Comparison of the Accula SARS-CoV-2 Test with a Laboratory-Developed Assay for Detection of SARS-CoV-2 RNA in Clinical Nasopharyngeal Specimens

Catherine A. Hogan, MD, MSc, Natasha Garamani, BSc, Andrew S. Lee, MD, PhD, Jack K. Tung, MD, PhD, Malaya K. Sahoo, PhD, ChunHong Huang, MD, Bryan Stevens, MD, James Zehnder, MD, Benjamin A. Pinsky, MD, PhD

1 Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA
2 Clinical Virology Laboratory, Stanford Health Care, Stanford, CA, USA
3 Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA

*Corresponding author:
Benjamin A. Pinsky
3375 Hillview, Room 2913
Palo Alto, CA 94304
Phone (650) 498-5575
Fax (650) 736-1964
bpinsky@stanford.edu

Running title: Performance of the Accula SARS-CoV-2 Test
Word count: 1,445 words
Keywords: SARS-CoV-2, COVID-19, Mesa Accula, Point-of-Care Test, Laboratory-developed Test
Abstract

Background: Several point-of-care (POC) molecular tests have received emergency use authorization (EUA) from the Food and Drug Administration (FDA) for diagnosis of SARS-CoV-2. The test performance characteristics of the Accula (Mesa Biotech) SARS-CoV-2 POC test need to be evaluated to inform its optimal use.

Objectives: The aim of this study was to assess test performance of the Accula SARS-CoV-2 test.

Study design: The performance of the Accula test was assessed by comparing results of 100 nasopharyngeal swab samples previously characterized by the Stanford Health Care EUA laboratory-developed test (SHC-LDT) targeting the envelope (E) gene. Assay concordance was assessed by overall percent agreement, positive percent agreement (PPA), negative percent agreement (NPA), and Cohen’s kappa coefficient.

Results: Overall percent agreement between the assays was 84.0% (95% confidence interval [CI] 75.3 to 90.6%), PPA was 68.0% (95% CI 53.3 to 80.5%) and the kappa coefficient was 0.68 (95% CI 0.54 to 0.82). Sixteen specimens detected by the SHC-LDT were not detected by the Accula test, and showed low viral load burden with a median cycle threshold value of 37.7. NPA was 100% (95% CI 94.2 to 100%).

Conclusion: Compared to the SHC-LDT, the Accula SARS-CoV-2 test showed excellent negative agreement. However, positive agreement was low for samples with low viral load. The false negative rate of the Accula POC test calls for a more thorough evaluation of POC test performance characteristics in clinical settings, and for confirmatory testing in individuals with moderate to high pre-test probability of SARS-CoV-2 who test negative on Accula.
Introduction

The importance of diagnostic testing for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has been strongly emphasized by both the World Health Organization (WHO) and the United States Centers for Disease Control and Prevention (CDC) (1-3). In the US, most SARS-CoV-2 testing has been conducted using high complexity molecular-based laboratory-developed tests (LDTs) that have received emergency use authorization (EUA) by the Food and Drug Administration (FDA) in centralized laboratories certified to meet the quality standards of the Clinical Laboratory Improvement Amendments of 1988 (CLIA) (4, 5). Currently, 3 CLIA-waived point-of-care tests (POCT) are EUA-approved for SARS-CoV-2 testing: the Cepheid Xpert Xpress, the Abbott ID NOW, and the Mesa Accula (6). Compared to high complexity LDTs, POCT have the potential to reduce turnaround time of testing, optimize clinical management and increase patient satisfaction (7). The Accula SARS-CoV-2 test is a POCT that requires only 30 minutes from sample to answer and utilizes the existing palm-sized Accula dock system originally developed for rapid influenza and RSV testing. Despite the multiple potential benefits of POC assays, concern has been raised regarding their lower sensitivity for COVID-19 diagnosis compared to standard high complexity molecular based tests (8-10). It remains unclear whether this decreased sensitivity is due to test validation studies being limited to in silico predictions and contrived samples using reference materials, as is the case currently for the Accula SARS-CoV-2 test.
Objectives

The aim of this study was to evaluate the test performance characteristics of the Accula SARS-CoV-2 test in a clinical setting against a high complexity reference standard.

