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Evaluation of the Abbott Panbio™ COVID-19 Ag rapid antigen test for the detection of SARS-CoV-2 in asymptomatic Canadians

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ABSTRACT

Several rapid testing methodologies have been approved for testing of symptomatic individuals but have not been validated for asymptomatic screening. We evaluated performance of the Abbott Panbio™ COVID-19 rapid antigen assay in the asymptomatic setting. We conducted a prospective study in an urban assessment center and in the context of long-term care staff screening. A total of 3014 individuals submitted paired nasopharyngeal samples, which were tested in parallel with the rapid antigen and laboratory-based, RT-PCR assays SARS-CoV-2 detection. There was 54.5% concordance in positive results between the rapid antigen assay and RT-PCR. All positive rapid antigen assay results were confirmed by RT-PCR. The negative predictive value of the rapid antigen assay minimally improved on the negative pre-test probability of SARS-CoV-2 infection. The Abbott Panbio™ COVID-19 rapid antigen test allowed for faster identification of infected individuals but cannot be used to rule-out SARS-CoV-2 infection.

1. Introduction

In response to laboratories being overwhelmed with requests for COVID testing during the early stages of the pandemic’s second wave in the Autumn of 2020, the Federal government of Canada secured procurement of millions of point-of-care tests, both molecular and antigen detection, for COVID-19. One of these tests, the Abbott Panbio™ COVID-19 Ag (hereafter simply referred to as “Panbio”), is a rapid, lateral flow antigen detection assay intended for point-of-care (POC) SARS-CoV-2 detection in symptomatic individuals, within the first 7 days of symptom onset (Canadian Public Health Laboratory Network Laboratory Directors Council, Canadian Public Health Laboratory Network Respiratory Virus Infection Working Group 2021). The assay targets the SARS-CoV-2 nucleocapsid encoded by the N gene (Fenollar et al., 2021). The main benefit of implementing rapid antigen detection tests is improved turn-around-times in remote locations or in fragile congregate settings such as long-term care centers. Additionally, many jurisdictions are interested in the use of POC COVID tests for screening and surveillance in asymptomatic populations, outside the recommended manufacturer use, to identify cases in the asymptomatic setting (Gill and Gray, 2020).

Prior to implementation of any new test in a diagnostic laboratory or POC environment, the manufacturer’s claims must be verified to ensure the test is performing as stated (CLSI, 2009; International Organization for Standardization 2016). Previous studies have demonstrated that the Panbio assay has a good sensitivity (95%) in symptomatic patients compared to qRT-PCR, when an arbitrary threshold for detection by qRT-PCR was set at a cycle threshold (Ct) of 32, but decreased significantly to 78.2% when all patients that were positive by qRT-PCR were considered (Gremmels et al., 2021). Another study showed similar findings, with a combined sensitivity of 75.5% in symptomatic patients but overall decreasing rates of positivity as Ct values increased (Fenollar et al., 2021). However, it has been demonstrated that asymptomatic patients can have detectable virus levels with low Ct values (median Ct values of 25.5 and 23.1 respectively) (Arons et al., 2020).

Based on the manufacturer’s recommendations and claims, asymptomatic screening is considered “off-label” for the Panbio and as such careful evaluation and validation of the test for this use case is required. Here, we evaluated the positive and negative concordance of results obtained with the Abbott Panbio™ COVID-19 Ag rapid antigen detection test and laboratory-based molecular assays for the detection of SARS-CoV-2 in nasopharyngeal swabs from asymptomatic individuals. Participants included staff in long-term care homes, as part of weekly surveillance testing, and individuals visiting one of the city’s COVID-19 assessment centres for asymptomatic screening.
Table 1

| Location            | Reason for testing            | Number of participants |
|---------------------|-------------------------------|------------------------|
| Assessment Centre   | Asymptomatic screening        | 6                      |
|                     | Pre-surgery screening         | 1                      |
|                     | Pre-travel screening          | 15                     |
|                     | Post-travel screening         | 2                      |
|                     | Screening for LTC entry       | 32                     |
| LTC home            | HCW surveillance screening    | 2999                   |

2. Methods

2.1. Participants

Individuals in each location (Table 1) provided verbal consent for an additional nasopharyngeal swab (NP) to be taken at the time of testing. The first nasopharyngeal swab was submitted in an appropriate transport media for laboratory-based molecular testing for the detection of SARS-CoV-2. The second nasopharyngeal swab was submitted in the Panbio kit-supplied buffer for immediate testing with the Panbio rapid antigen assay. Strict adherence to this collection order was enforced.

