1   **Rapid detection of SARS-CoV-2 antibodies in oral fluids**

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23   **Abstract**

24

25   Current commercially available methods for reliably detecting antibodies against

26   SARS-CoV-2 remain expensive and inaccessible due to the need for whole blood

27   collection by highly trained phlebotomists using personal protective equipment (PPE). We

28   evaluated an antibody detection approach utilizing the OraSure® Technologies' Oral

29   Antibody Collection Device (OACD) and their proprietary SARS-CoV-2 total antibody

30   detection enzyme-linked immunosorbent assay (ELISA). We found that the OraSure®

31   test for total antibody detection in oral fluid had comparable sensitivity and specificity to

32   serum-based ELISAs while presenting a more affordable and accessible system with the

33   potential for self-collection.

34   **Main text**

35

36   *Introduction*

37   The COVID-19 pandemic has had a severe impact on populations and economies

38   worldwide (1). While the relationship between antibodies and their protective role against

39   reinfection of SARS-CoV-2 is under investigation, it has been shown that antibodies

40   consistently develop as part of the immune response against the virus in some, if not

41   most, infected individuals (2). Neutralizing antibodies were effective and sustained for at

42   least two and potentially up to twelve years against SARS-CoV, the first SARS

43   coronavirus, strongly suggesting the potential for significant immunity conferred by

44   antibodies against SARS-CoV-2 and prompting extensive studies to identify neutralizing

45   antibodies for therapeutic development (3,4). Further, although residues of the receptor

46   binding domains (RBD) of SARS-CoV and SARS-CoV-2 differ by about 55% (18/33), it

47   has been shown that neutralizing antibodies against SARS-CoV are reactive against

48   SARS-CoV-2. This response indicates overlapping epitopes as the target of these

49   neutralizing antibodies, confirming similarity between antibodies developed against the

50   two SARS-CoVs and supporting the hypothesis of antibody efficacy against re-infection

51   by SARS-CoV-2 (5).

52   Since the introduction of SARS-CoV-2 in late 2019, individuals infected by the virus

53   have remained immune against re-infection in all but a few sporadic cases. Even in those

54   patients, it is still not clear whether it was a case of re-infection or re-activation of the

55   virus, suggesting that individuals who become infected with SARS-CoV-2 seem to have

56   at least some transient, temporal immunity (6). Given those findings, we have ample

3

57   reason to believe that the monitoring of antibodies is crucial to understanding global

58   disease spread, immunity against re-infection by the virus, and the effectiveness of

59   potential vaccine candidates.

60   The industry standard for antibody detection relies on the collection and analysis

61   of patient serum, requiring trained phlebotomists to draw the whole blood from a patient

62   following suspected viral infection (7). That process is time-consuming, costly, and

63   potentially hazardous to the phlebotomist. There is also a substantial need to protect

64   phlebotomists with personal protective equipment (PPEs) such as K95 masks, face

65   shields, gloves etc. that have had limited availability during the pandemic (8). In addition

66   to the health concerns, the requirement of a phlebotomist is a bottleneck for patient blood

67   collection which prevents easy antibody testing for widespread monitoring. Thus, a non-

68   invasive technology for self-collection is needed to improve access to antibody testing.

69   We have previously demonstrated that patient saliva, instead of serum, can serve

70   as a sufficient medium for the detection of antibodies against SARS-CoV-2 (9). However,

71   given the substantial need for sensitive and specific antibody detection, we did not feel

72   that our method of collection was adequately sensitive for practical manufacturing and

73   mass production for widespread testing. This led us to a collaboration with OraSure®

74   Technologies, who have previously demonstrated the capacity of their Food and Drug

75   Administration-approved OraSure® Oral Antibody Collection Device (OACD) to sample

76   oral fluid antibody against human immunodeficiency virus (HIV) and other pathogens.

