

1 Evaluation of the EUROIMMUN Anti-SARS-CoV-2 ELISA Assay for detection of IgA and
2 IgG antibodies.

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11 Running Head: EUROIMMUN Anti-SARS-CoV-2 IgA and IgG ELISA

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14 was determined by seniority.

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16

17 **Abstract.**

18 As the Coronavirus 2019 (COVID-19) pandemic evolves, the development of
19 immunoassays to help determine exposure and potentially predict immunity has
20 become a pressing priority. In this report we present the performance of the
21 EUROIMMUN enzyme-linked immunosorbent assay (ELISA) for semi-quantitative
22 detection of IgA and IgG antibodies in serum and plasma samples using recombinant
23 S1 domain of the SARS-CoV-2 spike protein as antigen. Specimens from patients, with
24 and without COVID-19 infection, were tested at the University of Chicago Clinical
25 Microbiology and Immunology Laboratory. Of 57 samples from COVID-19 PCR-
26 negative patients, including 28 samples positive for common human coronavirus strains,
27 53 tested negative and 4 tested positive for IgA (93.0% agreement) while 56 tested
28 negative and 1 tested positive for IgG (98.2% agreement). For COVID-19 PCR-positive
29 patients, 29 of 30 (96.7%) samples collected ≥ 3 days after positive PCR were positive
30 for IgA, and 28 of 28 samples collected ≥ 4 days after positive PCR were positive for
31 IgG.

32 The EUROIMMUN Anti-SARS-CoV-2 ELISA Assay demonstrates excellent sensitivity
33 for detection of IgA and IgG antibodies from samples collected ≥ 3 days and ≥ 4 days,
34 respectively, after COVID-19 diagnosis by PCR. This assay did not demonstrate cross
35 reaction in any of the 28 samples from patients with common human coronaviruses,
36 including types HKU1, NL63, CV229E, and OC43.

37

38 **Introduction**

39 In December 2019 a novel coronavirus emerged as the cause of severe respiratory
40 disease and quickly spread causing a worldwide pandemic. Severe Acute Respiratory
41 Coronavirus 2 (SARS-CoV-2) was determined to be the agent of coronavirus disease
42 2019 (COVID-19). The virus belongs to the Betacoronavirus genus of the Coronaviridae
43 family, which also includes Severe Acute Respiratory Syndrome Coronavirus 1 (SARS-
44 CoV-1) and Middle East respiratory Syndrome Coronavirus (MERS-CoV) (1). For
45 diagnostic purposes many nucleic acid amplification assays were quickly developed
46 and received Emergency Use Authorization (EUA) from the US Food and Drug
47 Administration (FDA). Multiple manufacturers are offering serological assays, but few
48 have received Emergency Use Authorization from the Food and Drug Administration
49 (FDA); the EUROIMMUN IgG assay has received Emergency Use Authorization.
50 Serological testing may be useful in conjunction with other laboratory tests and clinical
51 findings of COVID-19 infection for epidemiological monitoring and outbreak control. Of
52 the immunoassays currently available, choice of SARS-CoV-2 target antigens include
53 the spike protein (S) or the nucleocapsid (N) (2). IgA antibodies can show higher
54 sensitivity, while IgG antibodies typically have longer duration, better specificity, and are
55 better suited for serosurveillance studies (3-5).

56

57 **Materials and Methods**

58 The EUROIMMUN Anti-SARS-CoV-2 assay is an enzyme-linked immunosorbent assay
59 (ELISA) that provides semi-quantitative in vitro determination of human antibodies of
60 immunoglobulin classes IgA and IgG against severe acute respiratory syndrome
61 coronavirus 2 (SARS-CoV-2) in serum or EDTA plasma (6-7).

62 Each kit contains microplate strips with 8 break-off reagent wells coated with recombinant
63 structural protein of SARS-CoV-2. In the first reaction step, diluted patient samples are
64 incubated in the wells. In the case of positive samples, specific antibodies will bind to the
65 antigens. To detect the bound antibodies, a second incubation is carried out using an
66 enzyme-labelled antihuman IgA or IgG (enzyme conjugate) catalyzing a color reaction.

67 Results are evaluated semi-quantitatively by calculation of a ratio of the extinction of the
68 control or patient sample over the extinction of the calibrator. This ratio is interpreted as
69 follows:

- 70 • Ratio < 0.8 negative
- 71 • Ratio ≥ 0.8 to <1.0 borderline
- 72 • Ratio ≥ 1.1 positive

73 The IgG assay has received Emergency Use Authorization from the US Food and Drug
74 Administration (FDA).

75

76 The University of Chicago Medicine uses 2 different RT-PCR assays allowed by the
77 FDA under Emergency Use Authorization. The Roche cobas 6800 SARS-CoV-2 assay
78 relies on amplification of the SARS-CoV-2 specific ORF1 gene as well as a portion of

79 the E-gene conserved across the sarbecoviruses, a subgenus of coronaviruses which
80 includes SARS-CoV-2. The Cepheid Xpert Xpress SARS-CoV-2 assay also detects the
81 pan-sarbecovirus E-gene but uses the SARS-CoV-2 specific N-gene rather than ORF1
82 as its primary target. Samples tested include nasopharyngeal and nasal mid-turbinate
83 swabs transported in viral transport or liquid Amies media.

