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EARLY LABORATORY DIAGNOSIS OF COVID-19 BY ANTIGEN DETECTION IN BLOOD SAMPLES OF THE SARS-COV-2 NUCLEOCAPSID PROTEIN

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Running title: Diagnosis of COVID-19 by antigen detection in blood

ABSTRACT

The purpose of this study was to characterize the diagnostic performance of a newly developed enzyme-linked immunosorbent assay (ELISA) for detection of SARS-CoV-2 nucleocapsid protein (NP) in blood. Blood samples were collected during hospitalization of 165 inpatients with PCR-confirmed SARS-CoV-2 infection, and from 505 outpatients with relevant symptoms of COVID-19 simultaneously with PCR-testing. For the 143 inpatients who had their first blood sample collected within 2 weeks after PCR-confirmed infection, the diagnostic sensitivity of the ELISA was 91.6%. The mean NP concentration of the 131 ELISA-positive blood samples was 1,734 pg/ml (range: [10-3,840] pg/ml). An exponential decline in NP concentration was observed for 368 blood samples collected over the first 4 weeks after PCR-confirmed SARS-CoV-2 infection, and all blood samples taken later had an NP concentration below the 10 pg/ml diagnostic cut-off.

The diagnostic sensitivity of the ELISA was 81.4% for the 43 blood samples collected from outpatients with a simultaneous positive PCR-test, and the mean NP concentration of the 35 ELISA-positive samples was 157 pg/ml (range: [10-1,377] pg/ml). For the 462 outpatients with a simultaneous negative PCR-test, the diagnostic specificity of the ELISA was 99.8%.

In conclusion, the SARS-CoV-2 NP ELISA is a suitable laboratory diagnostic test for COVID-19. Particularly, for hospitals, where blood samples are readily available, screening of serum or plasma samples by ELISA can facilitate prevention of nosocomial infections and reduce the requirement for laborious swab sampling and subsequent PCR-analysis to confirmatory tests.

41 **INTRODUCTION**

42 The pandemic corona virus disease 2019 (COVID-19) caused by the severe acute respiratory
43 syndrome–related coronavirus 2 (SARS-CoV-2) virus has led to the rapid development and widespread
44 application of many laboratory diagnostic tests (1). According to the World Health Organization
45 (WHO), the standard confirmation of acute infections with SARS-CoV-2 is based on a nucleic acid
46 amplification test, such as real-time reverse-transcription PCR for the presence of unique sequences
47 of SARS-CoV-2 RNA (2). Testing for genomic RNA by PCR is widely supplemented by two other major
48 diagnostic test principles: testing for specific virus antigens and humoral immune response to the
49 infection. Like PCR, analyses for SARS-CoV-2 antigens are typically employed before the onset of
50 clinical symptoms of COVID-19 or during the anticipated acute phase of infection. In contrast,
51 immunoassays for humoral antibodies directed against components of SARS-CoV-2 should not be
52 applied until about 10 days after symptom onset, when the expected humoral immune response has
53 matured sufficiently to reach a detectable level (1).

54 These three fundamental *in vitro* diagnostic test principles have their individual advantages and
55 limitations, which partially are associated with their respective sampling techniques for appropriate
56 test material. For almost all PCR analyses for SARS-CoV-2 RNA and immunoassays for SARS-CoV-2
57 antigen, the hitherto preferred test material is extracted from swabs collected from the upper
58 respiratory tract (URT). In contrast to this heterogeneously composed, individually fluctuating, and
59 somewhat ill-defined test material, immunoassays for antibodies to SARS-CoV-2 rely on a blood
60 sample. In general, blood samples are by far the most used biological material in laboratory diagnostic
61 procedures, and consistencies and variations of this sample material are very well characterized.

62 Shortly after the severe acute respiratory syndrome (SARS) epidemic in 2002-2004, it was reported,
63 that the nucleocapsid protein (NP) of the original SARS corona virus (SARS-CoV) could be detected by
64 enzyme-linked immunosorbent assay (ELISA) in serum samples collected from 95% of infected
65 patients three days after symptom onset (3). The SARS-CoV-2 NP is highly conserved and 90.5%
66 identical to the primary structure of SARS-CoV NP, whereas the full proteome identity of these two
67 viruses is 77.1% (4). Inspired by these observations, a new ELISA has been developed and tested for
68 detection of SARS-CoV-2 NP antigen in blood samples collected from COVID-19 patients during the
69 early stages of SARS-CoV-2 infection (5)(6)(7). By using PCR analysis of URT swabs as reference, the
70 present clinical study reports the laboratory diagnostic characteristics and performance of this NP
71 ELISA, when used for SARS-CoV-2 antigen quantification in serum and plasma samples.

