Comparison of antigen test and polymerase chain reaction for SARS-CoV-2 in children younger than 12 years

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ABSTRACT
Stopping the spread of coronavirus disease 2019 (COVID-19) is critical and can be achieved through rapid and effective detection techniques. Our objective was to compare the diagnostic accuracy of rapid antigen tests (RAgT) and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and to describe amplification cycle thresholds (Cts). Participants were children aged 1 month to 11 years with symptoms for less than 7 days, who did not have a detectable result in the past 90 days, and were immunocompetent. A total of 1855 patients were included; the prevalence of COVID-19 was 4.7%. For the RAgT, overall sensitivity was 60.2% and specificity, 99.8%; in children older than 5 years, values were 69.8% and 99.8%, respectively. Ct values for discordant samples were higher. To conclude, the diagnostic accuracy indicated that the specificity of RAgT is similar to that of RT-qPCR, but its sensitivity is notably lower, especially in children younger than 5 years.
Key words: *SARS-CoV-2, antigen test, PCR, COVID-19, child.*

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INTRODUCTION

Rapid detection, effective isolation of confirmed cases, and close contact tracing are critical to stop the spread of coronavirus disease 2019 (COVID-19).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is the gold standard method for diagnosis.[1] Viral load of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) could be an important factor in determining the likelihood of dissemination.[2] Cycle thresholds (Ct) values for RT-qPCR are inversely related to viral load.[3]

Rapid antigen tests (RAgTs) emerged as an alternative point-of-care diagnostic tool, since they are easy to perform and allow a rapid identification of a confirmed case. Their cost is low and they do not require special equipment or specially trained personnel.[4] The recommended values for their use is a sensitivity ≥ 80% and a specificity > 97%.[5,6]

According to the information provided by the manufacturer, the Panbio™ COVID-19 Ag RAgT has a sensitivity of 98.1% with less than 7 days of symptoms. However, studies conducted in a symptomatic population under 16 years found sensitivity values ranging from 45.4%[7] to 77.8%.[8]

The assessment of the performance of RAgTs in the field is essential to understand their usefulness in clinical practice.

Our objective was to compare the diagnostic accuracy of the RAgT with that of RT-qPCR and to describe the amplification Cts of detectable RT-qPCR tests.

METHODS

This was a cross-sectional, prospective study conducted between June 14th and July 23rd, 2021. Participants included were children aged 1 month to 11 years, 11 months and 29 days for whom the hospital protocol did not establish doing a RAgT as diagnostic method and who required SARS-CoV-2 detection: patients with symptoms compatible with suspected COVID-19 cases according to the definition by the Ministry

of Health of the City of Buenos Aires[9] and asymptomatic children who met epidemiological criteria according to the current hospital protocol[10] (scheduled surgeries or tests that required anesthesia or patients who had to be hospitalized for other reasons). Children who had symptoms for 7 days or more or a detectable RT-qPCR result in the last 90 days or immunocompromise were excluded.

The study was approved by the Ethics Committee of Hospital General de Niños Pedro de Elizalde.

A pediatrician performed the physical examination, recorded clinical and epidemiological data, and obtained the signature of the informed consent. Then, a kinesiologist performed two swabs, one nasal and one nasopharyngeal, on each child.

The nasal swab was used to perform the RAgT. Although there are several commercial brands, we used the Panbio™ COVID-19 Ag test provided by the City of Buenos Aires as part of the community testing strategy implemented during the pandemic. The nasopharyngeal swab was sent to the Molecular Biology laboratory of the same hospital for RT-qPCR. The nucleocapsid (N), envelope (E), and RNA-dependent RNA polymerase (RdRp) genes were amplified.

The following variables were analyzed: RAgT result, RT-qPCR result, and Ct value of the amplification curve for each gene. Sensitivity, specificity, and predictive values for RAgT were calculated.

The sample size (n = 1705) was estimated based on an expected 90% sensitivity and 96% specificity and a 6% COVID-19 prevalence. Continuous variables were described as mean (standard deviation [SD]) and median (interquartile range [IQR]); and categorical variables, as percentage. A value of $p < 0.05$ was considered statistically significant. The kappa coefficient was used for the agreement analysis. Data were processed using the Stata® software; samples were processed in an independent, blinded manner.

RESULTS

A total of 1890 patients were seen during the study period. Of them, 35 did not meet the inclusion criteria. So, 1855 patients were analyzed (47% were females); participants' mean age was 4.3 years (SD ± 3.2). Symptoms were observed in 83%; the mean of symptom onset was 2.4 days (SD ± 1.4) (*Table 1*).