84

85 The BioFire FilmArray Respiratory Panel 2 (RP2) is a multiplex in vitro molecular
86 diagnostic test for the simultaneous and rapid detection of 21 pathogens, including 4
87 common human coronavirus strains, directly from nasopharyngeal swab (NPS)
88 samples.

89

90 Stored residual serum and plasma samples submitted to the University of Chicago
91 Medicine Clinical Laboratories for routine testing were recovered for this evaluation.

92 Percent agreement was determined and the hybrid Wilson/Brown method was used to
93 calculate 95% confidence intervals of proportions (95% CI). All statistical analyses were
94 performed using GraphPad Prism version 8.4.1.

95

96 **Results**

97 Fifty-seven samples were tested from patients thought to be negative for exposure to
98 SARS-CoV-2. Forty-one of the samples were from ambulatory patients at the University

99 of Chicago with negative results for SARS-CoV-2 by PCR. The remaining 16 samples
100 were collected in early 2019, prior to the current pandemic, and stored at -20°C.
101 Twenty-eight of the 57 samples were from patients who had tested positive by the
102 BioFire FilmArray RP2 respiratory viral panel for common coronavirus strains (6
103 samples positive for HKU1, 10 positive for NL63, 9 positive for OC43, 2 positive for
104 229E, and one positive for both OC43 and 229E).

105

106

107 Of these 57 samples, 53 tested negative and 4 tested positive for IgA (93.0%
108 agreement, 95% CI: 83.3-97.2) while 56 tested negative and 1 tested positive for IgG
109 (98.2% agreement, 95% CI: 90.7-99.9).

110 The sample that was positive for IgG was also positive for IgA. Chart review revealed
111 an episode 4 weeks prior of a fever of 103.5°F and diarrhea, without cough. This
112 clinical picture that could be consistent with COVID-19 disease. This positive
113 serological result and negative PCR could represent exposure to SARS-CoV-2 with
114 clearance of the virus.

115 Samples from sixty-seven unique specimens from 49 patients with PCR-positive SARS-
116 CoV-2 were tested. Of these samples, 55 tested positive and 12 tested negative for IgA
117 (82.1% agreement, 95% CI: 71.3-89.4) while 40 tested positive and 27 tested negative
118 for IgG (59.7% agreement, 95% CI: 47.7-70.6). Six borderline positives for IgA and 2
119 borderline positives for IgG were included in the positive results.

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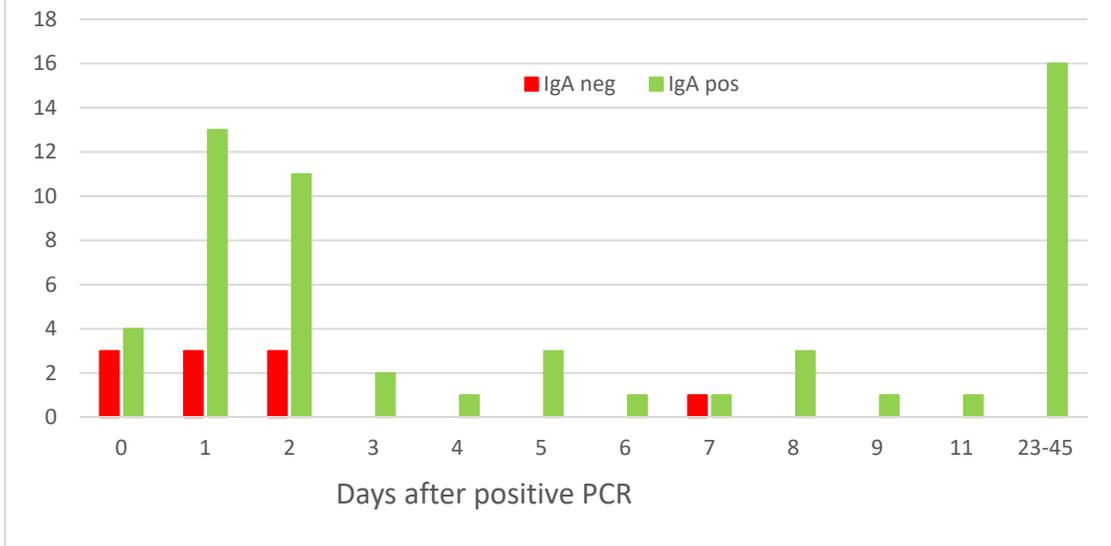
121 These 67 samples from PCR positive patients were collected 0 to 45 days after PCR
122 testing. The 12 IgA-negative samples and the 27 IgG-negative samples were collected
123 within 7 days of PCR testing. Since antibody development occurs after viremia and
124 requires time to reach a detectable concentration, it is likely that these samples were
125 collected too early in the course of disease to expect antibody production.