Study design

Nasopharyngeal (NP) swabs were collected in viral transport medium or saline from adult patients from SHC, and from pediatric and adult patients from surrounding hospitals in northern California. Testing for this study was performed at the SHC Clinical Virology Laboratory using samples collected between April 7, 2020 and April 13, 2020. The same NP specimen was used for both the reference assay (tested first) and Accula test for comparison (tested subsequently).

Clinical data on the presence of symptoms were extracted from the electronic medical record for individuals presenting to care at SHC or an affiliated hospital. This study was approved by the Stanford Institutional Review Board (protocol #48973).

RT-PCR assays

The reference assay for this study was the Stanford Health Care Clinical Virology Laboratory real-time reverse transcriptase polymerase chain reaction LDT (SHC-LDT) targeting the E gene (11-13). The Accula SARS-CoV-2 POCT (Mesa Biotech, Inc., San Diego, CA) is a sample-to-answer nucleic acid amplification test that can yield a diagnostic result within 30 minutes of specimen collection. This test uses RT-PCR to target the nucleocapsid protein (N) gene, and is read out via lateral flow (Figure 1) (14). The manufacturer’s instructions comprise the following steps: collection of nasopharyngeal (NP) swab, lysis of viral particles in SARS-CoV-2 buffer, transfer of nucleic acid solution to a test cassette which contains internal process positive and
negative controls, reverse transcription of viral RNA to cDNA, nucleic acid amplification, and
detection by lateral flow. Due to biosafety regulations and hospital-mandated protocols for
sample collection at SHC, NP swabs were directly placed into VTM or saline at the patient
bedside after collection. Each test was performed at the laboratory, where a volume of 10 µL of
VTM or saline was transferred to 60 µL of SARS-CoV-2 buffer and added to the test cassette.
These steps were performed within a biosafety cabinet to protect against aerosolization. All
remaining steps were followed as per the manufacturer’s instructions (14). Testing was repeated
once for invalid results on initial testing, and the second result was interpreted as final if valid.

Statistics
Overall percent agreement, positive percent agreement (PPA), negative percent agreement
(NPA) and associated 95% confidence intervals (CI) were calculated. Cohen’s kappa coefficient
(κ) of qualitative results (detected/non-detected) between the Accula SARS-CoV-2 test and the
SHC-LDT was also calculated with 95% CI. Cohen’s kappa values between 0.60 and 0.80 were
interpreted to indicate substantial agreement, and kappa values above 0.81 were interpreted as
excellent agreement (15). All analyses were performed using Stata version 15.1.

Results
We included 100 samples (50 positive, 50 negative) previously tested by the SHC LDT, and
subsequently tested with the Accula SARS-CoV-2 POCT. A total of 45 samples were collected
in VTM (21 positive, 24 negative), and 55 were collected in saline (29 positive, 26 negative).
Data on the presence of clinical symptoms were available for 26/50 individuals with positive
results. Of these, 24 individuals were symptomatic and 2 were asymptomatic and tested for
follow-up. Positive samples determined by the SHC-LDT included a range of cycle threshold (Ct) values, with a median Ct of 28.2 (IQR 20.4-36.3). A total of 3 samples were resulted as invalid on initial testing by Accula and were repeated once. One of these samples was detected for SARS-CoV-2 on repeat testing, and the other 2 samples were negative.

