 100.0%	98.1% - 100.0%	96.2% - 100.0%
(47.8%)					
Ag/Ac NS1/IgG/IgM	NS1	85.7% -100.0%	82.0% - 99.1%	98.5% - 100.0%	81.0% - 100.0%
3/23	IgG	100.0%	88.0% - 99.1%	97.1% - 99.3%	96.2% - 100.0%
(13.1%)	IgM	100.0%	88.0% - 99.1%	98.6% - 100.0%	98.6% - 100.0%

Source: Laboratory of Blood and Blood Products, 2019.
Ag: antigen; Ab: antibodies; IgG: immunoglobulin G; IgM: immunoglobulin M.

Of the total of nine unsatisfactory RTs, 44.0% (4/9) corresponded to RT for the detection of NS1, whose sensitivity obtained ranged from 62.9% to 82.0% compared to the declared 88.3% -99.5% by the manufacturers and the specificity of 97.8% -100.0% compared to the values of 98.3% to> 99.9% declared (Table 2).

The unsatisfactory RT for detection of IgG/IgM Ab corresponded to 22.2% (2/9), with 50.0% (1/2) unsatisfactory for sensitivity to IgM Ab and 50.0% (1/2), for sensitivity and specificity for IgM and IgG Ab (Table 2).

RT for detection of Ag/Ab NS1/IgG/IgM represented 33.3% (3/9) of unsatisfactory results, with 66.7% (2/3) unsatisfactory in sensitivity to Ag NS1 and 33.3% (1/3) unsatisfactory in sensitivity and specificity for NS1/IgM/IgG (Table 2).

Of the total of 33.3% (3/9) that corresponded to the unsatisfactory RT for detecting Ag NS1 and IgG/IgM Ab, the sensitivity for Ag NS1 ranged from 80.6% to 99.0%, being lower than that declared by the manufacturers from 90.8% to 100.0%. The sensitivity for IgG and IgM Ab was from 99.0% to 100.0% and the declared, from 99.5% to 100.0%. The specificity values for RT used in the research of Ag and Ab NS1/IgG/IgM for dengue were lower than those declared (Table 2).

The products that presented unsatisfactory results and/or clinical specificity in the analyzes carried out by INCQS, according to the current regulations, did not obtain product registration with Anvisa and, consequently, were not marketed in the country.

DISCUSSION

Early diagnosis of dengue cases is crucial in the treatment and implementation of effective public health actions to control the disease. The increase in the number of cases in the world stimulated the interest of the private sector in the development and launch of new products in the national and international market. Ideal characteristics of a diagnostic test, defined by the Assurance criteria, which include immunochromatographic RT are: accessibility, the production of few FN results (sensitivity) and FP results (specificity), simplicity in execution and the minimum training requirement, not requiring refrigeration for storage and equipment for execution, in addition to easy distribution to those who need a diagnosis in restricted access areas[46].

Among the methods available for the diagnosis of dengue, RT are widely used both for the detection of Ag NS1 and for the detection of IgM and IgG Ab individually or in combination (NS1/IgG/IgM). They are simple execution tests, do not require equipment, and can be performed in a maximum time of 20 min. Due to their applicability and scope, RT were the object of study, although all methodologies used in the diagnosis of dengue in the country are routinely evaluated by INCQS. The prior analysis of dengue diagnostic tests is an important step in the registration process and, consequently, in the availability of sensitive and specific products on the national market. As verified in performance evaluations carried out by independent international organizations, such as the WHO, in an attempt to determine the best commercially available diagnostic tests, the low performance of these products has been verified[46,47,48]. The studies carried out showed values of sensitivity and specificity below those declared by the producers in the instructions for use, challenging manufacturers in the search for improvement in the performance of these products[23].