77   OraSure® Technologies has adapted the use of this device for better collection of oral

78   specimens and developed an ELISA for detection of antibodies (IgG, IgM, IgA) against

79   SARS-CoV-2 in these specimens (10,11)

80

Results

82   To determine whether we could adequately detect antibodies against SARS-CoV-

83   2 in oral fluid self-collected under observation using the OraSure® OACD (Schematic 1),

84   we evaluated paired serum and oral fluid from individuals who had undergone SARS-

85   CoV-2 oral fluid molecular RNA testing (Fig. 1A). Participant specimens were collected

86   with informed consent from a diverse population of patients (Fig. 1B). It is important to

87   note that the antibody detection kits presented here do not claim to quantify antibody in

88   the sample but instead aim to provide a binary indicator of antibody presence. Thus, we

89   cannot derive any correlations here between antibody signal and any other factor, but can

90   use the results to determine the presence of an antibody response.

91   From positive participants, serum and oral fluid was collected at least 21 days after

92   the date of reported symptom onset to ensure adequate time for antibody seroconversion

93   (2). We tested participant serum specimens on the EuroImmun Anti-SARS-CoV-2 (IgG)

94   ELISA (12) to determine whether the participants were expressing antibodies against the

95   virus using previously described methods (9) (Fig. 2A). Paired oral fluid collected using

96   the OraSure® OACD from sixty-nine SARS-CoV-2 RNA- and serum antibody-positive

97   participants and eighty-one SARS-CoV-2 RNA- and serum antibody- negative

98   participants was evaluated on the OraSure® total antibody ELISA. Antibodies were

99   detected in sixty-four (92.8%) of sixty-nine serum antibody positive participant specimens

100   and zero (100% specificity) of eighty-one serum negative participant specimens (Fig. 2B).

101   We further investigated how well the OACD and ELISA performed when monitoring

102   paired specimens collected from the same participants at later time points post-infection.

103   To evaluate this, we selected sixteen out of our original sixty-nine positive participants

104   and collected their paired oral fluid and serum specimens at least 60 days after the original

105   collection date. We found that the oral fluid-based total antibody ELISA from OraSure®

106   Technologies performed with statistical equivelance in comparison to the serum-based

107   IgG antibody ELISA from EuroImmun, with all positive specimens passing the antibody

108   detection cutoff after both 21+ days and 60+ days (Fig 3.)

109   To evaluate the potential time and cost savings of using the OACD for antibody

110   detection instead of traditional serum-based methods, we estimated that a trained

111   phlebotomist operating at a full-capacity drive-thru center can only collect and process

112   blood serum from ~40 patients in a 5-hour period, resulting in collection of one patient

113   specimen per 7.5 minutes. Because oral fluid can potentially be self-collected using the

114   OACD, face-to-face specimen collection time can be eliminated. This dramatically

115   reduces the amount of time needed for each specimen to be collected, leading us to

116   estimate that we can collect devices from ~1,000 patients in a similar drive-thru setting

117   over the same 5-hour period, resulting in collection of one specimen in approximately 0.3

118   minutes. Note that this time does not include the actual time for collection of the sample,

119   which we are assuming individuals would perform while they are waiting in the drive-thru

120   line, thus highlighting the dramatic benefit of self-collected oral samples. This represents

121   approximately a 25-fold increase in sample collection throughput versus traditional blood

122   serum sample collection in the same time frame(Fig. 4).

123   To increase testing capacity and decrease turnaround time for antibody results,

124   we have automated the process for running the OraSure® Anti-SARS-CoV-2 total

125   antibody ELISA and will continue evaluating the efficiency and reliability of the test.

126   *Discussion*

127   While antibody testing has previously been limited by the requirement of trained

128   personnel for specimen collection, we have evaluated here a technology for non-invasive

129   self-collection which we believe will be able to be scaled up and mass-produced for

130   widespread testing. Based on the test efficacy and time-savings afforded by this method

131   of antibody detection, OraSure® Technologies has developed a more accessible and

132   affordable testing option for patients interested in understanding whether they have been

133   exposed to and developed antibodies against SARS-CoV-2. The simplicity of the OACD

134   allows us to speculate that this will enable specimens to be theoretically self-collected

135   and eliminate the need for a trained medical associate to administer the collection.

