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Evaluation of eleven immunochromatographic assays for SARS-CoV-2 detection: investigating the dengue cross-reaction

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ABSTRACT

COVID-19 disease is spread worldwide and diagnostic techniques have been studied in order to contain the pandemic. Immunochromatographic (IC) assays are feasible and a low-cost alternative especially in low and middle-income countries, which lack structure to perform certain diagnostic techniques. Here we evaluate the sensitivity and specificity of eleven different IC tests in 145 serum samples from confirmed cases of COVID-19 using RT-PCR and 100 negative serum samples from blood donors collected in February 2019. We also evaluated the cross-reactivity with dengue using 20 serum samples from patients with confirmed diagnosis for dengue collected in early 2019 through four different tests. We found high sensitivity (92%), specificity (100%) and an almost perfect agreement (Kappa 0.92) of IC assay, especially when we evaluated IgG and IgM combined after 10 days from the onset of symptoms with RT-PCR. However, we detected cross-reactivity between dengue and COVID-19 mainly with IgM antibodies (5 to 20% of cross-reaction) and demonstrated the need for better studies about diagnostic techniques for these diseases.

KEYWORDS: IC assays. COVID-19. Dengue. Serology.

INTRODUCTION

COVID-19 (Coronavirus disease 2019) is a human infectious disease caused by a new betacoronavirus SARS-Cov-2 or 2019-nCoV (Severe acute respiratory syndrome coronavirus 2), firstly reported in China with flu-like symptoms (December 26th, 2019) and now spread worldwide, affecting more than 522 million people globally according to the World Health Organization (May 22nd, 2022)[1-5].

The COVID-19 diagnosis is based on clinical and epidemiological features, image exams, and the analysis of nucleic acids through reverse-transcription polymerase chain reaction (RT-PCR), established as the gold standard for the COVID-19 disease[1,2,6]. However, the accuracy of this method depends on the viral load, on the collection site and on the time of symptom onset[7]. It presents limitations such as incorrect collection and processing of samples, the need of expensive equipment and reagents, trained operators, and delays for releasing the results[1,2,6,8].

Due to the false negative results of RT-PCR, the Chinese authorities recommended CT scans as a complementary parameter for the COVID-19 diagnosis[7,9]. However, CT scan diagnostics are not specific to COVID-19, in addition to being expensive[9]. Due to the rapid spread of the virus, the World Health Organization suggested that priority had to be given to Point of Care technologies[9].

The immunochromatographic (IC) assay is a feasible and low-cost alternative for epidemiological purposes; in this case, monitoring the spread of COVID-19 in the general population, especially in low and middle-income countries, which lack structure to perform certain diagnostic techniques. The evaluation of vaccination status, contact tracing, population survey including health workers, teachers, and students upon the resuming of classes are situations that may benefit from the use of serology, since the sensitivity of RT-PCR among the asymptomatic is low, ranging from 8 to 10%[1,2].

According to a meta-analysis about serological immunoassays (enzyme-linked immunosorbent assay, IC assay, and chemiluminescence immunoassay), the overall sensitivity and specificity for IgG evaluation was 85% and 99%; for IgM, 74% and 99%; and for evaluation of both immunoglobulins combined, 86% and 99%, respectively[10]. However, it is known that the stage of the disease interferes with these numbers.

Imai *et al.*[11] described a sensitivity of 17%, 33%, and 100% for samples collected within 1 week of symptoms, 1 to 2 weeks of symptoms, and more than 2 weeks after the symptom onset, respectively, using IC assays. Different studies corroborate this information, evidencing better sensitivity of IC assays when using samples collected after 10 days from the onset of symptoms, especially on the 15th day post-infection[12].

Like COVID-19, dengue is also an emerging disease, especially in tropical and subtropical countries, transmitted by the *Aedes aegypti* mosquito. Both diseases are similar in their clinical and laboratory features, which hampers the diagnosis[13-15]. Another matter is the temporal dynamic of both diseases in Brazil. The spread of dengue usually increases between March and April due to the rainy season, the same period when respiratory diseases are most common, and a period in which the number of COVID-19 confirmed cases started to rise[15].

