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1 # Correlation of ELISA based with random access serologic immunoassays for identifying
2 # adaptive immune response to SARS-CoV-2

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31 # Abstract

32 Public health emergency of SARS-CoV-2 has facilitated diagnostic testing as a related medical
33 countermeasure against COVID-19 outbreak. Numerous serologic antibody tests have become available
34 through an expedited federal emergency use only process. This paper highlights the analytical
35 characteristic of an ELISA based assay by AnshLabs and three random access immunoassay (RAIA) by
36 DiaSorin, Roche, and Abbott that have been approved for emergency use authorization (EUA), at a
37 tertiary academic center in a low disease-prevalence area. The AnshLabs gave higher estimates of sero-
38 prevalence, over the three RAIA methods. For positive results, AnshLabs had 93.3% and 100%
39 concordance with DiaSorin or Abbott and Roche respectively. For negative results, AnshLabs had 69.7%
40 and 73.0% concordance with DiaSorin and Roche or Abbott respectively. All discrepant samples that
41 were positive by AnshLabs and negative by RAIA tested positive by all-in-one step SARS-CoV-2 Total
42 (COV2T) assay performed on the automated Siemens Advia Centaur XPT analyzer. None of these
43 methods, however, are useful in early diagnosis of SARS-CoV-2.
44

45 # Introduction

46 The SARS-CoV-2 virus outbreak that began in late 2019 in Wuhan, has a mortality rate of approximately
47 6.1% worldwide [1-3]. Diagnostic testing is necessary for identifying and isolating infected individuals to
48 limit spread of disease. Molecular testing such as reverse-transcriptase polymerase chain reaction (rtPCR)
49 detects active infection; and serology testing helps identify those who were previously infected (including
50 asymptomatic infections) and have recovered [4, 5]. Nucleic acid detection using rtPCR has become the
51 confirmation test, due to its 99% specificity and 60-90% sensitivity within 7 days of exposure [6] but is
52 faced with numerous supply challenges [7]. The United States Food and Drug Administration (FDA)
53 issued an Emergency Use Authorization (EUA) approval for antibody testing as complementary to rtPCR,
54 leading to an explosion of new antibody methods, including rapid diagnostic test (RDT), enzyme-linked
55 immunosorbent assay (ELISA), virus neutralization assay (VNA), and chemiluminescent immunoassay

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56 (CLIA). These methods offer a range of sensitivities; the RDT provides results in less than 30 min for the
57 presence or absence of antibodies against the virus in a whole blood specimen but has the lowest
58 sensitivity, ELISA and CLIA can quantify antibodies to the virus in about 2-5 hours and 0.5-1 hour
59 respectively in either serum or plasma; while VNA can quantify presence of active antibodies that are
60 able to inhibit virus growth ex vivo, but requires 3-5 days [8, 9]. The best clinical utility of antibody
61 testing for efficient diagnosis at tertiary medical centers remains unclear for screening asymptomatic
62 patients and is being considered for identifying patients with adaptive immune responses for convalescent
63 plasma donor program, or for treating re-positive cases [10]. Additionally the relative performance of
64 many of these assays remains unclear.
65 We evaluated the performance of COVID-19 serology testing on three random access immunoassay
66 analyzers (RAIA) that are typically found in clinical laboratory across US - Architect i2000 (Abbott
67 Laboratories, Chicago IL), Cobas e601 (Roche Laboratories, Indianapolis, IN), and Liaison XL
68 (DiaSorin, Stillwater, MN) – comparing their performance to an ELISA assay (AnshLabs, Webster, TX)
69 and rtPCR test (Luminex Corporation, Austin, TX). The ELISA microtiter plate-based immunoassay,
70 was automated on Dynex DSX instrument (*Dynex Technologies*, Chantilly, VA, USA) for testing IgG and
71 IgM in serum or plasma.
72

73 Materials and methods

74 Specimen Selection

75 This project used 167 left-over and de-identified human serum specimens collected and stored at –20^{0}C.
76 This included patients who were either hospitalized with a confirmed COVID-19 diagnosis, seen in the
77 Emergency Department with symptoms for COVID-19, or were screened for COVID-19 before an
78 elective surgery procedure. Fifteen of the 167 samples were from patients that tested positive by rtPCR
79 with a confirmed COVID-19 clinical diagnosis. These samples were drawn >13 days after rtPCR testing.