136   Oral fluid and mucosal antibodies comprise the first line of defense against a

137   respiratory virus and such antibodies are known to typically contribute to sterilizing

138   immunity by neutralizing the virus prior to infection. This neutralization results in both

139   reduced viral load and impact of subsequent infection(13). The results of this study have

140   demonstrated the persistence of antibodies against SARS-CoV-2 in oral fluid of

141   convalescent humans, indicating the importance of having a widely accessible oral fluid

142   antibody detection system to help determine individual immunity in the population.

143   The OACD, when used with the Anti-SARS-CoV-2 total antibody ELISA kit

144   developed by OraSure® Technologies, has achieved sensitivity and specificity which are

145   equivalent to commercially available serum-based ELISAs(9). This represents a unique

146   and highly successful advancement in antibody detection technology. As we move closer

147   to the development and implementation of a successful vaccine against SARS-CoV-2,

148   we need reliable methods for monitoring antibody production on a wide scale and over

149   time. This antibody detection system allows us to overcome the limitations presented by

150   currently administered serum antibody tests, increasing accessibility to antibody testing

151   and enabling a better understanding of the trends of immune response against this virus

152   over time.

153 **Methods**

154 *Human serum and oral fluid specimen collection.*

155

156 *Post-COVID-19 serum and oral fluid specimens*

157 Clinical specimens were collected under the UCLA Institutional Review Board (IRB)

158 approved study protocol IRB#20-000703. The UCLA IRB determined the protocol was of

159 minimal risk and that verbal informed consent was sufficient for the research under 45

160 CFR 46.117(c). The study team complied with all UCLA policies and procedures, as well

161 as with all applicable Federal, State, and local laws regarding the protection of human

162 subjects in research as stated in the approved IRB. For this study, we obratined three

163 specimens: oral fluid for viral PCR testing, blood via venipuncture, and one oral fluid

164 specimens for antibody testing.

165

166 *Oral fluid swab for viral RNA:* This was obtained according to the reported guidelines for

167 Curative's oral fluid COVID-19 test (14). Briefly, participants coughed hard three times

168 while shielding their cough via mask and/or coughing into the crook of their elbow. They

169 then swabbed the inside of their cheeks, along the top and bottom gums, under the

170 tongue, and finally on the tongue, to gather a sufficient amount of oral fluid. Swabs were

171 placed in a tube containing RNA shield and transported at room temperature before

172 laboratory processing as described. Positive for viral RNA was determined as below 35

173 cycle threshold (CT).

174

175   *Blood sampling*: Participants underwent a standard venipuncture procedure. Briefly,

176   licensed phlebotomists collected a maximum of 15ml whole blood into 3 red-top SST

177   tubes (Becton-Dickinson, cat. number 367988). Once collected, the specimen was left at

178   ambient temperature for 30-60 minutes to coagulate and then was centrifuged at 2200-

179   2500 rpm for 15 minutes at room temperature. Specimens were then placed on ice until

180   delivered to the laboratory site where the serum was aliquoted to appropriate volumes for

181   storage at -80 ºC until use.

182

183   *OraSure® Oral Fluid Specimen Collection:* OraSure® Oral Antibody Collection Devices

184   (OACD) (item number 3001-3442-70, OraSure®, USA) were used as instructed(10,15).

185   The pad was brushed briefly on the lower and upper gums and then held between the

186   lower gum and the cheek for 2-5 minutes. The pad was then placed into the storage tube,

187   with the provided storage solution. Specimens were kept on ice until they reached the

188   lab. The specimens were processed as recommended by the manufacturer before being

189   aliquoted and stored at -80°C until use(16).

