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SHORT COMMUNICATION

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Usefulness of SARS-CoV-2 antigen test sample as input for SARS-CoV-2 RT-PCR analysis

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Abstract

SARS-CoV-2 rapid detection is of great interest to prevent viral dissemination. In that sense, antigen tests appeared as a very valuable tool to reach this goal. However, it is possible to obtain a negative result in those patients with low viral loads, and consequently, reverse transcription-polymerase chain reaction (RT-PCR) should be performed on samples from patients with a negative antigen test in which there is a strong suspicion of COVID infection. The common diagnostic algorithm involves taking a second sample for RT-PCR testing. This study evaluates the usefulness of the antigen test sample for carrying out RT-PCR analysis when necessary. Results obtained indicate that can be used a unique sample for both antigen test and RT-PCR. This data showed that it is possible to reduce excessive suspected individuals managing and so on increase staff security and patient comfort.

KEYWORDS
antigen test, COVID-19, RT-PCR, SARS-CoV-2

1 | INTRODUCTION

The SARS-CoV-2 pandemic has generated a great demand for useful and rapid tools for the diagnosis of this virus.[1] Although real-time reverse transcription-polymerase chain reaction (RT-PCR) is the technique considered the reference laboratory method to diagnose SARS-CoV-2 infection,[2] other tests such as rapid antigen (Ag) detection tests have been developed and many have received regulatory approval.[3] Also, these devices have been used to perform a rapid screening, thus avoiding the performance of RT-PCR, a much more laborious, expensive, and time-consuming technique, to those samples that give a positive result.[4,5]

Some studies[6] have shown a sensitivity of these devices that ranges from 90% to 95%, when the patient's viral load is high, cycle threshold (C_t) value < 30, to a sensitivity of 10%–40% when the viral load is low, C_t value ≥ 30. C_t values cannot be used to determine viral load or infectivity in an individual, but there is an inverse relationship between C_t value and the amount of genetic material present in specimens.[7] Therefore, all negative results must be verified using another technique in those patients deemed necessary.

Until now, the most widely used sample for the diagnosis of COVID-19 is the nasopharyngeal swab. The collection of this sample, although is not painful, is very unpleasant and irritating for the patient and represents a risk for the spread of the virus as respiratory secretions are the main route of infection.[8]

Therefore, to avoid patient discomfort as well as a duplicate exposure of the healthcare personnel, the objective of this study has been to study the possibility of using a single nasopharyngeal swab to perform both the Ag test and RT-PCR analysis when it is considered necessary.

2 | MATERIALS AND METHODS

Two antigen tests were employed, the PanBio COVID-19 Antigen test (Abbott Rapid Diagnostics Jena GmbH) and the CLINITEST Rapid COVID-19 Antigen test (Siemens Healthineers). CLINITEST Rapid COVID-19 Ag test was used to confirm the results obtained with the one commonly used in laboratory routine (PanBio COVID-19

Ag test). The CLINITEST Rapid COVID-19 Ag test was the device available at Microbiology Department at this time.

A total of 149 patients were included in the study. One hundred thirteen samples were analyzed by the PanBio COVID-19 Ag test and 36 by the CLINITEST Rapid COVID-19 Ag test. One or two nasopharyngeal swab specimens were collected from each patient. When only one sample was obtained, this was placed into an extraction tube provided in antigen test kits. The extraction tube was filled with the extraction buffer and immediately tested following the manufacturer's instruction. In 92 patients, two nasopharyngeal specimens were acquired (65 PanBio group and 27 CLINITEST group), one was processed as above described and the other one was kept in collection eSwab tubes for later RT-PCT processing.

After antigen testing, swab specimens (both extraction tubes and eSwab tubes) were sent to Microbiology Department for RT-PCR screening. RNA of each sample was extracted using automated EasyMag system (bioMérieux, Marcy l'Étoile, France), according to the manufacturer's instruction. Two hundred microliters were used as input volume and 50 µl as elution volume. All samples from antigen extraction tubes had a smaller than required volume. Therefore, it was necessary to add 150 µl of conjugate solution from the antigen test kit before starting the RNA extraction process.

Extracted RNA was used immediately or stored at −80°C until used.