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80 One hundred and fifty-two serum samples were from patients who tested negative by rtPCR, 134 of these
81 were collected on same day as rtPCR testing. For the remaining 18 samples, the interval between rtPCR
82 and sample collection ranged from 1–48 days. To avoid degradation, the specimens were tested by four
83 methodologies within 12-20 h of each other. Only samples having sufficient serum volume and rtPCR test
84 results were included in the evaluation project.

85 Instrumentation and analysis

86 Table 1 summarizes the characteristics of the four serologic assays we investigated.

87 **Table 1**. Characteristics summary of four serologic assays. CMIA = chemiluminescent microparticle
88 immunoassay; A450nm = absorbance at wavelength 450 nm; CLIA = chemiluminescent immunoassay;
89 ECIA = Electrochemiluminescent immunoassay. S/C = sample control index ratio; AU/mL = arbitrary
90 concentration units; COI = cutoff index.

	Abbott IgG	**AnshLabs IgG**	**Liaison IgG**	**Elecsys total**
Analyzer	Architect i2000SR	Dynex DSX	DiaSorin Liaison XL	Roche e601
Technique	Microparticles	ELISA	Solid phase	Double sandwich
Target	Nucleocapsid protein	Nucleocapsid & Spike proteins	Spike S1 & S2 proteins	Nucleocapsid protein
Antibody	IgG	IgG and IgM	IgG	IgG, IgM and IgA
Conjugate label	Acridinium	Peroxidase	Isoluminol	Ruthenium
Detection	CMIA	A450nm	CLIA	ECLIA
Calibration	2-points	3-points	2-points	2-points
Test run time	29 min	75 min	35 min	18 min
Positive cutoff	S/C ≥1.4	AU/mL of > 12	AU/mL ≥ 15	COI ≥1.0
EUA date	3/16/2020	4/10/2020	4/24/2020	5/2/2020

91

92 The AnshLabs SARS-CoV2 IgG assay is based on the ELISA technique that measures antibodies to spike
93 and nucleocapsid proteins. It is for in-vitro diagnostic use only and is performed on the Dynex automated
94 analyzer. Serum samples are diluted in a culture tube and transferred to the microtitration wells coated
95 with purified SARS-CoV-2 recombinant antigen. They are incubated for 30 min at 37°C along with
96 calibrators. The wells are washed and treated with the anti-human IgG antibodies conjugate labeled with
97 peroxidase. After a second incubation and washing step, the wells are incubated with the substrate
98 tetramethylbenzidine (TMB) chromogen solution to induce color change. An acidic stopping solution is

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99 added and the degree of enzymatic turnover of the substrate is determined by wavelength absorbance
100 measurement, with 450 nm as the primary filter and 630 nm as the reference filter. The intensity of color
101 change corresponds to arbitrary units of antibody-antigen complex concentration present in the specimen.
102 The analyzer calculates antibody concentration in arbitrary concentration units (AU/mL). Samples with
103 AU/mL of >12, 10–12, and <10 are considered positive, indeterminate and negative for IgG respectively.
104 It is the only test that uses a three-point calibration curve. The sensitivity and specificity are 95.0% and
105 98.3% respectively [11].

106 The Abbott SARS-CoV-2 IgG assay was run on the Abbott Architect i2000SR analyzer that measures
107 IgG antibodies to the nucleocapsid protein. The automated, two-step immunoassay uses
108 chemiluminescent microparticle immunoassay (CMIA) technology for qualitative detection of IgG
109 antibodies in human serum. The sample, SARS-CoV-2 antigen-coated paramagnetic microparticles, and
110 diluent are combined and incubated. The antibodies bind to the antigen-coated microparticles. The
111 mixture is washed and anti-human IgG acridinium-labeled conjugate is added. Following incubation, the
112 pre-trigger is added. The resulting chemiluminescent reaction is measured as a relative light unit (RLU).
113 The presence or absence of IgG antibodies is determined by dividing the sample RLU by the stored
114 calibrator RLU to find the IgG assay index (S/C), with a positive cutoff of ≥1.4. The sensitivity and
115 specificity are 100% and 99.63% respectively at ≥ 14 days post onset of symptoms [12].

