

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

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24

25 **Abstract**

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

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

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

37 **Results:** Overall percent agreement between the assays was 84.0% (95% confidence interval  
38 [CI] 75.3 to 90.6%), PPA was 68.0% (95% CI 53.3 to 80.5%) and the kappa coefficient was 0.68  
39 (95% CI 0.54 to 0.82). Sixteen specimens detected by the SHC-LDT were not detected by the  
40 Accula test, and showed low viral load burden with a median cycle threshold value of 37.7. NPA  
41 was 100% (95% CI 94.2 to 100%).

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

47 **Background**

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

66 ***Objectives***

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

69

70 ***Study design***

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

76

77 ***RT-PCR assays***

78 The reference assay for this study was the Stanford Health Care Clinical Virology Laboratory  
79 real-time reverse transcriptase polymerase chain reaction LDT (SHC-LDT) targeting the *E* gene  
80 (11-13). The Accula SARS-CoV-2 POCT (Mesa Biotech, Inc., San Diego, CA) is a sample-to-  
81 answer nucleic acid amplification test that can yield a diagnostic result within 30 minutes of  
82 specimen collection. This test uses RT-PCR to target the nucleocapsid protein (*N*) gene, and is  
83 read out via lateral flow (**Figure 1**) (14). The manufacturer's instructions are comprised of the  
84 following steps: collection of nasopharyngeal (NP) swab, lysis of viral particles in SARS-CoV-2  
85 buffer, transfer of nucleic acid solution to a test cassette which contains internal process positive  
86 and negative controls, reverse transcription of viral RNA to cDNA, nucleic acid amplification,  
87 and detection by lateral flow. Due to biosafety regulations and hospital-mandated protocols for  
88 sample collection at SHC, NP swabs were directly placed into VTM or saline at the patient

89 bedside after collection. Each test was performed at the laboratory, where a volume of 10  $\mu$ L of  
90 VTM or saline was transferred to 60  $\mu$ L of SARS-CoV-2 buffer and added to the test cassette.  
91 These steps were performed within a biosafety cabinet to protect against aerosolization. All  
92 remaining steps were followed as per the manufacturer's instructions (14). Testing was repeated  
93 once for invalid results on initial testing, and the second result was interpreted as final if valid.

94

### 95 *Statistics*

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

102

### 103 *Results*

104 We included 100 samples (50 positive, 50 negative) previously tested by the SHC LDT, and  
105 tested in parallel with the Accula SARS-CoV-2 POCT. A total of 37 samples were collected in  
106 VTM (13 positive, 24 negative), and 63 were collected in saline (37 positive, 26 negative).  
107 Positive samples determined by the SHC-LDT included a range of cycle threshold (Ct) values,  
108 with a median Ct of 28.2 (IQR 20.4-36.3). A total of 3 samples were resulted as invalid on initial  
109 testing by Accula and were repeated once. One of these samples was detected for SARS-CoV-2  
110 on repeat testing, and the other 2 samples were negative.

111

112 The Accula SARS-CoV-2 test correctly identified 34/50 positive samples and 50/50 negative  
113 samples, corresponding to an overall percent agreement of 84.0% (95% CI 75.3 to 90.6%),  
114 (**Table 1**). The positive percent agreement was 68.0% (95% CI 53.3 to 80.5%) and the Cohen's  
115 kappa coefficient was 0.74 (95% CI 0.61 to 0.87), indicating substantial agreement. The 16  
116 positive samples that were negative by the Accula test had a median Ct value of 37.7 (IQR 36.6  
117 to 38.2) by the SHC-LDT, consistent with lower viral loads. The NPA was 100% (95% CI 92.9  
118 to 100%). The lateral flow read-out on the Accular test was considered easy to interpret for all  
119 samples with the exception of a single known positive sample that showed a faint positive test  
120 line. Repeat testing of this sample showed the same faint test line, and was interpreted as  
121 positive.

122

### 123 *Discussion*

124 Although SARS-CoV-2 testing capacity has improved in many countries, a global shortage of  
125 diagnostic infrastructure and consumable reagents has limited testing efforts. Point-of-care tests  
126 offer the potential advantages of improved access to testing and reduced turnaround time of  
127 results. Of the multiple EUA assays for diagnosis of SARS-CoV-2, only the Xpert Xpress, the ID  
128 NOW, and the Accula are CLIA-waived (6). Recent data support the test performance of the  
129 Cepheid Xpert SARS-CoV-2 assay, with agreement over 99% compared to high-complexity  
130 EUA assays (8, 16, 17). In contrast, some studies have raised concern regarding the diagnostic  
131 accuracy of the ID NOW, with positive percent agreement ranging from 75-94% compared to  
132 reference assays (8-10, 18). Given the poor diagnostic performance of the ID NOW, and  
133 uncertainty regarding availability of Xpert Xpress cartridges, the Accula system has been touted  
134 as an interesting POCT alternative but data were previously lacking on its clinical performance.  
135 In this study, we showed that similar to ID NOW, the Accula SARS-CoV-2 test has a lower

136 sensitivity for diagnosis of COVID-19 compared to an EUA LDT. The false negatives obtained  
137 from the Accula SARS-CoV-2 test were predominantly observed with low viral load specimens.