According to Blacksell[49], until large-scale evaluations are carried out, many products from different continents are marketed worldwide with little or no verification regardless of performance. The quality of the validations is often questioned for the incorrect performance with the occurrence of failures related to the number of samples evaluated, inconsistencies in the methodologies, interpretation errors, failures in the execution of the tests, or even the title of Ab employed[23,50]. It should be noted that the values of sensitivity and specificity declared by the manufacturers, in the instructions for use to date, have guided the national and

Table 2. Sensitivity and specificity values (%) obtained through the previous analysis and the values declared by the manufacturers for unsatisfactory rapid tests for the detection of Ag (NS1), Ab (IgG/IgM) and Ag/Ab NS1/IgG/IgM of dengue.

Rapid Tests	Markers	Unsatisfactory (n = 9)			
		Sensibility		Specificity	
		Obtained	Declared	Obtained	Declared
Ag NS1					
4/9	NS1	62.9%-82.0%	88.3%-99.5%	97.8%-100.0%	98.3 ->99.9%
(44.0%)					
Ac IgG/IgM	IgG	100.0%	98.6% - 100.0%	80.4% - 88.5%	97.76% - 99.0%
2/9	IgM	88.4% - 94.1%	97.4% - 97.9%	80.4% -96.8%	98.29% - 99.0%
(22.2%)					
Ag/Ac NS1/IgG/IgM	NS1	80.6% - 99.0%	90.8% -100.0%	91.5% - 98.7%	92.1% - 100.0%
3/9	IgG	99.0% -100.0%	99.5% -100.0%	91.5% - 98.7%	98.5% - 100.0%
(33.3%)	IgM	99.0% -100.0%	99.5% -100.0%	91.5% - 98.7%	98.5% - 100.0%

Source: Laboratory of Blood and Blood Products, 2019.
Ag: antigen; Ab: antibodies; IgG: immunoglobulin G; IgM: immunoglobulin M.

international market for the acquisition and use of these products, which reinforces the need for constant evaluation.

Aiming to control the quality of products offered in the national market, the parameters of TR diagnostic sensitivity for the detection of Ag NS1, IgG and IgM Ab and AgNS/AbIgG/IgM were evaluated, using serological panels of clinical samples collected during the epidemic period of the dengue disease (2010 to 2013) in Rio de Janeiro. Specificity was verified against non-reactive serum/plasma samples characterized as TN and commercial panels, the WHO reference standard for dengue and interfering samples.

In compliance with the requirement for prior analysis issued by Anvisa since its implementation, 32 different RTs were analyzed in the period evaluated, the highest amount being received in 2017, the year following the implementation of the prior analysis carried out in May 2016. The amount of RT made available on the national market reflects the outbreaks of the disease that occurred in the last decades and in the four major epidemics that occurred in Brazil, associated with the alternation of the predominant viral serotype: DENV-1, DENV-3, DENV-2, and DENV-4, reaching an accumulated total number of 12,171,009 probable cases of the disease in the years 1998, 2002, 2008 and 2010, 2013, 2014, 2017[44].

The highest percentage of RT considered unsatisfactory corresponded to national products for the detection of Ag NS1. With regard to origin, if one or more critical stages of the product manufacturing process are carried out in Brazil, the product is registered as national, according to current legislation, even if they are used in manufacturing Ag and/or Ab and imported components[50].

NS1 protein, identified as an early marker of acute dengue, is present between the first and the ninth day after the onset of the disease, however, after seroconversion, it can be difficult to detect it in the serum. Different groups in several countries have reported low sensitivity in NS1 assays compared to molecular methods, especially in populations that have suffered sequential outbreaks of this disease. As this finding is definitely more pronounced in secondary infections, it has already been suggested that Ag NS1 could be sequestered in IgG immunocomplexes. In a study carried out in the Brazilian population, at least 68% of patients had a secondary dengue infection, which could explain a lower performance (less than 40% positivity) of the test[26,28,51,52].

The performance of RT for the detection of Ag NS1, alone or combined with the detection of IgM or IgG Ab, has been evaluated[26,28]. Although the results are not always consistent across different cohorts and tests, several general comments can be made. Sensitivity is highest in primary infections, when the test occurs shortly after the onset of symptoms and when IgG is not detectable. Variations in sensitivity depend on the serotype of the samples evaluated and on the sensitization by Ab or Ag used.

