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Short communication

Performance of a qualitative rapid chromatographic immunoassay to diagnose COVID-19 in **patients** in a middle-income country

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ABSTRACT

Objectives: We evaluated a rapid chromatographic immunoassay (IgG/IgM antibodies) and an ELISA assay to diagnose COVID-19 in **patient s**at two Brazilian hospitals.
Methods: A total of 122 subjects with COVID-19 were included: 106 SARS-COV-2 RT-PCR-positive patients and 16 RT-PCR-negative patients with symptoms and chest computed tomography (CT) consistent with COVID-19. Ninety-six historical blood donation samples were used as controls. Demographic and clinical characteristics were retrieved from electronic records. Sensitivity and specificity were calculated, as were their 95% binomial confidence intervals using the Clopper-Pearson method. All analyses were performed in R version 3.6.3.
Results: The sensitivity of the chromatographic immunoassay in all RT-PCR-positive **patients**, irrespective of the timing of symptom onset, was 85.8% (95% binomial CI 77.7% to 91.9%). This increased with time after symptom onset, and at >14 days was 94.9% (85.9% to 98.9%). The specificity was 100% (96.4% to 100%). 15/16 (94%) RT- PCR-negative cases tested positive. The most frequent comorbidities were hypertension and diabetes mellitus and the most frequent symptoms were fever, cough, and dyspnea. All RT-PCR-negative patients had pneumonia. The most frequent thoracic CT findings were ground glass changes (n = 11, 68%), which were bilateral in 9 (56%) patients, and diffuse reticulonodular infiltrates (n = 5, 31%).
Conclusions: The COVID-19 rapid chromatographic immunoassay evaluated in this study had a high sensitivity and specificity using plasma, particularly after 14 days from symptom onset. ELISA and qualitative rapid chromatographic immunoassays can be used for the diagnosis of RT-PCR-negative patients.

1. Background

Brazil is the epicenter of the COVID-19 pandemic in Latin America. As of 29th May 2020, there had been 555,383 confirmed cases and 31,199 deaths [1–2]. There is little data on the use of serology assays for the investigation of patients with COVID-19 in Brazil [3]. The current gold standard for COVID-19 diagnosis is real-time reverse transcription polymerase chain reaction (RT-PCR)[4–5]. The accuracy of this method depends on the viral load at the collection site and on time from symptom onset [4–6].

Detection of antibodies, especially IgA and IgM, which are produced early after the onset of infection, may be an important tool when

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combined with RT-PCR to improve sensitivity and diagnostic accuracy [5–10]. Several qualitative rapid chromatographic immunoassays (RCI) have been developed and used in different countries. However, the test characteristics vary widely, with reported sensitivities ranging from 20% to 93%[11–12].

The aim of this study was to evaluate an RCI (Wondfo-China) to diagnose COVID-19 in inpatients in two Brazilian hospitals. We further compared the results with those obtained with an ELISA (Euroimmun – Germany).

2. METHODS

2.1. Study design

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

2.2. Patient population

We included a group of hospitalized patients and healthcare workers (not requiring hospitalization) with a positive SARS-CoV-2 RT-PCR, as well as a group of patients with negative RT-PCR but a clinical COVID-19 diagnosis based on highly suggestive symptoms and chest computed tomography (CT) findings. Demographic and clinical characteristics – including age, sex, comorbidities and presenting symptoms – were retrieved from electronic health records. The database was built using the Epi Info software (CDC, Atlanta, GA).

3. RT-PCR

Respiratory samples were obtained from both the nasopharynx and oropharynx using rayon swabs. RNA was extracted from clinical samples with an automated method using magnetic beads (mSample Preparation System RNA, Abbott, Illinois, USA). SARS-CoV-2 RNA reverse transcription, amplification, and detection were performed using an adapted protocol, as described elsewhere [11–12]. An assay detecting the E gene was used as the first-line screening tool, followed by confirmatory testing with an assay detecting the N gene.

3.1. Serology

We tested all patient and control samples using an ELISA (Euroimmun-Lübeck, Germany) that detects anti-SARS-CoV-2 IgA and IgG antibodies, as well as an RCI (Wondfo-China) that detects anti-SARS-CoV-2 IgG/IgM.

3.2. ELISA assay

The ELISA assays, which detect anti-SARS-CoV-2 S1 IgG and IgA, were performed according to the manufacturer's protocol. We detected optical density (OD) at 450 nm and calculated a ratio of the reading of each sample to the reading of the calibrator (included in the kit). Results were interpreted according to the manufacturer's recommendation: a ratio <0.8 as negative, between 0.8 and 1.1 as borderline, and ≥ 1.1 as positive.

