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1 **Detection of SARS-CoV-2 nucleocapsid antigen from serum can aid in timing of**
2 **COVID-19 infection**

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13

14 **Abstract**

15 SARS-CoV-2 RNA can be detected in respiratory samples for weeks or even months after
16 onset of COVID-19 disease. Therefore, one of the diagnostic challenges of PCR positive
17 cases is differentiating between acute COVID-19 disease and convalescent phase. Recently,
18 the presence of SARS-CoV-2 nucleocapsid antigen in serum samples of COVID-19 patients
19 was published [Le Hingrat et al. Detection of SARS-CoV-2 N-antigen in blood during acute
20 COVID-19 provides a sensitive new marker and new testing alternatives, *Clinical*
21 *Microbiology and Infection*, 2020].

22 Our study aimed to characterize the analytical specificity and sensitivity of an enzyme-linked
23 immunosorbent assay (Salocor SARS-CoV-2 Antigen Quantitative Assay Kit[®] (Salofa Ltd,
24 Salo, Finland)) for the detection of SARS-CoV-2 antigen in serum, and to characterize the
25 kinetics of antigenemia. The evaluation material included a negative serum panel of 155
26 samples, and 126 serum samples from patients with PCR-confirmed COVID-19.

27 The specificity of the Salocor SARS-CoV-2 serum N antigen test was 98.0%. In comparison
28 with simultaneous positive PCR from upper respiratory tract (URT) specimens, the test
29 sensitivity was 91.7%. In a serum panel in which the earliest serum sample was collected two
30 days before the collection of positive URT specimen, and the latest 48 days after (median 1
31 day post URT sample collection), the serum N antigen test sensitivity was 94% within 14
32 days post onset of symptoms. The antigenemia resolved approximately two weeks after the
33 onset of disease and diagnostic PCR.

34 The combination of simultaneous SARS-CoV-2 antigen and antibody testing appeared to
35 provide useful information for the timing of COVID-19. Our results suggest that SARS-CoV-
36 2 N-antigenemia may be used as a diagnostic marker in acute COVID-19.

37

38 **Introduction**

39 SARS-CoV-2 laboratory diagnostics relies primarily on molecular diagnostic techniques [1-
40 3]. More recently, a variety of tests for SARS-CoV-2 antigen detection from upper
41 respiratory tract (URT) specimens have established a complementary role [4]. While less
42 sensitive, they benefit from being rapid, cheap, and performable outside centralized
43 laboratory facilities.

44 Recent articles and preprints demonstrate SARS-CoV-2 nucleocapsid (N) antigenemia in
45 COVID-19 patients [5,6]. While posing new questions on the pathophysiology of acute
46 COVID-19, this may offer a novel diagnostic approach. Only limited information is available
47 on the performance of serum antigen tests as a diagnostic method for SARS-CoV-2.

48 The aim of this study was to characterize the analytical specificity and sensitivity of an
49 enzyme-linked immunosorbent assay (ELISA) for the detection of SARS-CoV-2 antigen in
50 serum, namely Salocor SARS-CoV-2 Antigen Quantitative Assay Kit[®] (Salofa Ltd, Salo,
51 Finland; later Salocor N-antigen EIA) and to characterize the kinetics of antigenemia.

52 **Materials and methods**

53 Serum samples were originally sent for diagnostic purposes to the Department of Virology
54 and Immunology, Helsinki University Hospital Laboratory HUSLAB, Finland. Research
55 permit HUS/157/2020-44 (Helsinki University Hospital, Finland) was obtained from the local
56 review board.