Participants were self-described as asymptomatic at the time of testing. Individuals seeking testing in the context of a contact tracing investigation were excluded. A total of 3014 asymptomatic individuals participated in this study, yielding 3007 paired antigen detection and qRT-PCR assays. The location and reason for testing are summarized in Table 1. This study was approved by the research ethics board of the Ottawa Hospital Research Institute (Protocol #20200749-01H).

2.2. Laboratory-based molecular assays

Nasopharyngeal swabs collected in appropriate media for viral nucleic acid stability were tested by qRT-PCR or transcription mediated amplification (TMA) on high-throughput automated platforms. In order to optimize turn-around time and overall efficiency of SARS-CoV-2 testing for the local health integrated unit, formal laboratory qRT-PCR testing was performed on a variety of instruments. Testing for the majority of samples was performed at the Eastern Ontario Regional Laboratory Association (EORLA), virology laboratory using 3 RT-PCR on 4 extraction platforms (Table 2): Seegene Allplex™ SARS-CoV-2 Assay (E gene, N gene and RdRP gene detection), the Cobas® SARS-CoV-2 Test using the Cobas® 6800 platform (E gene, Orf1a gene), the Panther Fusion® SARS-CoV-2 Assay (Orf1a and Orf1b gene regions) and the Aptima® SARS-CoV-2 Assay (TMA)(Orf1a and Orf1b gene regions). For the Seegene Allplex™ assay, nucleic acid extraction and amplification occurs on separate platforms. At EORLA, sample extraction for the Allplex™ assay is done on automated platforms using the Seegene STARMag universal nucleic acid extraction kits as per manufacturer instructions, while amplification is performed on Biorad CFX instruments in 96-well plates. 395 samples submitted to the Public Health Ontario Laboratory (PHO) were tested using a lab-developed SARS-CoV-2 RT-PCR targeting the E gene based on the protocol published by Corman et al. (Corman et al., 2020).

2.3. Abbott Panbio POC test

Following collection, Panbio nasopharyngeal swabs were inserted into an extraction tube containing 300 μL of extraction buffer, swirled 5 times following which the extraction tube was squeezed around the swab tip to allow release of the specimen into the buffer. The specimens were processed as per instructions within 2 hours of collection and results were interpreted visually after 15–20 minutes of incubation at room temperature. A result was considered positive if both a test and control line were seen and considered negative if no test line was seen in the presence of a control line. In the absence of a control line, the test was considered invalid.

2.4. Data analysis

Data were analyzed using Microsoft Excel. Positive and negative concordance, negative predictive value and positive predictive value were calculated for the Abbott Panbio™ COVID-19 Ag rapid antigen test, considering the laboratory RT-PCR method as the gold standard comparator. The cycle threshold values (Ct) for specimens measured positive by RT-PCR and relative light unit (RLU) values for samples measured by TMA were also listed.

Table 2

| POC Ag PANBIO | LAB Assay | Ct values (qRT-PCR) | RLU (MA) |
|---------------|----------|---------------------|----------|
| Result        | Platform | E                   | RdRP     | N       | Orf1a/1b |
| POS           | POS      | cobas               | 19.23    |         |         |
| POS           | POS      | PHO                 | 28.48    |         |         |
| POS           | POS      | Allplex             | 19.42    | 20.31   | 20.7    |
| POS           | POS      | Allplex             | 18.48    | 20.08   | 20.24   |
| POS           | POS      | PHO                 | 24.33    |         |         |
| POS           | POS      | Allplex             | 22.51    | 24.04   | 25.64   |
| NEG           | NEG      | Aptima              | N/D      | N/D     | 34.4    |
| NEG           | NEG      | Allplex             | 33.16    | 34.95   | 35.52   |
| NEG           | NEG      | Allplex             | 31.93    | 33.2    | 32.57   |
| NEG           | NEG      | PHO                 | 34.44    |         |         |
positive predictive value of the Panbio is 100% and the negative predictive value is 99.8%. Overall, the incidence of SARS-CoV-2 detection in the test population was 0.37%.

