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Post-market monitoring of rapid diagnostic tests for COVID-19: confronting the pandemic

Monitoramento pós-mercado dos testes rápidos para COVID-19: enfrentamento da pandemia

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ABSTRACT

Introduction: In December 2019, the first group of patients with symptoms of atypical pneumonia was discovered in Wuhan, China. On January 7, 2020, the etiologic agent was identified; it was a new betacoronavirus, genetically similar to SARS-CoV-1, consisting of a simple RNA strand, an enveloped virus of 50-200nm in diameter, which was called SARS-CoV-2. Soon after, the disease was named COVID-19. On January 30, WHO declared a Public Health Emergency of International Importance due to the spread of the coronavirus. Tests for serological detection of IgM and IgG antibodies are those that provide an estimate of the immune response to SARS-CoV-2, highlighting the Rapid Diagnostic Tests (RDT), simple and accessible with a result within 5-30 minutes, based on sensitization of antigens/antibodies conjugated to colloidal gold capturing specific proteins present in the infected serum, plasma or blood. **Objective:** This work aims to show the analysis carried out with RDT for COVID-19 diagnosis in compliance with the current legislation from 02.04 to 18.08.2020. **Method:** In March of 2020, 25 serum/plasma samples were donated, without any identification. These samples were the remaining samples of tests performed on individuals with a confirmed diagnosis of SARS-CoV-2 infection by the RT-PCR technique from health services (National Institute of Infectious Diseases Evandro Chagas - INI and State Institute of the Brain Paulo Niemeyer - IEC) located in the metropolitan region of the state of Rio de Janeiro. The samples obtained in order to become a serological panel were stored at -20ºC until the moment of use. Simultaneously, a panel of samples with confirmed reactivity for IgM and IgG antibodies from COVID-19 was being made, throughout the pandemic and the samples used were evaluated against three Rapid Tests, of different antigenic compositions or different brands; two ELISA tests for IgM and IgG; two chemiluminescence tests and when applicable, a molecular test. In order to assess the specificity of the products sent, surplus donation plasma samples were selected, known to be negative for HIV, HTLV, hepatitis b and c, chagas and syphilis, collected between 2013 and 2014, in the southern regions of the country, period in which SARS-CoV-2 was nonexistent in the world. In addition to True Positive (VP) and True Negative (VN) samples, interfering serum or plasma samples with reactivity for HIV, HCV, HTLV, HBsAg, chagas disease, syphilis and dengue were also included in the evaluation. **Results:** Out of 178 TR lots, 74.1%, 132 lots were from China and 25.9%, 46 TR lots were from Brazil; Germany; South Korea; Canada; USA; Singapore; Ireland and Switzerland. The analytical result showed that 57.0%, 101 TR lots obtained a Satisfactory result and 43%, 77 lots had Unsatisfactory results, when compared to the Sensitivity and Specificity values declared by the manufacturer, in the Instructions for Use. **Conclusions:** The results obtained show the need for constant monitoring of TRs for COVID-19 with the primary purpose of guaranteeing the quality of products sold in the country, one of the National Health Surveillance System pillars of action.

KEYWORDS: COVID-19; SARS-CoV-2; Rapid Diagnostic Test; Quality Monitoring

RESUMO

Introdução: Em dezembro de 2019, foi descoberto na cidade de Wuhan, China, um primeiro grupo de pacientes com sintomas de uma pneumonia atípica. Em 7 de janeiro de 2020, o seu agente etiológico foi identificado: tratava-se de um novo betacoronavírus, geneticamente similar ao SARS-CoV-1, constituído de fita simples de RNA, vírus envelopado de 50-200 nm de diâmetro designado como SARS-CoV-2, e a doença foi denominada COVID-19. Em 30 de janeiro, a Organização Mundial da Saúde declarou Emergência de Saúde Pública de Importância Internacional em razão da disseminação desse novo vírus. Os testes para detecção sorológica de anticorpos IgM e IgG fornecem uma estimativa da resposta imune ao SARS-CoV-2, com destaque para os Testes Rápidos (TR) que são simples e acessíveis fornecendo resultados em 5-30 min. Esses testes são sensibilizados com antígenos/anticorpos conjugados ao ouro coloidal, capturando proteínas específicas presentes no soro, plasma ou sangue de pacientes infectados. **Objetivo:** Demonstrar a análise efetuada nos TR para diagnóstico da COVID-19, em atendimento a legislação vigente, no período de 2 de abril a 18 de agosto de 2020. **Método:** Durante o mês de março de 2020, foram cedidas 25 amostras de soro/plasma, sem qualquer identificação, excedentes dos testes efetuados em indivíduos com diagnóstico confirmado de infecção pelo SARS-CoV-2 pela técnica de RT-PCR provenientes de serviços de saúde (Instituto Nacional de Infectologia Evandro Chagas – INI e Instituto Estadual do Cérebro Paulo Niemeyer – IEC) localizados na região metropolitana do estado do Rio de Janeiro. The samples obtained for the preparation of the serological panel were stored at -20ºC until use. Concomitantemente, um painel de amostras com reatividade confirmada para anticorpos IgM e IgG da COVID-19 foi sendo confeccionado ao longo da pandemia e as amostras utilizadas foram avaliadas frente a três TR, de diferentes composições antigênicas ou diferentes marcas; dois testes ELISA para IgM e IgG; dois testes de quimioluminescência e quando aplicável, um teste molecular. Para avaliação da especificidade dos produtos encaminhados, foram selecionadas amostras de plasma excedentes de doação, sabidamente negativas para HIV, HTLV, hepatite B e C, doença de chagas e sífilis coletadas nos anos de 2013 e 2014, na Região Sul do país, período no qual o SARS-CoV-2 era inexistente. Além de amostras Verdadeiro Positivas (VP) e Verdadeiro Negativas (VN), ainda foram incluídas na avaliação amostras de soro ou plasma interferentes com reatividade para HIV, HCV, HTLV, HBsAg, doença de chagas, sífilis e dengue. **Resultados:** Dos 178 lotes de TR, 74,1% foram provenientes da China e 25,9%, do Brasil, da Alemanha, da Coreia do Sul, do Canadá, dos EUA, da Cingapura, da Irlanda e da Suíça. O resultado analítico demonstrou que 57,0% dos TR obtiveram resultados satisfatórios e 43,0%, resultados insatisfatórios, quando comparados aos valores de sensibilidade e especificidade declarados pelo fabricante na instrução de uso. **Conclusões:** Há necessidade de constante monitoramento dos TR para COVID-19, com finalidade precípua de garantir a qualidade dos produtos comercializados no país, um dos pilares das ações do Sistema Nacional de Vigilância Sanitária.

