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Comparative study between an antigen test and the reverse transcription-polymerase chain reaction (RT-PCR) test for the diagnosis of COVID-19 in pediatrics

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ABSTRACT

Introduction. The COVID-19 pandemic has brought to light the need for rapid diagnostic tests. The gold standard test is reverse transcription-polymerase chain reaction (RT-PCR). RT-PCR requires equipment and trained personnel, and results may take a long waiting time. The BD Veritor® System is a rapid chromatographic method used for the detection of severe acute respiratory syndrome coronavirus 2 antigen in symptomatic individuals. The primary objective of this study is to assess the sensitivity and specificity of the antigen test (AT) compared to the RT-PCR in the pediatric population.

Population and methods. Prospective study with a diagnostic test. All children younger than 17 years in the first 5 days of symptom onset, who consulted between July 2021 and February 2022, were included. A minimum of 300 specimens was estimated to achieve an accuracy of ±8.76% and ±3.68% for sensitivity and specificity, respectively. Specimens were analyzed in parallel using both methodologies.

Results. Of 316 paired samples, 33 were positive by both methods; 6 were positive only by RT-PCR. The specificity of the AT was 100%; sensitivity was 84.6%, with a positive and negative predictive value of 100% and 98%, respectively.

Conclusions. The AT proved to be useful in the diagnosis of pediatric patients with COVID-19 in the first 5 days of symptom onset, although those with a negative AT and high clinical suspicion should confirm their result with a RT-PCR.

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INTRODUCTION

In December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in China and, as early as March 2020, the World Health Organization (WHO) declared an international health emergency. The pandemic has brought to light the need for rapid diagnostic tests to detect positive cases and stop the spread of disease.

The gold standard technique suggested by the WHO for the diagnosis of SARS-CoV-2 is the detection of viral RNA by reverse transcription-polymerase chain reaction (RT-PCR). According to the recommendations, the RT-PCR should be performed on specimens obtained from the upper airway, such as the nasopharynx, nose (anterior or middle turbinates) or saliva.[1,2]

The RT-PCR has limitations because it requires equipment and trained personnel; in turn, the result may take a long waiting time (up to 24 hours in our hospital). Such delay in diagnosis leads to an unnecessary increase in preventive isolation measures and the consequent impact on work and school absenteeism.

An alternative to speed up diagnosis is the rapid antigen test (AT), which can demonstrate the presence of the virus in a few minutes.[3] The AT is suitable because of its sensitivity, speed, and lower cost compared to molecular methods for assessing patients who make an early consultation.

The BD Veritor® System is a digital chromatographic immunoassay used for the direct and qualitative detection of SARS-CoV-2 antigen in symptomatic individuals. It detects SARS-CoV-2 in upper airway specimens during the acute phase of infection.[4]

Comparative studies between the AT and the RT-PCR have been published, but most were conducted in adults and few have focused on the pediatric population.[5,6] Moreover, the results are heterogeneous due to the multiplicity of AT available in the market. A previous study carried out in the laboratory of our hospital compared both methods in symptomatic adults and found an 89% sensitivity and a 100% specificity.[7]

Our hypothesis was that the AT has similar sensitivity and specificity to the RT-PCR in the symptomatic pediatric population in the acute phase and is a suitable diagnostic method for the detection of SARS-CoV-2.

The primary objective of this study was to assess the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the BD Veritor® AT in comparison with the RT-PCR in the pediatric population.

In addition, the secondary objective was to describe the association between the result and the day of symptom onset, age, vaccination status, and RT-PCR cycle threshold (Ct). Other respiratory viruses were also studied in patients who tested negative for COVID-19 in low-prevalence months.

POPULATION AND METHODS

This was a prospective study with a diagnostic test carried out at the teaching hospital of CEMIC between July 2021 and February 2022.

The study included pediatric patients aged 0 to 16 years in the first 5 days of symptom onset who met the criteria for suspected COVID-19, according to the definition by the Ministry of Health of the City of Buenos Aires[8] who visited the Emergency Department of CEMIC's teaching hospital, in the Saavedra location, during the study period. Any patient and/or caregiver who refused to give consent to participate in the study, asymptomatic patients, or patients with symptoms for more than 5 days were excluded.

The study was approved by the Ethics and Research Committee of CEMIC. All adult caregivers of patients younger than 7 years gave their consent prior to study participation; children aged 7-13 years gave their assent together with their adult caregivers' consent; and children aged 14-17 years gave their consent together with their adult caregivers' assent.

