1    Development and validation  of a highly sensitive and specific electrochemical assay to quantify

2    anti-SARS-CoV-2 IgG antibodies to facilitate pandemic surveillance and  monitoring of vaccine

3    response.

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13   Running Head: Electrochemical assay for anti-SARS-CoV-2 IgG

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# Abstract

19    Amperial™ is a novel assay platform that uses immobilized antigen in a conductive polymer gel

20    followed by an electrochemical detection. A highly specific and sensitive assay was developed to

21    quantify levels of  IgG antibodies to SARS-CoV-2 in saliva. After establishing linearity and limit

22    of detection we established a reference range of 5 standard deviations above the mean. There

23    were no false positives in 667 consecutive saliva samples obtained prior to 2019. Saliva was

24    obtained from 34 patients who had recovered from documented COVID-19 or had documented

25    positive serologies. All of the patients with symptoms severe enough to seek medical attention

26    had positive antibody tests and 88% overall had positive results.

27

28    We obtained blinded paired saliva and plasma samples from 14 individuals.  The plasma was

29    analyzed  using an EUA-FDA cleared ELISA kit and the saliva was analyzed by our Amperial™

30    assay. All 5 samples with negative plasma titers were negative in saliva testing. Eight of the 9

31    positive plasma samples were positive in saliva and 1 had borderline results. A CLIA validation

32    was performed as a laboratory developed test in a high complexity laboratory.

33

34    A quantitative non-invasive saliva based SARSCoV-2 antibody test was developed and validated

35    with sufficient specificity to be useful for population-based monitoring and monitoring of

36    individuals following vaccination.

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**Introduction**

A novel corona virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused a global pandemic causing major disruptions world-wide (*1*). Multiple high-throughput PCR based tests have been developed that are reasonably sensitive and specific, however the same cannot be said for antibody testing, prompting The Center for Disease Control (CDC) to issue guidelines entitled "Interim Guidelines for COVID-19 Antibody Testing" (*2*). This publication describes the variability of in-home antibody tests and the lack of specificity required to make home-based antibody testing a valuable tool for epidemiologic surveillance.

Having a reliable self-collection antibody test may be of enormous help in epidemiologic studies of background immunity, testing symptomatic individuals without RNA based testing during their acute illness, and screening health care providers and first responders to establish prior COVID-19 infection. Such a test may also be valuable in following vaccinated patients to assess the kinetics of anti-SARS-CoV-2 antibody production following inoculation. Multiple serological tests based on serum or plasma have been developed and marketed, with ELISA and lateral flow methods predominating. However, many methods suffer from low sensitivities and specificities (*2-6*).

Antibodies begin appearing in the first week following the development symptoms. IgG, IgM, and IgA are detectable with IgA appearing somewhat earlier than IgG and IgM. Most patients seroconvert by 2 weeks following symptoms. Unlike IgA and IgM, IgG persists for several months following infection (*7-9*).

In a published study of 1,797 Icelandic individuals recovered from qPCR documented COVID-19 disease, 91% were IgG seropositive and antibody levels remained stable for 4 months after initial symptoms (*10*). Notably 2.3% of individuals quarantined due to exposure but

64    untested for virus, with negative qPCR results, tested positive for IgG antibodies. Of 18,609

65    patients who were both unexposed and asymptomatic, the seropositivity rate was 0.3% (*11*).

66          Since health care systems are burdened with care for COVID-19 patients, having a test

67    that does not require phlebotomy would be extremely beneficial. To that end, investigations have

68    been carried out using home finger prick blood sampling and even some home blood spot testing

69    lateral flow strips (*5-7*). However, home finger stick is invasive and not acceptable to some

70    individuals, and requires a health care professional to administer the test to vulnerable

71    individuals such as the elderly and children. In addition, home blood collection tests are less

72    accurate than phlebotomy, with specificities less than 98%. In a low prevalence disease, the

73    positive predictive value for a test with 98% specificity is less than 50% (*7, 11*).

74          Saliva is an oral fluid that is obtained easily and non-invasively. Proteomic studies show

75    that the immunoglobulin profile in saliva is nearly identical to that of plasma (*12*). Therefore,

76    saliva is an excellent medium for COVID-19 antibody measurement. There are several

77    commercially available collection devices to facilitate saliva collection, stabilization of IgG, and

78    transport.

79          A recently published study demonstrated excellent correlation between levels of COVID-

80    19 antibodies in serum and saliva (*13*). In order to be useful in population-based screening and

81    to determine individual immunity in exposed populations, a SARS-CoV-2 antibody test must be

82    highly specific because of the low seroprevalence rate in the population (*2, 14*). In addition, the

83    ability to quantify antibody levels is important for vaccine development and in monitoring for

84    waning immunity (*2,14*).  The only published saliva based assay for SARS-CoV-2 antibodies

85    had only  89% sensitivity with 98% specificity (*13*), leading to a positive predictive value of only

86    49% in a population with a 2% prevalence of COVID-19 exposure.

87      Our goal was to develop a non-invasive saliva based quantitative test for COVID-19

88   antibodies with exquisite sensitivity. We reviewed existing literature to find the SARS-CoV-2

89   antigen domain with the highest specificity and the ability to distinguish between the COVID-19

90   virus and other related Coronaviruses. The S1 domain is the most specific in terms of cross

91   reactivity with other Corona and other respiratory viruses. As recombinant S1 antigen is readily

92   available from at least 2 vendors, we chose the S1 antigen for our assay development.

93      Levels of IgM and IgA deteriorate rapidly following recovery from COVID-19 infection;

94   IgG levels remain detectable for several weeks to months (*10*). Since the intended use of our

95   assay is for population-based screening and vaccine efficacy monitoring, we chose to assay IgG

96   only.

97      The Amperial™ technology, formerly known as Electric Field Induced Release and

98   Measurement (EFIRM™), is a novel platform capable of performing quantitation of target

99   molecules in both blood and saliva (*13-16*). We developed quantitative Amperial™ assays for

100   IgG, IgM, and IgA antibodies to the S1 spike protein antigen of SARS-CoV-2. This test is highly

101   sensitive (>88%) and specific (>99.85%) for patients with COVID-19 infections and correlates

102   well with plasma ELISA analysis. The unique assay described in this article is completely non-

103   invasive, allows home-collection, is quantitative, and has shown no false positives in 667

104   unexposed individuals, leading to a specificity of at least 99.6%.  The widespread use of this test

105   may be of great value in identifying individuals with prior exposure to SARS-CoV-2, to follow

106   patients longitudinally to determine the kinetics of diminishing antibody concentration, and may

107   be of special value in the longitudinal monitoring of vaccinated individuals to assess continued

108   serologic immunity.

109

**Materials and Methods**

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111       The schematic of the Amperial™ SARS-CoV-2 IgG antibody is shown in Figure 1. The

112    principle of the Amperial™ platform is that a biomolecule (in this case SARS-CoV-2 Spike

113    protein S1 antigen) is added to a liquid pyrrole solution that is then pipetted into the bottom of

114    microtiter wells containing a gold electrode at the bottom of each well. After the solution is

115    added to each well, the plate is placed into the Amperial™ Reader and subjected to an electric

116    current leading to polymerization. This procedure results in each well becoming coated with a

117    conducting polymer gel containing the S1 antigen. Following the polymerization, diluted saliva,

118    plasma, or serum is added to the well. Specific anti-S1 antibodies bind to the S1 antigen in the

119    polymer. After rigorous washing procedures, the bound antibody is detected by using

120    biotinylated anti-human IgG and then the signal is amplified by a standard streptavidin /

121    horseradish peroxidase reaction that produces an electric current measured by the Amperial™

122    Reader in the nanoampere (nA) scale. The instrument is capable of accurately measuring current

123    in the picoampere (pA) range, so the measurement is well within the ability of the instrument

124    (*13-16*). The measurement of current rather than optical absorbance, as is done in the typical

125    ELISA, has two important advantages over standard ELISA. Firstly, it allows precise

126    quantitation of the amount of bound antibody and secondly, the measurement of current rather

127    than optical absorbance allows increased sensitivity. Since antibody levels in saliva are lower

128    than in plasma (*13,15*), this increased sensitivity is crucial. The precise details of the assay are

129    described in the next paragraph.