190

191   *Serum ELISA*

192   EuroImmun's FDA-approved SARS-CoV-2 (IgG) ELISA for serum (catalog #EI 2606-

193   9620, EuroImmun, New Jersey, USA) targeting spike (S) protein was run according to the

194   manufacturer provided protocol on the Thunderbolt (Gold Standard Diagnostics, Davis,

195   CA) automated instrument (12). Briefly, serum was diluted 1:101 in each well with the

196   provided sample buffer and then incubated at 37ºC for 1 hour. Sample wells were washed

197   three times with a provided wash buffer (10X dilution with ddH$_2$O, 0.35ml per well), before

198   the provided conjugate solution was added (0.1ml per well) and incubated at 37ºC for 30

199   minutes. After a second wash step, the provided substrate solution was added (0.1ml per

200   well) and incubated at ambient temperature for 30 minutes. The provided stop solution

201   was then added (0.1ml per well) and absorbance of sample wells measured immediately

202   at 450 nm and 630 nm, with output reports generated with optical density (O.D.) at 630nm

203   subtracted from O.D. at 450nm.

204   Data were then analyzed as recommended by the manufacturer and results reported as

205   a ratio (**Equation 1**). Specimens whose ratio exceeded or was equal to 1.1 were

206   considered positive, while specimens with a ratio greater than 0.8 and less than 1.1 were

207   considered equivocal and specimens with a ratio less than or equal to 0.8 were

208   considered negative. Specimens with ratios in the equivocal range were recommended

209   to be rerun, but this did not apply to any specimens in this study.

210

211   **Equation 1.** Determination of specimen absorbance ratio based on specimen O.D.

212   divided by the average O.D. of the calibrators.

213    $$Ratio = \frac{O.D. \, of \, the \, control \, or \, clinical \, specimen}{O.D. \, of \, the \, calibrator}$$

214

215   *Oral Fluid ELISA*

216   OraSure® Technologies Anti-SARS-CoV-2 Total Antibody ELISA for oral fluid specimen

217   (item number 3001-3317-70, catalog #1125SB) targeting coronavirus spike protein

218   antigens S1 and S2 was run manually according to the manufacturer provided

219   protocol(11). 25uL of provided sample diluent buffer was added to the desired wells of

220   the plate followed by 100uL of each specimen. Specimens were incubated at ambient

11

221   temperature for 1 hour. Sample wells were washed six times with a provided wash buffer

222   (20X dilution with ddH$_2$O, 0.35ml per well), before the provided conjugate solution was

223   added (0.1ml per well) and incubated at ambient temperature for 1 hour. After a second

224   wash step, the provided substrate solution was added (0.1ml per well) and incubated at

225   ambient temperature for 30 minutes. The provided stop solution was then added (0.1ml

226   per well) and absorbance of sample wells measured immediately at 450 nm and 630 nm,

227   with output reports generated with O.D. at 630nm subtracted from O.D. at 450nm.

228   Data were analyzed as recommended by the manufacturer and results reported as a

229   specimen to cutoff (S/CO) ratio (**Equation 2**). Specimens whose ratio was equal to or

230   exceeding 1.0 were considered positive, while specimens with a ratio greater than 0.8

231   and less than 1.0 were considered equivocal and specimens with a ratio less than or

232   equal to 0.8 were considered negative. Specimens with ratios in the equivocal range were

233   recommended to be rerun, but this did not apply to any specimens in this study.

234

235   **Equation 2.** Determination of specimen absorbance ratio based on specimen O.D.

236   divided by the averaged O.D. of the cutoff calibrators.

237
$$S/CO\ Ratio = \frac{O.D.\,of\ the\ control\ or\ clinical\ specimen}{Averaged\ O.D.\,of\ the\ cutoff\ calibrator}$$

238

239   *ELISA Automation*

240   Using the automated Dynex DSX 5 plate ELISA Processing System, we developed a

241   quick two-step automated method for SARS-CoV-2 antibody detection involving: 1)

242   centrifugation of the oral fluid collection devices in a secondary tube to transfer oral fluid

243   in the device and 2) processing of the collected saliva specimens on OraSure®

244   Technologies SARS-CoV-2 Total Antibody ELISA plates.

245

246   *Statistical analysis*

247   ROC curves were generated in GraphPad Prism (GraphPad Prism Version 8.4.3, San

248   Diego, USA), with a 95% confidence interval. Area under the curves was also calculated.

249   Correlation between serum and oral fluid specimen IgG values were calculated using a

250   Pearson correlation computing R between the two datasets, with a 95% confidence

251   interval.