Trained pediatricians collected 2 specimens from each patient: a nasal specimen for AT obtained with a flexible swab placed in a dry tube and processed within 1 hour of sample collection and a nasopharyngeal specimen for RT-PCR obtained with a flexible swab placed in virus transport medium. Both specimens were processed according to the process currently in place in our hospital. The analysis and interpretation of the results were performed by laboratory personnel specialized in virology. The RT-PCR used were RealStar® altona Diagnostics, which detects the *E* and *S* genes, and the Discovery Detection Kit® reagent, which amplifies the *ORF1ab* and *N* genes.

The sample size was estimated considering an expected sensitivity and specificity of 90% for an estimated disease prevalence of 15%, using the RT-PCR as control test. A minimum of 300 specimens was estimated to achieve an accuracy of ±8.76% and ±3.68% for sensitivity

and specificity, respectively.

In turn, we conducted a retrospective study of SARS-Cov-2 negative samples stored at -70 °C during low-prevalence months (July, August, and September 2021). The following respiratory viruses were tested by RT-PCR (RealStar® altona Diagnostics): respiratory syncytial virus (RSV), influenza A, influenza B, adenovirus (hADV), parainfluenza 1–4 (PIV), rhinovirus (hRV), and metapneumovirus. The nucleic acid extraction was performed using an automated method (BIOER).

Continuous variables were described as mean (standard deviation [SD]) and median (interquartile range); and categorical variables, as percentage. *P* values were estimated using the χ^2 test or Wilcoxon test, as applicable. A value of $p < 0.05$ was considered statistically significant. Data were analyzed using the Stata 13 statistical software package.

RESULTS

A total of 676 patients were invited to participate and 320 of them accepted. Of these, 4 were excluded from the analysis (2 due to errors in the date of symptom onset and 2 for not meeting the diagnostic criteria).

A total of 316 paired samples were included in the analysis; 33 were positive by both methods; 6 were positive only by RT-PCR. The SARS-CoV-2 positivity rate during the study period was 12.3%.

The AT has a specificity of 100% (95% confidence interval [CI]: 98.6–100) and a sensitivity of 84.6% (95% CI: 70.3–92.7), a PPV of 100% (95% CI: 89.6–100), and a NPV of 98% (95% CI: 95.5–99).

Two sub-analysis were performed: the first analysis included the samples obtained in the period between December 2021 and February 2022, during which the positivity rate was 58.2% (55 specimens, 32 positive by RT-PCR, 5 false negative results). In this analysis, the following values were observed: sensitivity of 84.4% (95% CI: 68.2–93.1), a specificity of 100% (95% CI: 85.7–100), a PPV of 100% (95% CI: 87.5–100), and a NPV of 82.1% (95% CI: 64.4–92.1). The second analysis included the specimens obtained between July and November 2021, with a positivity rate of 2.7% (261 specimens, 7 positive by RT-PCR, 1 false negative result). In this analysis, the following values were observed: sensitivity of 85.7% (95% CI: 48.7–97.4), a specificity of 100% (95% CI: 98.5–100), a PPV of 100% (95% CI: 61–100), and a NPV of 99.6% (95% CI: 97.8–99.9).

Of the 277 negative specimens, 205 were tested for other respiratory viruses and 138 (67.3%) were positive. Findings corresponded mainly to hRV in 84 (60.9%), RSV in 53 (38.4%), and PIV in 1 (0.7%). In 6 cases, 2 viruses were detected together: RSV-hRV in 4 patients, RSV-hADV in 1 patient, and hRV-hADV in 1 patient.

The demographic and clinical characteristics of patients are shown in *Table 1*.

At least 1 risk factor for severe COVID-19 was observed in 7.3% (n = 23) of children: 9 had asthma/recurrent obstructive bronchitis, 4 had congenital heart disease, 3 had active cancer, 2 had encephalopathy, and the following were observed in 1 each: obesity, cystic fibrosis, Down syndrome, chronic liver disease.

Table 2 shows the mean Ct values for both RT-PCR tests. The Ct values of samples showing inconsistent results were significantly higher than of those with consistent results when the Discovery Detection Kit was used. Of the 39 positive results, 4 had a Ct of 30 or higher, of which 3 corresponded to the group with false-negative results as per the AT. The fourth patient was immunocompromised due to cancer.