3.3. Rapid chromatographic immunoassay

The qualitative RCI was performed using 10 μL of serum or plasma, pipetted into the sample cavity of the test device. 2-3 drops of buffer solution (80 μL) were added to the cavity below the sample cavity. The result was read in 15 minutes by three people that had received appropriate training. The color change was compared to the assay standard.

Table 1
Demographic and clinical characteristic of 122 subjects: 75 COVID-19 patients (59 RT-PCR positive, 16 RT-PCR negative) from two Brazilian hospitals and 47 health care workers with RT-PCR-confirmed COVID-19

Patient characteristics	RT-PCR positive inpatients n=59 (%) **HSL**	RT-PCR positive outpatient health care workers N = 47(%)	RT-PCR negative inpatients n=16 (%) **HC-FMUSP**
Age (years), median (range)	61 (32-90)	44 (21-62)	55 (36-77)
Sex			
Male	41 (70)	20 (43)	6 (38)
Female	18 (31)	27 (57)	10 (63)
Any comorbidity	44 (75)	NA	11 (69)
Specific comorbidities			
Diabetes mellitus			
Hypertension	15 (26)		3 (19)
Kidney	21 (36)		6 (38)
Transplantation	1 (1.6)		NA
Dyslipidemia	7 (12.1)		NA
Neurovascular disease	6 (10)		NA
Obesity	5 (8.6)		3 (19)
Cardiac disease	4 (6.9)		NA
Cancer	4 (6.9)		NA
Alcohol dependency	NA		2 (13)
Hypothyrodism	NA		1 (6)
Symptoms onset (days), mean (range)	10.7 (4-23)	32.0 (16-42)	8 (2-15)
Symptoms**			
Fever	34 (60)	27 (61)	13 (81)
Cough	38 (67)	35 (79)	16 (100)
Coryza	7 (12)	10 (23)	1 (6)
Sore Throat	6 (11)	16 (36)	1 (6)
Dyspnea	30 (53)	12 (27)	15 (94)
Myalgia	6 (11)	18 (41)	3 (19)
Asthenia	6 (11)	8 (18)	NA
Headache	4 (7)	27 (61)	2 (13)
GI symptoms*	5 (9)	17 (38)	3 (19)
Hemoptysis	3 (5)	NA	NA
Dysgeusia	1 (1.8)	2 (4.5)	2 (13)
Anosmia	NA	7 (15)	2(13)

*GI (gastrointestinal) symptoms: nausea, vomiting and diarrhoea. HSL: hospital Sírio-Libanês, HC-FMUSP: Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, NA: Not Applicable. Symptoms**: only 44 HCW reported their symptoms.

3.4. Statistical analysis

Specificity was calculated as the number of negative test results divided by the total number of negative samples tested. The sensitivity was the number of positive test results divided by the number of known-positive samples tested. 95% binomial confidence intervals were calculated using the Clopper-Pearson method. All analyses were performed in R version 3.6.3.

3.5. Ethical approval

This study was approved by the Brazilian national ethics review board (CONEP), protocol number 30701920200000068.

4. RESULTS

A total of 122 subjects with COVID-19 were evaluated, including 106 SARS-COV-2 RT-PCR-positive patients and 16 RT-PCR-negative patients with a clinical COVID-19 diagnosis. Fourteen of the 16 RT-PCR-negative patients had a second negative RT-PCR. Demographic and clinical characteristics are shown on Table 1. All RT-PCR-negative patients had pneumonia. The most frequent thoracic CT findings were ground glass changes (n = 11, 68%), which were bilateral in 9 (56%) patients, and diffuse reticulonodular infiltrates (n = 5, 31%). Six (38%) patients were

Table 2
Qualitative rapid chromatographic immunoassay results using peripheral blood among cases and controls and by time between symptom onset and blood collection

Rapid test result	Controls n = 100	All PCR-positive cases n = 106	PCR-positive cases (symptoms <14 days) n = 38	PCR-positive cases (symptoms ≥14 days) n = 59	PCR-positive cases (unknown symptom onset) n = 9	All PCR-negative cases n = 16
Positive	0(0)	91 (86)	27 (71)	56 (95)	8 (89)	15 (94)
Negative	100 (100)	15 (14)	11 (29)	3 (5)	1 (11)	1 (6)

intubated (Table 1).

The sensitivity of the RCI for all SARS-CoV-2 RT-PCR positive inpatients, regardless of time from symptom onset, was 85.8% (95% binomial CI 77.7% to 91.9%). Sensitivity increased with time from symptom onset. Among those with ≤14 days from symptom onset sensitivity was 71.1% (54.1% to 84.6%), versus 94.9% (85.9% to 98.9%) among those with >14 days from symptom onset. Among COVID-19 RT-PCR-negative patients the sensitivity of the RCI was 93.7 (69.8% to 99.8%) (Table 2). The specificity among historical (February 2019) blood donors was 100% (96.4% to 100%). The distribution of signal-to-cut off values for the ELISA assay (IgG and IgA) according to the RCI result is shown in the supplemental figure.