Due to the high number of IC assays available and the readiness to perform the diagnosis pertaining the advantages involving their use, we evaluated the sensitivity and specificity of eleven different IC assays in serum samples from confirmed cases of COVID-19 through RT-PCR, and negative serum samples from blood donors collected in February 2019. Considering the endemic situation of dengue in Brazil, we also evaluated the cross-reactivity with dengue using serum samples from patients with confirmed diagnosis for dengue collected in early 2019 using four different tests.

MATERIALS AND METHODS

Study design

This is a prospective multicenter study of COVID-19 hospitalized patients in two Brazilian Hospitals: Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo (HC-FMUSP), a public teaching hospital with 2,000 beds; and Hospital Sirio-Libanes (HSL), a private 400-bed hospital. Both hospitals are located in Sao Paulo State.

Ethical approval

This study was approved by the Brazilian national ethics review board (CONEP), protocol Nº 30701920200000068.

Sample collection

The positive serum samples used in this study were collected between March 5th and March 24th, 2020 at HSL and HC-FMUSP, from 121 symptomatic patients with confirmed COVID-19 diagnosis through RT-PCR[11] before the 10th day of symptoms; additionally, 24 samples collected from patients with more than 10 days of symptoms were also evaluated. The serum samples were stored at -20 °C until the time for testing. In addition, 100 serum samples collected in February 2019 from blood donors at Fundacao Pro-Sangue – Hemocentro de Sao Paulo (Sao Paulo State, Brazil) were used as negative controls for experiments. These samples were also stored at -20 °C until the time for testing. To evaluate the cross reactivity with dengue, we analyzed 20 samples collected from patients with confirmed diagnosis for dengue using the ELISA technique between February and March/2019.

Immunochromatographic assays for antibodies against SARS-CoV-2

In this study, positive and negative samples from Brazilian subjects were evaluated through eleven qualitative IC assays performed according to the manufacturer instructions summarized in Table 1. The samples were tested according to the number of kits available at the moment of evaluation. The following kits were used: Wondfo, China; Thermogenesis, China; Luxus, China; Camtech, Singapore; Bioclin, Brazil; TBG, Taiwan; Ecotest, Brazil; MedTest,

China; Lepu, China; Advagen, Brazil and MedNet Wuhan, China. The samples were considered positive when they demonstrated the presence of IgG or IgM antibody line in the addition of the control line after the incubation time. The samples that demonstrated only the control line after the incubation time were considered negative.

Cross-reactivity between dengue and COVID-19 serology

To evaluate the cross-reactivity of COVID-19 disease and dengue serology, 20 samples of confirmed dengue cases diagnosed through the ELISA technique in early 2019 were evaluated using four IC assays for detection of antibodies against SARS-CoV-2: Wondfo, Camtech, Advagen, and MedNet Wuhan, according to the manufacturer instructions. In addition, 40 samples from COVID-19 confirmed cases were evaluated using the PANBIO DENGUE IgM CAPTURE ELISA (Abbott, USA – batch 01P20E014). We also evaluated the cross-reactivity using IC assays for detection of antibodies against dengue in 33 samples of COVID-19 confirmed cases using the ALERE DENGUE DUO-NS1 IgG and IgM (Abbott, USA – batch 11DDE008A-A and 11DDE007A-A).

Statistical analysis

The validity of the tests was measured through sensitivity (true positive / true positive + false negative = %) and specificity (true negative / true negative + false positive = %) calculations[16]. The sensitivity and specificity calculations were performed based on the RT-PCR results. The concordance analysis was performed by Cohen's (two raters) and Fleiss' (three or more raters) Kappa methods comparing the agreement between the gold standard (RT-PCR) and each one of the eleven tested IC assays; the confidence interval was of 95%[17]. The Kappa's interpretation was performed according to that described by Landis and Koch: < 0 – Poor agreement; 0.01-0.20 – Slight agreement; 0.21-0.40 – Fair agreement; 0.41-0.60 – Moderate agreement; 0.61-0.80 – Substantial agreement; 0.81-1.00 – Almost perfect agreement[18].

RESULTS

Altogether, 245 serum samples were collected for this study; among them, 145 samples were collected from confirmed COVID-19 patients through RT-PCR, 121 of these patients' samples were collected before the 10th day

Table 1 - Instructions of eleven immunochromatographic (colloidal gold) tests.