MATERIALS AND METHODS

Patients and blood samples

Venous blood was collected from patients at two Danish university hospitals and prepared as either serum or EDTA plasma according to the Standard Operating Procedures in Bio- and GenomeBank, Denmark (8). All blood samples were collected from patients, who had not been COVID-19 vaccinated.

Serum samples were obtained from two different patient groups: Inpatients with symptoms of COVID-19 and a confirmatory PCR-positive test result admitted to a COVID-19 specific department at University Hospital Rigshospitalet, Copenhagen, and outpatients referred for testing for SARS-CoV-2 infection at an outpatient testing facility at University Hospital Rigshospitalet. Only outpatients with symptoms of upper respiratory tract infection (e.g., fever, sore throat, cough) were included in the study.

Plasma samples were obtained from two different patient groups: Inpatients with symptoms of COVID-19 and a confirmatory PCR-positive test result admitted to COVID-19 specific departments at Aalborg University Hospital, and outpatients referred for testing for SARS-CoV-2 infection at an outpatient testing facility at Aalborg University Hospital including both persons with and without symptoms of SARS-CoV-2 and persons who had been exposed.

For each inpatient, 1 to 10 blood samples collected within the interval from the day of their first PCR-positive URT swab (Day 0) until Day 201 were all included in the study. For outpatients, only the blood sample collected simultaneously with their URT swab (Day 0) was included in the study.

The serum and plasma samples were stored at -20°C or -80°C until testing by ELISA.

92 All patients provided written statement being part of Bio- and Genome Bank, Denmark. For
93 participants under the age of 18 years, a parent or legal guardian provided the consent. The present
94 methodology study was approved by the Central Denmark Region Committees on Biomedical
95 Research Ethics (IORG-number: 0005129).
96
97 **PCR analysis**
98 For all in- and outpatients included in this study, the reference laboratory diagnosis of COVID-19 was
99 performed at the involved hospitals by PCR analysis of URT swabs for the presence of unique
100 sequences of SARS-CoV-2 RNA. All URT swabs were collected according to Danish national guidelines
101 (9) by health professionals and taken as oropharyngeal samples. The swabs were then processed as
102 routine samples and analyzed using a real-time reverse-transcription PCR assay. Two different PCR
103 test kits were used, either the "Cobas® SARS-CoV-2 Test" on a Cobas 6800 system (Roche, Basel,
104 Switzerland) or the "RealStar® SARS-CoV-2 RT-PCR Kit" (Altona, Hamburg, Germany). The result of PCR
105 analysis was reported as positive or negative for SARS-CoV-2 genomic RNA.
106
107 **Quantification of NP in blood samples**
108 The quantification of NP concentration [NP] in serum or plasma was accomplished in approximately 2
109 hours by the "Solsten SARS-CoV-2 Antigen ELISA Kit" from Solsten Diagnostics International (Aarhus,
110 Denmark) according to the manufacturer's guidelines. Up to 12 strips of each 8 wells precoated with
111 antibody to SARS-CoV-2 NP were mounted in each 96-well frame. First, 50 µl of biotin-conjugated
112 antibody was added to each well and then directly supplemented with 50 µl/well of either internal NP

113 calibrator, serum, or plasma. Hereafter, the wells were incubated for 1 hour at 37°C, washed,
114 incubated with 100 µl/well of peroxidase-conjugated streptavidin for 30 minutes at 37°C, washed, and
115 then incubated with the provided substrate for 15 minutes at 37°C before stopping the chromogenic
116 enzyme reaction and measuring the absorbance photometrically. Standard curves based on ELISA
117 results of the 5 internal calibrators were used for quantification of [NP] between 0 and 160 pg/ml, as
118 defined by the manufacturer.
119 All samples were analyzed twice by the ELISA on different days. The first NP-quantification was done
120 blinded for the characteristics of the individual sample except for being serum or plasma. Similarly,
121 the second ELISA analysis was done blinded for COVID-19 status, but with insight into the [NP]
122 determined by the first ELISA run. This allowed the appropriate dilution of samples with previously
123 determined [NP] higher than 100 pg/ml. Serum and plasma samples, which after a 24-fold dilution,
124 produced an ELISA absorbance value higher than the highest NP calibrator (160 pg/ml) were not
125 further diluted for precise quantification but registered as having an [NP] of 3,840 pg/ml.
126

