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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23   Test

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 μL of

90   VTM or saline was transferred to 60 μL 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   (κ) 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 calues 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 tauted

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

179   *Conflicts of Interest*

180   The authors declare no conflicts of interest.

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182

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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

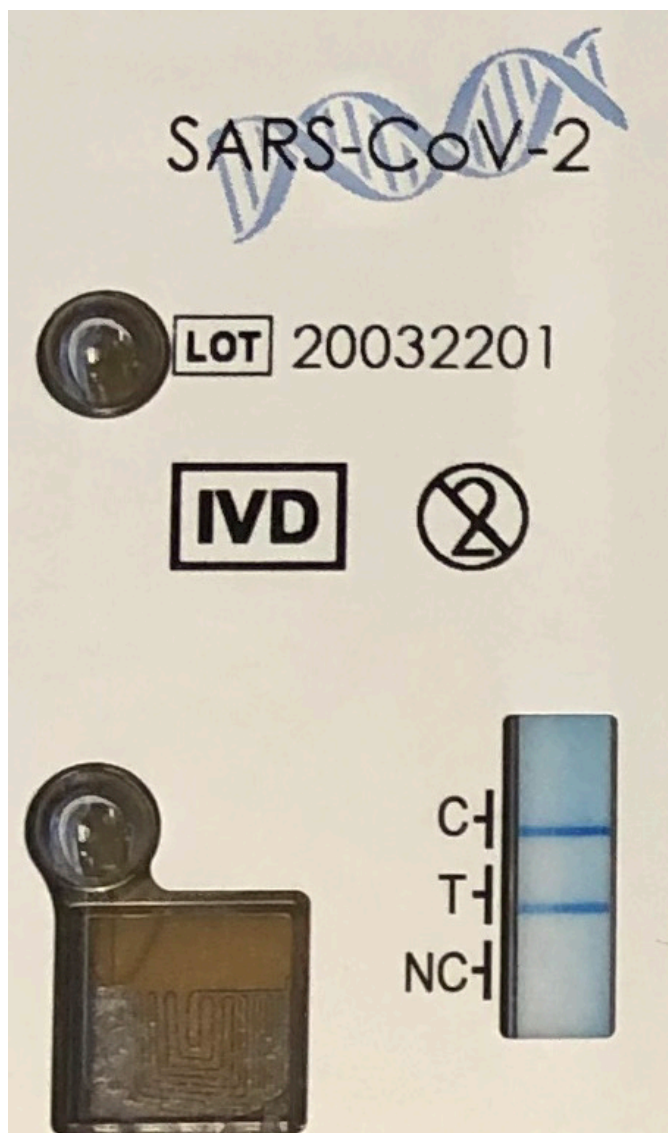251 test; NC, internal negative process control.

252

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		
		Detected	Not Detected	Total
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