57 The negative panel (N=155; 148 cases) consisted of 144 serum samples collected in 2019,
58 and 11 samples (positive for Aspergillus antigen) in 2020. Of the 155 specimens, 37 were
59 sent for respiratory virus antibody testing; 32 samples were positive for anti-nuclear
60 antibodies; 16 for phospholipase-A2-receptor antibodies; 8 for antineutrophil cytoplasmic (C-

61 ANCA, P-ANCA and parallel C- and P-ANCA), and 4 for glomerular basement membrane
62 antibodies. Two samples were from patients with Human coronavirus (HCoV) OC43
63 diagnosis (by PCR) and five with primary Epstein-Barr virus (EBV) infection. We also
64 included serum specimens with a positive microbial antigen test as follows: 22 with Dengue
65 virus NS1 antigen, 17 with hepatitis B virus surface antigen, 11 with Aspergillus antigen, and
66 one with HIV p24 antigen. The median age of the negative panel cases was 53 years (range 2-
67 89); 45% were males (66/148).

68 There were two separate serum specimen panels from PCR confirmed COVID-19 patients
69 (panel A and panel B), who were previously SARS-CoV-2 PCR positive from a URT
70 specimen. They were also tested for SARS-CoV-2 IgG by both Abbott SARS-CoV-2 IgG (N
71 antigen) and Euroimmun SARS-CoV-2 IgG (S1 antigen) according to manufacturer's
72 instructions.

73 Panel A comprised 70 serum samples from 62 cases (median age 54 years, range 24-86 years;
74 28/62 (45%) males). The earliest serum sample was collected 2 days before the collection of
75 the positive URT specimen, and the latest 48 days after (median 1 day post URT sample
76 collection). The samples were previously tested with SARS-CoV-2 microneutralization
77 (MNT) [7]. The date of onset of symptoms was available for 55/62 cases. Adjusted p-values
78 for comparison of antigen and MNT result combinations were determined using Kruskal-
79 Wallis test (GraphPad Prism 8.0.1).

80 Panel B comprised 56 serum samples from 27 cases (median age 50 years, range 27-65 years;
81 4/27 males); 2-4 consecutive serum samples from each. At least one serum from all 27 cases
82 was SARS-CoV-2 IgG positive in both Abbott and Euroimmun tests. The earliest serum
83 sample was collected 5 days before the collection of the positive URT specimen, and the

84 latest 207 days after (median 101 days post URT specimen collection). The date of onset of
85 symptoms and MNT result were not available for this panel.

86 The PCR tests were carried out with one of the following methods: a laboratory-developed
87 test based on Corman et al.; cobas® SARS-CoV-2 test kit on the cobas® 6800 system (Roche
88 Diagnostics, Basel, Switzerland); and the Amplidiag® COVID-19 test on the Amplidiag®
89 Easy platform (Mobidiag, Espoo, Finland). The performance of these tests in our laboratory
90 is reported elsewhere [8].

91 The here-evaluated Salocor N-antigen ELISA (Salofa) is based on a double antibody
92 sandwich ELISA test. The assay protocol is described in the Supplement.

93 The 95% Clopper-Pearson confidence intervals were calculated for sensitivity and specificity
94 (IBM SPSS statistical program package, version 25).

95 **Results**

96 The specificity of the Salocor N antigen ELISA was 98.0% (145/148) (Clopper-Pearson 95%
97 confidence interval 94.2-99.6%) as determined by the negative panel. The three positive
98 samples were collected in 2019. One was from a patient with EBV primary infection (5
99 pg/ml). Two were originally sent for respiratory virus antibody screening; one with reported
100 myocarditis (84.5 pg/ml) and one without any reported clinical outcome (4 pg/ml).

101 There was a simultaneous serum and PCR positive URT specimen available in 24 cases: N
102 antigen was positive in 22/24, rendering 91.7% sensitivity (Clopper-Pearson 95% confidence
103 interval 73.0-99.0%). The negative specimens were retrieved at one day and at 16 days post
104 disease onset. The PCR cycle threshold values did not appear to associate with the N antigen
105 concentrations (Figure 1a).

106 Using COVID-19 panel A, we calculated test sensitivity in relation to disease onset (Table 1).
107 The sensitivity with specimens retrieved at ≤ 14 days post onset was 94%, and decreased to
108 50% with specimens retrieved 15-21 days post onset (Table 1). The N antigen concentrations
109 decreased over time from symptom onset (Figure 1b). The furthest time point for a positive N
110 antigen was observed at 47 days post disease onset (3.5 pg/ml) (Figure 1b).