PALAVRAS-CHAVE: COVID-19; SARS-CoV-2; Teste Rápido; Monitoramento da Qualidade

INTRODUCTION

In December 2019, the first group of patients with symptoms of an atypical pneumonia not etiologically identified was discovered in the city, in the Hubei province of Wuhan, China[1,2]. On January 7, 2020, the etiological agent was identified: it was a new betacoronavirus, genetically similar to SARS-CoV-1, consisting of a single strand of RNA, an enveloped virus of 50-200 nm in diameter, designated as and the disease was named COVID-19[2]. The disease spread rapidly, reaching more than 150 countries in three months, initially across the Asian continent, with reports in Thailand, Japan, and South Korea on January 13, 15, and 20, respectively, and then in other countries and continents[3].

On January 22, 2020, it was discussed by an emergency committee organized by the World Health Organization (WHO) whether or not this event constituted a Public Health Emergency of International Concern (PHEIC). This situation represents a formal declaration by the WHO of "an extraordinary event, which has implications for public health beyond the border of the affected state, through the international spread of disease and may require an immediate and coordinated international response"[4].

Subsequently, on January 30, the WHO declared, after meeting with experts, the occurrence of a PHEIC due to the spread of the coronavirus[4]. On March 11, due to the occurrence of more than 118,000 cases of the disease distributed in more than 110 countries and territories around the world, a pandemic was declared. This occurs, according to the WHO, when a disease has the ability to infect people easily, to spread from person to person, efficiently and sustainably, in various regions[4,5,6].

According to the WHO, on February 19, 2021, at 12:05 pm, there were 109,594,835 confirmed cases of COVID-19 in the world and 2,424,060 registered deaths[7]. In Brazil, according to data from the Ministry of Health, on February 6, 2021, at 6:30 pm, almost 12 months after the first case occurred on February 26, 2020, in São Paulo, the country had 9,497,795 confirmed cases and 231,012 deaths[8]. The highest number of new cases (87,843 cases) occurred on January 7, 2021, and the highest number of deaths (1,595 deaths) on July 29, 2021[8]. The Southeast Region was the region with the highest incidence of new cases (135,053) and the highest mortality rate in the country was in the North Region, with the state of Amazonas presenting 212.3 deaths/100 thousand inhabitants[8].

SARS-CoV-2 belongs to lineage B of the beta-coronavirus family, of zoonotic origin, genetically similar to the 2002 coronavirus (SARS-CoV-1)[2], consisting of single-stranded RNA, enveloped

virus 50-200 nm in diameter. Seven species can infect humans, three of which can produce serious diseases, SARS-CoV-2, SARS-CoV, the agent of the 2002-2003 pandemic, and MERS- CoV, which causes Middle East respiratory syndrome[2,3,9]. The SARS-CoV-2 genome is similar to that of the bat SARS-CoV-1 and the MERS-CoV[10] virus, composed of five proteins: *spike* (S), nucleocapsid protein (N), hemagglutinin-esterase protein dimer (HE), envelope protein (E), and membrane protein[9,10,12].

The disease can be transmitted from human to human and has an average incubation period of approximately five days (ranging from two to 14 days), with symptoms appearing approximately 12 days after infection (ranging from eight to 16 days)[10,11,13], however there are cases in the literature with an incubation period longer than 19 days[9,11,13].

Transmission can occur before potentially infected individuals develop symptoms, being considered pre-symptomatic individuals. In addition, a portion of infected individuals, who will never develop specific symptoms of the infection, may significantly contribute to the transmission of the disease[11].

Laboratory diagnosis

From 1970 onwards, among the different methodologies developed and applicable to the diagnosis of diseases, the following stand out: the molecular test for the detection of nucleic acid in real time (Nucleic Acid Amplification Technology - NAT), which was developed in 1988 by Kary Mullis, considered as the gold standard mainly in the case of COVID-19, and the serological tests, developed from 1971 by Peter Perlmann and Eva Engwell[13].