127 **Statistical analysis**

128 The statistical uncertainty of the estimates of diagnostic accuracy for the SARS-CoV-2 NP ELISA,
129 including sensitivity, specificity, and predictive values of positive and negative results were reported
130 as 95% confidence intervals (95% CI). The mean [NP] ± standard deviation (SD) of serum and plasma
131 were compared by two-tailed t-tests. A p-value less than 0.05 was considered statistically significant.

132 **RESULTS**

133 **Patients and blood samples**

134 The 670 individuals included in this study comprised 414 females aged 14 to 102 years (mean ± SD: 52
135 ± 19 years) and 256 males aged 20 to 100 years (mean ± SD: 62 ± 20 years). According to PCR-analysis
136 of URT swabs, 208 of these individuals were infected with SARS-CoV-2. The COVID-19 patients
137 comprised 97 females aged 22 to 96 years (mean ± SD: 62 ± 19 years) and 111 males aged 28 to 100
138 years (mean ± SD: 70 ± 16 years).

139 A total of 914 human blood samples were collected from the 670 individuals between March 3, 2020,
140 and February 2, 2021, and prepared as either serum (n=439) or plasma (n=475). Of these, 447 (49%)
141 blood samples were from 165 COVID-19 inpatients and 43 COVID-19 outpatients, including 173 serum
142 samples and 231 plasma samples collected from 38 and 127 inpatients, respectively, between 0 and
143 201 days after their first confirmatory PCR-positive URT swab. Furthermore, 15 serum samples and 28
144 plasma samples were collected from outpatients simultaneously with their first confirmatory PCR-
145 positive URT swab. Most of the blood samples from COVID-19 patients (n=324, 72%) were collected
146 within 14 days of the first PCR-positive URT swab confirming the patient's infection with SARS-CoV-2,
147 and the remaining blood samples (n=123, 28%) were collected from COVID-19 inpatients more than 2
148 weeks after their first PCR-positive test. All other blood samples (n=467, 51%) were collected from
149 462 outpatients without COVID-19 according to a simultaneously collected PCR-negative URT swab
150 and comprised 251 serum samples from 251 outpatients, and 216 plasma samples from 211
151 outpatients.

152 A schematic overview of patients and samples of the study is provided in Supplemental Material
153 (**Figure S1**).
154
155 **Diagnostic performance of the SARS-CoV-2 NP ELISA**
156 Based on the Receiver Operating Characteristic (ROC) curve in **Figure 1**, and prioritization of a low
157 false positive rate, the manufacturer's recommended diagnostic cut-off value of 10 pg/ml NP was
158 confirmed. When using this cut-off value, the specificity of the SARS-CoV-2 NP ELISA was 99.8% (95%
159 CI: 99.4% - 100%), as 1 of 462 outpatients without COVID-19 had a false positive blood sample with an
160 [NP] of 12 pg/ml. All 462 blood samples were collected simultaneously with a PCR-negative URT swab.
161 The diagnostic sensitivity of the SARS-CoV-2 NP ELISA was determined at patient level by using only
162 the [NP] measured for the first blood sample after collection of the confirmatory PCR-positive URT
163 swab. According to results for 160 COVID-19 inpatients, the ELISA sensitivity varied with the time gap
164 from confirmatory PCR-positive URT swab to blood sampling (**Figure 2**).
165 When the first blood sample was collected from COVID-19 inpatients within 1 and 2 weeks from PCR-
166 confirmed infection, the diagnostic sensitivity of the SARS CoV-2 NP ELISA was 92.9% (n=99; 95% CI:
167 87.9% - 98.0%) and 91.6% (n=143; 95% CI: 87.1% - 96.2%), respectively (**Table 1**). The average [NP] (±
168 SD) of the true ELISA-positive blood samples collected within the first 2 weeks from 131 COVID-19
169 inpatients was 1,734 ± 1,560 pg/ml (range: [10 - 3,840] pg/ml, median: 1,184 pg/ml). The average
170 [NP] (± SD) of the true ELISA-positive blood samples collected at Day 0 from 35 COVID-19 outpatients
171 was 157 ± 294 pg/ml (range: [10 - 1,377] pg/ml, median: 52 pg/ml).

