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Short communication

Panbio antigen rapid test is reliable to diagnose SARS-CoV-2 infection in the first 7 days after the onset of symptoms

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ABSTRACT

Background: RT-qPCR is the current recommended laboratory method to diagnose SARS-CoV-2 acute infection, several factors such as requirement of special equipment, time consuming, high cost and skilled staff limit the use of these techniques. A more rapid and high-throughput method is essential.
Methods: We analyzed clinical data and nasopharyngeal samples, collected during September 2020, from patients attended at the emergency department of a secondary hospital and in two primary healthcare centers in Madrid. The performance of the Panbio™ COVID-19 AG Rapid Test Device for the detection of SARS-CoV-2 antigen was compared to RT-qPCR.
Results: 255 nasopharyngeal swabs, including 150 from the emergency department and 105 from primary helthcare centers, were tested. 184 patients were symptomatic (72.1 %). Amongst the 60 positive RT-qPCR samples, 40 were detected by the rapid antigen test, given an overall sensitivity of 73.3 %. All the samples detected positive with the rapid antigen test were also positive with RT-qPCR. The median cycle threshold was 23.28 (IQR 18.5–30.16). Patients with less than seven days onset of symptoms showed a higher viral load, and sensitivity for rapid antigen test (86.5 %), compared to those with more days (sensitivity of 53.8 %)($p < 0.004$).
Conclusions: The rapid antigen test evaluated in this study showed a high sensitivity and specificity in samples obtained during the first week of symptoms and with high viral loads. This assay seems to be an effective strategy for controlling the COVID-19 pandemic for the rapid identification and isolation of SARS-CoV-2 infected patients.

1. Introduction

Ensuring accurate diagnosis is essential to limit the spread of SARS-CoV-2 and for the clinical management of COVID-19. Although real-time reverse transcription polymerase chain reaction (RT-qPCR) is the currently recommended laboratory method to diagnose SARS-CoV-2 acute infection, several factors such as requirement of special equipment, time consuming, high cost and skilled staff limit the use of these molecular techniques. A more rapid and high-throughput method is in growing demand [1,2].

Until now, the use of antigen detection tests alone had been ruled out and not recommended due to their low sensitivity [3–5]. Previously in the first wave, several easy to perform rapid antigen detection tests were developed as the first line of diagnostic. However, the results obtained were not good enough [6–9].

2. Material and methods

2.1. Population and study period

Study was conducted between September 10, 2020 and September 15, 2020 at Prínice de Asturias Hospital in Alcalá de Henares, Madrid and its area of influence. The cumulative incidence rate of active cases in the last 14 days (Confirmed Cases per 100,000 inhabitants) in this area

Abbreviations: COVID-19, Coronavirus disease 2019; CT, cycle threshold; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; NP, nucleocapsid protein; LFA, lateral flow assay; FDA, Food and Drug Administration; RT- qPCR, real-time reverse transcription polymerase chain reaction; RDT, rapid diagnostic test.

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was 518.8 [10]. We included patients that were attended at our emergency department and in two of our primary healthcare centers:

Emergency department (ED) patients: we included 135 symptomatic patients that were admitted in our ED with clinical suspicion or COVID-19 and 17 asymptomatic patients with history of contact with another COVID-19 patient.

Primary healthcare (PH) patients: 50 symptomatic patients and 55 asymptomatic patients attendend in two of our primary healthcare centres.

A total of two consecutive nasopharyngeal swabs were obtained from each patient. One of them was employed to perform the antigenic rapid test and the other sample was employed to carry out the RT-PCR.

2.2. Diagnostic procedures

RT-PCR: one automatic extractor was employed to obtain viral RNA from clinical samples: Hamilton Microlab Starlet (Hamilton Company, Bonaduz, Switzerland). RNA amplification was made using Allplex SARS-CoV-2 assay (Seegene, Seoul, South Korea).

Antigenic rapid test: we applied the Panbio COVID-19 Ag Rapid Test Device (Abbott Rapid Diagnostic Jena GmbH, Jena, Germany). This test is a qualitative membrane-based immunoassay (immunochromatography) for the detection of Nucleocapsid protein of SARS-CoV-2 in nasopharyngeal samples.

2.3. Clinical data

Demographic (age and sex) and clinical variables of the study population were obtained from the medical records. We also recorded the time from the onset of symptoms and the story of prior contact with COVID-19 patients.

2.4. Statistical analysis

Specificity and sensitivity with 95 % confidence intervals (95 %CI) were calculated using the RT-PCR results as gold standard. Sensitivity was evaluated globally and also according to the time from the onset of symptoms ($\leq$ 5days, $<$ 7 days and $>$ 7 days). Agreement between techniques was evaluated using Cohen's kappa score [11].