116 The LIAISON SARS-Cov-2 S1/S2 IgG is a chemiluminescent immunoassay (CLIA) for detection of anti-
117 S1 and anti-S2 spike glycoprotein specific to SARS-CoV-2 in human serum or plasma on the DiaSorin
118 XL analyzer (Stillwater, MN). Specimen, calibrator, control, coated magnetic particles and diluent are
119 incubated in reaction cuvettes. The antibodies bind to the solid phase through the recombinant S1 and S2
120 antigens. A second incubation links recombinant S1 and S2 antigens to an isoluminol-antibody conjugate.
121 The starter reagents are then added, and a flash chemiluminescence reaction induced. The light signal, and
122 hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier and result
123 converted to arbitrary concentration, AU/mL. Samples with AU/mL of ≥15 are considered positive for

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124 IgG antibodies. The sensitivity and specificity are 90-97% and 98% respectively ≥ 14 days post onset of
125 symptoms [13].

126 The Elecsys Anti-SARS-CoV-2 assay is performed on the Roche cobas e601 analyzer for total antibodies
127 specific for IgG, IgM and IgA which target nucleocapsid protein, in human serum or plasma. A 20uL
128 sample and biotinylated SARS-CoV-2 specific recombinant antigen labeled with ruthenium bind in the
129 first incubation. In the second incubation, streptavidin-coated solid phase microparticles are added to help
130 bind the complex to the solid phase via interaction between biotin and streptavidin. The reaction mixture
131 is aspirated into cells where microparticles are captured on the surface of electrode, and the unbound
132 substances are washed out with ProCell solution. The ruthenylated-labeled antigen mediates detection via
133 electrochemiluminescence, which is measured by a photomultiplier tube. Results are calculated by
134 software, comparing the electrochemiluminescence signal of the sample to the cutoff value of the
135 calibration as a cutoff index (COI). Samples with COI ≥1.0 are considered reactive or positive for anti-
136 SARS-COV-2 antibodies. The sensitivity and specificity are 65.5-100% and 99.81% respectively [14].

137 Precision and specificity analysis

138 The precision studies were carried out by testing pooled positive and negative patient specimens for 5
139 days in duplicate. No discrepant results were noted, i.e. all positive and negative were consistent. The test
140 specificity towards the common cold coronavirus was evaluated by testing 100 prepandemic plasma
141 samples that were collected in October 2019 and stored at -80ºC. All samples were from asymptomatic
142 patients who were being evaluated for thyroid disorder.

143 Dilution studies

144 In order to rule out non-specific binding, samples that tested positive by ELISA assay were diluted using
145 sample diluent provided in the AnshLabs assay kit. We made and reran samples for a 1:2, 1:3 and 1:4
146 dilution, and calculated percent recovery.

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147 ### Third party adjudication studies

148 All ELISA and RAIA discordant result samples were evaluated against the FDA emergency used
149 approved all-in-one step SARS-CoV-2 Total (COV2T) assay performed on the automated Siemens Advia
150 Centaur XPT analyzer.

151 ### Statistical analysis

152 All test results were collated using a Microsoft Excel (Microsoft, Redmond, WA) spreadsheet.
153 Concordance was calculated using the macro formula in Excel.

154

155 ## Results

156 The specificities of the validated in-house AnshLabs SARS-CoV-2-IgG and IgM are listed in Table 2.
157 The cross reactivity to anti-influenza B IgG (5 samples), anti-respiratory syncytial virus IgG (5 samples),
158 anti-nuclear antibodies (5 samples), rheumatoid factors (5 samples), anti-influenza A IgG (5 samples),
159 anti-HCV IgG (5 samples), anti-HBV IgG (5 samples), anti-Haemophilus influenza IgG (5 samples) and
160 anti-HIV (5 samples) was determined by testing 45 patient samples obtained before the pandemic and
161 were positive for these analytes. No cross-reactivity was noted for either SARS-CoV-2-IgG or IgM. The
162 clinical sensitivity and specificity using rtPCR results as the gold standard were found to be 86.7and
163 91.2% respectively. All samples used for the sensitivity and specificity evaluation were collected from
164 symptomatic patients, either hospitalized inpatients or treated in Emergency Department. The interval
165 between rtPCR confirmation and serology testing ranged from 2-12 days. The specificity toward common
166 cold coronavirus is shown in Table 3. Three of 100 prepandemic samples tested positive for IgG by
167 ELISA and none tested positive by RAIA methods, thereby giving a calculated specificity of 97% and
168 100% for ELISA and RAIA respectively.