RT-PCR was performed using the FTD SARS-CoV-2 Assay reagents (Siemens Healthineers), following the manufacturer's instructions. Results were interpreted as positive if the C_t (crossing point) value was ≤40, as negative if no value or $C_t > 40$.

3 | RESULTS AND DISCUSSION

Six out of the 113 samples from the PanBio COVID-19 Ag test collection tube were inhibited in the PCR assay thus comparative studies were carried out only with 143 (antigen vs. PCR of antigen collection tubes) and 92 (PCR eSwab vs. PCR antigen collection tubes) samples.

Seven out of the 36 samples analyzed by the CLINITEST Rapid COVID-19 Ag test and 30 out of the 107 samples analyzed with the PanBio COVID-19 Ag test were positive by both Ag test and the RT-PCR carried on with antigen collection tube. Twenty-one CLINITEST Rapid COVID-19 Ag test and 69 PanBio COVID-19 Ag test samples were negative by both techniques. The remaining 16 samples showed different results in these two tests being only one out of this 16 positive by antigen testing and negative by the PCR. The other fifteen samples were positive only by PCR with C_t values ranging 34–40 with samples from CLINITEST Rapid COVID-19 Ag test and 29–39 with those of PanBio COVID-19 Ag test (Table 1).

The two tests used to show different sensitivity, being PanBio more sensitive than CLINITEST. Even so, the results obtained show that both antigen test samples can be used as input for the SARS-CoV-2 RT-PCR analysis.

These results are in agreement with previously published data that indicate that RT-PCR is more sensitive than Ag test and, therefore, samples with low viral load ($C_t > 30$) can often be detected by RT-PCR but not by Ag test.[9,10]

There was only one sample positive by antigen test (CLINITEST Rapid COVID-19 Antigen test) and negative by RT-PCR. This sample was reported as "weak positive" as the intensity of the line in the immunochromatography test was very low and consequently, it could be a false positive of the Ag test.

TABLE 1 Comparative results of SARS-CoV-2 antigen test and PCR using same collection tube

	CLINITEST antigen collection tubes ($n = 36$)		PanBio antigen collection tubes ($n = 107$)	
Ag/PCR results	Samples (n)	C_t range	Samples (n)	C_t range
Negative/negative	21	NA	69	NA
Positive/positive	7	23–34	30	14.5–37
Positive/negative	1	NA	0	NA
Negative/positive	7	35–40	8	28.5–39

Abbreviation: NA, not applicable.

TABLE 2 Comparative results of SARS-CoV-2 PCR using different collection tube

	CLINITEST antigen collection tubes vs. eSwab ($n = 27$)		PanBio antigen collection tubes vs. eSwab ($n = 65$)	
PCRs results	Samples (n)	C_t range	Samples (n)	C_t range
Negative/negative	17	NA	55	NA
Positive/positive	4	17–38	6	19.5–37
Positive/negative	5	34–39.5	3	35–38
Negative/positive	1	ND	1	ND

Note: Positive was obtained only with the Panther system (no C_t).

Abbreviations: NA, not applicable; ND, not determined.

Although the results obtained were in agreement with those already published, as the medium used to collect the sample is not recommended for SARS-CoV-2 PCR testing, the assay was also carried out with the same samples collected in eSwab tubes. Both samples were taken at the same time and by the same person to reduce the differences related to the sampling.

The results obtained (Table 2) showed a high degree of concordance between both PCRs.

The relatively small number of samples tested, mainly with the CLINITEST device, should be considered as a potential limitation of this study. Even so, these data provide clear evidence that a single sample could be enough to perform both determinations, the initial antigenic test, and a subsequent RT-PCR to confirm negative results. This protocol saves both, time and reagents and it is more comfortable for the patient and safer for sanitary personal.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Cristina García-Salguero: Investigation, validation formal analysis, original draft preparation. **Esther Culebras**: Conceptualization, data analysis, review, and editing. **Paloma Merino**: Sample collection, methodology. **Elvira Baos**: Methodology, validation formal analysis. **Alberto Delgado-Iribarren**: Project administration, reviewing, and editing. All authors reviewed subsequent drafts and approved the final version.

DATA AVAILABILITY STATEMENT

The authors confirm that the main data supporting the findings of this study are available within the manuscript. Any additional information is available on request from the corresponding author (CGS).

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