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“The effectiveness of rapid antigen test-based for SARS-CoV-2 detection in nursing homes in Valencia, Spain”

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ARTICLE INFO

Keywords:
SARS-CoV-2
Pandemics
Diagnostic
Rapid techniques

ABSTRACT

The usefulness of the PANBIO™ COVID-19 Ag rapid test for SARS-CoV-2 infection detection has not been widely studied, especially in specific population groups such as the elderly who are institutionalized. Rapid diagnostic tests have the potential to benefit testing strategies, as they have short turnaround times, they are cheap, simple to perform and can be used in decentralized testing. The objective of this study is to show the performance of the PANBIO™ COVID-19 Ag Rapid test device conducted at geriatric institutions and to compare results to those obtained from RT-PCR. A total of 448 individuals were enrolled in the study, including both residents and employees. Nasopharyngeal swabs were collected for both PANBIO™ COVID-19 Ag Rapid test and RT-PCR testing. All the samples were analyzed by specialized microbiologists. A total of 117 out of 448 individuals (26%) tested positive by RT-PCR, of whom 99 (85%) returned positive Antigen test results. There were 18 Antigen negative cases with positive RT-PCR results. Accordingly, concordance between RT-PCR and Antigen test results was acceptable (κ index, 0.89; 95% IC 0.8455–0.9345). Overall sensitivity and specificity of Antigen test was 85% and 100%, respectively. When defining RT-PCR CT positivity on a cut-off value of 35, LFA sensitivity was 90%. In case a cut-off value of 30 was used, LFA would increase up to 99%. In this real-life evaluation of the PANBIO™ COVID-Ag rapid test, the assay reliably identified SARS-CoV-2 infected individuals with low CT-values by RT-PCR. False negative results were observed only at high CT-values, meaning low viral loads in nasopharyngeal samples.

1. Introduction

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has undoubtedly created an emerging disease of topmost public health priority spilling throughout the globe. The diagnosis currently relies on a multiplex of criteria including the epidemiology, clinical manifestations and in vitro diagnostics [1].

The reference test for detection of acute SARS-CoV-2 infection is reverse transcriptase polymerase chain reaction (RT-PCR) [2]. RT-PCR requires expensive laboratory equipment, as well as specific laboratory supplies and qualified and trained laboratory technicians, which supposes a challenge to answer the demanding testing capacity and short turnaround times [3].

Lateral Flow Assay (LFA) -based point of care tests (POCT) for rapid antigen detection using antibodies are cheap, easy-to-use tools that do not require laboratory instrumentation and generate results within a short period of time (20 min) [4].

Several rapid antigen tests have been developed, usually showing high specificity results but low or varying sensitivity [5,6].

Articles published show good specificity results but variable results when it comes to sensitivity. The manufacturer of the PANBIO™ COVID-19 Ag rapid test reported a very high sensitivity. However, real-life data is available from small cohorts, especially from Spain. There is no data available yet on the diagnostic performance of this test in
institutionalized elder individuals with or without symptoms. This population is of special interest, considering their vulnerability and the devastating effects SARS-CoV-2 has in nursing homes.

Testing and subsequent isolation is crucial to halt transmission, especially in those institutions where the residents live close together. Rapid diagnostic tests have the potential to benefit testing strategies, as they have short turnaround times, they are cheap, simple to perform and can be used in decentralized testing.

The objective of this study is to show the performance of the PANBIO™ COVID-19 Ag Rapid test device (Abbott Diagnostic GmbH, Jena, Germany) conducted at nursing homes and to compare results to those obtained from RT-PCR.

2. Methods and materials

2.1. Study population

A total of 448 individuals were enrolled in this retrospective study. They were residents in any of the seven nursing homes located in Valencia (Spain) that belonged to the health department of Consorcio Hospital General Universitario de Valencia. The study took place in November and December 2020, coinciding with the second wave of SARS-CoV-2 infection in Spain.

Participants were either residents or caretakers living with them, meaning they all had close contact every day. In all of the nursing homes selected for the study, there had been at least two cases of SARS-CoV-2 (symptomatic or asymptomatic). The study was approved by the Research Ethics Committee of the Hospital General Universitario de Valencia.

2.2. SARS-CoV-2 testing

Nasopharyngeal swabs (NP) for Rapid Antigen Diagnostic and RT-PCR testing were collected by experienced nurses at each geriatric institution each time. Antigen testing was carried out at point of care (POC) immediately after sampling and validated by a Microbiologist present at the site. RT-PCRs were performed within 24 h of specimen collection at the Microbiology department of Consorcio Hospital General Universitario de Valencia.

After collection, swabs were transferred into 3 ml Universal Transport Medium (COPAN diagnostics, Italy) until further processing. Nucleic acid extraction, RT-PCR and results interpretation were performed according to the instructions of the manufacturer (Seegene, Seoul, South-Korea). In short, RNA was isolated and purified using the Seegene STARlet IVD, which works from primary sample tube to nucleic acid extraction and PCR setup. Amplification was performed in a single tube assay using the Allplex 19-nCoV multiplex platform for detection of SARS-CoV-2 (Seegene, Seoul, South-Korea), and results were interpreted with Seegene Viewer data analysis software. The assay uses fluorescent Taqman probes for three SARS-CoV-2 genes (E [Envelope], N [Nucleocapsid], and RdRp [RNA dependent RNA Polymerase] genes). Amplification and detection were performed for 45 cycles on a BioRad CFX96 thermocycler (BioRad Laboratories, Hercules, California, United States), the threshold Cycle (Ct) was automatically determined by the manufacturer’s software. A positive result was defined as amplification of any of the three SARS-CoV-2 genes. If not all targets showed a positive result, this always corresponded with Ct-values above 35, suggesting low levels of SARS-CoV-2 RNA.