252

253   *Data availability*

254   The data that support the findings of this study are available from the authors upon

255   reasonable request.

261 **Author Contributions**

262 M.M and designed and ran experiments, analyzed and interpreted data, and drafted the

263 manuscript, P.C. automated the experimental system and drafted the manuscript, A.M.

264 and S.D. analyzed and interpreted data, F.E.T, V.S., and conceptualized the project, A.I.

265 designed experiments, oversaw data collection and analysis, edited the manuscript and

266 maintained correspondence with OraSure® Technologies for the duration of these

267 experiments.

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Figures

Schematic 1. OraSure® Technologies OACD Collection Method
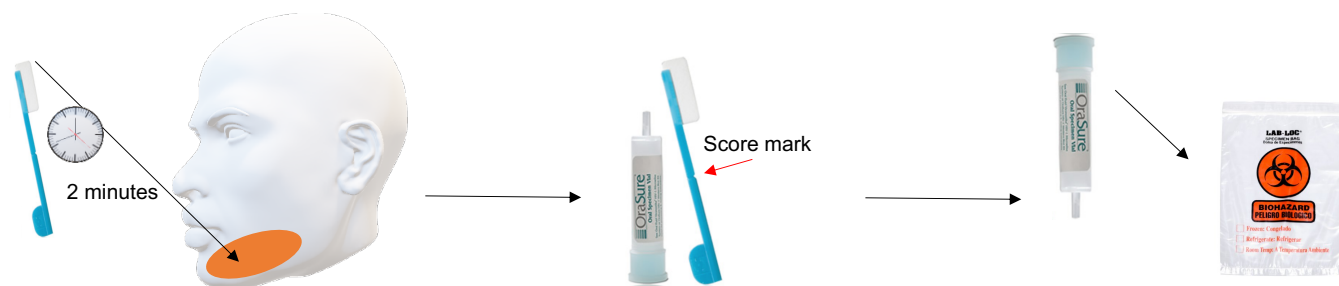
Step 1. Insert collection pad inside the mouth between the lower gum and cheek and gently rub the pad back and forth along the gum line 5 times. Repeat on the other side of the mouth and on both upper gums. Move the pad to the lower gum and leave the pad stationary against the lower gum for 2 minutes.

Step 2. Open the collection vial while ensuring enclosed liquid does not spill. Insert collection pad with pad facing down into the liquid in the vial. Bend collection pad handle to break at score mark, discarding the upper half of the stick and place it into the vial. Place the cap on top of the vial and push down firmly until it snaps into place.