Although reverse transcriptase polymerase chain reaction (RT-PCR) based viral RNA detection has been widely used in the diagnosis of COVID-19, it cannot be applied to monitoring the progress of disease stages or assessing immunity[13,14,15].

Serological tests, as complementary to molecular tests, are based on the detection of IgM and IgG antibodies that can provide an estimate of the immune response to SARS-CoV-2 in the population[11,15]. There are four types of serological tests: rapid diagnostic tests, which are immunochromatographic; enzyme linked immunosorbent assay (ELISA); chemiluminescence immunoassay – CLIA; and neutralization tests, the latter must be carried out in more complex laboratories and requires three to five days to obtain the results[11,15].

Among the tests of simple execution and accessibility, the rapid test, typically qualitative (positive or negative), whose result can be obtained between 5-30 min, is based on the sensitization of antigens/antibodies conjugated to colloidal gold that capture immunoglobulins and proteins specific for SARS-CoV-2 present in the serum, plasma, or blood of infected individuals, forming an antigen-antibody complex that migrates by capillarity along the nitrocellulose membrane[11,13,16]. The nitrocellulose membrane is arranged in a polyethylene device, commonly called a test device or cassette[11,16].

As the result of immunochromatography, the complexes between antigens and antibodies are captured by the anti-human IgM and/or IgG antibodies fixed to the nitrocellulose strip to form the test line (T). The marker (colloidal gold) specifically binds to the area intended to control the reaction to form the control line (C)[11,16], as shown in Figure 1.

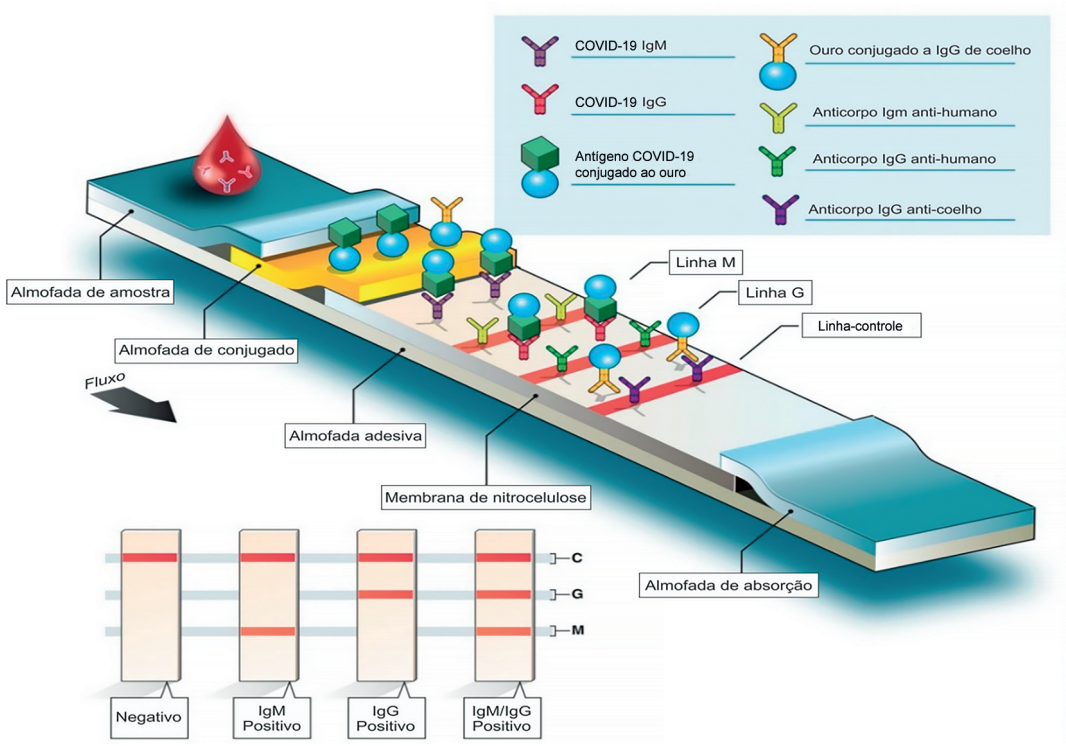
Antibody detection is indicated by visible lines, which appear on the test strip, or by fluorescence, which can be identified using a reading device. Many of these tests are known as colloidal gold-based immunoassays because they use the virus antigen conjugated to gold nanoparticles[17,18]. In the case of SARS-CoV-2, the seroconversion of the acute phase of the infection has not yet been fully determined, however IgA and IgM antibodies have already been detected on the 5th day of symptoms with an interquartile range of 3 to 6 days, respectively, and, as for IgG anti-SARS-CoV-2 antibodies, the mean time of appearance was on the 14th day of infection, with an interquartile range of 10 to 18 days[16,18,19,20,21] (Figure 2).

In any infectious disease outbreak, an accurate and affordable diagnostic test should be one of the pillars of health control measures policies to understand and minimize the spread of diseases[1]. In this context, the National Institute for Quality Control in Health (INCQS), belonging to the Oswaldo Cruz Foundation (Fiocruz) and technically subordinated to the Brazilian National Health Surveillance Agency (Anvisa), acts as a reference for scientific and technological issues related to the quality control of products, environments and services linked to Health Surveillance.