Continuous variables were presented as median and interquartile range (IQR) and categorical variables as proportions. We used the Mann-Whitney U-test, $\chi 2$ test, or Fisher's exact test to compare differences between survivors and non-survivors where appropriate. For these comparisons, a p value of 0.05 or below was considered significant. Statistical analysis was performed with SPSS v20.0 (IBM Corp., Armonk, NY, USA) (Table 1).

3. Results

255 nasopharyngeal swabs collected from symptomatic and asymptomatic patients were tested. 60 (23,5 %) positive RT-qPCR samples, 44 (17,2 %) were detected by the rapid antigen test. In our study population, the overall sensitivity was 73.3 % (95 % IC: 62.2–83.8).

Considering only symptomatic patients with <5 days, <7 days or ≥7days since onset, the sensitivity was 85.3 % (95 % IC: 73.4–97.2) (Cohen's kappa score = 0.897), 86.5 % (95 % IC: 75.5–97.5) (Cohen's kappa score = 0.904) and 53.8 % (95 % IC: 26.7–80.9) (Cohen's kappa score = 0.617)(Fisher's exact test was $p < 0.001$ independently of group of patients) respectively. Specificity was always 1.0 % (Table 2).

The RDT evaluated in this study showed a high sensitivity and specificity in samples mainly obtained during the first week of symptoms and with high viral loads (Tables 2 and 3). The range of cycle threshold (Ct) values was 0–37.8 (median 23.28 IQR 18.5–30.16). There were statistically significant differences when comparing the Ct gene N of patients with <7 days onset compared to the rest ($p < 0.004$) (Fig. 1, Table 4).

Table 1
Patients.

Characteristics	Emergency department	Primary care	p-value
No. patients	150 (58.8 %)	105 (41.2 %)	
Demographic			
Gender (female)	75 (50.0 %)	56 (53.3 %)	0.596
Age (years)	515 (37,0–71,8)	39,0 (25,0–56,0)	<0.001
Asymptomatic patients with close contact	13 (8.6 %)	54 (51.4 %)	<0.001
Days from close contact*	2 (1–3)	6 (4–8)	<0.001
Symptomatic patients	134 (89.3 %)	50 (47.6 %)	≤0.001
Days from the onset of symptoms	2 (1–5)	4 (2–7)	≤0.001
Fever (> 38.0 °C)	41(27.3 %)	15(14.3 %)	0.013
Myalgia	3(2.0 %)	7(6.7 %)	0.552
Headache	14(9.3 %)	17(16.2 %)	0.099
Sore throat	9(6.0 %)	17(16.2 %)	0.008
Cough	23(7 %)	19(18.1 %)	0.558
Dyspnoea	40(26.7 %)	1(1.0 %)	<0.001
Anosmia and/or ageusia	0	3(2.8 %)	0.037
Diarrhea	13(8.7 %)	4(3.8 %)	0.126
confusional syndrome	6(4.0 %)	1(1.0 %)	0.143

* Only asymptomatic patients. Days from close contact with a confirmed COVID-19 case*.

Considering asymptomatic patients with close contact the overall sensitivity was 54.5 % (95 % IC: 0,25-0,84) (Cohen's kappa score = 0.667). However in asymptomatic patients with less of seven days from close contact with a confirmed COVID-19 case it was 1.0 %, although only three positive RT-qPCR samples were included.

4. Discussion

As of September 18, 2020, the Food and Drug Administration (FDA) has approved more tan 150 SARS-CoV-2 RNA detection kits [12,13]. The lateral flow assay (LFA) is user-friendly, cheap, and easily mass-produced. In addition, it may be the optimal method for in-field detection of SARS-CoV-2 antigens if accurate, easy-to-use, rapid, and cost-effective [14]. In fact this is the first study carried out in primary care and shows the usability of the technique at this level of care.

Our results suggest that Panbio™ COVID-19 AG Rapid Test Device can rapidly identify SARS-CoV-2-infected individuals with moderate to high viral loads. Antigenic tests have shown 1.0 % specificity in all types of patients and can be a powerful tool of high positive predictive value to control the COVID pandemia. It could be used as a substitute technique for PCR in this type of patients to shortcut delays and intensive labour costs generated by the massive use of PCRs.

In the group of symptomatic patients with less than 7 days of evolution, the test reaches a sensitivity of 86.5 %, low to that described in the technical data sheet of the test. It is necessary to carry out studies with more patients to assess the usefulness that this technique could have in evaluating the infective potential of close contacts with PCR positive patients [15,16]. When testing with antigen tests, it must be considered that the infection prevalence and the clinical context of the recipient of the test affects at the pretest probability of the result being correct. Also, rapid antigen tests could be used for screening in high-risk clusters settings to identify quickly persons with a SARS-CoV-2 infection and prevent the transmission by repeat testing.

The role for this antigen-detecting rapid test can be considered in areas that are experiencing widespread community transmission, where the health system may be overburdened and where it may not be possible to test all suspect cases by RT- qPCR. In these situations, primary care may be the appropriate place to use it.