3.2. Cycle threshold (Ct) value analysis

Ct or RLU values for genes detected by laboratory molecular assays were available for the 11 positive specimens. These are shown in Table 2. Public Health Ontario laboratories provide result interpretations based on Ct values, where Ct < 35 are results as “COVID-19 detected”, Ct values between 35 and 38 are resulted as “COVID-19 detected at low level” and Ct values > 38.01 are resulted as “COVID-19 indeterminate” (Coronavirus Disease, 2019, Ontario Agency for Health Protection and Promotion). Based on the obtained Ct values, none of the positive samples tested fell into the “low level” or “indeterminate” categories. Of note, results for the TMA assay are expressed in relative light units (RLU). For reference, results with RLU < 1000 indicate low levels of detection. In general, higher Ct values were associated with absence of detection by the Panbio COVID-19 Ag POC test. The highest Ct value detected by qRT-PCR that was associated with positive antigen detection was 28.48 (Table 2).

4. Discussion

In response to the ongoing pandemic, pressure has been mounting in Canada to allow the use of rapid detection assays for the diagnosis of SARS-CoV-2 infections in the asymptomatic population or for screening purposes. In Ontario, the Ministry of Long-Term Care has mandated the use of the SARS-CoV-2 rapid antigen detection assays as regular screening tools for long-term care staff, volunteers and visitors. While this guidance recommends the use of Health Canada approved assays, it should be noted that the Panbio COVID-19 Ag is only approved by Health Canada in the asymptomatic setting (Canadian Public Health Laboratory Network Laboratory Directors Council, Canadian Public Health Laboratory Network Respiratory Virus Infection Working Group 2021). In accordance to the manufacturer’s intended use for this device, previously published studies have been conducted in symptomatic individuals and/or asymptomatic close contacts (Albert et al., 2021a; Linares et al., 2020; Torres et al., 2021a). To the best of our knowledge, our study represents the largest attempt at evaluating the performance of this assay in a real life, low incidence, asymptomatic setting with over 3000 paired samples collected.

In the current study, the Panbio COVID-19 Ag exhibited a 54.5% positive concordance with laboratory-based molecular assays. This is in keeping with sensitivity data derived from previous subsets of asymptomatic patients previously published: in a study by Torres & al, 79 individuals (12.4%) who were asymptomatic close contacts of patients infected with SARS-CoV-2 tested positive for the virus by qRT-PCR, but only 38 (48.1%) of these had detectable signal on the Panbio assay (Torres et al., 2021a). Similarly, Fenollar & al showed a 45.4% sensitivity (10/22 cases) in asymptomatic close contacts (Fenollar et al., 2021). The high specificity of the assay was also confirmed, as there were no false positive results. The highest corresponding Ct value for any Panbio positive sample was 28.48. This is also in keeping with previously published data. In the interim guidance published in the January 2021 Canada Communicable Disease Report (Canadian Public Health Laboratory Network Laboratory Directors Council, Canadian Public Health Laboratory Network Respiratory Virus Infection Working Group 2021), the highest Ct value for a sample generating a positive result with the Panbio was 28.9. The same findings were noted in a head-to-head comparison of rapid and automated antigen detection tests, where none of the samples with Ct values above 30 were detected by the Panbio assay and the overall sensitivity for samples with Ct values between 25 and 30 was 52.6% (Favresse et al., 2021).

There is ongoing debate surrounding the link between Ct values and SARS-CoV-2 infectivity, with an important study conducted at the Public Health Agency of Canada National Microbiology Laboratory demonstrating the lack of cell culture infectivity by SARS-CoV-2 from clinical samples with Ct values < 24 (Bullard et al., 2020). These data must however be interpreted with caution. Cell culture is known to suffer from an overall lack of sensitivity and is not an effective tool for the diagnosis of human coronavirus infections (Gadsby and Templeton, 2019). Accordingly, Bullard et al. does not formulate specific recommendations with regards to patient isolation in the context of specific Ct values. Furthermore, there are no data to convincingly demonstrate that risk of infectivity disappears at higher Ct values and there is no consensus as to what represents a safe Ct to infer a lack of contagiousness.

Part of the interest in rapid testing methodologies for the detection of SARS-CoV-2 stems from their expected high negative predictive value in low incidence populations, and screening tools for immediate identification of new cases. The data set presented here did reveal a very high negative predictive value for a negative result with the Panbio assay (99.8% NPV), but this number needs to be evaluated in the context of the pre-test likelihood of a negative result. Positive or negative predictive values for any assay are only valuable if they significantly improve on the pre-test probability of a positive or negative result. In the patient population tested here, an incidence of SARS-CoV-2 of 0.37% was measured (11 cases out of 3007 tests). The pre-test likelihood of a negative result was therefore 99.63%, indicating that the negative predictive value measured only minimally improved on the negative pre-test probability. Furthermore, rapid antigen tests are being deployed for use with minimal training provided and with no laboratory oversight for quality assurance. These realities may result in a further reduction in sensitivity than was demonstrated here, where all staff performing testing received hands-on training from a medical laboratory technologist and laboratory quality assurance oversight was in-place. A study from the UK demonstrated reduced sensitivity of POCT rapid antigen tests when performed by non-laboratory trained individuals compared to laboratory-trained staff (Preliminary report from the Joint, 2020). It is therefore clear that while there is advocacy for greater access to these assays, including in Canada, by non-medical personal in essential service settings (schools, manufactures, etc) (Schwartz et al., 2021), the data we have accumulated here does not support this approach unless appropriate training and oversight are available for those performing testing.