130       COVID-19 Spike-1 Antigen (Sanyou-Bio, Shanghai, China) was diluted to a

131    concentration of 6.25 µg / mL, added to each well of the microtiter plate, and co-polymerized

132    with pyrrole (Sigma-Aldrich, St. Louis, MO) onto the bare gold electrodes by applying a cyclic

133    square wave electric field at 350 mV for 1 second and 1100 mV for 1 second. In total,

134    polymerization proceeded for 4 cycles of 2 seconds each. Following this electro-polymerization

135    procedure, 6 wash cycles were performed using 1x PBS with 0.05% Tween-20 (PBS-T) using a

136    96-channel Biotek 405LS plate washer programmed to aspirate and dispense 400 µL of solution

137    per cycle.

138         Following the application of the polymer layer, 30 µL of saliva diluted at a 1:10 ratio in

139    Casein/PBS (Thermo-Fisher, Waltham, MA) was pipetted into each well and incubated for 10

140    minutes at room temperature.  Unbound components were removed by performing 6 wash cycles

141    of PBS-T using the plate washer.

142         Biotinylated anti-human IgG secondary antibody (Thermofisher, Waltham, MA) at a

143    stock concentration of 1.5 mg / mL was diluted 1:500 in Casein/PBS and 30 µL pipetted to the

144    surface of each well and incubated for 10 minutes at room temperature followed by 6 wash

145    cycles using PBS-T. Subsequently, 30 µL of Poly-HRP80 (Fitzgerald Industries, Acton, MA) at

146    a stock concentration of 2 µg / mL was diluted 1:25 in Casein/PBS, added to the wells, and

147    incubated at 10 minutes at room temperature. Following a final wash using 6 cycles of PBS-T,

148    current generation is accomplished by pipetting 60 µL of 1-Step Ultra TMB (Thermofisher,

149    Waltham, MA) to the surface of the electrode and placing the plate into the Amperial™ reader

150    where current is measured at -200 mV for 60 seconds. The current in nA is measured 3 times for

151    each well. The process for reading the entire 96 well plate requires approximately 3 minutes.

152    **Plasma Quantitative Amperial™ Assay for SARS-CoV-2 IgG**

153         The protocol is similar to the Amperial™ SARS-CoV-2 IgG antibody for saliva samples.

154    Following the application of the polymer layer, 30 µL of plasma diluted at a 1:100 ratio in

155    Casein/PBS (Thermo-Fisher, Waltham, MA) was pipetted into each well and incubated for 10

156     minutes at room temperature. The standard curve for plasma contains the following points: 300

157     ng / ml, 150 ng / ml, 75 ng / ml, 37.5 ng / ml, 18.75 ng / ml, and 0 ng / ml.

158     **Plasma SARS-CoV-2 ELISA Assay**

159     We purchased FDA EUA ELISA kits EUROIMMUN Anti-SARS-CoV-2 ELISA Assay

160     for detection of IgG antibodies (EUROIMMUN US, Mountain Lakes, NJ). We processed

161     samples exactly as described in the package insert.

162     **Human Subjects**

163     Volunteers, with prior positive qPCR tests for COVID-19 infection or positive antibody

164     tests using currently available FDA EUA-cleared antibody tests were consented and responded to

165     a questionnaire regarding severity of symptoms, onset of symptoms, and method of diagnosis

166     (UCLA IRB #06-05-042). Severity of symptoms were self-graded on the following 7-point scale:

167     0: Asymptomatic

168     1: Mild (Barely noticed, perhaps slight fever and cough)

169     2: Moderate (felt moderately ill but did not need to seek medical care)

170     3: Sought medical Care but was not admitted to hospital

171     4: Hospitalized

172     5: Admitted to ICU

173     6: Placed on Ventilator

174     A set of 13 paired saliva and plasma samples were provided by the Orasure™ Company.

175     **Saliva Collection**

176     All COVID-19 samples were obtained using the Orasure™ FDA-cleared saliva collection

177     device and used according to manufacturer instructions. The Orasure™ collection device

178     consists of an absorbent pad on the end of a scored plastic wand. The individual places the pad

179   between cheek and gum for a period of 2 – 5 minutes. Subsequently the wand and pad are placed

180   into a tube containing transport medium, the top of the stick is broken off, and the tube is sealed

181   for transport. The sealed tube is placed into a zip-lock bag and shipped by any standard method.

182   According to the package insert, samples are stable at ambient temperature for 21 days (see

183   results below and Orasure™ website). An alternate sample collection method involves the

184   individual swabbing the pad 4 times in the gingival tooth junction prior to placing the pad

185   between the cheek and gum. This method has been shown to improve IgG yield in some patients

186   with low antibody levels (personal communication).

187        Samples collected pre-2012 were used as controls. Saliva was collected from healthy

188   individual volunteers at meetings of the American Dental Association between 2006 and 2011.

189   Consent was obtained under IRB approval UCLA IRB #06-05-042. Both  male and females,

190   mostly non-smokers, 18-80 years of age, and a differing ethnicities were included. All subjects

191   were consented prior to collection. Each subject expectorated ~ 5 mL of whole saliva in a 50cc

192   conical tube set on ice. The saliva was processed within 1/2 hour of collection. Samples were

193   spun in a refrigerated centrifuge @ 2600 X g for 15 minutes at 4°C. The supernatant (cell-free

194   saliva) was then pipetted into two-2 mL cryotubes and 1.1 µL Superase-In (Ambion, Austin, TX)

195   was added as a preservative. Each tube was inverted to mix. The samples were frozen in dry ice

196   and later stored in -80°C.

197                                        **Results**

198   **Linearity**

199        Figure 2 demonstrates the dynamic range and linearity of the assay. In these experiments

200   varying amounts of monoclonal human anti-S1 IgG was added to a saliva sample from a healthy

201   volunteer and subjected to the assay. Figure 2A shows a range of 0.2 to 6 ng/ml. The Y-axis

202    shows amperage measured in nA. The X-axis represents spike-in concentrations of IgG. The

203    assay begins to become saturated at about 3 ng / ml. Panel 2B shows dilutions down to 0.03 ng /

204    ml to 0.6 ng / ml and shows linearity in that range. This allows us to create a standard curve

205    containing the following points: 3 ng / ml, 1.5 ng / ml, 0.75 ng / ml, 0.375 ng / ml, 0.1875 ng /

206    ml, and 0 ng / ml.

207    **Inhibition Assay**

208        In order to demonstrate the specificity for the assay on actual clinical samples, we used

209    the saliva from 3 recovered patients who had high levels of SARS-CoV-2 antibodies and added

210    exogenous S1 antigen in varying amounts prior to analysis on the Amperial™ assay. The

211    exogenous S1 antigen should compete for binding sites and therefore extinguish the nA signal.

212    Figure 3 shows the results of this experiment. The red, purple, and green represent 3 different

213    patients. The X-axis demonstrates increasing concentration of exogenous S1 added to the saliva

214    before subjecting it to the assay. As shown, saliva pre-incubated with S1 antigen extinguishes the

215    detectable IgG signal proportionately, therefore demonstrating the specificity of the assay to S1

216    antigen in clinical samples.

217    **Matrix Effects**

218        Since we are be comparing samples collected by various methods, it is vital to determine

219    if any significant matrix effects could interfere with data interpretation.  We examined the 3

220    different collection methods used in this study: Expectoration/centrifugation, Orasure™ without

221    swabbing and Orasure™ with swabbing.

222        Two methods of collection using the Orasure™ Oral Fluid Collection Device were tested.

223    The first method (non-swabbing) collects saliva by placing an absorbent pad into the lower gum

224    area for 2-5 minutes and then placing the saturated collection pad into a preservative collection

225    tube. The second method (swabbing) adds the step of first gently rubbing the collection pad

226    along gum line, between the gum and cheek, 5 times, before placing the device in the lower gum

227    area for 2-5 minutes, and then immersing the saturated collection pad into the collection tube.