The test was performed on the same day of symptom onset (day 0) in 10% of patients; on day 1 in 30%; on day 2 in 32%; on day 3 in 21%; on day 4 in 5%; and on day 5 in 2%. Among patients with a positive result, the test was performed on the same day of symptom onset (day 0) in 31% of patients; on day 1 in 26%; on day 2 in 28%; and on day 3 in 10%. No patient had a positive swab on days 4 and 5 after symptom onset. Of the 6 false negative results, 3 had been tested on the same day of symptom onset.

In addition, 90% (n = 284) of patients had not received any COVID-19 vaccine at the time of the test; 5% (n = 16) had received 2 doses of a mRNA vaccine (Pfizer or Moderna); 2.5% (n = 8), 2 doses of an inactivated vaccine (Sinopharm); 1 patient, 1 dose of the Sinopharm vaccine; and 1 patient, 1 dose of the Pfizer vaccine; no data were available on the vaccine history of 6 patients (2%).

Table 3 shows the characteristics of patients with false negative results as per the AT.

DISCUSSION

This study assessed the diagnostic accuracy of the BD Veritor® AT compared to the RT-PCR for the diagnosis of COVID-19 in pediatrics; the

Table 1. Demographic and clinical data of patients

	Total	Negative RT-PCR	Positive RT-PCR	*P*
Patients (%)	316 (100)	277 (87.7)	39 (12.3)	-
Demographic data				
Age (interquartile range)	8 (3–12)	8 (3–12)	11 (2–14)	0.17
Age range	0–16	0–16	0–16	-
Male sex (%)	185 (58.5)	167 (60)	18 (46)	0.0934
Clinical data				
Days since symptom onset (standard deviation)	1.9 (1.13)	2 (1.11)	1.2 (1)	**0.0002**
Range of days since symptom onset	0–5	0–5	0–3	-
Cough (%)	215 (68)	195 (70.4)	20 (51.3)	**0.02**
Nasal congestion (%)	205 (64.9)	193 (69.7)	12 (30.8)	**0**
Fever (%)	177 (56)	148 (53.4)	29 (74.4)	**0.01**
Odynophagia (%)	115 (36.4)	101 (36.5)	14 (35.9)	0.9453
Headache (%)	71 (22.5)	60 (21.7)	11 (28.2)	0.3592
Vomiting (%)	34 (10.8)	31 (11.2)	3 (7.7)	0.5091
Diarrhea (%)	28 (8.9)	23 (8.3)	5 (12.8)	0.3527
Myalgia (%)	24 (7.6)	21 (7.6)	3 (7.7)	0.9804
Respiratory distress (%)	21 (6.6)	19 (6.9)	2 (5.1)	0.6845
Anosmia/dysgeusia (%)	6 (1.9)	6 (2.2)	0 (0)	0.3534
Mean number of symptoms (interquartile range)	3 (2–3)	3 (2–3)	3 (2–3)	0.16
Range of number of symptoms	1–6	1–6	1–6	-

findings showed a sensitivity of 84.6% and a specificity of 100%, which is consistent with other published studies.[3] The data obtained in this study comply with the WHO guidelines recommending a sensitivity > 80% and a specificity > 97% as minimum requirements for AT, considering the most reliable results in a context of a prevalence > 5%.[9]

At the time of study enrollment, SARS-CoV-2 variants circulating in Argentina among patients with no history of travel were predominantly Gamma, Lambda, and Alpha. The Delta variant emerged by the end of July 2021, with a significant increase in early September. By mid-October 2021, more than 80% of cases corresponded to the Delta variant. Omicron emerged in the first week of December 2021 and, in January 2022, Omicron cases rose to more than 85%; it displaced Delta and remained prevalent, accounting for almost 100% of cases until the end of February 2022.[10]

The characteristics of the 6 patients with false negative results as per the AT were analyzed.

Regarding the association between the result and the day of symptom onset, based on published studies, it is known that the viral load curve should be above the detection threshold for AT from the day of symptom onset to approximately day +7, with a peak on days 1–2 from symptom onset.[11] Our hypothesis was that false negative results would be observed in patients who were swabbed after more days since symptom onset, but it was not possible to establish this because none of the positive patients was swabbed beyond day 3. An observational and/or recall bias may be present in this study because

Table 2. Analysis of amplification cycle thresholds (Ct)

	Discovery			Altona	
	Consistent (n = 28)	Inconsistent (n = 5)		Consistent (n = 5)	Inconsistent (n = 1)
***ORF1AB* gene. Mean (SD)**	21.4 (4.79)	31.36 (4.30)	***E* gene. Mean (SD)**	23.34 (2.46)	22
***N* gene. Mean (SD)**	19.5 (4.9)	30.7 (4.82)	***S* gene. Mean (SD)**	21.98 (2.97)	21

SD: standard deviation.