This institute, through the Laboratory of Blood and Hemoderivatives, has since 2000 routinely evaluated by prior, inspection, and control analysis, as provided for in legislation, products for *in vitro* diagnostic use belonging to risk class IV and, more recently, risk class III, different methodologies and markers, with a view to assessing the quality of pre- and post-market products.

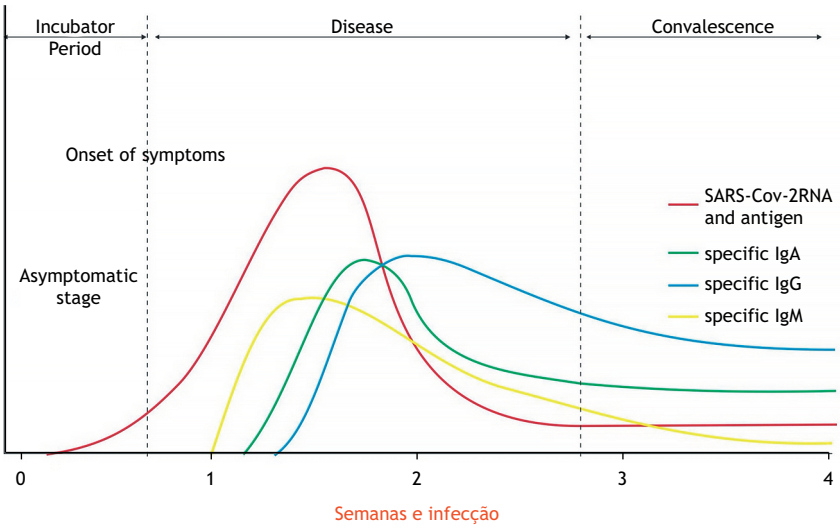
Also according to current legislation, Law No. 5,991, of December 17, 1973, and Law No. 6,360, of September 23, 1977, the analyzes provided for are defined as follows: prior – carried out on certain products under sanitary surveillance, in order to verify whether they can be registered; control – carried out on products under the sanitary surveillance regime, after their release for consumption, and intended to prove the conformity of the product according to the specifications established at the time of the registration request; and inspection – carried out on the products submitted to the system established by the legislation, on a routine basis, for verification of infraction or verification of fortuitous or eventual occurrence[22,23,24,25].

Currently, the guidelines for the registration of diagnostic kits are based on Resolution of the Collegiate Board of Directors (RDC) No. 36, of August 26, 2015, which aims to establish risk classification, control, registration and registry, and the labeling requirements and instructions for use of products for *in vitro* diagnostics, including their instruments[25].



Source: Adapted[1].

Figure 1. Schematic of the rapid test confection.



Source: Adapted[1].

Figure 2. Schematic of the course of seroconversion after SARS-CoV-2 infection.

With the declaration of the COVID-19 pandemic by the WHO and the need to make tests for the diagnosis of SARS-CoV-2 available in the national market, the General Coordination of Analysis of the Contracts of Strategic Inputs for Health of the Ministry of Health published, on March 17, 2020, the public call notice aimed at inviting companies to supply the portfolios of supplies for the diagnosis of COVID-19 to the General Coordinator of Public Health Laboratories (CGLAB) of the Ministry of Health[26]. This notice registered around 20 companies to present their products for control analysis.

Subsequently, Anvisa published RDC No. 379, of April 30, 2020[27], which amended RDC No. 356, of March 23, 2020, currently revoked, which provides, on an extraordinary and temporary

basis, on the requirements for the manufacture, import, and acquisition of priority medical devices for use in health services, due to the international public health emergency identified as related to SARS-CoV-2. This RDC, in its 7th item of art. 9, establishes that:

> Those responsible for importing diagnostic kits under the terms of the caput must send, within a maximum period of 5 (five) days, counted from the date of clearance of the cargo, a sample of at least 100 units of each imported batch for analysis by the National Institute for Quality Control in Health (INCQS)[28].

Thus, this work aimed to present the analysis performed on the rapid tests (immunochromatographic) for the diagnosis of COVID-19 received for analysis, as determined in the public call notice of the Ministry of Health and compliance with RDC nº 379/2020, in the period from April 2, 2020, to August 18, 2020.

METHOD

In compliance with art. 9 of RDC No. 379/2020, in the period from April 2, 2020, to August 18, 2020, 277 batches of COVID-19 diagnostic kits were received for analysis at the INCQS, with sampling of 100 tests for each batch of different methodologies such as: rapid tests (RT), ELISA, chemiluminescence, and RT-PCR. In this work, only the RT intended for the detection of IgM and IgG antibodies for COVID-19 were considered, although the laboratory also received the RT for the detection of antigens and for isothermal amplification of nucleic acids, totaling 178 batches of RT evaluated for the attributes of sensitivity and specificity, as well as the technical performance of the cassettes or test devices, a tool that includes the nitrocellulose strip intended for the tests[26].