228    Healthy donors (n=5) collected their saliva using these two different methods. The control pre-

229    2012 samples were collected with an expectoration protocol for whole saliva collection (falcon

230    tubes), processing (centrifuge), stabilization, and storage. Five samples collected by each of the 3

231    methods and were analyzed in duplicate. The results are shown in Figure 4 under the heading

232    "No spike in." There are no differences among 3 sample types. We then added monoclonal

233    human anti-S1 IgG to each sample and again ran them in duplicate (Figure 4) above caption

234    Spike-in 1.5 ng / ml IgG. A non-parametric Student t-test was performed with no significant

235    differences between any of the collection methods.

236    **Stability**

237    The Orasure™ collector is an FDA-cleared device for the analysis of anti-HIV IgG. The

238    package insert describes a 21-day stability at ambient temperature. We wished to establish the

239    stability of anti COVID-19 IgG using this collector. Passive whole saliva was collected from

240    four healthy individuals using 50 mL falcon tubes and spiked with anti-Spike S1 IgG to reach a

241    final concentration of 300 ng / ml. Aliquots of 1.75 mL of saliva were placed into 50 mL tubes

242    and then the sponge of the Orasure™ collector was submerged into the saliva for five minutes

243    and processed as described in Methods.  The collected saliva was then aliquoted into PCR tubes

244    and left at ambient temperature (21°C) for 0, 1, 3, 7, and 14 days before storage at -80°C.  After

245    14 days, samples were thawed and assayed using the anti-Spike S1 IgG Amperial™ assay to

246    assess stability. At 14 days, 95% of the original signal remained, demonstrating the 14-day

247    stability of anti-SARS-CoV-2 antibodies collected in Orasure™ containers (data not shown).

**Specificity and Reference Range**

249    Once we established no significant differences between the tube collection method and

250    the Orasure™ collector method, we analyzed a series of 667 samples collected between 2006 and

251    2009 at the annual meeting of the American Dental Association. Scatter plots of these data for

252    both nA and ng / ml are shown in Figure 5A and 5B. We established the mean and standard

253    deviation for both raw nA values and concentration in ng / ml. In order to maximize specificity,

254    we selected a reference range > 5 SD above the mean. A 5 sigma level would lead to a specificity

255    of 99.9994%. In fact, we have never seen a healthy sample above the 5 sigma level. As will be

256    seen, the sensitivity of the assay remains greater than 88% even with this rigorous specificity.

**Recovered COVID-19 Patients**

258    Figure 6 displays the scatter plot for 667 healthy controls and 34 volunteer patients who

259    recovered from COVID-19 infection. All patients were a minimum of 14 days post onset of

260    symptoms and some patients were as many as 15 weeks post symptoms. The 5 sigma cutoff is

261    shown by the green dotted line. A more detailed discussion of the recovered patients appears in

262    the following section. The data show that all healthy patients are negative and 30 of the 34

263    recovered patients are positive. These data demonstrate a sensitivity of 88% and a specificity of

264    > 99.985%. It is important to note that not all recovered patients have detectable antibody (10) so

265    the 4 patients with undetectable antibody may be biologically negative and not the result of lack

266    of sensitivity of the assay.

267    Figure 7 demonstrates the relationship of anti-S1 IgG levels to severity of symptoms.

268    Table 1 is a tabular summary of these data. All patients who had severity indexes ≥3 (sought

269    medical attention, admitted to hospital, admitted to ICU, on ventilator) had positive antibody

270    levels. Although 4 patents with mild symptoms had antibody levels in the normal range, both

271 asymptomatic patients had appreciable antibody levels. These patients were close contacts of

272 more severely affected patients. The highest antibody level recorded is severity index level 2

273 patient (moderate symptoms, did not seek medical care). It is important to note that both

274 asymptomatic patients had easily detectable antibody levels in saliva, suggesting this test may be

275 useful in general population screening.

276 **Paired Saliva and Plasma Samples**

277 We obtained 14 paired, blinded plasma and saliva samples. The plasma was analyzed by

278 an FDA EUA-cleared ELISA test purchased from EUROIMMUN (see Methods).  The saliva

279 samples, collected in Orasure™ buffer, were analyzed by the Amperial™ assay described in

280 Methods. After unblinding, we discovered  8 recovered COVID patients and 5 healthy patients in

281 this series. All 5 healthy patients were negative in both the saliva and plasma assays. In 7 of the 8

282 recovered patients, both plasma and saliva tests were positive. There was one sample with a

283 discrepancy between saliva and plasma, with the plasma positive and the saliva in the

284 indeterminate range.

285 The EUROIMMUNE ELISA assay is a semi-quantitative assay and yields an absorbance

286 ratio rather than a quantity.  Figure 8 demonstrates the relationship between the saliva

287 quantitative results and plasma absorbance ratio for the paired plasma and saliva samples. There

288 is a clear relationship between the 2 levels, with the higher plasma absorbance ratios associated

289 with higher saliva quantitation.

290 We developed a research quality assay to quantify anti-SARS-CoV-2 IgG levels in

291 plasma (see Methods). We analyzed the 13 plasma samples using this assay.  The results of this

292 experiment are shown in Figure 9. Panel A shows a log / log plot of plasma versus saliva levels

293 showing a clustering  with high plasma levels associated with high saliva levels. Panel B shows

294    the box plot of these values, demonstrating that plasma levels are approximately 50X those of

295    saliva. This observation explains the necessity for an extremely sensitive assay such as the

296    Amperial™ assay in order to detect antibodies in saliva. Of note, the publication regarding saliva

297    SARS-CoV-2 IgG detection reports levels of 25 – 60 mcg / ml, 1000 times less sensitive than our

298    assay.

299    **Longitudinal Tracking of Antibody Levels**

300    Three of our volunteers supplied samples at weekly intervals so we could determine the

301    stability of their antibody levels. Results appear in Figure 10. The 5 standard deviation cutoff is

302    again shown with the dashed green line. All 3 patients continued to have detectable levels for

303    more than 12 weeks, with the longest interval of 15 weeks. All tests were positive in all patients

304    and antibody levels in all 3 patients remained clearly positive during the time interval studied.

305    Patients C1 and C3 seem to have a rise in antibody level between 11 and 12 weeks post initial

306    symptoms followed by a return to baseline level. Patient C2 might also have had a spike in

307    antibody levels at 10 weeks. This may be result of the amnestic B-cell population becoming

308    established. There is insufficient data at this time to determine if this is a generalized pattern.

309    **CLIA Evaluation**

310    We performed a full CLIA laboratory developed test evaluation for the Amperial™

311    COVID-19 IgG Antibody test. The validation assayed 72 unaffected patients and 30 recovered

312    patients and demonstrated 100% sensitivity and specificity. The intra-assay and inter-assay

313    variability were 9.28% and 16.2% respectively.

314    **Discussion**

315    We have developed an exquisitely specific, sensitive, non-invasive saliva based

316    quantitative assay for anti-SARS-CoV-2 IgG antibodies. Our goal was to create a quantitative

317    assay with sufficient positive predictive value to be useful to inform individuals regarding

318    previous infection with COVID-19. By establishing a reference range of 5 sigma above than the

319    mean we have a theoretical analytical specificity of 99.9999994%. We plan to repeat the analysis

320    of all positive samples to further increase analytical specificity. Since our test is non-invasive

321    with home-collection we can also offer repeat testing on a second sample to further increase

322    specificity. These procedures will minimize the false positives due to purely technical issues.

323    There is still the possibility of biological false positives, however, due to cross reactivity with

324    other infectious or environmental agents. The S1 antigen appears to be specific for SARS-CoV-2

325    (*2, 3, 10*) and in our series of 667 samples collected prior to 2019 we observed no false positive

326    results.

327         Many investigations of neutralizing antibodies use antibodies directed to a different

328    epitope, the Ribosomal Binding Domain (RBD). Therefore, we tried to assay the RNA binding

329    domain (RBD) but found a false positive in the initial 10 unaffected controls indicating

330    significant cross reactivity between the RBD and other viral species, disqualifying RBD for our

331    purposes.