The Accula SARS-CoV-2 test correctly identified 34/50 positive samples and 50/50 negative samples, corresponding to an overall percent agreement of 84.0% (95% CI 75.3 to 90.6%), (Table 1). The positive percent agreement was 68.0% (95% CI 53.3 to 80.5%), the Cohen’s kappa coefficient was 0.74 (95% CI 0.61 to 0.87), indicating substantial agreement, and the NPA was 100% (95% CI 92.9 to 100%). The positive percent agreement varied by Ct values and transport medium used, with higher performance in samples with low Ct samples and in VTM (Table 2). The 34 samples that were detected by both assays had a median Ct value of 23.5 (IQR 19.7-28.7). The 16 samples that were positive by SHC-LDT but negative by the Accula test had a median Ct value of 37.7 (IQR 36.6 to 38.2), consistent with lower viral loads. Restricting the analysis to the 24 symptomatic individuals, the positive percent agreement was 66.7% (95% CI 44.7-84.4%), and the median Ct value was 26.5 (IQR 19.8-37.3). The lateral flow read-out on the Accula test was considered easy to interpret for all samples with the exception of a single known positive sample that showed a faint positive test line. Repeat testing of this sample showed the same faint test line, and was interpreted as positive.

**Discussion**

Although SARS-CoV-2 testing capacity has improved in many countries, a global shortage of diagnostic infrastructure and consumable reagents has limited testing efforts. Point-of-care tests offer the potential advantages of improved access to testing and reduced turnaround time of
results. Of the multiple EUA assays for diagnosis of SARS-CoV-2, only the Xpert Xpress, the ID NOW, and the Accula are CLIA-waived (6). Recent data support the test performance of the Cepheid Xpert SARS-CoV-2 assay, with agreement over 99% compared to high-complexity EUA assays (8, 16, 17). In contrast, some studies have raised concern regarding the diagnostic accuracy of the ID NOW, with positive percent agreement ranging from 75-94% compared to reference assays (8-10, 18). Given the poor diagnostic performance of the ID NOW, and uncertainty regarding availability of Xpert Xpress cartridges, the Accula system has been tauted as an interesting POCT alternative but data were previously lacking on its clinical performance. In this study, we showed that similar to ID NOW, the Accula SARS-CoV-2 test has a lower sensitivity for diagnosis of COVID-19 compared to an EUA LDT. The false negatives obtained from the Accula SARS-CoV-2 test were predominantly observed with low viral load specimens. The exact reason for the low sensitivity of the Accula is unclear at present. The primer and probe sequences are not publicly available for this assay to identify which region of the N gene is targeted; previous comparative data support similarly high sensitivity of the N2 and E gene targets, but lower sensitivity of the N3 target, for the diagnosis of SARS-CoV-2 (19).

Given the accumulating evidence on lower diagnostic performance with 2 of the 3 CLIA-waived SARS-CoV-2 assays, it is now important to consider how best to integrate these tests in diagnostic workflows and to identify groups of individuals for whom POCT use should be prioritized. Furthermore, reagents and kits have been limited, which limits POCT capacity. Certain groups such as individuals requiring urgent pre-operative assessment including transplantation, patient-facing symptomatic healthcare workers, and individuals waiting for enrollment in a SARS-CoV-2 therapeutic trial have been identified as key groups in whom to...
prioritize POCT. However, for each of these scenarios and depending on the POCT used, the risk of missing a case due to low sensitivity must be considered. In individuals with moderate to high pre-test probability of SARS-CoV-2, reflex testing of negative samples on a separate EUA assay should be performed. Education of health care professionals on the limitations of SARS-CoV-2 POCT should also be implemented to ensure optimal interpretation and management of negative results.

Our study has several limitations. First, NP swabs were placed in VTM or saline at the patient bedside before loading the Accula test cassette, which may have decreased sensitivity by diluting the viral inoculum. Although this is discordant with the best recommended practice by the manufacturer, it is in line with the practice at multiple institutions with clinical laboratories that have assessed SARS-CoV-2 POCT due to biosafety concerns from risk of aerosolization (8-10, 18, 20). Second, it is possible that the use of saline instead of VTM led to poorer performance of the Accula. However, aliquots from the same sample were used for parallel testing with the EUA method, which minimizes sources of variation, and represents a pragmatic comparison given widespread VTM shortages. Finally, the lateral-flow read-out of the Accula test is generally easy to interpret; however, faint lines may be more challenging to interpret and lead to result discrepancies.