A total of 88 RT-qPCR detectable results (COVID-19 prevalence: 4.7%) and 56 RAgT positive results (3%) were obtained; 53 were positive in both tests. There were 38 discordant results: 35 RAgT(-)/RT-qPCR(+) and 3 RAgT(+)/RT-qPCR(-). The other results were negative according to both methods.

TABLE 1. *Clinical and epidemiological characteristics of included patients (n = 1855)*

	Median (IQR)	**Mean (SD)**
Age (years old)	3.4 (1.6–6.4)	4.3 (3.2)
Days since symptom onset	2 (1–3)	2.3 (1.4)
	Total	**Percentage (%)**
Sex		
Female	872	47
Reason for consultation		
Symptoms	1538	83
Protocol (asymptomatic)	306	16.5
Close contact	11	0.5
Clinical presentation		
Fever	920	49
Cough	947	51
Odynophagia	332	18
GI manifestations	272	15
Headache	192	10
Dysgeusia and/or anosmia	9	0.5
Rhinorrhea	277	15

IQR: interquartile range; SD: standard deviation; GI: gastrointestinal.

The level of agreement between both tests was 0.73 (95% confidence interval [CI]: 0.64–0.81).

Table 2 shows the diagnostic accuracy parameters for the RAgT. The prevalence of COVID-19 was 2.9% in children younger than 5 years and 8% in children older than 5 years. No statistically significant differences were observed in terms of RAgT sensitivity and specificity in children in whom ≤ 3 days had elapsed since symptom onset compared to those with > 3 days.

Table 3 shows mean Ct values. Ct values for the samples with discordant RAgT(-)/RT-qPCR(+) results were significantly higher than those for non-discordant RAgT(+)/RT-PCR(+) results.

DISCUSSION

In this study, we assessed the accuracy of RAgT for the detection of SARS-CoV-2 in nasal swabs compared to RT-qPCR in nasopharyngeal swabs (gold standard).

The overall sensitivity found is consistent with published data.[7,8] Although it is higher in children older than 5 years (69.8%), it is different from that reported by the manufacturer (98.1%).

The specificity found was high, which would indicate a low probability that isolation in children was not well indicated; moreover, the agreement between both tests was adequate.

The differences found in the sensitivity of the RAgT based on age could be due to the fact that the viral load of children older 5 years would be higher and, consequently, the RAgT would have a better performance in this age group. Such differences can be taken into account by pediatricians, especially in contexts of high prevalence of COVID-19, so that they may consider using other diagnostic methods if the RAgT result is negative.

Age older than 5 years and a low prevalence of COVID-19 could be a good scenario to consider the exclusive use of the studied test.

No differences were found in the diagnostic accuracy of the RAgT in relation to days since symptom onset, as opposed to the results obtained by Linares et al.[11] The reference to days since symptom onset is a subjective assessment of the accompanying adult at the time of consultation, which could explain the absence of differences due to observation bias.

It is known that the RT-qPCR can remain detectable for weeks or months after the initial infection,[12] so a detectable result would not always indicate dissemination capability. In pediatric patients, discordant RAgT(-)/RT-qPCR(+) results (with Ct values between 29 and

TABLE 2. *Diagnostic accuracy of the Panbio™ COVID-19 Ag Rapid Test in children stratified by age; mean (95% CI)*

	Overall	Children < 5 years (n = 1209)	Children ≥ 5 years (n = 656)
Se	60.2% (49.2–70.3)	47.1% (30.1–64.6)	69.8% (55.5–81.2)
Sp	99.8% (99.4–99.9)	99.8% (99.3–99.9)	99.8% (99.7–99.9)
PPV	94.6% (84.2–98.6)	88.9% (63.9–98.1)	97.4% (84.6–99.7)
NPV	98.1% (97.3–98.6)	98.4% (97.4–99.0)	97.4% (95.7–99.5)

CI: confidence interval; Se: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value.

TABLE 3. Analysis of amplification cycle thresholds

	Ct n = 88	Discordant RAgT Ct values n = 35	Non-discordant RAgT Ct values n = 53
E gene (n = 69) Mean (SD)	24.0 (6.8)	31.0 (4.4)	20.4 (4.7)
N gene (n = 84) Mean (SD)	25.4 (7.1)	31.5 (4.3)	19.9 (4.3)
RdRp gene (n = 74) Mean (SD)	25.6 (6.4)	31.7 (4.2)	22.0 (4.5)

Ct: cycle threshold.