332         We cannot predict the eventual clinical specificity of this assay. At a minimum, the

333    specificity is 667 / 668 or 99.985% assuming the next control sample tested would be a false

334    positive, but the specificity is likely to be higher. Our current sensitivity is 100% for patients

335    with symptoms severe enough to seek medical care. For all patients, including mildly

336    asymptomatic patients, our clinical sensitivity is 88%. Since the Amperial™ assay only requires

337    6 μL of collection fluid, several assays can be performed from the same sample. This allows all

338    positives to be repeated to confirm the positive results, This further increasing the specificity of

339    the assay. We will offer testing of a second, independent sample for all patients testing positive.

340    Since saliva collection is easily be performed at home, obtaining a second sample is not difficult.

341        For any laboratory test, the PPV is proportional to the prevalence of positivity in the

342    population. A recent study demonstrated a prevalence of between 4.4% to 6% in Britain (*16*).

343    Using the minimum specificity of 99.985% and a prevalence of 6% the Amperial™ saliva assay

344    would have a minimum PPV of 96%. In contrast, a published saliva antibody detection assay

345    reported  a specificity of 98% with a similar sensitivity (89%). This specificity leads to PPV of

346    only 69% making it an ineffective tool for population screening.

347        Our data demonstrate that the Imperial™ assay is appropriate for longitudinal screening

348    of antibody levels, a particular utility in vaccine trials and in population monitoring following

349    mass immunization. Since this assay is quantitative and levels appear to be stable with time,

350    patients may be monitored from home at frequent intervals. If antibodies raised in response to

351    vaccination do not include IgG antibodies to S1 antigen, it is easy to rapidly develop Amperial™

352    antibody tests to any antigen. This requires adding the new antigen to the pyrrole solution and

353    does not require significant alteration of assay conditions.

354        A particular advantage of this assay is convenience. The Orasure™ collector is simple

355    and easy to use and does not require professional monitoring for adequate collection. Home

356    collection relieves the burden to an already stressed health care system. Vulnerable populations

357    such as children and the elderly can be guided through the collection process by parents or other

358    adults. It is possible to obtain repeat samples to confirm positives and to perform longitudinal

359    testing since the only requirement for testing is shipping th collecting kit.

360        The Amperial™ IgG test is plate-based and high-throughput. An entire plate is easily

361    processed in 2 hours, leading to rapid turnaround time once the sample enters the laboratory.

362    There is no pre-processing of the sample required; samples are taken directly from the collection

363    vial and placed into the assay. With standard liquid handlers, the assay may be easily automated

364    allowing for extremely high-throughput since the Amperial™ reader is only required for the

365    polymerization step of less than a minute at the beginning of the assay and 3 minutes for the

366    measurement phase at the end of the assay.

367         Published data (*13*) and our own demonstrate a correlation between blood results and

368    saliva results indicating that the IgG present in saliva is most likely derived from the plasma

369    through filtration. Our data shows that saliva IgG levels are approximately 50-fold less than

370    those in plasma necessitating a highly sensitive assay in order to detect the IgG levels in saliva.

371         There is some discussion in the literature of the role antibody testing may have in

372    managing the COVID-19 epidemic. Alter and Seder published an editorial in the New England

373    Journal of Medicine arguing, "Contrary to recent reports suggesting that SARS-CoV-2 RNA

374    testing alone, in the absence of antibodies, will be sufficient to track and contain the pandemic,

375    the cost, complexity, and transient nature of RNA testing for pathogen detection render it an

376    incomplete metric of viral spread at the population level. Instead, the accurate assessment of

377    antibodies during a pandemic can provide important population-based data on pathogen

378    exposure, facilitate an understanding of the role of antibodies in protective immunity, and guide

379    vaccine development. (14)"

380         In this article, we describe the development of a non-invasive, home collection based,

381    exquisitely specific, and acceptably sensitive test for the presence of anti-SARS-CoV-2

382    antibodies in saliva. This may be an important tool in controlling the pandemic and facilitating

383    and understanding of the role of antibody production in COVID-19 immunity. Longitudinal

384    monitoring of anti-SARS-CoV-2 IgG levels could also play a valuable role in vaccine

385    development and deployment by allowing longitudinal quantitative assessment of antibody

386    levels. If the presence of detectable anti-COVID-19 IgG is shown to be an indicator of immunity

387    to reinfection, measurement of these antibodies could allow individuals to safely return to work,

388    school and community. The Amperial™ SARS-CoV-2 assay fulfills the requirements for all of

389    these applications.

390    **Acknowledgements**

393    **Disclosures**

394        CS is a founder and CEO of Liquid Diagnostics, LLC and holds equity in the company.

395    MT is an employee and an equity holder in Liquid Diagnostics. RB is the CMO of Liquid

396    Diagnostics and holds equity in the company. DW is an equity holder in Liquid Diagnostics, LLC.

397    and a consultant to Mars Wrigley and Colgate-Palmolive

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469    1.

470

| Severity Index | Positive IgG (no. patients) | Negative IgG (no. patients) | Sensitivity (%) |
|---|---|---|---|
| 0: Asymptomatic | 2 | 0 | 100 |
| 1: Mild Flu-Like Symptoms | 3 | 3 | 50 |
| 2: Moderate Flu-Like Symptoms | 9 | 1 | 90 |
| 3: Sought Medical Attention | 10 | 0 | 100 |
| 4: Admitted to Hospital | 3 | 0 | 100 |
| 5: Admitted to ICU | 1 | 0 | 100 |
| 6: Placed on Ventilator | 2 | 0 | 100 |
| Total | 30 | 4 | 88.24 |

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473    Table 1. Correlation of Amperial™ anti-SARS-CoV-2 IgG levels in saliva with severity of

474    symptoms in 34 COVID positive subjects.

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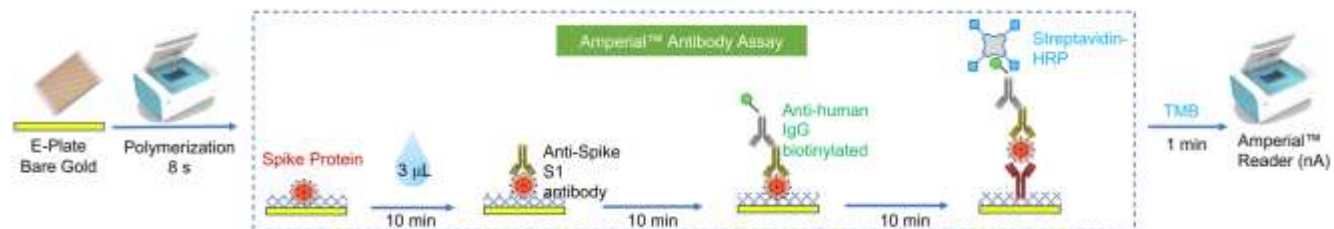


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479   Figure 1. Schematic of the Amperial™ saliva anti-SARS-CoV-2 IgG assay. See methods for

480   description.