In summary, this study demonstrated that the Accula POCT lacks sensitivity compared to a reference EUA SARS-CoV-2 LDT. Careful consideration should be given to balance the potential advantages of rapid POCT to lower diagnostic accuracy. Individuals with moderate to high pre-test probability of SARS-CoV-2 should be reflex tested on a separate EUA assay.
high pre-test probability who initially test negative on the Accula test should undergo confirmatory testing with a separate EUA assay.

**Acknowledgments**

We would like to thank the members of the Stanford Health Care Clinical Virology Laboratory, Department of Emergency Medicine, and Department of Medicine, Division of Infectious Disease for their hard work and dedication to patient care.

**Funding**

None

**Conflicts of Interest**

The authors declare no conflicts of interest.
References

1. Bedford J, Enria D, Giesecke J, Heymann DL, Ihekweazu C, Kobinger G, Lane HC, Memish Z, Oh MD, Sall AA, Schuchat A, Ungchusak K, Wieler LH, Strategic WHO, Technical Advisory Group for Infectious H. 2020. COVID-19: towards controlling of a pandemic. Lancet 395:1015-1018.

2. Centers for Disease Control and Prevention (CDC). 2020. Evaluating and Testing Persons for Coronavirus Disease 2019 (COVID-19). https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-criteria.html. Accessed May 13 2020.

3. World Health Organization. 2020. Coronavirus disease (COVID-19) technical guidance: Laboratory testing for 2019-nCoV in humans. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance. Accessed May 7 2020.

4. Food and Drug Administration. 2020. Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised). https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-coronavirus-disease-2019-tests-during-public-health-emergency-revised. Accessed May 7 2020.

5. Sharfstein JM, Becker SJ, Mello MM. 2020 Mar 9. doi: 10.1001/jama.2020.3864. [Epub ahead of print]. Diagnostic Testing for the Novel Coronavirus. JAMA doi:10.1001/jama.2020.3864.

6. Food and Drug Administration. 2020. Emergency Use Authorizations. https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations. Accessed

7. Sheridan C. 2020. Fast, portable tests come online to curb coronavirus pandemic. Nat Biotechnol doi:10.1038/d41587-020-00010-2.

8. Zhen W, Smith E, Manji R, Schron D, Berry GJ. 2020. Clinical Evaluation of Three Sample-To-Answer Platforms for the Detection of SARS-CoV-2. J Clin Microbiol doi:10.1128/JCM.00783-20.

9. Harrington A, Cox B, Snowdon J, Bakst J, Ley E, Grajales P, Maggiore J, Kahn S. 2020. Comparison of Abbott ID Now and Abbott m2000 methods for the detection of SARS-
10. Hogan CA, Sahoo MK, Huang C, Garamani N, Stevens B, Zehnder J, Pinsky BA. 2020. Five-minute point-of-care testing for SARS-CoV-2: Not there yet. Journal of Clinical Virology 128:104410.

11. Hogan CA, Sahoo MK, Pinsky BA. 2020. Sample Pooling as a Strategy to Detect Community Transmission of SARS-CoV-2. JAMA doi:10.1001/jama.2020.5445.

12. Hogan CA, Sahoo MK, Huang C, Garamani N, Stevens B, Zehnder J, Pinsky BA. 2020. Comparison of the Panther Fusion and a Laboratory-developed Test Targeting the Envelope gene for Detection of SARS-CoV-2. Journal of Clinical Virology doi:https://doi.org/10.1016/j.jcv.2020.104383:104383.

13. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, Bleicker T, Brunink S, Schneider J, Schmidt ML, Mulders DG, Haagmans BL, van der Veer B, van den Brink S, Wijsman L, Godeurski G, Romette JL, Ellis J, Zambon M, Peiris M, Goossens H, Reusken C, Koopmans MP, Drosten C. 2020. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 25.