35) may be due to the persistence of viral RNA and symptoms may be due to other respiratory viruses. Some authors who assessed the viability of SARS-CoV-2 in cultures[13] did not observe virus isolation in all samples with discordant results. This might suggest that the patients are unlikely to be infectious.

This study has the following limitations:

- Sample size calculation: this was performed based on a high RAgT sensitivity according to the manufacturer's information, so the final statistical power was lower.
- Viral culture was not performed to assess the infectivity of discordant samples, nor was a search for other respiratory viruses that could explain the symptoms.

To conclude, the diagnostic accuracy indicated that the specificity of RAgT is similar to that of RT-qPCR, but its sensitivity is considerably lower, especially in children younger than 5 years. ■

REFERENCES

1. Tang YW, Schmitz JE, Persing DH, Stratton CW. Laboratory Diagnosis of COVID-19: Current Issues and Challenges. *J Clin Microbiol.* 2020; 58(6):e00512-20.
2. Walsh KA, Jordan K, Clyne B, Rohde D, et al. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. *J Infect*. 2020; 81(3):357-71.
3. Aranha C, Patel V, Bhor V, Gogoi D. Cycle threshold values in RT-PCR to determine dynamics of SARS-CoV-2 viral load: An approach to reduce the isolation period for COVID-19 patients. *J Med Virol.* 2021; 93(12):6794-7.
4. Mina MJ, Parker R, Larremore DB. Rethinking COVID-19 test sensitivity - A strategy for containment. *N Engl J Med*. 2020; 383(22):e120.
5. World Health Organization. Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays: Interim guidance, 11 September 2020. [Accessed on: May 15th, 2021]. Available at: https://apps.who.int/iris/handle/10665/334253
6. Argentina. Ministerio de Salud. Consenso sobre el uso de pruebas diagnósticas para SARS-CoV-2. Versión 2. Mayo 2021. [Accessed on: May 20th, 2021]. Available at: https://bancos.salud.gob.ar/recurso/consenso-sobre-el-uso-de-pruebas-diagnosticas-para-sars-cov-2
7. Villaverde S, Domínguez-Rodríguez S, Sabrido G, Pérez-Jorge C, et al. Diagnostic Accuracy of the Panbio Severe Acute Respiratory Syndrome Coronavirus 2 Antigen Rapid Test Compared with Reverse-Transcriptase Polymerase Chain Reaction Testing of Nasopharyngeal Samples in the Pediatric Population. *J Pediatr*. 2021; 232:287-89.e4.
8. González-Donapetry P, García-Clemente P, Bloise I, García-Sánchez C, et al. Think of the Children: Evaluation of SARS-CoV-2 Rapid Antigen Test in Pediatric Population. *Pediatr Infect Dis J*. 2021; 40(5):385-88.
9. Argentina. Ministerio de Salud del Gobierno de la Ciudad Autónoma de Buenos Aires. Protocolo manejo frente a casos sospechosos y confirmados COVID19 en pediatría. 2021. [Accessed on: May 10th, 2021]. Available at: https://www.buenosaires.gob.ar/sites/gcaba/files/protocolo_de_manejo_de_casos_en_pediatria.pdf
10. Comité de Emergencia COVID 19. Protocolo de manejo hospitalario durante la fase de contención/mitigación. Buenos Aires: Hospital General de Niños Pedro de Elizalde; 8 de mayo de 2020.
11. Linares M, Pérez-Tanoira R, Carrero A, Romanyk J, et al. Panbio antigen rapid test is reliable to diagnose SARS-CoV-2 infection in the first 7 days after the onset of symptoms. *J Clin Virol.* 2020; 133:104659.
12. Herrero-Hernando C, Amadeo-Álvarez J, Elizari-Saco MJ, Martínez-Nadal S, Vila-Cerén C. Test de PCR a SARS-CoV-2 persistentemente positivo. No siempre la detección del virus es COVID-19. *An Pediatr* (Barc). 2020; 93(4):264-5.
13. Albert E, Torres I, Bueno F, Huntley D, et al A. Field evaluation of a rapid antigen test (Panbio™ COVID-19 Ag Rapid Test Device) for COVID-19 diagnosis in primary healthcare centres. *Clin Microbiol Infect*. 2021; 27(3):472.e7-10.