The kits, according to § 7 of RDC No. 379/2020, were received accompanied by the batch release certificate issued by the quality control; copy of Annex I, Term of Responsibility as provided for by law; complete production and quality control dossier, emphasizing the stability test, in addition to the instructions for use in Portuguese, provided for in § 5 of art. 9 of the same RDC[26].

During the month of March, were kindly provided 25 excess serum/plasma samples from tests carried out on individuals with a confirmed diagnosis of SARS-CoV-2 infection using the RT-PCR technique from health services (Evandro Chagas National Institute of Infectious Diseases - INI and Paulo Niemeyer State Brain Insitute - IEC) located in the metropolitan region of the state of Rio de Janeiro. The samples obtained for the preparation of the serological panel were stored at -20ºC until use.

In addition, a panel of samples with confirmed reactivity for IgM and IgG antibodies to COVID-19 was being made throughout the pandemic, and the samples used were evaluated against at least three RT of different antigenic compositions or different brands; two ELISA tests for IgM and IgG; two chemiluminescence tests and, where applicable, a molecular test.

To evaluate the specificity of the products sent, samples of excess plasma from donation were selected, known to be negative for: HIV, HTLV, hepatitis B and C, Chagas disease, and syphilis, in the years 2013 and 2014, period in which SARS-CoV-2 was still non-existent. In addition to true positive (TP) and true negative (TN) samples, interfering serum or plasma samples with reactivity for: HIV, HCV, HTLV, HBsAg, Chagas disease, syphilis, and dengue were also included in the evaluation.

During the period of analysis, standards and/or international panels or standard sera for COVID-19 had not yet been made available on the national and international market, so they were not included in this work. The analyzes were carried out strictly following the instructions for use that came with the products. The percentage values of the sensitivity and specificity attributes obtained in the batches of the evaluated products were compared with the values declared in the instructions for use that accompanied the rapid tests. Clinical sensitivity is understood as the percentage of positive results obtained when the analyte is present in the sample, recognizing the presence of a certain disease or condition, and clinical specificity, as the ability of an analytical method to determine only the analyte against other substances present in the analyzed sample[25]. A false negative is the negative result obtained in samples from an infected individual and a false positive result is a false positive result obtained in samples from non-infected individuals[25].

The sensitivity value of each product was obtained based on the number of TP samples for IgM and IgG analyzed and correctly identified by the evaluated test. It was calculated according to the 2 x 2 contingency table, with the following equation: TP results divided by the sum of TP results with false negative results multiplied by 100. Specificity was calculated according to the following equation: TN results divided by the sum of TN results and false positive results multiplied by 100[25,28].

As this is a new worldwide infection, together with the absence of international standards, the values of the specification declared by the manufacturer in the instructions for use that accompany the product were adopted as a reference value. The reference value is defined as a theoretical value or established in scientific principles that serve as a reference for comparison with the result obtained. Thus, tests whose sensitivity and/or specificity values were greater than or equal to those declared by the manufacturer, in the instructions for use, were considered satisfactory and those with lower values, unsatisfactory[25].

The sampling of 100 tests per lot of product received for analysis was distributed as follows to perform each analysis: a) positive samples for COVID-19 IgM and/or IgG: 25% to 30%; b) negative samples (samples collected between 2013 and 2014, free from HIV; HTLV; HCV; HBsAg; syphilis; dengue; chikungunya and zika, previously analyzed and proven negative, as well as samples free

from COVID-19, as they were collected before the 1st confirmed case in the country, which occurred in February 2020): 60% to 65%; c) interfering samples for HIV, HTLV, HBsAg, HCV, Chagas disease, syphilis, and dengue: 5% to 10%, as established in the analytical procedure.

In addition, quality deviations were identified and quantified regarding the cassettes or test devices received for analysis, such as: a) presence of flaws in the marking of the control line of the cassette or test device; b) failures in marking the test line; c) strips of nitrocellulose displaced from the display of the cassette or test device; d) other cassette quality deviations found during the tests. As it is a visual reading and depends on the visual acuity of each professional, as well as a destructible analyte, the reading of the results was performed by more than one professional and photographed for registration.

RESULTS AND DISCUSSION

The 178 batches received for analysis were distributed as follows: 150 (84.2%) kits intended for control analysis; 24 (13.6%) kits collected by state and/or municipal Health Surveillance for inspection analysis; and four (2.2%) kits intended for forensic counterproof samples. The forensic counterproof is the appeal filed by the company, in accordance with Law No. 6,437, of August 20, 1977, when it disagrees with the result obtained from the inspection analysis[24].

The collections intended for inspection analysis corresponded to the following states: Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo, Paraná, Rio Grande do Sul, Santa Catarina, and Goiás. When evaluating this distribution, 149 (83.7%) kits received corresponded to the control analysis in response to the public call of the Ministry of Health and mainly to RDC nº 379/2020, which made it mandatory for companies to forward the kits for analysis during customs clearance.

Another fact that is worth mentioning corresponded to the amount of 24 batches collected for inspection analysis and five (3.0%) batches destined for the forensic counterproof, promoted by Anvisa through the quality monitoring program of COVID-19 diagnostic kits, an essential tool for product quality control.