111 The median days from disease onset for subgroups in panel A were as follows: N antigen
112 positive (≥ 2.97 pg/ml), MNT antibody negative (< 40 titer) (6 days; 25 cases); N antigen
113 positive, MNT antibody positive (10 days; 31 cases); N antigen negative, MNT antibody
114 positive (37 days; 10 cases) (Figure 2).

115 There appeared to be a decreasing trend in N antigen concentration over increasing
116 Euroimmun anti-S1 IgG test result (Figure 1c), but no such trend was observed over
117 increasing Abbott anti-N IgG test result (Figure 1d), nor the increasing MNT titer (Figure 1e).

118 Of the COVID-19 panel B, the only N antigen positive serum was retrieved on the date of
119 positive PCR. All other 69 panel B serum specimens were N antigen negative; one was
120 retrieved 5 days before positive PCR in URT, and the others between 12 and 207 days
121 (median 107 days) after positive PCR in URT.

122 **Discussion**

123 SARS-CoV-2 RNA can be detected for even 4-6 weeks post disease onset in URT samples
124 [9,10]. Subsequently, one of the challenges in contact tracing of PCR positive cases is
125 differentiating between acute COVID-19 disease and convalescent phase. The present study
126 brings forward a potential tool to aid in timing of COVID-19 disease: detection of SARS-
127 CoV-2 N antigen in serum.

128 Only two previous reports are available on SARS-CoV-2 antigen detection in serum [5,6].
129 Some antigen tests for the detection of SARS-CoV have been described [11,12]. We showed
130 a very good specificity (98.0%), and a reasonable sensitivity (91.7%; in comparison with
131 simultaneous positive PCR from URT specimens) for the Salocor N antigen test. SARS-CoV-
132 2 N antigenemia appeared to resolve within the first two weeks of illness. Particularly the
133 combination of simultaneous SARS-CoV-2 antigen and antibody testing may provide useful
134 information for timing of disease (Figure 2). These tests can be performed from a single
135 serum specimen in high throughput platforms. While this approach does not replace detection
136 of SARS-CoV-2 RNA from URT, it may provide an additional aid.

137 Only a limited number of serum specimens was available with information on disease onset,
138 particularly for the convalescent phase. This diminishes the ability of this study to provide a
139 complete timeline for antigenemia in COVID-19. Potential cross-reactivity with seasonal
140 coronaviruses was not assessed in our study.

141 More data is needed to clarify whether serum N antigen testing could be used to supplement
142 current testing strategies for acute COVID-19. One interesting question is whether there is an
143 association between antigenemia and viral shedding. Besides diagnostic differentiation
144 between acute and convalescent COVID-19, N antigen testing from serum could potentially
145 be deployed in epidemiological screening of asymptomatic infections, e.g. in recently
146 vaccinated populations.

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158 original draft, SKU: Conceptualization, Formal analysis, Resources, Writing – original draft,
159 Writing – review and editing, Supervision, Project administration, SK: Investigation, Writing
160 –review and editing, ML: Resources, Writing – review and editing, Supervision, Project
161 administration HJ: Conceptualization, Formal analysis, Visualization, Resources, Writing –
162 review and editing, Supervision, Project administration, AJJ: Conceptualization,
163 Investigation, Formal analysis, Data curation, Writing – original draft, Supervision, Project
164 administration