Label	Test name/Batch	Manufacturer country	Detection	Sample quantity	Amount of Reagent	Incubation period
Advagen	Kit COVID-19 IgG/IgM LF (L20183-02)	Brazil	IgG/IgM	10 µL	40 µl	15 min
Bioclin	Rapid test COVID-19 IgG/IgM BIO (0010)	Brazil	IgG/IgM	10 µL	2 drops (60~80 µL)	10 ~ 15 min
CamTech	COVID-19 IgM/IgG Rapid Test Kit (NF3170)	Singapore	IgG/IgM	10 µL	2 drops (60 µL)	15 min
Ecotest	COVID-19 IgG/IgM ECO TEST (202005043)	Brazil	IgG/IgM	10 µL	3 drops (90 µL)	10 ~ 15 min
Lepu	SARS-Cov-2 antibody detection test (20CG2518X)	China	IgG/IgM	10 µL	2 drops (80 µL)	10 ~ 20 min
Luxus	SARS-COV-2 IgM/IgG Antibody Test Kit (SYG202010)	China	IgG/IgM	20 µL	80 µl	3 min
MedNet Wuhan	COVID-19 (SARS-CoV-2) IgM/IgG antibody Test kit (20030501)	China	IgG/IgM	10 µL	2 drops (70 µL)	15 min
MedTest	MedTest Coronavirus (COVID) IgG/IgM (COV20030081)	China	IgG/IgM	10 µL	2 drops (80 µL)	10 min
TBG	SARS-CoV-2 IgG / IgM Rapid Test Kit (FRS20041K)	Taiwan	IgG/IgM	10 µL	2 drops	15 min
Thermogenesis	SARS-COV-2 (COVID-19) IgG/IgM Antibody Fast Detection (SYG202010)	China	IgG/IgM	20 µL	80 µL	3 min
Wondfo	One Step COVID-19 test (W19500341)	China	Total IG	10 µL	2~3 drops (80 µL)	15 ~ 20 min

of symptoms onset, and 24 samples were collected after 10 days from the beginning of symptoms. In addition, 100 samples collected from blood donors in February 2019 were used as negative control. The sensitivity and specificity as well as agreement ratio of eleven commercial qualitative IC assays were evaluated; the results were obtained through tests performed between May 2020 and July 2020.

Sensitivity of IC assay

A panel containing 121 positive samples for COVID-19 through RT-PCR and 100 negative samples was used to evaluate the sensitivity of eleven IC tests. As summarized in Table 2, among the tests, ten of them separately described the detection of IgG and IgM antibodies, and one of them, Wondfo (China), the presence of total immunoglobulins.

The greater sensitivity for detection of only IgG antibody was observed in the TBG (Taiwan) IC test at 93%, followed by Ecotest (Brazil) and Lepu (China) at 90%; Bioclin (Brazil) at 82%; MedTest (China) and Thermogenesis (China) at 80%; MedNet (China) at 78%; and Advagen (Brazil) and Luxus (China) at 75%. The Camtech (Singapore) test presented the lower sensitivity among the tests at 70%.

The sensitivity for detection of only the IgM antibody was also evaluated, the best result was achieved by MedTest (China) at 93%, followed by Ecotest (Brazil) and Lepu (China) at 90%; Camtech (Singapore), 88%; TBG (Taiwan), Thermogenesis (China), and MedNet (China), 80%; Luxus (China), 73%; Bioclin (Brazil), 61%; and finally, Advagen (Brazil) at 36%. The evaluation of total immunoglobulins presented a sensitivity of 76% as observed in the Wondfo test (China).

Specificity of IC assay

The assay specificity was evaluated according to Table 2. In IgG IC assays, the specificity was equal (100%) in all tests except for MedNet (China) at 98%. In the evaluation of IgM detection, the assays sensitivity was 100% for Ecotest (Brazil), Lepu (China), Luxus (China), MedTest (China), TBG (Taiwan), and Thermogenesis (China). Bioclin (Brazil) and Camtech achieved a specificity of 98%, Advagen (Brazil) at 97%, and MedNet (China) at

Table 2 - Quality measurements of immunochromatographic assays.