4.1. Discussion

This is the first multicenter study conducted in a middle-income country to validate a qualitative rapid chromatographic immunoassay for the diagnosis of COVID-19. Our findings show that the Wondfo RCI, when used with plasma or serum, has a high sensitivity and specificity, especially after 14 days from symptom onset. Furthermore, the performance of the rapid test was superior to that of RT-PCR, as it detected 15 of 16 PCR-negative cases. RT-PCR is not usually available in low-resource settings, which makes the rapid test (using plasma or serum) suitable as an alternative diagnostic modality. However, it is important to note that RCIs such as the Wondfo assay may have a lower sensitivity (23% to 90%) when used with capillary blood [3,9,10,15,16]

The Wondfo RCI detects SARS-CoV-2 IgG/IgM antibodies. Anti-SARS-CoV-2 antibodies present in the sample bind to recombinant antigens coated on colloidal gold particles and form an antigen-antibody/colloidal gold complex. Despite the high sensitivity using plasma or serum, a limitation of the assay is that it cannot differentiate acute disease from past infection.

Our study was conducted in patients with a wide spectrum of disease, including different age groups, periods from symptom onset and comorbidities. Our results show that serology assays are useful for diagnosing SARS-COV-2, even in PCR-negative patients where there is a strong suspicion of COVID-19 based on presenting symptoms and CT findings.

The Euroimmun ELISA has the advantage of being automated, and therefore can be performed on a large scale. Furthermore, it can differentiate the antibody class, such as IgA that becomes positive from the fifth symptomatic day [13–14]. In contrast, the Wondfo RCI – albeit with the disadvantage of having to use plasma or serum to achieve a good sensitivity – can be used in primary care with results available within 15 minutes. However, it is important to note that both tests are most sensitive after 14 days of symptoms.

4.2. Conclusion

We found a high sensitivity and specificity for the Wondfo RCI for the diagnosis of COVID-19 using plasma samples. Sensitivity was highest after 14 days from symptom onset. ELISA and qualitative rapid chromatographic immunoassays can also be used for the diagnosis of RT-PCR-negative patients.

Declaration of Competing Interest

The authors report no declarations of interest.

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CRediT authorship contribution statement

Silvia Figueiredo Costa: Conceptualization, Formal analysis, Resources, Writing - original draft, Writing - review & editing. **Lewis Buss:** Writing - original draft. **Evelyn Patrícia Sanchez Espinoza:** . **Jose Mauro Vieira:** Supervision. **Lea Campos de Oliveira da Silva:** Methodology. **Regina Maia de Souza:** Methodology. **Lauro Perdigão Neto:** Supervision. **Ana Paula Matos Porto:** Methodology. **Carolina Lazari:** Methodology. **Vera Aparecida dos Santos:** Methodology. **Alberto da Silva Duarte:** Formal analysis, Funding acquisition. **Ana Catharina Nastri:** Resources, Supervision. **Gabriel Fialkovitz da Costa Leite:** Supervision. **Erika Manuli:** Methodology. **Maura Salaroli de Oliveira:** Supervision. **Daniella Bosco Zampelli:** Supervision. **Laerte Pastore:** Supervision. **Aluísio Cotrim Segurado:** Conceptualization, Formal analysis, Funding acquisition. **Anna S. Levin:** Conceptualization, Formal analysis, Funding acquisition, Resources, Writing - review & editing. **Ester Sabino:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2020.104592.

References

[1] WHO situation report.
[2] Coronavirus disease 2019 (COVID-19) Situation Report - 136 2020, World Health Organization, 2020. Accessed June 4, 2020, https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200324-sitrep-64-covid-19.pdf?sfvrsn=703b2c40_2.
[3] V.A. dos Santos, M. Matias Rafael, E. Cerdeira Sabino, A.J. da Silva Duarte, Sensitivity of the Wondfo One Step COVID-19 test using serum samples, Clinics 75 (2020), e2013.
[4] N. Chen, M. Zhou, X. Dong, J. Qu, F. Gong, Y. Han, Y. Qiu, J. Wang, Y. Liu, Y. Wei, J. Xia, T. Yu, X. Zhang, L. Zhang, Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study, Lancet 395 (10223) (2020) 507–513.
[5] R. Patel, E. Babady, E.S. Theel, G.A. Storch, B.A. Pinsky, K. St George, T.C. Smith, S. Bertuzzi, Report from the American Society for Microbiology COVID-19 International Summit, 23 March 2020: Value of Diagnostic Testing for SARS-CoV-2/COVID-19, mBio 11 (2) (2020), e00722. March 26.
[6] W. Zhang, R. Du, B. Li, X. Zheng, X. Yang, B. Hu, Y. Wang, G. Xiao, B. Yan, Z. Shi, P. Zhou, Molecular and serological investigation of 2019-nCov infected patients:

implication of multiple shedding routes, Emerging Microbes & Infections 9 (2020) 386–389.
[7] L. Guo, L. Ren, S. Yang, M. Xiao, D. Chang, F. Yang, C.S. Dela Cruz, Y. Wang, C. Wu, Y. Xiao, L. Zhang, L. Han, S. Dang, Y. Xu, Q. Yang, S. Xu, H. Zhu, Y. Xu, Jin Qi, L. Sharma, L. Wang, J. Wang, Profiling early humoral response to diagnose novel coronavirus disease (COVID-19), Clin Infect Dis XX (XX) (2020) 1–8.
[8] J. Zhao, Q. Yuan, H. Wang, W. Liu, X. Liao, Y. Su, X. Wang, J. Yuan, T. Li, J. Li, S. Qian, C. Hong, F. Wang, Y. Liu, Z. Wang, Q. He, Z. Li, B. He, T. Zhang, Y. Fu, S. Ge, L. Liu, J. Zhang, N. Xia, Z. Zhang, Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019, Clinical Infect Dis (2020), ciaa344.
[9] Z. Li, Y. Yi, X. Luo, N. Xiong, Y. Liu, S. Li, R. Sun, Y. Wang, B. Hu, W. Chen, Y. Zhang, J. Wang, B. Huang, Y. Lin, J. Yang, W. Cai, X. Wang, J. Cheng, Z. Chen, K. Sun, W. Pan, Z. Zhan, L. Chen, F. Ye, Development and clinical application of a rapid IgM-IgG combined antibody test from SARS-CoV-2 infections diagnosis, J Med Virol (2020), https://doi.org/10.1002/jmv.25727.
[10] I. Cassaniti, F. Novazzi, F. Giardina, F. Salinaro, M. Sachs, S. Perlini, R. Bruno, F. Mojoli, F. Baldanti, Members of the San Matteo Pavia COVID-19 Task Force. Performance of VivaDiag COVID-19 IgM/IgG Rapid Test is inadequate for diagnosis of COVID-19 in acute patients referring to emergency room department, . Journal of Medical Virology (2020), https://doi.org/10.1002/jmv.25800.
[11] Accessed January 17, 2020. https://www.who.int/docs/defaultsource/coronaviruse/protocol-v21.pdf.
[12] V.M. Corman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D.K. Chu, T. Bleicker, S. Brünink, J. Schneider, M.L. Schmidt, D.G. Mulders, B.L. Haagmans, B. van der Veer, S. van den Brink, L. Wijsman, G. Goderski, J. Romette, J. Ellis, M. Zambon, M. Peiris, H. Goossens, C. Reusken, M.P. Koopmans, C. Drosten, Detection of 2019 novel coronavirus (2019- nCoV) by real-time RT-PCR, Euro Surveill (3) (2020), 2000045.
[13] N.M.A. Okba, M.A. Müller, W. Li, C. Wang, C.H. GeurtsvanKessel, V.M. Corman, M. M. Lamers, R.S. Sikkema, E. de Bruin, F.D. Chandler, Y. Yazdanpanah, Q. Le Hingrat, D. Descamps, N. Houhou-Fidouh, C.B. Reusken, B. Bosch, C. Drosten, M.P. G. Koopmans, B.L. Haagmans, Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease 2019 Patients, Emerg Infect Dis 26 (7) (2020).
[14] A. Padoan, L. Sciacovelli, D. Basso, D. Negrini, S. Zuin, C. Cosma, D. Faggian, P. Matricardi, M. Plebani, IgA-Ab response to spike glycoprotein of SARS-CoV-2 in patients with COVID-19: A longitudinal study, Clin Chim Acta 507 (2020) 164–166.
[15] N. Kohmer, S. Westhaus, C.R.S. Ciesek, H.F. Rabenau, Brief clinical evaluation of six high-throughput SARS-CoV-2 IgG antibody assays, Journal of Clinical Virology 129 (2020), 104480.
[16] M.F. Silveira, A.J.D. Barros, B.L. Horta, L.C. Pellanda, G.D. Victora, O. A. Dellagostin, C.J. Struchiner, M.N. Burattini, A.R.M. Valim, E.M. Berlezi, J. M. Mesa, M.L.R. Ikeda, M.A. Mesenburg, M. Mantesso, M.M. Dall'Agnol, R. A. Bittencourt, F.P. Hartwig, A.M.B. Menezes, F.C. Barros, P.C. Hallal, C.G. Victora, Population-based surveys of antibodies against SARS-CoV-2 in Southern Brazil, Nat Med (2020), https://doi.org/10.1038/s41591-020-0992-3. Jul 8, Online ahead of print.