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- 205
- 206
- 207

208

209 **Table 1** Sensitivity of serum antigen test in relation to time post symptom onset

| Time post symptom onset (days) | Time post symptom onset (days, mean [SD]) | N | Sensitivity (% [CI 95%] ^a) |
|--------------------------------|---|----|--|
| ≤7 | 4.6 (2.3) | 26 | 96.2 (80.4-99.9) |
| 8-14 | 10.4 (1.7) | 24 | 91.7 (73.0-99.0) |
| ≤14 | 7.4 (3.5) | 50 | 94.0 (83.5-98.7) |
| 15-21 | 16.3 (1.9) | 6 | 50.0 (11.8-88.2) |
| ≤21 | 8.4 (4.4) | 56 | 89.3 (78.1-96.0) |
| ≥7 | 16.2 (12.5) | 41 | 75.6 (59.7-87.6) |
| ≥14 | 28.6 (14.9) | 14 | 42.9 (17.7-71.1) |
| ≥21 | 41.1 (10.3) | 7 | 28.6 (3.7-71.0) |

210 ^aClopper-Pearson confidence interval

211

212

213 **Figure legends**

214 **Figure 1.** Serum antigen concentrations in relation to the timing of sampling and other tests.
215 Serum antigen concentrations of >180 pg/ml are depicted as 180 pg/ml. A. Serum antigen
216 concentrations compared to the Ct-value of a URT PCR sample obtained on the same day. B.
217 Serum antigen concentrations relative to the timing of sampling after the onset of symptoms.
218 C. Correlation of serum antigen concentration with the Euroimmun IgG assay results. The
219 vertical line indicates the cut-off value of the Euroimmun assay D. Antigen concentrations in
220 comparison with the Abbott IgG assay results. The vertical line indicates the cut-off value of
221 the Abbott assay E. Serum antigen concentrations relative to the microneutralization test titer.
222 MNT titers of <40 (negative) are depicted as 0 and MNT titers of >2560 as 2560. Figure
223 created using GraphPad Prism 8.0.1 software.

224

225 **Figure 2** Simultaneous N antigen and MNT test results in relation to symptom onset. N
226 antigen positive and MNT negative, median 6 days. N antigen positive, MNT positive,
227 median 10 days. N antigen negative and MNT positive median. 37 days. Adjusted p-values
228 (Kruskal-Wallis test): *p=0.0317; **p 0.0015, and ***p=<0.0001. Figure created using
229 GraphPad Prism 8.0.1 software.