Label	Detection	Nº of positive samples (RT-PCR)	Nº of positive detected	Nº of negative samples (RT-PCR)	Nº of negative detected	Sensitivity	Specificity	Cohen's Kappa
Advagen	IgG	121	91	59	59	75%	100%	0.67
Advagen	IgM	121	43	59	57	36%	97%	0.24
Bioclin	IgG	49	40	50	50	82%	100%	0.82
Bioclin	IgM	49	30	50	49	61%	98%	0.59
CamTech	IgG	40	28	40	40	70%	100%	0.70
CamTech	IgM	40	35	40	39	88%	98%	0.85
Ecotest	IgG	20	18	20	20	90%	100%	0.90
Ecotest	IgM	20	18	20	20	90%	100%	0.90
Lepu	IgG	10	9	10	10	90%	100%	0.90
Lepu	IgM	10	9	10	10	90%	100%	0.90
Luxus	IgG	40	30	40	40	75%	100%	0.75
Luxus	IgM	40	29	40	40	73%	100%	0.73
MedNet Wuhan	IgG	40	31	40	39	78%	98%	0.75
MedNet Wuhan	IgM	40	32	40	38	80%	95%	0.75
MedTest	IgG	15	12	10	10	80%	100%	0.76
MedTest	IgM	15	14	10	10	93%	100%	0.92
TBG	IgG	15	14	10	10	93%	100%	0.92
TBG	IgM	15	12	10	10	80%	100%	0.76
Thermogenesis	IgG	40	32	40	40	80%	100%	0,80
Thermogenesis	IgM	40	32	40	40	80%	100%	0,80
Wondfo	Total IG	74	56	100	100	76%	100%	0.78

95%. The evaluation of total immunoglobulins presented a specificity of 100% for Wondfo (China).

Agreement rate

To evaluate the agreement among the tests and the gold standard RT-PCR, we analyzed the Cohen's kappa value described in Table 2. Among the evaluated tests for detection of IgG antibody, four of them presented an almost perfect agreement level (0.81-1.00): TBG (Taiwan) 0.92, Lepu (China) and Ecotest (Brazil) 0.90, and finally, Bioclin (Brazil) 0.82; followed by a substantial agreement (0.61-0.80) of Thermogenesis (China) 0.80, MedTest (China) 0.76, Luxus (China), and MedNet (China) 0.75, Camtech (Singapore) and Advagen, 0.70 and 0.67, respectively.

The agreement of IgM detection tests was almost perfect for three of the evaluated tests: Ecotest (Brazil) and Lepu (China) 0.90, followed by Camtech (Singapore) 0.85. Four tests presented a substantial agreement (0.61-0.80): Thermogenesis (China) 0.80, TBG (Taiwan) 0.76, MedNet (China) 0.75, and Luxus (China) 0.73. Bioclin (Brazil) 0.59 demonstrated a moderate agreement (0.41-0.60) followed by a fair agreement (0.21-0.40) of Advagen (Brazil) 0.24.

Evaluation according to the stage of the disease

Considering the temporal dynamic of antibodies, we evaluated the sensitivity of tests in samples from patients collected more than 10 days after symptom onset according to Table 3. The sensitivity observed for IgG assays was 92% in MedNet (China) and 79% in Bioclin (Brazil). Evaluating the agreement ratio between MedNet (China) and Bioclin (Brazil) compared to RT-PCR, the Kappa value of 0.92 and 0.79 was obtained, respectively. For IgM assays, the MedNet (China) sensitivity was 92% while Bioclin (Brazil) was 71%; the Kappa value agreement ratio obtained between MedNet (China) and RT-PCR was of 0.92, and Bioclin (Brazil), 0.71.

Fleiss' Kappa analysis was performed considering the results of combined immunoglobulins, total IG, and RT-PCR; Cohen's Kappa evaluation of assays was also performed individually against RT-PCR, obtaining an almost perfect result and the sensitivity of 92%, 92% and 83% for Wondfo, MedNet and Bioclin respectively.