The TR for the detection of Ab IgG/IgM showed the best sensitivity performance among the evaluated products. Tests for the detection of IgM and IgG Ab are routinely used in clinical laboratories and can differentiate between primary and secondary infections. The combined use of IgM and IgG has been shown to increase sensitivity in detecting DENV infection. According to the Pan American Health Organization (PAHO), 80% of all dengue cases have detectable IgM Ab on the fifth day and in 93% to 99% of the cases between the sixth and tenth days. IgM levels increase rapidly and reach their peak in about two weeks, remaining detectable for two to three months, which makes these Ab indicators of recent infections[27,49]. IgG Ab starts to be detected in the primary response, a few days after IgM Ab, being detectable from the fifth day of illness and remaining for life. The secondary response is characterized by a rapid increase in IgG almost immediately after the onset of symptoms[31]. IgM levels in the secondary response are considerably lower than in the primary response. It is known that the divergent values of sensitivity and specificity obtained may be associated with the different populations analyzed, with the number of samples, stage of infection, or with previous infections by different serotypes[53].

In the Americas, the existence of local transmission of multiple arboviruses and cross-reactivity between *flavivirus*, in particular dengue and zika, are known, and the identification of infectious agents is a challenge. Although the cross-reactivity between *Flavivirus* is described, zika-reactive samples (IgM or IgG) were not included in the evaluation, because, in the proposed analysis period (2016-2018), they were not yet found commercially available serological performance panels or international standards for zika are, therefore, a limitation of the study[19].

Currently, RT used in Brazil for the diagnosis of dengue are used in primary care settings and clinical laboratories. The use of tests with lower than expected performance or even without proper validation challenges the health system. The use of RT with unsatisfactory performance for the detection and management of dengue cases can lead to an increase in FN or FP results and a consequent increase in lethal cases of the disease due to inadequate treatment or absence of treatment[54]. In this way, we warn that the frequent evaluation of these products is necessary, not only at the time of registration (prior analysis) but through the performance of a control analysis (post-marketing).

The evaluation of the stability of the products through the storage and transport conditions, although it was not the subject of this work, must be taken into account, as Brazil is a country of continental dimensions with varying temperatures and relative humidity. In a study carried out by Sengvilaipaseuth et al.[55], it was found that components (Ab) of an RT were affected by storage at a high temperature, reducing the sensitivity of the product.

CONCLUSIONS

Due to their applicability and scope, RT are widely used in the diagnosis of dengue both for the detection of Ag NS1 and for the detection of IgM and IgG Ab. When purchasing such products, sensitivity and specificity parameters stated in the instructions for use guide the national and international market. Aiming to control the quality of the products offered in the national market, the parameters of sensitivity and clinical or diagnostic specificity

of the immunochromatographic used in the serological diagnosis of dengue were evaluated, as part of the process of obtaining registration with Anvisa. The results obtained were compared to the values declared in the instructions for use of the products for these attributes. Of the 32 RT sent for analysis, 23 showed satisfactory results for the sensitivity and specificity parameters. RT for the detection of IgG/IgM Ab showed the best sensitivity performance among the evaluated products. A total of nine RT were considered unsatisfactory and corresponded to products intended for the detection of Ag NS1. Unsatisfactory tests were not registered and, consequently, were not commercialized in the country.

The laboratory evaluation prior to product registration makes it possible to make sensitive and specific RTs available on the national market, expanding the quality, safety, and reliability of products intended for the diagnosis of dengue sold in Brazil.

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Authors' Contribution

Borges HCBG - Conception, planning (study design), acquisition, analysis, data interpretation, and writing of the work. Adati MC - Conception, planning (study design), data interpretation, and writing of the work. Vigo DC, Mendonça VF, Issobe MA - Analysis and interpretation of results. Santos FB, Zamith HPS - Writing of the work. All authors approved the final version of the work.

Conflict of Interests

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