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169 **Table 2.** Specificity of AnshLabs SARS-CoV-2 IgG and IgM assays before and during COVID-19
170 outbreak for asymptomatic and negative individuals

Subjects	**No of samples**	**IgG (-)**	**IgM (-)**
Asymptomatic adults (during COVID-19 outbreak)	40	39/40= 97.5%	40/40= 100%
Presumed negative adults (prepandemic)	100	100/100= 100%	100/100= 100%
Presumed negative pediatric (prepandemic)	39	39/39= 100%	39/39= 100%
Total	179	178/179= 99.4%	179/179= 100%

171

172 **Table 3.** Observed specificities towards common cold coronavirus

Pre-pandemic Sample Testing (n=100)		
Test Name	**Number of Positive**	**% Specificity**
AnshLabs ELISA IgG	3	97%
Architect i2000	0	100%
Elecsys e601	0	100%
Liaison XL	0	100%

173

174 Table 4 shows the concordance between ELISA and RAIA results for samples that were confirmed
175 positive for SARS-CoV-2 by rtPCR. These samples were collected from symptomatic patients > 13 days
176 post rtPCR confirmation. ELISA assay correlated best with Total Antibody assay on Roche Elecsys e601
177 analyzer. This could possibly be attributed to the measurement of IgG antibodies directed towards
178 multiple antigenic proteins (nucleocapsid & spike) by ELISA or measurement of total antibodies (IgG,
179 IgM, and IgA) on Roche Elecsys e601 analyzer.

180 **Table 4.** Concordance of 15 rtPCR positive samples between a) ELISA and RAIA systems and b) among
181 three RAIA platforms

Concordance Between ELISA and RAIA for samples from rtPCR positive patients (N = 15)	
AnshLabs IgG vs Architect i2000	93.3%
AnshLabs IgG vs Elecsys e601	100%

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AnshLabs IgG vs Liaison XL	93.3%
Concordance Among RAIA platforms for samples from rtPCR positive patients (N = 15)	
Architect i2000 vs Liaison XL	100%
Architect i2000 vs Elecsys e601	93.3%
Liaison XL vs Elecsys e601	93.3%

182

183 Table 5 shows the concordance between ELISA and RAIA for samples from patients that tested negative
184 for SARS-CoV-2 by rtPCR. The ELISA assay showed a concordance ranging from 69.7–73% with
185 different RAIA methodologies: 34, 1, 7, and 5 patients that had tested negative by rtPCR tested positive
186 for antibodies by ELISA, Architect i2000, Liaison XL and Elecsys e601 methodology respectively. All
187 samples that tested positive by ELISA also test positive by Siemens all-in-one step SARS-CoV-2 Total
188 (COV2T) assay Siemens Advia Centaur XPT analyzer. Thus a higher rate of sero-prevalence is observed
189 by ELISA versus RAIA.

190 **Table 5.** Concordance of 152 rtPCR negative samples between a) ELISA and RAIA systems and b)
191 among four RAIA platforms

Concordance Between ELISA and RAIA for samples from rtPCR negative patients (n=152)	
AnshLabs IgG vs Architect i2000	73.0%
AnshLabs IgG vs Elecsys e601	73.0%
AnshLabs IgG vs Liaison XL	69.7%
Concordance Among RAIA platforms for samples from rtPCR negative patients (n=152)	
Architect i2000 vs Liaison XL	96.1%
Architect i2000 vs Elecsys e601	97.4%
Liaison XL vs Elecsys e601	94.7%

192

193 The concordance of ELISA and RAIA results with rtPCR is shown in Table 6. All patient tested positive
194 by rtPCR also tested positive by ELISA and Elecsys e601 total antibody. Architect i2000 SARS-CoV-2-
195 IgG and Liaison XL were unable to detect antibodies in one sample. All RAIA methodologies showed
196 high correlation with nucleic acid test for patient samples that tested negative by rtPCR, with

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197 concordances ranging from 95.39–99.34 %. The ELISA assay on the other hand showed a concordance of
198 only 72.36% for these rtPCR negative samples.