Step 3. After securing the vial cap, place the vial into a plastic biohazard collection bag and label with patient name and date. This bag will be transported or collected for processing.
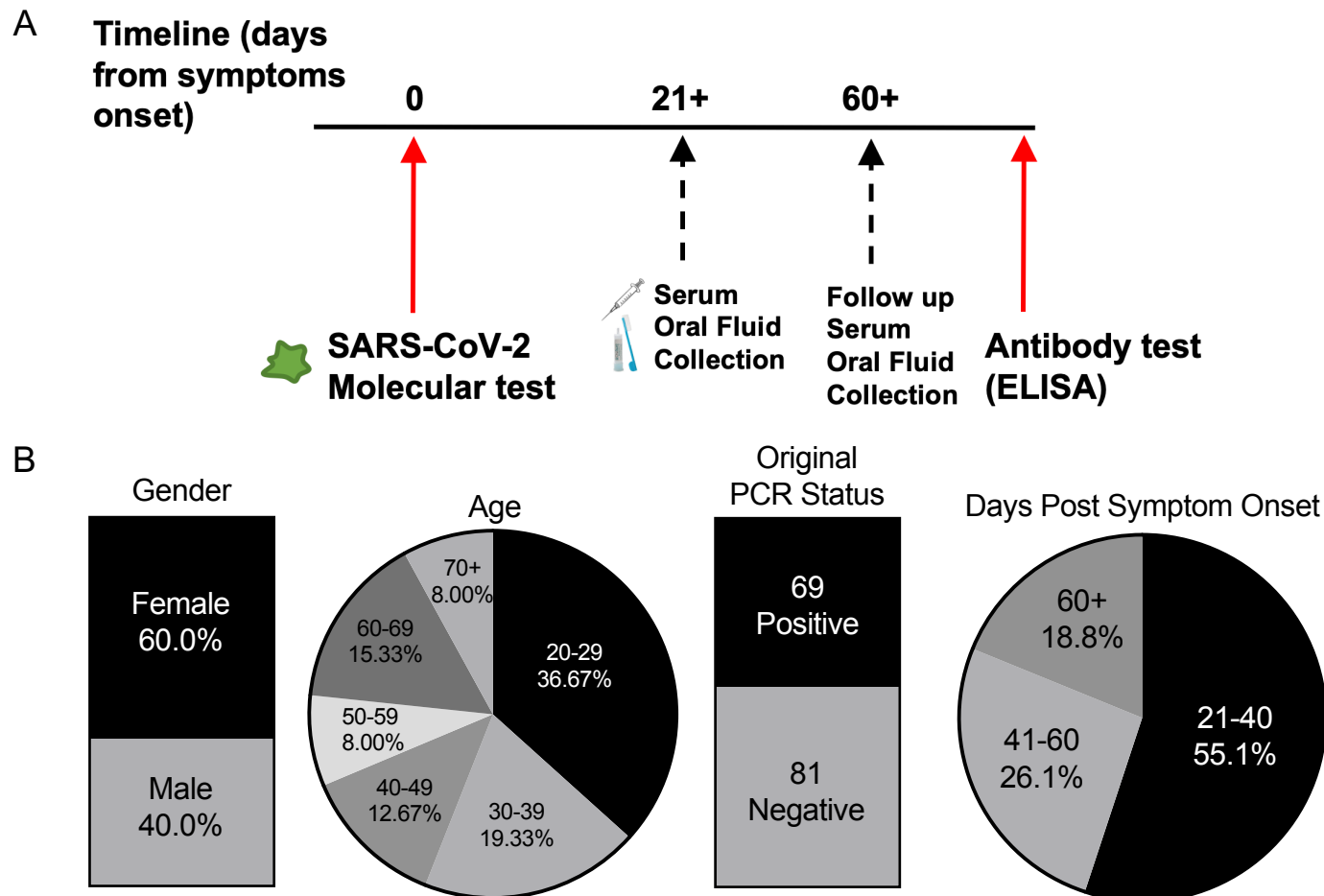
18