14. Mesa Biotech. Document Library for Accula SARS-CoV-2 Test. https://www.mesabiotech.com/coronavirusdocuments. Accessed May 7 2020.

15. Landis JR, Koch GG. 1977. The measurement of observer agreement for categorical data. Biometrics 33:159-74.

16. Lieberman JA, Pepper G, Naccache SN, Huang ML, Jerome KR, Greninger AL. 2020. Comparison of Commercially Available and Laboratory Developed Assays for in vitro Detection of SARS-CoV-2 in Clinical Laboratories. J Clin Microbiol doi:10.1128/JCM.00821-20.

17. Moran A, Beavis KG, Matushek SM, Ciaglia C, Francois N, Tesic V, Love N. 2020. The Detection of SARS-CoV-2 using the Cepheid Xpert Xpress SARS-CoV-2 and Roche cobas SARS-CoV-2 Assays. J Clin Microbiol doi:10.1128/JCM.00772-20.

18. Rhoads DD, Cherian SS, Roman K, Stempak LM, Schmotzer CL, Sadri N. 2020. Comparison of Abbott ID Now, Diasorin Simplexa, and CDC FDA EUA methods for the detection of SARS-CoV-2 from nasopharyngeal and nasal swabs from individuals diagnosed with COVID-19. J Clin Microbiol doi:10.1128/JCM.00760-20.
19. Nalla AK, Casto AM, Huang MW, Perchetti GA, Sampoleo R, Shrestha L, Wei Y, Zhu H, Jerome KR, Greninger AL. 2020. Comparative Performance of SARS-CoV-2 Detection Assays using Seven Different Primer/Probe Sets and One Assay Kit. J Clin Microbiol doi:10.1128/JCM.00557-20.

20. Kaiser Health News. 2020. Abbott’s Fast COVID Test Poses Safety Issues, Lab Workers Say. https://khn.org/news/abbotts-fast-covid-test-poses-safety-issues-lab-workers-say/. Accessed April 25 2020.
Figure Legend

Figure 1. Images of the Accula SARS-CoV-2 Lateral Flow Readout. (A) positive patient specimen; (B) negative patient specimen. C, internal positive process control; T, SARS-CoV-2 test; NC, internal negative process control.
Table 1. Comparison of the Stanford Health Care SARS-CoV-2 Laboratory-Developed Test and the Accula SARS-CoV-2 PCR Test

| Accula SARS-CoV-2 PCR Test | Detected | Not Detected | Total |
|---------------------------|----------|--------------|-------|
| **SHC-LDT**               |          |              |       |
| Detected                  | 34       | 16           | 50    |
| Not Detected              | 0        | 50           | 50    |
| **Total**                 | 34       | 66           | 100   |

LDT: Laboratory-developed test; PCR: polymerase chain reaction; SARS-CoV-2: severe acute respiratory syndrome coronavirus; SHC: Stanford Health Care

Table 2. Positive Percent Agreement of the Accula SARS-CoV-2 PCR Test Compared to the Stanford Health Care SARS-CoV-2 Laboratory-Developed Test, Stratified by Cycle Threshold Values and Transport Medium Type

| Ct value | Saline PPA | VTM PPA | Overall PPA |
|----------|------------|---------|-------------|
| <30      | 100% (11/11) | 100% (16/16) | 100% (27/27) |
| 30-35    | 50.0% (3/6) | 100% (3/3) | 66.7% (6/9) |
| >35      | 8.3% (1/12) | 0% (0/2) | 7.1% (1/14) |
| **Total** | 51.7% (15/29) | 90.5% (19/21) | 68.0% (34/50) |

Ct: cycle threshold; PPA: positive percent agreement; SARS-CoV-2: severe acute respiratory syndrome coronavirus; VTM: viral transport medium