Of the 178 batches of RT received for analysis in the aforementioned period, the manufacturers corresponded to 73 companies in three continents: 13 (17.9%) from the American continent, 55 (75.3%) from the Asian continent, and five (6.8%) from the European continent. The origin of the products involved nine countries in the world and 73 manufacturing companies distributed as follows: 49 (67.1%) companies from China; nine (12.3%) companies in Brazil; five (6.8%) from South Korea; three (4.1%) from Germany; three (4.1%) from the USA; one (1.40%) from Canada, Ireland, Singapore, and Switzerland, respectively. It is worth mentioning the five cities in China that contributed most of the companies that imported kits for the diagnosis of COVID-19: Hangzhou, seven (33.5%) companies; Shanghai, six (28.5%) companies; Beijing, four (19.0%) companies; Guangzhou and Shenzhen, two (9.5%) companies each. Of the nine national companies, four (44.5%) companies are located in São Paulo; two (22.2%) in Minas Gerais; two (22.2%) in Rio de Janeiro, and one (11.1%) in Paraná.

In this regard, it is worth mentioning China, with 49 companies and, of these, 21 located in five Chinese provinces, ratifying the country as an Asian tiger in the trade of products, among these, RT for the diagnosis of COVID-19. Another highlight is Brazil, which in this work was represented with nine companies, demonstrating the industrial park installed in the country, as well as the need to manufacture and distribute national products, intended for the Brazilian population.

Regarding the distribution of origin of the 178 batches of RT kits for COVID-19 received for analysis: 132 (74.1%) batches came from China and 46 (25.9%) corresponded to other countries such as: Brazil, with 16 (9.0%) batches; Germany, with nine (6.2%); South Korea, with nine (5.0%); Canada, with four (2.20%); USA, with three (1.7%), Singapore, Ireland, and Switzerland, one (0.6%) per country. The strong participation of China as well as Brazil is evident. In addition, 122 importing or distributing companies in the country or public applicants for analysis were evidenced, to market the 178 batches of COVID-19 RT: 94 (77.0%) marketed the products from China; nine (7.4%) sold national products; seven (5.7%) sold products from South Korea; three (2.5%), products from Germany, USA, and Canada, and one (0.8%), products from Ireland, Singapore, and Switzerland. The highlight once again goes to China with 94 companies that imported and marketed their products in the country, as shown in Figure 3.

When evaluating the number of batches of kits received from April 2 to August 18, it was found that 75 (42.1%) batches were received in June, followed by 65 (36.5%) batches in July; 16 (9.0%) batches in August; 14 (7.9%) batches in May, and eight (4.5%) batches in April, at the beginning of the analyses.

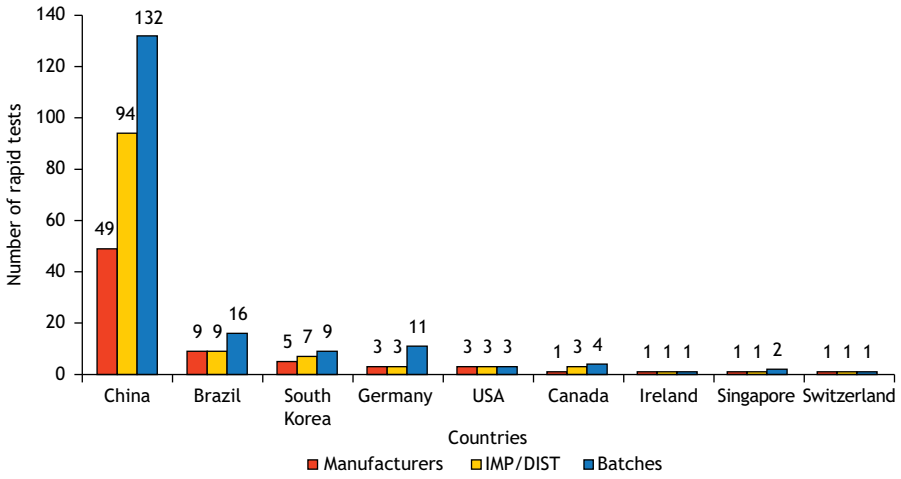
As for the type of analysis, samples were received under three modalities: control analysis, inspection analysis, and forensic counterproof analysis[23,24].

The forensic counterproof analysis is previously scheduled with Anvisa and the Sanitary Surveillance that collected the product. Subsequently, the company is notified, and the analysis is carried out in accordance with the following rite recommended by current legislation: analytical procedure strictly similar to that carried out in the inspection analysis and preparation of minutes containing all the information relevant to this activity, in front of the representatives appointed by the company, to witness this forensics[24]. The result of the forensic counterproof goes directly to the local Sanitary Surveillance and Anvisa, for the appropriate administrative and sanitary measures[25].