We compared the sensitivity of assays in Table 4 using samples collected before and after the 10th day of symptoms. According to our results, all the assays evaluated presented a better sensitivity, except Bioclin (Brazil) in detection of IgG, which evidenced a lower sensitivity after the 10th day of symptoms (79%) than before the 10th day of symptoms (82%).

Cross-reactivity between dengue and COVID-19 detection

Forty samples of COVID-19 confirmed cases were evaluated using tests for detection of antibodies against

Table 3 - Evaluation of samples collected more than 10 days after the beginning of symptoms.

Label	Detection	Nº of positive samples (RT-PCR)	Nº of positive detected	Sensitivity	Specificity	Cohen's Kappa
Bioclin	IgG	24	19	79%	100%	0.79
MedNet Wuhan	IgG	24	22	92%	100%	0.92
Bioclin	IgM	24	17	71%	100%	0.71
MedNet Wuhan	IgM	24	22	92%	100%	0.92
Bioclin	IgG and IgM	24	20	83%	100%	0.83
MedNet Wuhan	IgG and IgM	24	22	92%	100%	0.92
Wondfo	Total IG	24	22	92%	100%	0.92

Table 4 - Comparison of IC assays' sensitivity before and after 10 days of symptoms.

Label	Detection	Nº of positive samples (<10 days)	Sensitivity	Nº of positive samples (>10 days)	Sensitivity
Bioclin	IgG	49	82%	24	79%
Bioclin	IgM	49	61%	24	71%
MedNet Wuhan	IgG	40	78%	24	92%
MedNet Wuhan	IgM	40	80%	24	92%
Wondfo	Total IG	74	76%	24	92%

Table 5 - Evaluation of cross-reactivity between Dengue and COVID-19.

Assay	Label	Detection	Nº of samples	Specificity
Dengue	PANBIO DENGUE ELISA	IgM	40	100%
Dengue	NS1 Dengue	IgG	33	100%
Dengue	NS1 Dengue	IgM	33	100%
COVID-19	Advagen	IgG	20	100%
COVID-19	Advagen	IgM	20	85%
COVID-19	Camtech	IgG	20	100%
COVID-19	Camtech	IgM	20	85%
COVID-19	MedNet	IgG	20	95%
COVID-19	MedNet	IgM	20	80%
COVID-19	Wondfo	Total IG	20	95%

dengue; the ELISA PANBIO DENGUE CAPTURE ELISA (Abbott, USA) achieved 100% specificity. The IC assay ALERE DENGUE DUO-NS1 IgG and IgM (Abbott, USA) demonstrated 100% specificity for both IgM and IgG antibodies using 33 samples of COVID-19 patients.

In the analysis of immunochromatographic tests for COVID-19 diagnosis, Advagen (Brazil) and Camtech (Singapore) reached 100% specificity for IgG antibody detection using 20 dengue samples while MedNet (China) achieved 95% (19/20). For IgM detection, the specificity was 85% (17/20) for Advagen (Brazil) and Camtech (Singapore); MedNet (China), 80% (16/20); while Wondfo, 95% (19/20) for detection of total antibodies, as described in Table 5.

DISCUSSION

In this study, we evaluated eleven IC colloidal gold qualitative-based assays and analyzed the quality measurements of each one of them. According to our results, most of the IC assays provide high sensitivity and specificity compared to the gold standard RT-PCR, especially after 10 days since the disease onset, with an excellent agreement ratio compared to the gold standard for evaluating total antibodies against SARS-CoV-2.

The COVID-19 pandemic impacted the world health system in an unprecedented way. In Brazil, the first reported case occurred on February 27th, 2020, and since then more than 1 million Brazilian individuals tested positive for COVID-19[4,19]. Besides COVID-19, Brazil also faces an endemic situation regarding dengue, and considering the importance of both diseases, we evaluated the cross-reactivity of dengue and COVID-19 using different tests.