172 According to all patients (n=520) with a blood sample collected simultaneously with the URT swab i.e.,
173 at Day 0, the PCR-defined point prevalence of COVID-19 was 11.2%: 15 PCR-positive inpatients and
174 505 outpatients, including 43 PCR-positive. For the SARS-CoV-2 NP ELISA, at this timepoint and
175 prevalence, the specificity and sensitivity were 99.8% (95% CI: 99.4-100%) and 82.8% (95% CI: 73.0-
176 92.5%), respectively, whereas the positive predictive value (PPV) and negative predictive value (NPV)
177 were 98.0% (95% CI: 94.0-100%) and 97.9% (95% CI: 96.6-99.2%), respectively.
178 For COVID-19 outpatients, who had their blood sample collected at Day 0 i.e., simultaneously with the
179 confirmatory PCR-positive URT swab, the diagnostic sensitivity of the SARS-CoV-2 NP ELISA was 81.4%
180 (n=43; 95% CI: 69.8% - 93.0%). For the present study, where the point prevalence of COVID-19 for the
181 analyzed group of 505 outpatients according to PCR analysis was 8.5%, the probability of having
182 COVID-19 was 97.2% for ELISA-positive outpatients (n=36; 95% CI: 91.9% - 100.0%), and the
183 probability of not being infected with SARS-CoV-2 was 98.3% for ELISA-negative outpatients (n=469;
184 95% CI: 97.1% - 99.5%).
185
186 **Serum and plasma analysis by the SARS-CoV-2 NP ELISA**
187 According to 368 blood samples from 160 COVID-19 inpatients, the correlation between SARS-CoV-2
188 ELISA and PCR confirmed SARS-CoV-2 infection varied with the time gap from confirmatory PCR-
189 positive URT swab to blood sampling (**Figure 3**).
190 For 131 serum and 150 plasma samples collected from COVID-19 inpatients within 2 weeks after their
191 confirmatory PCR-positive URT swab, the correlation was 89.3% (95% CI: 84.0% - 94.6%) and 86.7%
192 (95% CI: 81.2% - 92.1%), respectively (**Table 2**). For collection within the first week only, the

193 corresponding correlations were higher: 92.3% (n=65; 95% CI: 85.8% - 98.8%) for serum and 91.7%
194 (n=84; 95% CI: 85.8% - 97.6%) for plasma.
195 The average [NP] (± SD) of 65 serum samples and 84 plasma samples collected from COVID-19
196 inpatients within the first week from their confirmatory PCR-positive URT swab was 1,041 ± 1,332
197 pg/ml (range: [3 - 3,840] pg/ml, median: 337 pg/ml) and 1,631 ± 1,553 pg/ml (range: [3 - 3,840] pg/ml,
198 median: 1,036 pg/ml), respectively. The median [NP] decreased exponentially with the time gap from
199 collection of the PCR-positive URT swab to blood sampling (**Figure 4**). No systematic difference was
200 observed in the [NP] levels between serum and plasma samples collected within the first week from
201 COVID-19 inpatients (p=0.0577).
202 For outpatients with SARS-CoV-2 infection according to PCR, the average apparent [NP] (± SD) of 15
203 serum samples and 28 plasma samples were 54 ± 69 pg/ml (range: [2 - 274] pg/ml, median: 28 pg/ml)
204 and 169 ± 328 pg/ml (range: [3 - 1,377] pg/ml, median: 31 pg/ml), respectively. No systematic
205 difference was observed in the [NP] levels between serum and plasma samples of COVID-19
206 outpatients (p=0.418).
207 For outpatients without SARS-CoV-2 infection according to PCR, the average apparent [NP] (± SD) of
208 251 serum samples and 216 plasma samples were 2.1 ± 1.3 pg/ml (range: [0 - 12] pg/ml, median: 2.4
209 pg/ml) and 2.5 ± 1.3 pg/ml (range: [0 - 7.4] pg/ml, median: 2.4 pg/ml), respectively.
210
211 **Individual dynamics in [NP] levels of blood samples**
212 The individual progression in [NP] during the first month after PCR-based diagnosis was observed for
213 40 COVID-19 inpatients, who had at least 3 blood samples collected within 30 days from their first