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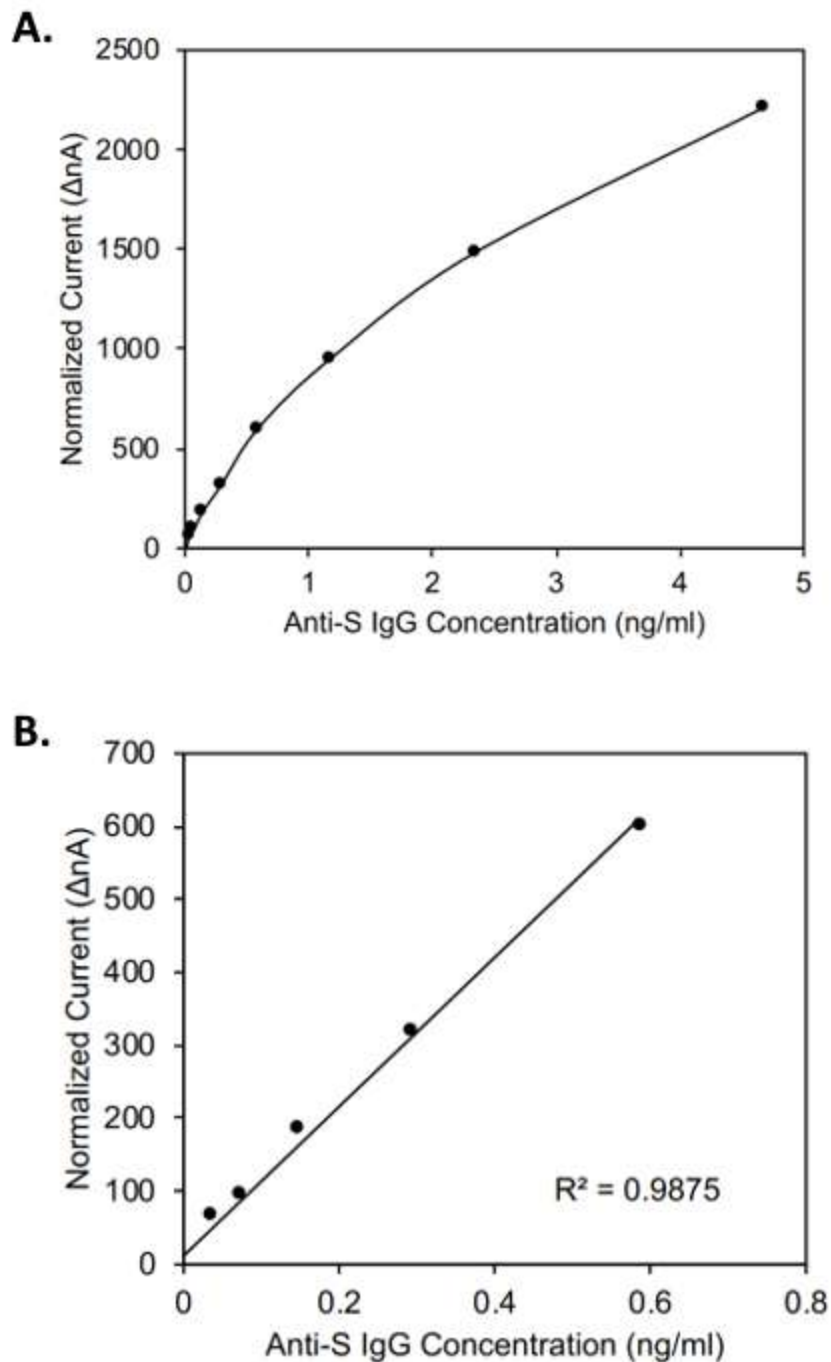
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483  Figure 2. Dynamic range and linear range of Amperial™ anti-Spike S1 IgG assay. X-axis:

484  Amount of spike in anti-SARS-CoV-2 IgG in ng / ml. Y-axis: Normalized current in nA. Panel

485  A: 0 – 5 ng / ml Panel B: 0.1 – 0.7 ng / ml

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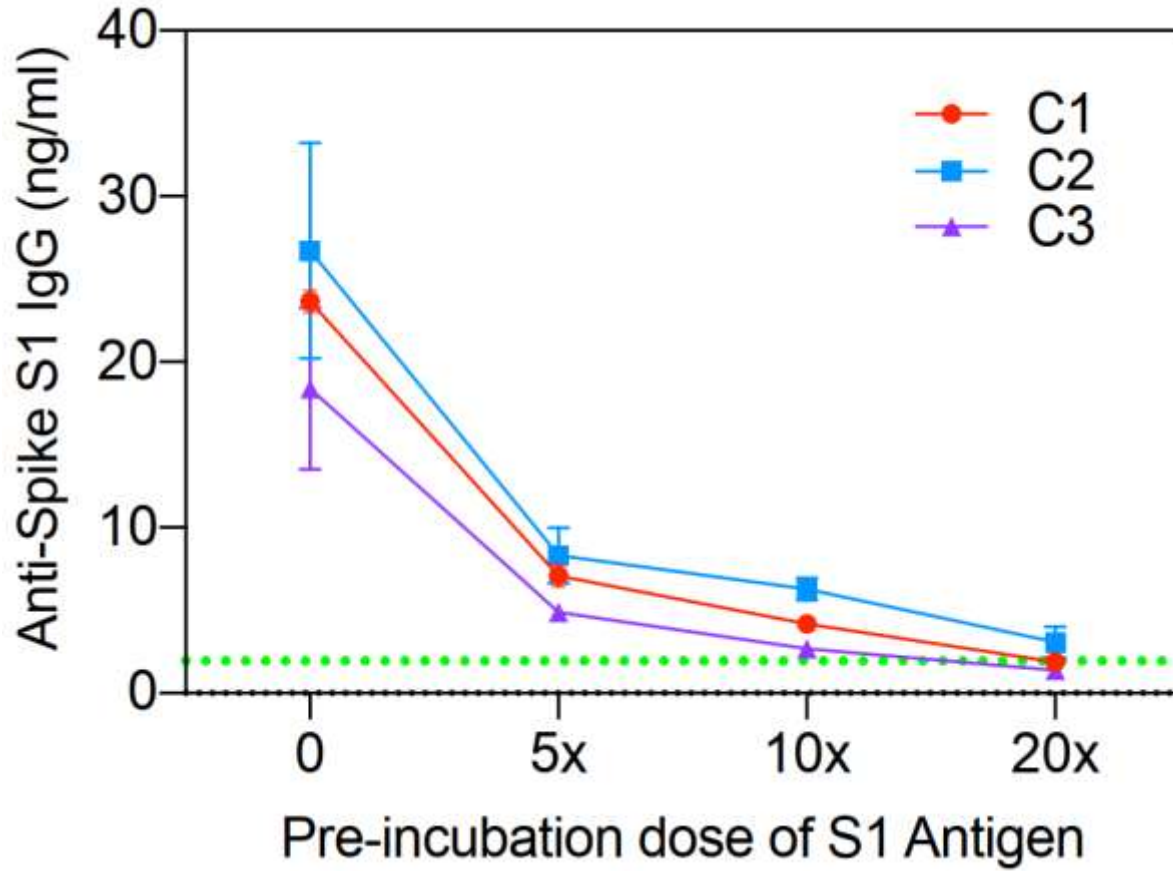
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489 Figure 3. Competition assay of three COVID-19 patients: C1, C2, and C3. Varying amounts of

490 exogenous anti-SARS-CoV-2 IgG added to saliva of 3 different recovered COVID-19 patients.

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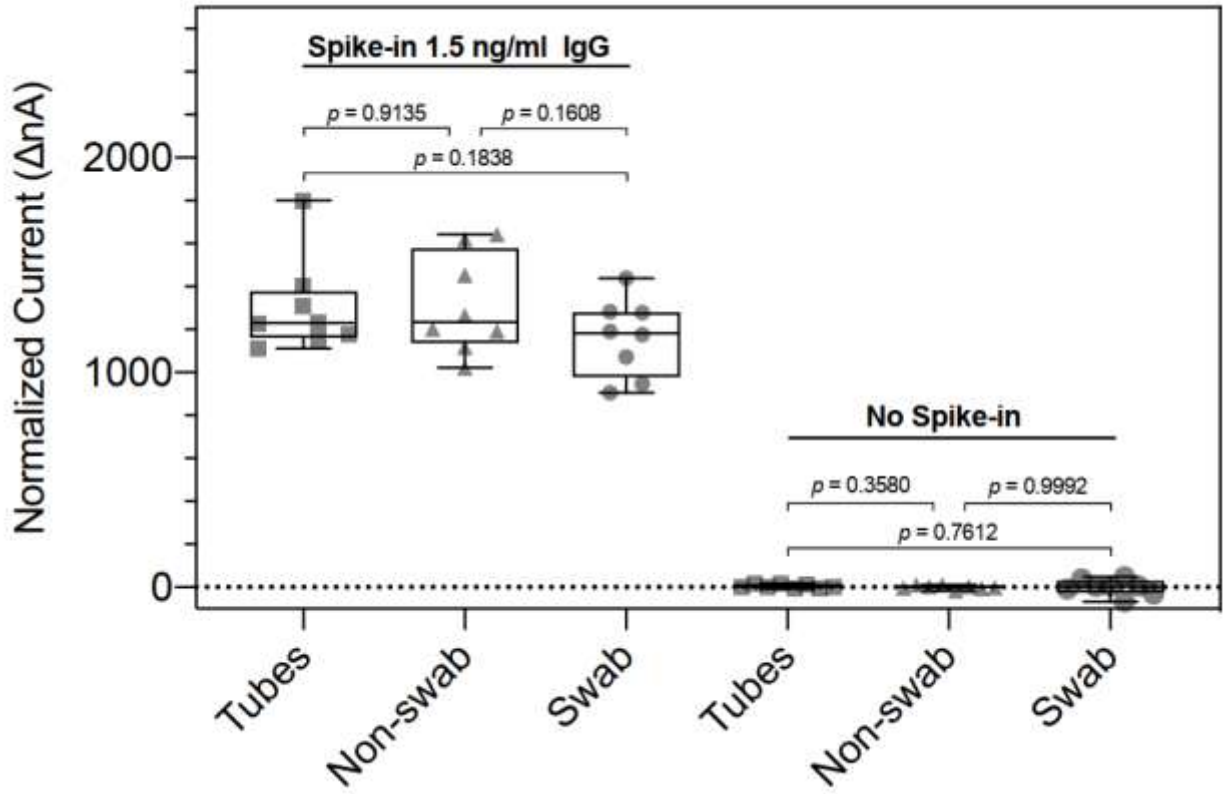
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494    Figure 4. Box plot of saliva matrix experiments with saliva from healthy subjects. Green dashed