The 178 batches sent for analysis were distributed as follows: 149 (84%) batches intended for control analysis, in compliance with the public call of the Ministry of Health and RDC No. 379/2020;

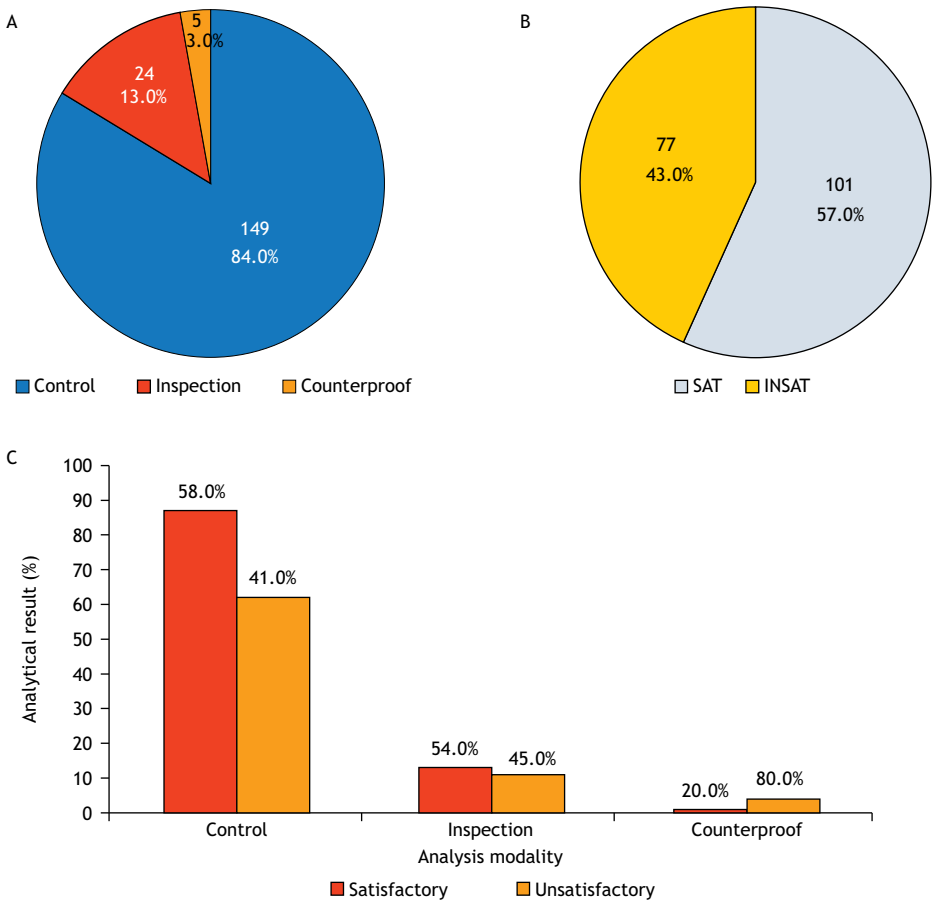
24 (19%) batches collected for inspection analysis, in compliance with Law No. 6,437/1977 and five (3%) batches destined for the forensic counterproof[23,24,26] (Figure 4A).

The sensitization of the solid phase of the test, nitrocellulose strip, was performed as follows: 100 (56.3%) batches of RT were sensitized with anti-human IgM/IgG antibodies, followed



Source: Laboratory of Blood and Hemoderivatives, 2020.
IMP: importers; DIST: distributors.

Figure 3. Demonstration of the importation and/or commercialization of rapid tests for COVID-19.



Source: Laboratory of Blood and Hemoderivatives, 2020.

Figure 4. Distribution of the analysis modality and results obtained. (A) Distribution of samples received by analysis modality; (B) Analytical results obtained; (C) Analytical result by analysis modality.

by 36 (20.2%) batches sensitized with anti-IgM/IgG monoclonal antibodies; 33 (18.5%) sensitized with SARS-CoV-2 recombinant antigen, and nine (5.0%) batches with SARS-CoV-2 specific recombinant proteins.

As for the instructions for use, 142 (80.0%) batches of products were translated into Portuguese and 36 (20.0%) were still in English. However, it is worth noting that most of the instructions for use were translated into Portuguese in a precarious manner, making it difficult to understand the procedure and the performance of the products. A rigid analysis of the instructions for use will be the subject of a specific article.

The analysis matrix was also evaluated. Of the 178 batches of products analyzed, 165 (92.7%) declared serum or plasma or blood by finger puncture as the analysis matrix, in the instructions for use, and 13 (7.3%) only accept human serum and plasma.

Of the 178 batches received for analysis, 17,800 tests were performed. Of these, approximately 4,900 tests used positive samples and 12,900 tests used negative and interfering samples. Among these 178 lots of RTs received for analysis, five (2.8%) corresponded to kits linked to strip reading equipment, which are intended to eliminate the bias of visual reading of the tests, and 173 (97.2%) maintained visual reading.

In the analysis of the technical performance of the cassettes, the defects found during the analysis were addressed and, in this case, 500 tests referring to cassettes with reading by equipment were excluded. Therefore, in this evaluation, the sampling was performed in 17,300 tests and in approximately 12,543 tests negative samples were used and in 4,757 tests positive samples were used.

Of the 17,300 cassettes analyzed, 13,512 cassettes showed no defects and 3,788 cassettes showed defects such as: 125 invalid; 133 spotted; 37 with the strips offset from the display; 815 with faulty control-line marking; 394 with very weak, almost imperceptible control-line marking; 2,284 with test-line staining for COVID-19 IgM or IgG very weak, almost imperceptible. Very weak test-line markings imply the appearance of false negative results and such defects found confirm the final analytical result.

Among the 178 RT batches analyzed, the following results were obtained: 101 (57.0%) batches obtained satisfactory results and 77 (43.0%) obtained unsatisfactory results, when the results were compared with the values assigned to the sensitivity and specificity attributes declared by the manufacturer, in the instructions for use (Figure 4B).