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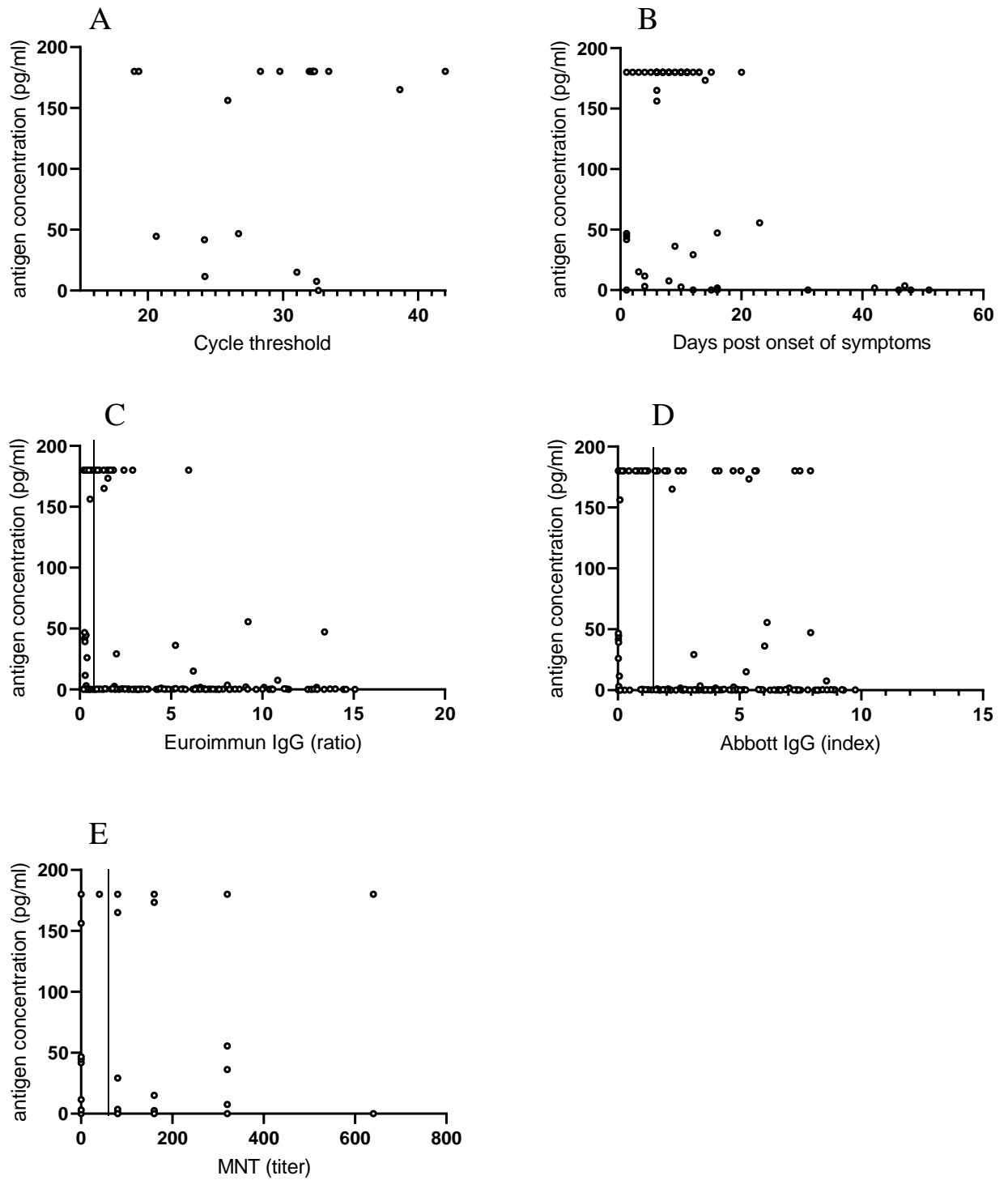
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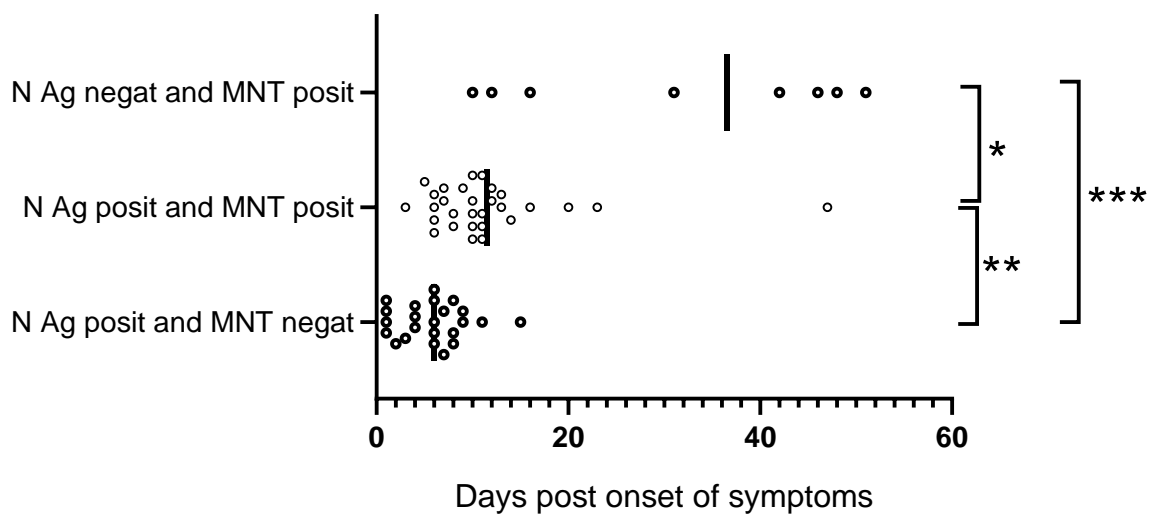
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Figure 1



236 **Figure 2**



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