Based on our experience within clinical practice and results from viral studies [7] we used a cut-off Ct-value of 35 to determine clinically relevant levels of SARS-CoV-2 RNA.

2.3. Lateral flow assay

The PANBIO™ COVID-19 Ag rapid test device by Abbott (Abbott Diagnostic GmbH, Jena, Germany) is a membrane-based immunochromatography assay which detects the nucleocapsid protein of SARS-CoV-2 in nasopharyngeal samples. Collected swabs were transferred into dedicated sample collection tubes containing a sampling buffer. All the samples were analyzed within a maximum of 15 min after collection. Test results were recorded after 15 min of assay initiation, always by specialized microbiologists.

2.4. Statistical analyses

Population characteristics are reported as mean or median values. Concordance, specificity and sensitivity with 95% confidence intervals, and positive predictive value and negative predictive value (PPV/NPV) of the LFA were calculated using the RT-PCR results as the reference test.

3. Results

3.1. Overall performance

Contacts were tested at a median of 2 days (range 1–7) after diagnosis of the index case.

We observed no variability in interpretation and no bands were classified as unclear by the independent observers.

False negative LFA results were mostly observed in subjects with RT-PCR CT-values above 35, reflecting low viral load levels in nasopharyngeal material.

The mean CT-values for E-gene, N-gene and RdRp-gene were 21, 22 and 21, respectively.

A total of 117 out of 448 individuals (26%) tested positive by RT-PCR, of whom 99 (85%) returned positive LFA test results. There were 18 LFA negative cases with positive RT-PCR results (RNA detected found in Nursing homes with less than five positive cases detected in the previous 48 h. No false negative results were found in those nursing homes with five or more positive cases detected in the previous 48 h. Accordingly, concordance between RT-PCR and LFA results was acceptable (K index, 0.89; 95% IC 0.8455–0.9345).

Overall sensitivity and specificity of LFA was 85% and 100%, respectively. When defining RT-PCR CT positivity on a cut-off value of 35, LFA sensitivity was 90%. In case a cut-off value of 30 was used, LFA would increase up to 99%. Results are shown in Table 2. In Table 1 population characteristics are shown.

Most of the individuals were female, and the median age was very similar in both groups also. Previous contact with positive SARS-CoV-2 individuals was uncertain for most of them, as only in the case of roommates or residents sharing caretakers the link was well defined. Most of them were asymptomatic and only 26% showed symptoms at the moment of the diagnosis (mainly employees).

4. Discussion

In this real-life evaluation of the PANBIO™ COVID-Ag rapid test in institutionalized individuals and workers, where most of them were asymptomatic, the assay reliably identified SARS-CoV-2 infected individuals with low CT-values by RT-PCR (infections with a high viral load in nasopharyngeal specimens). In our study, specificity was 100%;
overall sensitivity was 85% and 90% when using a CT-value of 35 as cut-off. Although in other studies it has been shown that in NP with CT values above 30 or even 25 the virus cultures are negative [8], this cut-off value was established in order to be more cautious because of the vulnerability of the population studied.

At the moment, this new LFA test has not been evaluated extensively [9].

In a study performed in Madrid by Linares et al. [10], LFA sensitivity was directly related to the magnitude of SARS-CoV-2 RNA load in nasopharyngeal samples, just as the results from our study. In the study performed by Fenollar et al. [11], sensitivity is really low for asymptomatic patients, unlike our results which show a high sensitivity in both symptomatic and asymptomatic individuals.

In our study cohort, false negative results were observed only at high CT-values, meaning low viral loads in nasopharyngeal samples. This may occur very early in the infection (presymptomatic stage) before viral replication peaks, or in a late stage of infection when replication has decreased.

According to our results and following WHO recommendations [12], PANBIO™ COVID-Ag rapid test is a useful tool for SARS-CoV-2 diagnosis in settings where prevalence is high, which is the case of the nursing homes being suspected of having an outbreak.

In our study we observed that in those institutions where there were less than five cases in 48 h, most of the PANBIO™ COVID-Ag rapid test results were negative and RT-PCR resulted more reliable. However, in those institutions where the tests were performed after having diagnosed more than five cases in 48 h, the PANBIO™ COVID-Ag rapid test obtained a lot of positive results, meaning that the higher the prevalence and the number of days since the first case appeared, the higher the sensitivity of the test. Regarding the ability of the test to detect SARS-CoV-2 variants, a recent study showed that it effectively detected B.1.1.7, B.1.351 and P.1 variant infections [13].

From a public healthcare point of view, not diagnosed infections with the LFA in patients with high CT-values in a late stage of infection may have limited impact, as these individuals are less likely to contribute to transmission. This is supported by findings that SARS-CoV-2 cultures were not possible to obtain at CT-values above 35 [7].

However, in the case of geriatric institutions, it is of paramount importance to detect absolutely all cases of COVID-19 infection that could appear. For this reason, even though it seems a good strategy to perform rapid LFA, because it could help re-organize rooms and move residents from isolation or vice versa, it is necessary to also perform RT-PCR for those LFA negative cases at the beginning of the outbreak where CT values are high but may be infectious. The strength of the current study is that it shows the real-life performance of the LFA test in geriatric institutions, which have been severely damaged by COVID-19, even in asymptomatic individuals. Among its limitations, as it has been said before, the false negative results which means, in terms of organization, that residents in this situation cannot be moved out from isolation until RT-PCR results are obtained.

Financial support
This work received no public or private funds.

Declaration of Competing Interest
The authors declare no conflicts of interest.

Acknowledgment
We want to thank everyone working at the Microbiology Department of Consorci Hospital General Universitari de Valencia for their commitment in the fight against COVID-19.

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