199 **Table 6.** Concordance of a) serology systems for rtPCR positives confirmed more than 13 days and b)
200 serology systems for all rtPCR negatives

a. CONCORDANCE FOR ALL rtPCR POSITIVE SAMPLES DRAWN > 13 days after rtPCR result (N=15)	
rtPCR vs ELISA SARS-CoV-2-IgG	100%
rtPCR vs Architect i2000 SARS-CoV-2IgG	93.3%
rtPCR vs Liaison XL SARS-CoV-2 IgG	93.3%
rtPCR vs Elecsys e601 total antibody	100%
b. CONCORDANCE FOR ALL rtPCR NEGATIVE SAMPLES (N=152)	
rtPCR vs ELISA SARS-CoV-2 IgG	72.4%
rtPCR vs Architect i2000 SARS-CoV-2 IgG	99.3%
rtPCR vs Liaison XL SARS-CoV-2 IgG	95.4%
rtPCR vs Elecsys e601 total antibody	96.7%

201

202 The non-specific binding dilution data of the AnshLabs assay showed five samples with various
203 concentration levels of IgG were serial diluted to 1:2, 1:4: 1:8 and 1:16. All samples gave a consistent
204 dilution pattern and expected 90-100% recovery of neat sample in AU/mL units (Fig 1).

205

206 Discussion

207 All RAIA methods correlated well with ELISA and rtPCR for samples collected >13 days post rtPCR
208 confirmation. There were no significant differences among the methods which tested for IgG targeted to
209 one or both nucleocapsid and spike proteins, or tested for total antibodies.

210 ELISA detected higher sero-prevalence in rtPCR negative samples than the RAIA methods. This may be
211 due to i) higher analytical sensitivity or a lower cutoff by ELISA, which triggered more positive results;
212 ii) cross reactivity to other coronavirus; iii) non-specific binding of other antibodies, for example
213 autoimmune antibodies or deposition of detection antibody on the microtiter well which led to increased
214 absorbance causing false positives

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215 ELISA assays are generally known for low detection limits in sub ng/mL to low pg/mL because of their
216 increased incubation time thereby allowing antigen-antibody to reach reaction equilibrium and extra
217 washing steps [15, 16]. The Dynex DSX analyzer used for ELISA assay provided optimization flexibility
218 and automation, which is not available on RAIA due to throughput constraint. Cross-reactivity to other
219 coronavirus was evaluated by testing 100 prepandemic samples and found to be 3% and 0% for ELISA
220 and RAIA respectively. The differences in cross-reactivity may account for one or two false positive
221 results, but not for all 34 and 15 positives picked up by ELISA. Non-specific deposition of other
222 antibodies in patient samples or detection antibody was ruled out by dilution studies for ELISA. Recovery
223 of 90–110% ruled out non-specific binding as a possible cause for false positives (Fig 1). The difference
224 in results for positive and negative samples by RAIA methods may also be due to a higher threshold for
225 positivity. The rtPCR assay is used as the gold standard in maximizing analytical sensitivity and
226 specificity during method development which is the most accurate in the early days of the infection when
227 antibody development is low and results in the reported sensitivity of 10-60% on samples collected <14
228 days post rtPCR confirmation [17-19].

229 We believe that higher rate of positivity observed for ELISA i.e. 34 versus 1 by Architect, 7 by Liaison
230 XL and 5 by Elecsys e601, is the net effect of extra washing and longer incubation times used by ELISA
231 or a higher S/C cutoff set in RAIA assays. These are not false positives as claimed in other studies [17,
232 18] but are true positives not picked up by RAIA. This inadvertently decreases identification of infected
233 patients 5-10 days post infection. The recently released all-in-one step SARS-CoV-2 Total (COV2T)
234 assay performed on the automated Siemens RAIA - Advia Centaur XPT analyzer has resolved some of
235 these issues and it correlates well with our in-house ELISA assay by detecting all 34 samples that were
236 missed by other RAIA as positives.