Figure 1. (A) Timeline of sample collection, methods and sample metrics from clinical trial participants.
(B) Visualization of the age, gender, and original PCR status (n = 150) of our cohort of clinical samples, as well as days post symptom onset of our positive participant samples (n = 69).
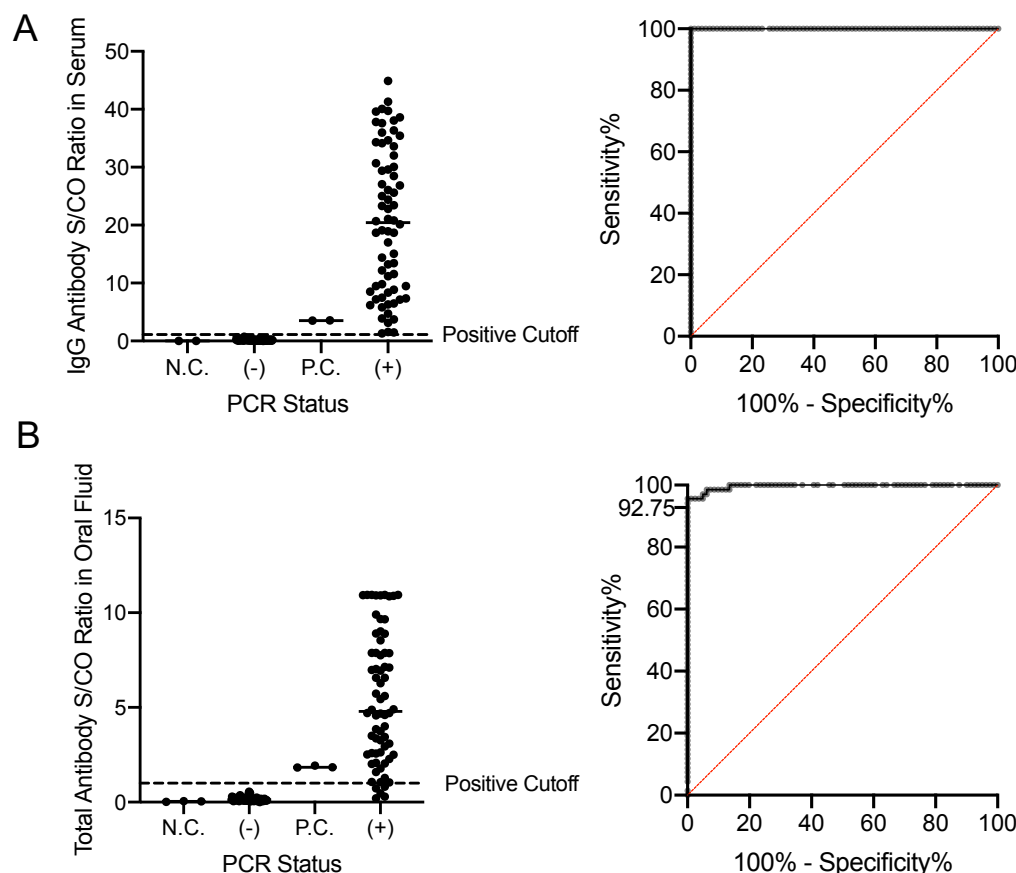
Figure 2. A) Detection of antibodies against SARS-CoV-2 in participant-derived serum samples collected from participants who previously tested negative or positive for SARS-CoV-2 RNA by an oral-fluid PCR test. The manufacturer provided positive cutoff value of 1.1 is represented by the dotted line. N.C. = negative control; P.C. = positive control; S/CO = Specimen/Cutoff Ratio. ROC analysis of this dataset in serum results in 100% sensitivity and 100% specificity with an area under the ROC curve (AUC) of 1. **B)** Detection of antibodies against SARS-CoV-2 in paired oral fluid specimens collected using the OraSure® Technologies OACD from the same participants whose serum was tested. The manufacturer provided positive cutoff value of 1.0 is represented by the dotted line. N.C. = negative control; P.C. = positive control; S/CO = Specimen/Cutoff Ratio. ROC analysis of this dataset in oral fluid reveals 92.75% sensitivity and 100% specificity with the manufacturer provided cutoff value of 1.0 total antibody units and an AUC of 0.9965.
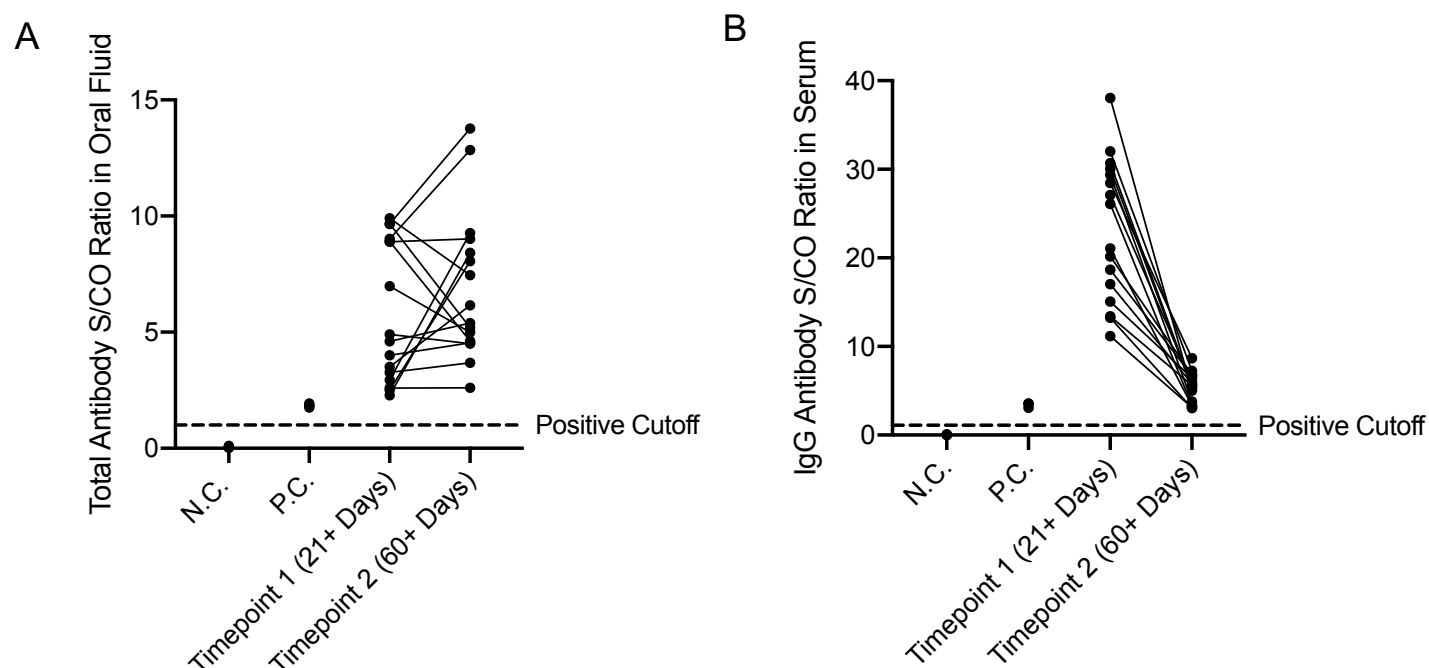
20