138

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

152

153 Our study has several limitations. First, NP swabs were placed in VTM or saline at the patient  
154 bedside before loading the Accula test cassette, which may have decreased sensitivity by diluting  
155 the viral inoculum. Although this is discordant with the best recommended practice by the  
156 manufacturer, it is in line with the practice at multiple institutions with clinical laboratories that  
157 have assessed SARS-CoV-2 POCT due to biosafety concerns from risk of aerosolization (8-10,  
158 18, 19). Second, it is possible that the use of saline instead of VTM led to poorer performance of

159 the Accula. However, aliquots from the same sample were used for parallel testing with the EUA  
160 method, which minimizes sources of variation, and represents a pragmatic comparison given  
161 widespread VTM shortages. Finally, the lateral-flow read-out of the Accula test is generally easy  
162 to interpret; however, faint lines may be more challenging to interpret and lead to result  
163 discrepancies.

164  
165 In summary, this study demonstrated that the Accula POCT lacks sensitivity compared to a  
166 reference EUA SARS-CoV-2 LDT. Careful consideration should be given to balance the  
167 potential advantages of rapid POCT to lower diagnostic accuracy. Individuals with moderate to  
168 high pre-test probability who initially test negative on the Accula test should undergo  
169 confirmatory testing with a separate EUA assay.

170

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175

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177 None

178

### 179 *Conflicts of Interest*

180 The authors declare no conflicts of interest.



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247 **Figure Legend**

248

249 **Figure 1.** Images of the Accula SARS-CoV-2 Lateral Flow Readout. (A) positive patient  
250 specimen; (B) negative patient specimen. C, internal positive process control; T, SARS-CoV-2  
251 test; NC, internal negative process control.

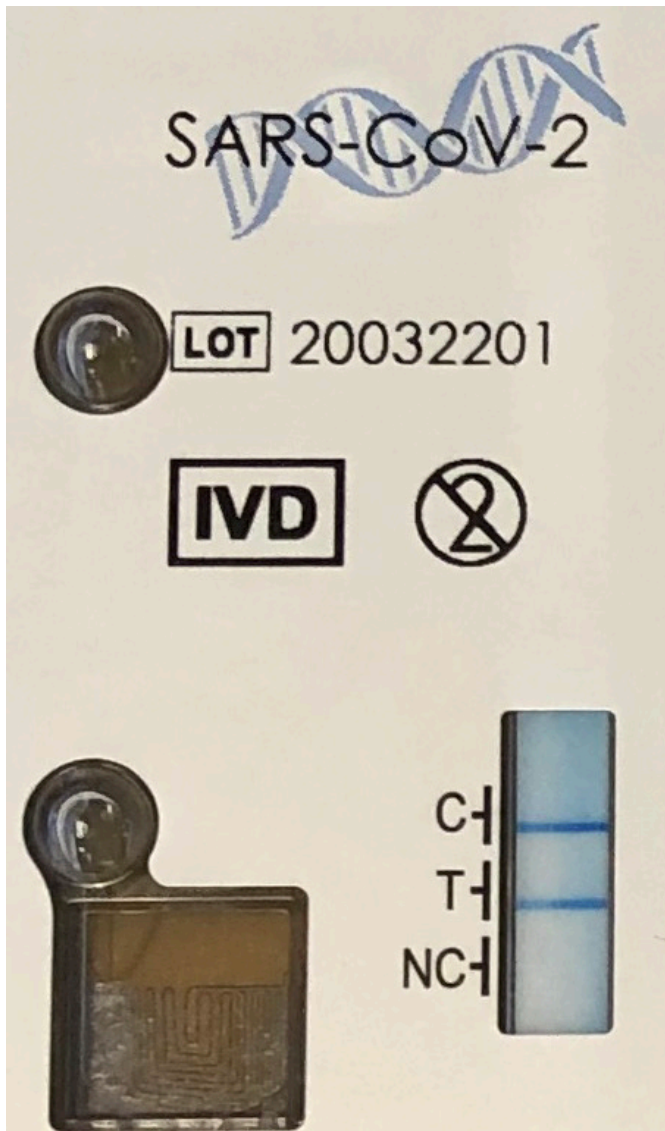
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253 **Table 1.** Comparison of the Stanford Health Care SARS-CoV-2 Laboratory-Developed Test and  
254 the Accula SARS-CoV-2 PCR Test

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

255  
256 LDT: Laboratory-developed test; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; SHC: Stanford  
257 Health Care  
258

A.



B.