237 Project Limitations

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238 Our quality assurance project has some notable limitations. At this stage of the disease, true clinical
239 sensitivity and specificity for different methodologies is difficult to determine because of our limited
240 understanding of the disease process and kinetics. Secondly, our assumption that ELISA has better limits
241 of detection is based on circumstantial evidence, as certified standards quantifying limits of detection on
242 different platforms are not available. Third, the cutoffs provided by manufacturers were relied on which
243 may not have undergone extensive validation. Establishing laboratory specific cut-off is akin to
244 establishing reference ranges, which is highly dependent on prevalence of disease in local population.
245

246 Conclusion

247 All of the assays we investigated would work well for epidemiological sero-prevalence studies. Among
248 rtPCR negative patients, ELISA gave higher estimates of sero-prevalence in our dataset and would
249 probably do so in population-based epidemiological surveys using serological testing. RAIA methods
250 could however offer other advantages over ELISA which includes i) faster turnaround time; ii) random
251 access to allow immediate testing; iii) longer calibration stability, obviating the need to perform daily
252 calibration as required by ELISA; iv) the ability to perform other immunoassay testing concurrently; and
253 v) higher test throughput and walk away capabilities. However in conclusion, no serological method
254 tested has sensitivity and specificity greater than or equal to 99% for one to 5 days post exposure, limiting
255 their use in early diagnosis.

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260

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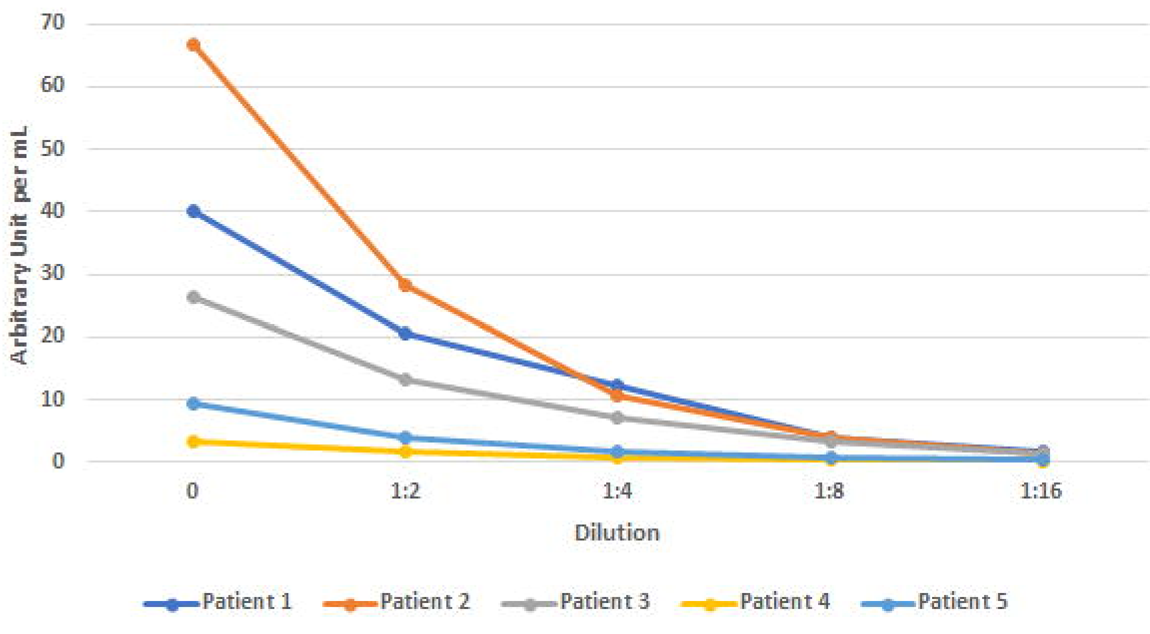
316

317

318 # Figure Legend

319 **Fig 1.** Graph of 5 patient samples diluent sets (1:2, 1:4, 1:8, and 1:16) versus AU/mL levels, ruling out
320 non-specific binding in AnshLabs ELISA assay


70
60
50
40
30
20
10
0
Arbitrary Unit per mL
0
1:2
1:4
1:8
1:16
Dilution
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5