There is no such thing as a perfect assay to detect SARS-CoV-2, all currently available tests have their limitations. Overall, our findings highlight that proper validation of any assay is critical, especially when the desired use-cases are “off-label”, i.e., outside of the manufacturer-defined use case. A significant investment in human resources is however required to conduct these types of studies and recruitment of trained personal has been challenging for clinical laboratories during this pandemic. This evaluation was initially projected to detect the standard minimum of 20-25 COVID-19 positive patients to inform our evaluation of the assay, however, given the demands of a limited skilled labor force that were required for clinical care, it was unsustainable to maintain dedicated staff to this evaluation, which would have required thousands more to recruit and test to meet our minimum target. It is also important to consider how assays, in-laboratory and POCT, will perform in detecting the emerging variants of concern. Reduced or a lack of sensitivity for the variants of concern could have devastating consequences, and ongoing validation studies to confirm sensitivity of assays for variants is important. Of note, this assay is intended for use in the point-of-care setting, but recent findings by Public Health England have highlighted that the buffer supplied with the kits does not inactivate SARS-CoV-2 (Public Health England 2020). This was reiterated by Public Health Ontario in a later document along with recommendations for safe
use in the point-of-care setting (Ontario Agency for Health Protection and Promotion (Public Health Ontario) 2020). This limitation also highlights the need for laboratory oversight to ensure safety and quality assurance practices are in-place.

Limitations to our study include a small number of positive patients despite a large number of paired samples. This is not surprising given the expected low incidence in the population tested and demonstrates why validating the use of these devices in these settings is a complex endeavor. Our data also only provides an overview of the behavior of the assay when used as a single, isolated screening tool, and does not consider the impact of repeat testing, which is the strategy being implemented in Ontario long-term care homes for surveillance. While it makes sense that varying degrees of viral shedding on different days may impact positively or negatively viral detection by an antigen assay, to our knowledge there are no data that demonstrate repeat testing in the asymptomatic population will lead to an overall increase in sensitivity. Finally, our study only assesses the use of nasopharyngeal swabs and does not provide concordance data for the use of other samples such as deep nasal swabs, throat swabs, or saliva. A recently published systematic review and meta-analysis does reiterate that nasopharyngeal remain the gold standard for diagnosis of SARS-CoV-2 infections (Lee et al., 2021).

While the Panbio antigen assay can be used as a strategy for faster identification of infected individuals than the gold-standard, laboratory-based molecular assays, as stated by the manufacturer this assay should not be used to rule-out infection with SARS-CoV-2. Faster detection will allow for faster initiation of isolation and contact tracing; however, care should be taken to ensure that negative results generated through screening and surveillance programs do not breed complacency with regards to proper social distancing and infection prevention and control measures. Given that these devices are now being deployed in the most vulnerable setting, complacency could lead to more outbreaks and dire consequences.

5. Conclusions

The Abbott Panbio™ COVID-19 Ag rapid antigen detection lateral flow assay showed reduced positive concordance with the reference, laboratory-based molecular assays for the diagnosis of SARS-CoV-2 infections in the asymptomatic population compared to the data available in the literature for symptomatic patients. While the negative predictive value is high, it does not represent an improvement over the pre-test probability of a negative result in this setting. The assay allows for faster identification of SARS-CoV-2 infected individuals, but care should be taken to ensure that use of the assay does not negatively impact adherence to recommended infection prevention and control measures.

Author statement

JLV Shaw, V. Deslandes and M. Desjardins conceived the idea for the study, analyzed the data and prepared the manuscript, J. Smith trained all LTC staff and performed Panbio testing.

All authors have approved the revised manuscript.

Funding

No funding was received for this work; however, the Abbott Panbio tests were provided by Ontario Health at no cost.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. J. Shaw has served as an Advisory Board Member for Abbott

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