126

127 The 67 serological samples from SARS-CoV-2 PCR-positive patients were stratified by
128 the number of days following the positive PCR result. For samples collected ≥ 3 days
129 after positive PCR, 29 of 30 (96.7%, 95% CI: 83.3-99.8) were positive for IgA (see chart
130 1); all 21 samples collected after one week were IgA positive. For samples collected ≥ 4
131 days after positive PCR, 28 of 28 (100%, 95% CI: 87.9-100) were positive for IgG (see
132 chart 2).

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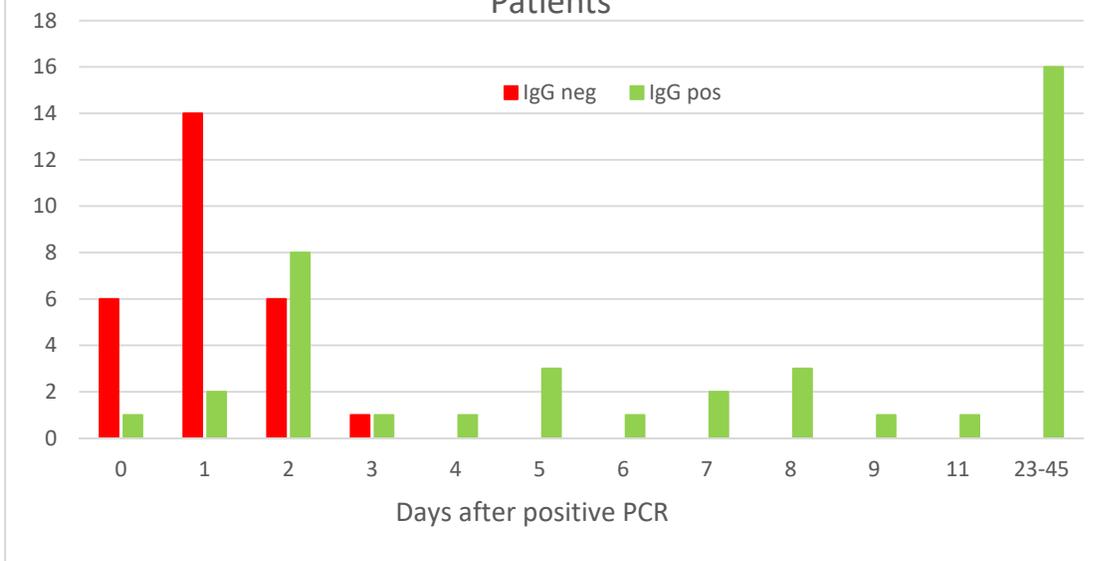
Chart 1. Timeline of IgA Results on PCR Positive Patients



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Chart 2. Timeline of IgG Results from PCR Positive Patients



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139 **Cross-reactivity**

140 A total of 28 unique samples that tested positive by BioFire FilmArray RP2 panel for
141 common human coronaviruses including types 229E, NL63, HKU1 and OC43 were
142 tested for cross reactivity. All 28 tested negative for both IgA and IgG antibodies.

143

144 **Discussion**

145 A positive or negative test for the SARS-CoV2 antibody is difficult to interpret at this
146 time. It is not yet known if antibodies serve as an indication of the presence or absence
147 of protective or sustained immunity. Negative SARS-CoV-2 antibody results do not rule
148 out SARS-CoV-2 infection, particularly in those who have been in contact with the virus.
149 Results from antibody testing should not be used as the sole basis to diagnose or
150 exclude SARS-CoV-2 infection or to inform infection status. Our evaluation of the
151 EUROIMMUN assay did not demonstrate any cross reaction with samples from patients
152 positive for common human coronaviruses.

153

154 **Conclusion**

155 The EUROIMMUN Anti-SARS-CoV-2 ELISA Assay demonstrated excellent sensitivity
156 for detection of IgA and IgG antibodies from samples collected >2 days and >3 days,
157 respectively, after COVID-19 diagnosis by PCR. The EUROIMMUN Anti-SARS-CoV-2
158 ELISA Assay demonstrated excellent specificity and did not demonstrate cross reaction
159 with common human coronaviruses, including types HKU1, NL63, CV229E, and OC43.

160

161 Limitations of this study include a small sample size and a lack of specimens collected
162 more than 45 days following a positive PCR.

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