The RDT evaluated in this study showed a high sensitivity and specificity in samples mainly obtained during the first week of symptoms and with high viral loads (Ct<25). Its massive use, as suggested by

Table 2
Agreement between antigenic test and PCR.

⩽ 5 days				< 7 days				⩾ 7 days			
		PCR				PCR				PCR	
		+	−			+	−			+	−
Antigen	+	29	0	Antigen	+	32	0	Antigen	+	7	0
	−	5	102		−	5	104		−	6	29

Table 3
Color scale stepped from white to gray of median of Cycle threshold (Ct), number of positive results for antigen test and PCR test and sensitivity of antigen test considering days since onset of a) symptoms or contact and b) only symptoms. *Number of positive results.

a)

Days since onset	0	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	27
Ct Gene N	26	20	27	21	23	27	19	21	21	36	31	20	37	-	32	31	35
Antigen*	3	9	9	5	3	3	2	2	3	0	1	2	0	0	0	1	0
PCR*	3	10	11	5	3	5	2	3	5	1	1	2	1	1	2	1	1
Sensitivity antigen	100	90	82	100	100	60	100	67	60	0	100	100	0	0	0	100	0

b)

Days since onset	0	1	2	3	4	5	6	7	8	10	12	13	14	15	16
Ct Gene N	27	20	27	19,0	23	27	19	19	21	36	20	37	-	32	31
Antigen*	2	8	9	4	3	3	2	2	2	0	2	0	0	0	1
PCR*	2	9	11	4	3	5	2	2	3	1	2	1	1	2	1
Sensitivity antigen (%)	100	89	82	100	100	60	100	100	67	0	100	0	0	0	100

the new protocols [5,17], can change the course of the pandemic. This assay seems to be an effective strategy for controlling the COVID-19 for the rapid identification and isolation of SARS-CoV-2 infected patients [5, 17,18].

Informed consent

We also made sure that oral informed consent was obtained from each patient to participate on a voluntary basis in the study and a questionnaire with complete epidemiological and clinical questions was filled out. Oral informed consent was recorded in each questionnaire and was obtained from the next of kin, caretakers, or guardians on behalf of the minors/children enrolled in this study. No economic compensation was granted for participating in the study. All the data were treated confidentially and anonymized.

Ethical approval

The study was conducted according to the ethical requirements established by the Declaration of Helsinki. The Ethics Committee of Hospital Universitario Príncipe de Asturias (Madrid) approved the study.

Author contributions

Study concept and design: ML, RPT and JCG.
Patients' selection and clinical data acquisition: ML, RPT, AC, JR, FPG, PGH and JCG.
Sample processing: ML, RPT, AC, JR, FPG, PGH, TA and JCG.
Statistical analysis and interpretation of data: ML and RPT.
Writing of the manuscript: ML, RPT, JR, FPG, PGH, TA and JCG.
Critical revision of the manuscript for relevant intellectual content:

Table 4
Sensitivity of antigen test and percentage of patients with less of seven days from the onset of symptoms according to the viral loads represented by Cycle Threshold Values (Ct) of N gene.

Ct N group	Symptomatic patients<7days*	Sensitivity
Ct < 25 (n = 34)	26 (76.5 %)	97.1 %
Ct<30 (n = 9)	9 (1.0 %)	77.8 %
Ct<35 (n = 10)	1 (10 %)	30 %
Ct<40 (n = 7),	1 (14 %)	14 %

* Days from the onset of symptoms<7days.

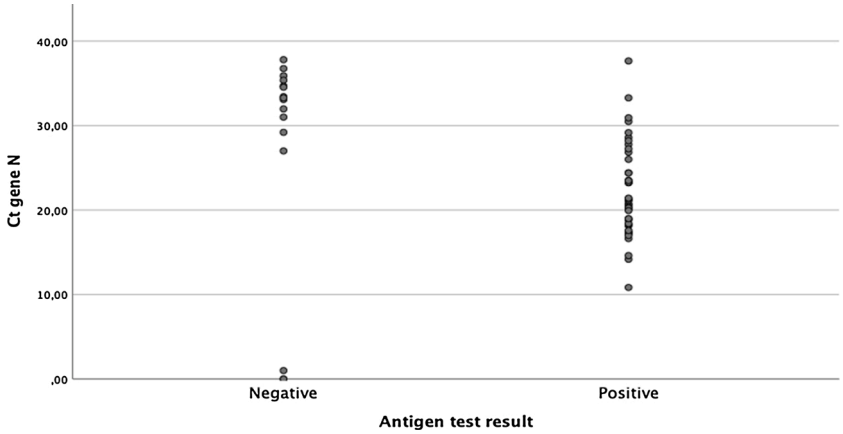

Fig. 1. COVID-19 antigen results according to viral load.

ML, RPT, AC, JR, FPG, PGH, TA and JCG.
Supervision and visualization: JCG.
All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors report no declarations of interest.

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