According to our results, the specificity in detection of IgG antibodies was greater in comparison to IgM detection (5 to 20% of cross-reaction) when using IC assays for detection of COVID-19 in dengue patients' samples. It is widely known that, before the high-affinity response by IgG antibodies to pathogens, the first defense provided by organisms occurs through IgM molecules[20]. Our results suggest that the evaluation of total antibodies (IgG and IgM) is a useful tool to broaden the range of detection, enabling the evaluation of acute phase through IgM detection and convalescent phase by the presence of IgG. The evaluation of combined antibodies helps not only the discrimination of dengue and COVID-19 but to also avoid false negative results for COVID-19, and contributes to the establishment of control measures. On the other hand, the use of IC assays that detect IgM may have cross-reaction with dengue and lead to a false positive result that might impact the clinical management of the patient.

Immunochromatographic is an easy and affordable method for diagnosis, enabling prompt results on a large scale. According to our results, the agreement ratio for detection of only IgG antibodies before the 10th day of symptoms using IC technique in comparison with RT-PCR was substantial in most used tests (70%), followed by an almost perfect agreement (30%).

In contrast, for only IgM detection, the agreement ratio between IC and RT-PCR was almost perfect in 40% of tests, followed by substantial agreement in 40%. The literature suggests that any Kappa below 0.60 represents little confidence and is not adequate[17]; among our results, 20% of tests presented a Kappa value below 0.60.

The evaluation of total antibodies using the Wondfo test demonstrated better results using samples collected after the 10th day of symptoms (almost perfect agreement) than before this period (substantial agreement). According to the literature, it is possible to find the detection of immunoglobulin on the 5th day from the disease onset. However, this detection is greater from the 8th day onwards[21]. Our results are in accordance with the literature,

demonstrating an increase in sensitivity levels especially using the combined assessment of IgG and IgM antibodies.

Different studies have been performed aiming to evaluate the efficacy of IC assays. Rivera-Olivero *et al.*[22] described a sensitivity and specificity of 79.4% and 100% for IgG, and 67.6% and 97.5% for IgM, using samples collected in the 15th day of symptoms and tested with Camtech assay. Another study using Lepu assay in 286 nasopharyngeal swabs samples demonstrated an overall sensitivity of 89.2%[23]. The TBG assay reached a sensitivity and specificity of 99.8%, using samples after the 15th day of symptoms[24]. The Wondfo and Bioclin assays were evaluated by Conte and colleagues in finger prick samples derived from Brazilian healthcare workers, the sensitivity was 47.62% and 85.7%, respectively[25]. Another Brazilian study that evaluated different IC assays reported a sensitivity of 81.71% and 84.15%, and specificity of 78.38% and 100% for Ecotest and Medtest, respectively[26]. Changes in the sensitivity and specificity rate varies according to study, and can be affected by the period of infection and collection date or type of sample used.

Our study has limitations, such as sample size; however, to our knowledge, the analysis of SARS-CoV-2 IC tests and their cross-reaction with dengue in the Brazilian population is extremely important considering the proportion of both diseases in the country. In 2019, Brazil had more than 2 million possible dengue cases[27]. In 2020, there was a decrease in the number of cases, totaling 979,764 of confirmed cases[28]. Studies in endemic dengue Asian countries demonstrated a serological cross-reaction between the dengue virus and SARS-CoV-2, especially when using rapid serology-based tests, resulting in misdiagnosis and uncertainties in the numbers of cases for both diseases, COVID-19 and dengue[14,29,30].

Thus, our findings can be useful in countries with high prevalence of dengue, to alert to the possible cross-reactivity of the SARS-CoV-2 IC assays, mainly IgM, with dengue antibodies.

Limitations of the study

In this study, only a small number of samples were tested due to the limited availability of tests at the beginning of the COVID-19 pandemic in Brazil. Studies with more samples are needed to ensure the validity of the results. Another limitation is the cross-reactivity with different diseases. During assay evaluations, there were no other SARS-CoV-2 variants described within the Brazilian population and also no vaccines available for population[31]; however, further studies about sensitivity and specificity of assays considering different SARS-CoV-2 variants and other respiratory diseases (influenza, SARS, and MERS) should be performed.

CONCLUSION

In general, we found high sensitivity and specificity and good agreement of IC assays, especially after 10 days from the symptoms onset. Our results also evidenced the importance of evaluating total immunoglobulins to increase sensitivity and specificity of IC assays. On the other hand, we detected cross-reactivity between dengue and COVID-19, and demonstrated the need for better studies and improvements in diagnostic techniques for these diseases.

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