Figure 3. A) Detection of antibodies against SARS-CoV-2 in participant-derived serum samples collected from participants who previously tested positive for SARS-CoV-2 RNA by an oral-fluid PCR test both 21+ days post symptom onset and 60+ days post symptom onset (n = 16). All samples passed the positive cutoff value of 1.1 set by the manufacturer and represented by the dotted line. N.C. = negative control; P.C. = positive control; S/CO = Specimen/Cutoff Ratio. ROC analysis of this dataset in serum results in 100% sensitivity and 100% specificity with an area under the ROC curve (AUC) of 1. **B)** Detection of antibodies against SARS-CoV-2 in paired oral fluid samples collected using the OraSure® Technologies OACD from the same participants whose serum was tested (n = 16). The manufacturer provided positive cutoff value of 1.0 is represented by the dotted line. N.C. = negative control; P.C. = positive control; S/CO = Specimen/Cutoff Ratio. ROC analysis of this dataset in oral fluid reveals 100% sensitivity and 100% specificity with the manufacturer provided cutoff value of 1.0 total antibody units and an AUC of 1.
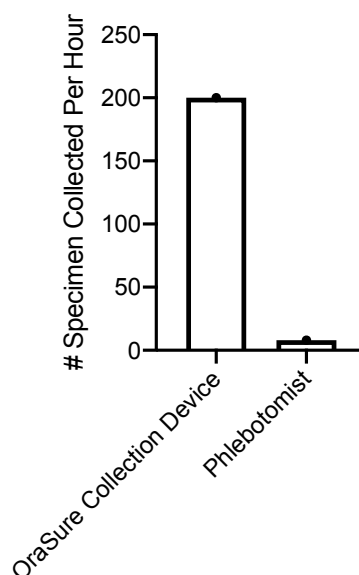
Figure 4. Number of antibody specimens which can be collected in a drive-thru testing center per hour. Oral fluid specimens (200 per hour) are able to be observed self-collected using the OraSure® OACD and simply handed off to a test site employee, while blood serum specimens (8 per hour) must be collected through direct contact with a phlebotomist, thus highlighting the benefit of testing by self-collection.