495    line represents 5 standard deviations above the mean.

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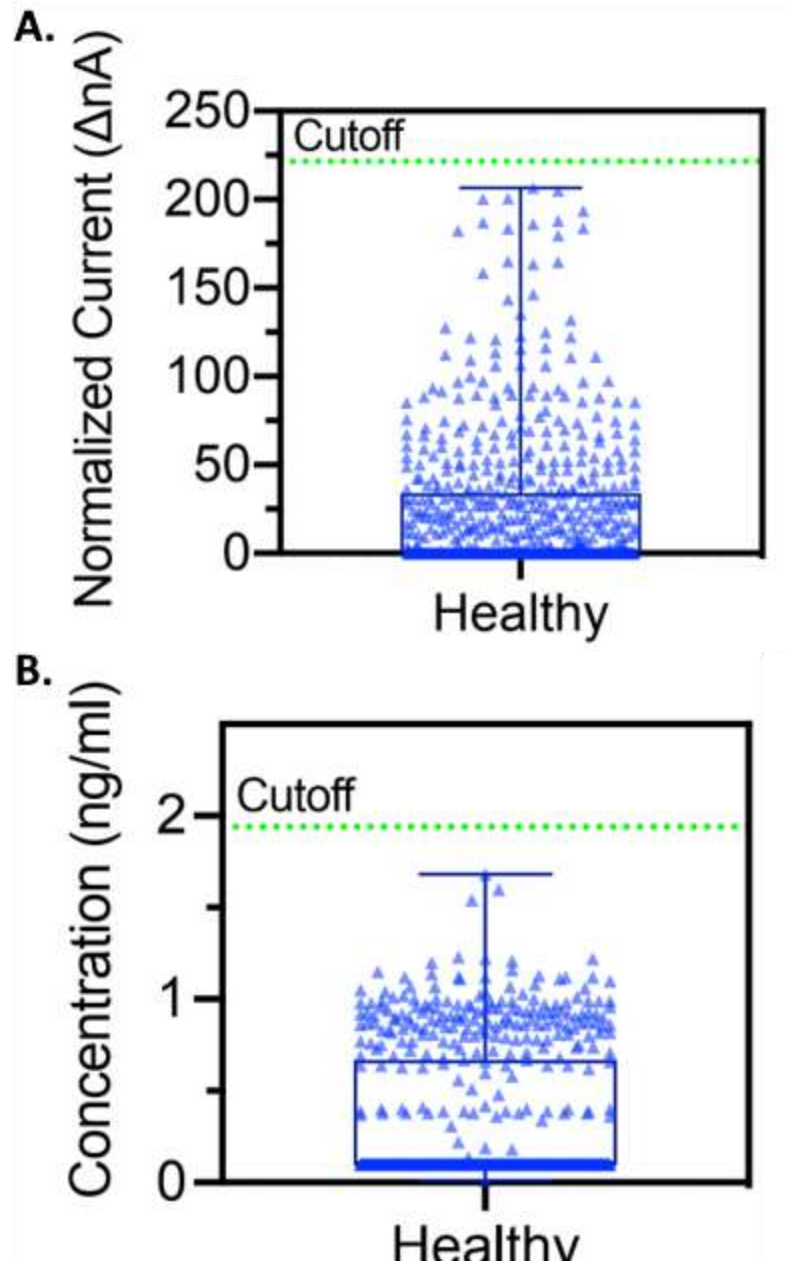
Figure 5. Healthy reference range of Amperial™ saliva anti-SARS-CoV-2 IgG assay of 667 unexposed subjects in (A) normalized current (ΔnA) with mean=24.38 and cutoff=221.47 and (B) concentration (ng / ml) with mean=0.33 and cutoff=1.19.
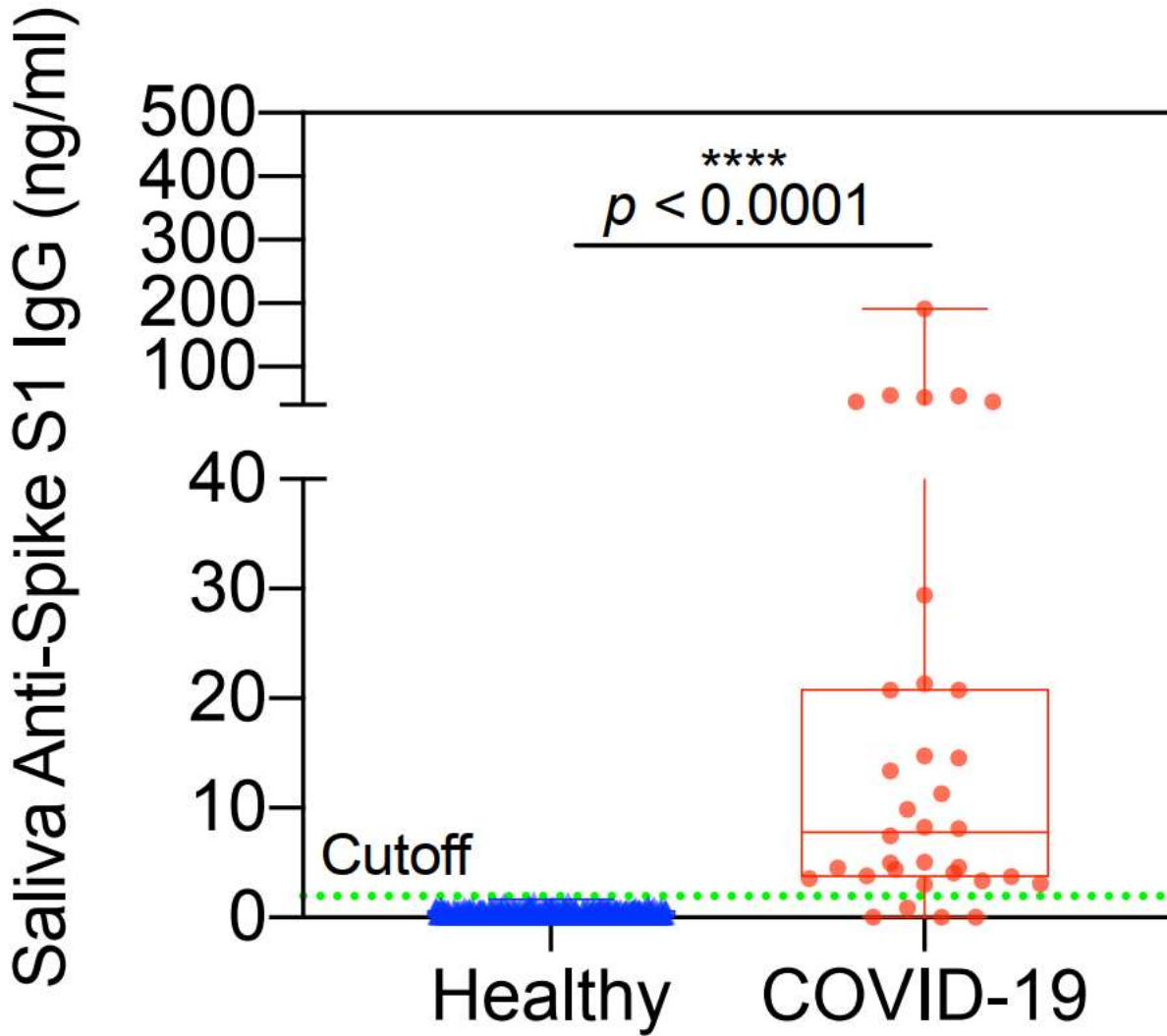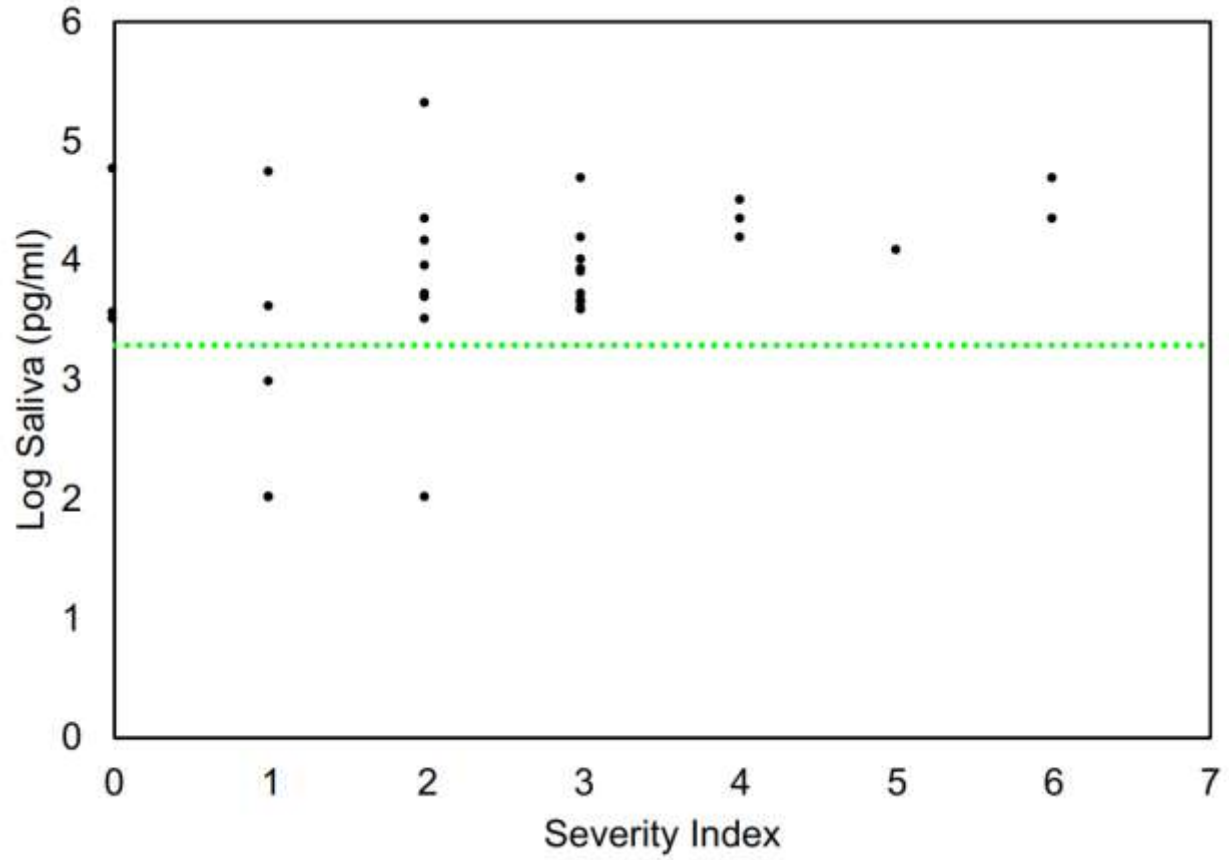
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504 Figure 6. Amperial™ detection of anti-Spike S1 IgG in saliva of COVID-19 (n=34) and healthy