Table 3. Characteristics of patients with false negative results in the antigen test (AT)

Age	Sex	DSO	Ct	Vaccination	Comorbidities	Symptoms
12 years	F	3	27.8/26.1	NO	NO	Cough, odynophagia, nasal congestion
16 years	F	2	22/21	2 doses of Pfizer	NO	Odynophagia, vomiting, diarrhea, nasal congestion
2 years	M	0	35/32.5	NO	Repaired complex congenital heart disease. Anticoagulant therapy	Cough, nasal congestion
9 years	M	0	25.8/25	NO	Biliary atresia on transplant waiting list	Fever, headache, nasal congestion
12 days	F	0	35.2/35.2	NO	Congenital heart disease not repaired	Respiratory distress, tachypnea/dyspnea, chest wall retraction
10 years	F	1	33/34.7	2 doses of Sinopharm	NO	Fever

M: male; F: female; DSO: days since symptom onset; Ct: RT-PCR cycle threshold.

the day of symptom onset was referred by the accompanying adults and sometimes it was difficult for them to accurately establish the time of onset of some subtle symptoms.

Regarding the age of patients, a study conducted by Euser et al. found that children younger than 12 years had lower viral loads and this may affect the AT's detection capability.[12] In our study, 4 of the 6 patients with false negative results were under 12 years of age; however, given our small sample size, it is not possible to establish a pattern as to whether age influenced the false negative result.

Although the study was started and carried out mostly in the period prior to the introduction of COVID-19 vaccines in the Argentine pediatric population, 90% of the enrolled patients had not received any vaccine and only 7.5% had a complete vaccination schedule. It would be interesting to conduct studies to compare the diagnostic accuracy among vaccinated and unvaccinated patients.

Of the 6 patients with false negative results as per the AT, 3 had a Ct value over 30 and 5 had a Ct value over 24. The Ct is the first significant increase in the amount of RT-PCR product and is a determinant of the viral load present at the start of the amplification reaction; it displays a reverse relationship: high Ct values (> 30) account for low viral loads and low or no infectivity, according to studies comparing a viral culture to the RT-PCR. In one of these studies, with Ct values > 24, infectivity decreased significantly or was null.[13] Therefore, if the AT shows a false negative result in a patient with low or no infectivity, the risk for misdiagnosing that patient is very low for society and would highlight the usefulness of the AT as a rapid and simple screening test. It would be important to conduct studies with a larger number of patients.

Another characteristic of false negative results was that 5 of them were observed during January 2022, when more than 85% of isolated variants corresponded to Omicron. Studies have described that the AT has a lower sensitivity to diagnose this variant, especially when the Ct value is greater than or equal to 25.[14]

This study has certain limitations. The main limitation was the collection of 2 different specimens, which we believe affected the decision of some patients and their caregivers to participate. When the study was planned, the prevalence of positivity in our hospital was approximately 30%, but when enrollment began, it coincided with the period of lower prevalence of infection in the City of Buenos Aires, so that, despite having enrolled 316 patients, the number of positive samples was low. However, the sensitivity was very similar in the sub-analyses of both high and low prevalence periods. In addition, other limitations were the use of 2 different reagents for the RT-PCR and that no viral culture was performed to assess the infectivity of inconsistent samples.

However, as a strength of the study, we believe that, despite the low prevalence during

the study period, no false positive results were detected. The setting in which the study was conducted was a real-life clinical setting, with more homogeneous inclusion criteria than those observed in some validation studies, and only in children, in whom such studies are rarely conducted.

Having searched for other respiratory viruses during the months of lower prevalence of COVID-19 confirms that patients were symptomatic and contributes to the epidemiological knowledge of the circulation of respiratory viruses in children since the emergence of COVID-19.

CONCLUSION

The BD Veritor® AT proved to be useful for the simple, rapid, and specific diagnosis of pediatric patients with COVID-19 in the first 5 days of symptom onset. Its sensitivity and specificity were within the parameters accepted by the WHO. However, patients with a negative AT and high clinical suspicion should confirm their result with a RT-PCR, especially if the predominant variant is Omicron. ■

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