When evaluating the analytical result against the different modalities of analysis, the following results were observed: of the 149 batches of control analysis, 87 (58.3%) obtained satisfactory results and 62 (41.7%), unsatisfactory. As for the inspection analysis, 24 batches were analyzed, with the following results: 13 (54.2%) had satisfactory results and 11 (45.8%) had unsatisfactory results. The unsatisfactory results obtained in samples collected for inspection analysis imply the recourse of counterproof forensic, filed by the importing or distributing company, therefore, five (2.80%) samples were object of the forensic counterproof and obtained the following results: one (20.0%) sample was considered satisfactory and four (80.0%) samples had an unsatisfactory result when compared to the values declared by the manufacturer for sensitivity and specificity in the instructions for use (Figure 4C).

The satisfactory analytical result in the analysis of the forensic counterproof corresponded to the company that submitted an alteration of the information in the technical dossier to the Management of Diagnostic Products of Anvisa's General Management of Technology and Health Products to present new studies of product performance, increasing the sample size, presenting new values for the attributes of sensitivity and specificity, and also including the 95% confidence interval.

This change in the sensitivity and specificity values declared in the instruction for use provided the approval of the results when compared to the updated values. The products that obtained unsatisfactory results, in the forensic counterproof, were directly sent to the Health Surveillance that collected the samples and to Anvisa, so that the appropriate measures, provided for in Law No. 6,437/1977, could be taken. It should be noted that these actions are the pillars of the Sanitary Surveillance of products.

From the satisfactory results of the attributes of sensitivity and specificity, the distribution of the frequency of the values obtained, grouped as follows:

1. Sensitivity: 69 (68.3%) results in the range of 80% to 95%; 27 (26.7%) results in the range of 95.1% to 99.9%, and five (5.0%) in 100%;

2. Specificity: five (5.0%) results in the range of 80% to 95%; 72 (71.3%) in the range of 95.1% to 99.9%, and 24 (23.7%) in 100%.

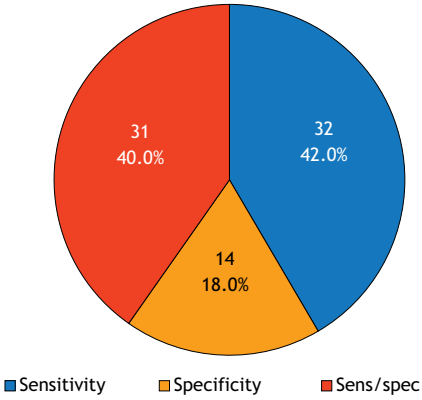
By observing these results, it is possible to infer that the sensitivity attribute range was from 80% to 95%, represented by 69 results of the analyzed RTs. This percentage mainly implies the type of sensitization of the solid phase of the product, as well as the seroconversion period, not yet fully defined.

When analyzing the specificity, as we found 72 (71.3%) RT with results in the range of 95.1% to 99.9%, in addition to 24 (23.7%) with 100% specificity, we can observe in this sample that the analyzed products were more specific than sensitive[29].

The unsatisfactory results represented 77 (43.0%) RT batches, which showed: 32 (42.0%) batches were unsatisfactory for sensitivity; 14 (18.0%) batches with an unsatisfactory result for specificity, and 32 (40.0%) batches with unsatisfactory results for sensitivity and specificity. These results are justified when compared to the technical defects of the cassettes found during the analysis, such as, for example, very weak marking of the test line, implying false negative results (Figure 5).

CONCLUSIONS

RTs, due to their applicability, simplicity, and scope, are tools widely used in the serological diagnosis of COVID-19.



Source: Laboratory of Blood and Hemoderivatives, 2020.
Sens: sensitivity; Spec: specificity.

Figure 5. Distribution of unsatisfactory analytical results.

However, in the acquisition of such products, the specification of the sensitivity parameters and the specificity declared in the instructions for use still support the national and international market. Aiming to control the quality of the products offered in the national market, the parameters of sensitivity and diagnostic specificity of the RTs used in the serological diagnosis of COVID-19 were evaluated, as part of the import process, by exceptionality, in compliance with RDC No. 379/2020. Of the 178 RTs received for analysis, 101 showed satisfactory results for the sensitivity and specificity parameters when compared to the specification stated in the instructions for use accompanying the product. The RTs that obtained unsatisfactory results were not distributed in the national market.

In view of the analytical results obtained, the need for constant monitoring of the quality of products for the diagnosis of COVID-19 is evident, with the primary purpose of guaranteeing the quality of the products marketed in the country, one of the pillars of the actions of the National Health Surveillance System, and a contribution to the country's public health, during a pandemic.

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Author's Contributions

Adati MC, Ribeiro AS, Borges HCBG - Conception, planning (study design), data interpretation, and writing of the work. Cirilo CA, Vigo DC, Passo DCD, Macedo GPS, Castro JRN, Teixeira LV, Silva MM, Guimarães PCM, Maria RIF, Maia RM, Passo RM, Cunha RS, Mendonça VF, Ribeiro YR - Planning (study design) and data acquisition. Araújo ACMM, Silva JG, Aquino NC - Planning (study design) and reviewing 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.