505 subjects (n=667). Green dashed line indicates 5 SD reference range cutoff.
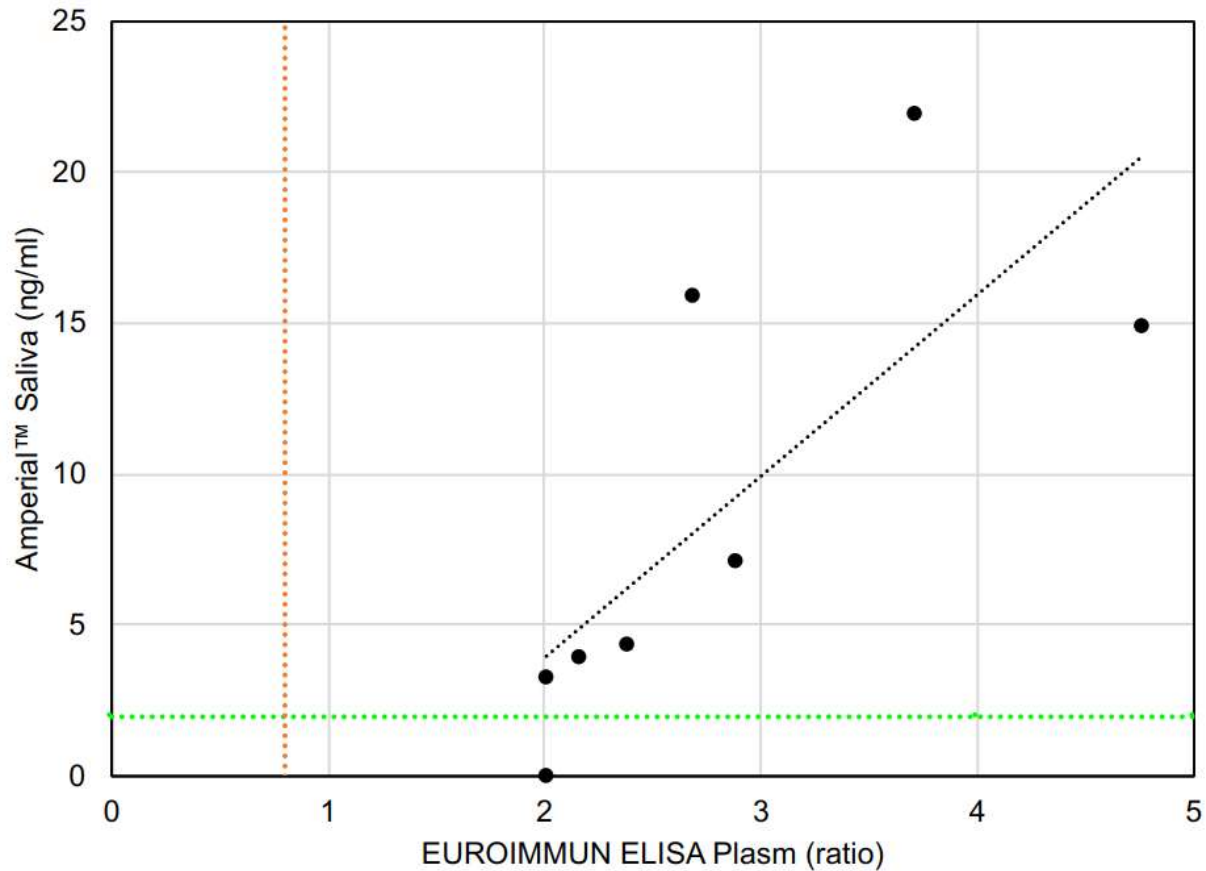
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508    Figure 7. Clinical severity index and anti-Spike S1 IgG level in saliva.

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511

512    Figure 8. COVID-19 antibody level in paired saliva and plasma of COVID-19 (n=8) subjects in a
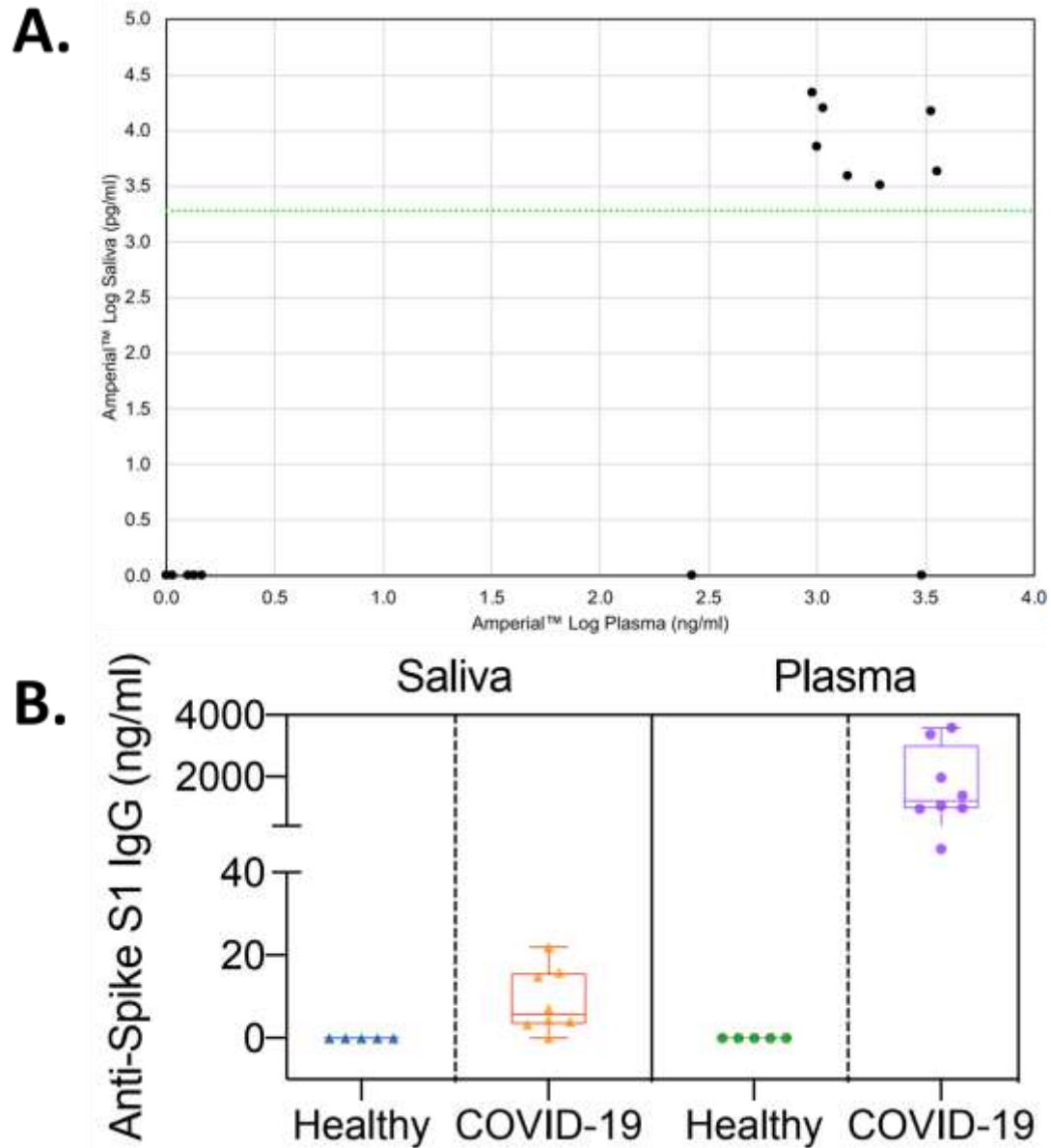
513    blinded randomized cohort. Plasma antibodies level are measured by EUROIMMUN ELISA

514    reported in ratio (proportion of OD of calibrator to OD of sample) and saliva antibodies are

515    measured by Amperial™ in pg / ml. Green dashed line indicates 5 SD reference range cutoff of

516    Amperial™ test and red dashed line is reference range for EUROIMMUN ELISA.

517

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519

520    Figure 9.  Relationship of plasma anti-SARSCoV-2 IgG levels to saliva levels measured by

521    Amperial™ assays. (A) Panel A shows a log / log plot of plasma versus saliva levels showing a

522    clustering of the positive values with high plasma levels associated with high saliva levels on the
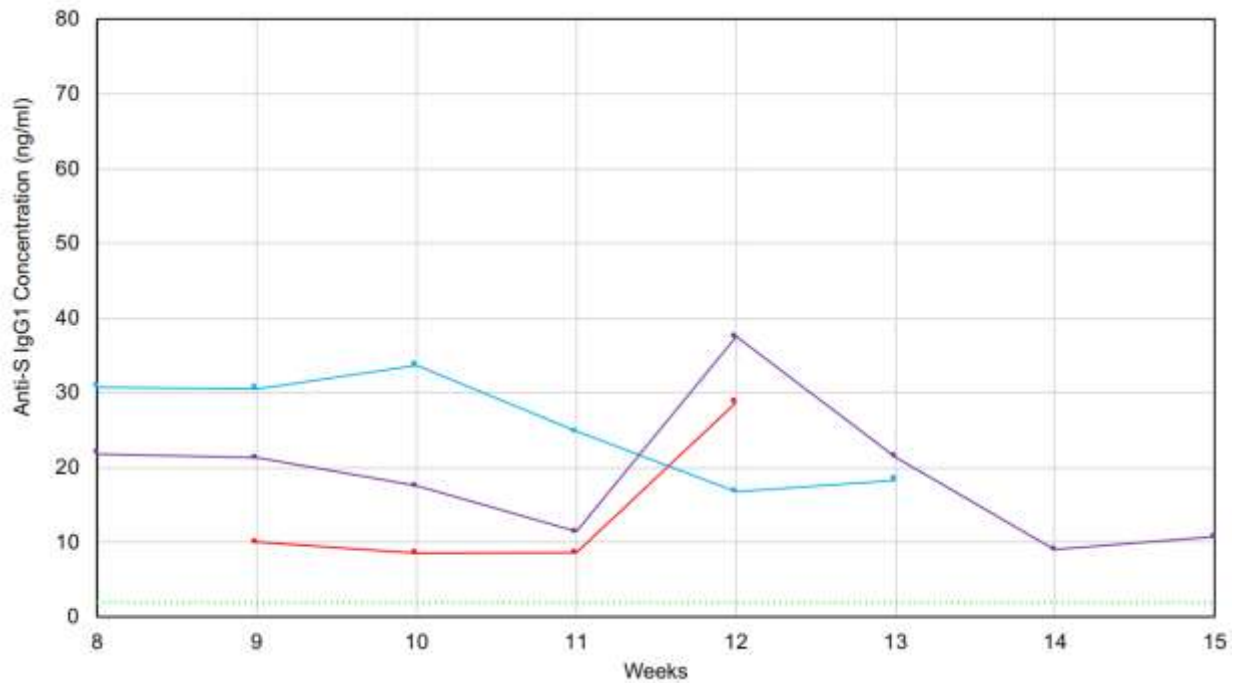
523    Amperial™ platform. (B) Box plot of COVID-19 (n=8) and healthy (n=5) subjects

524    demonstrating that the normalized plasma levels are approximately 50X those of saliva.

525

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527

528       Figure 10. Longitudinal Measurement of saliva anti-SARS-CoV-2 IgG levels in 3 recovered

529    patients. X-axis: Time after initial onset of symptoms (in weeks). Y-axis: IgG levels measured in

530                       saliva.

531