PCR-positive URT swab (**Figure 5**). For 4 of these inpatients (10%), none of their blood samples (total n=20) reached an [NP] above the diagnostic cut-off value of 10 pg/ml. In two of these cases, the earliest blood samples were collected more than 2½ weeks after their PCR-based diagnosis, when a substantial humoral immune response to infection was measured (**Table S1** and **Figure S2** in Supplemental Material). For all the remaining 36 COVID-19 inpatients (90%), at least their first blood sample had an [NP] above the diagnostic cut-off value. Despite the clear individual tendency of decline in [NP] over time, all blood samples (total n=122) collected from 23 of the 40 COVID-19 inpatients (58%) were positive according to the NP ELISA.

None of the 34 plasma samples collected from 10 COVID-19 inpatients 27-201 days after their first PCR-positive URT swab had an [NP] above the diagnostic cut-off value of 10 pg/ml (**Figure 6**), even though 1 to 3 plasma samples collected earlier from each of these patients were clearly positive for NP (mean ± SD: 1,444 ± 1,448 pg/ml; range: [34 – 3,840] pg/ml).

227 **DISCUSSION**

228 The outbreak of COVID-19 has caused an unparalleled worldwide requirement for laboratory
229 diagnostic tests for virus infection, and PCR analysis for genomic RNA of SARS-CoV-2 in extracts of
230 swabs collected from the upper respiratory tract has proven very suitable for early detection of
231 infection, even in patients with mild or no clinical symptoms. Still, the characterization of PCR as the
232 gold standard laboratory diagnostic test for COVID-19 (1), and its wide application as a reference test
233 in performance evaluation of other laboratory diagnostic methods is debated (10). This is particularly
234 due to concerns of false-negative PCR results caused by a low viral load at the chosen time and site of
235 URT sample collection, inadequate URT swabbing technique of some operators, failing storage
236 conditions during specimen transportation, laboratory error, and/or mutation of the viral target RNA
237 (11). These concerns have intensified the search for improved and less resource-demanding
238 laboratory test procedures for COVID-19 and has led to the development of complementing and
239 supplementing screening methods, which will contribute to diagnostic triage procedures relying on a
240 final confirmation of positive results by PCR-analysis.

241 In the present study, we have characterized the first ELISA test kit for quantification of the SARS-CoV-
242 2 NP antigen in serum and plasma samples. When used for blood samples collected from COVID-19
243 inpatients within 2 weeks after PCR-confirmed SARS-CoV-2 infection, the diagnostic sensitivity of the
244 ELISA was 91.6% (95% CI: 85.6% - 95.2%).

245 The group of 505 outpatients in this study had an 8.5% point prevalence of SARS-CoV-2 infection, and
246 for outpatients with an ELISA-positive blood sample, the probability of having COVID-19 was 97.2%
247 (n=36; 95% CI: 91.9% - 100.0%), whereas those with an ELISA-negative blood sample had a 98.3%

248 probability of not being infected with SARS-CoV-2 (n=469; 95% CI: 97.1% - 99.5%). During this early
249 stage of infection, the SARS-CoV-2 NP ELISA thereby prove to be a very reliable predictor of COVID-19.
250 The individual NP concentrations of COVID-19 patients varied considerably even for blood samples
251 collected within the first week of PCR-confirmed SARS-CoV-2 infection, and probably reflected the
252 disease severity. The [NP] in blood samples collected at Day 0 from 15 COVID-19 inpatients (median:
253 1,237 pg/ml, mean ± SD: 1,792 ± 1,687 pg/ml, range: [3-3,840] pg/ml) was at a substantially higher
254 level than the [NP] in blood samples collected from 43 COVID-19 outpatients simultaneously with
255 their PCR-positive URT swab (median: 29 pg/ml, mean ± SD: 129 ± 271 pg/ml, range: [2-1,377] pg/ml).
256 Despite the variability in [NP] between patients and over time, the individual progressions in [NP]
257 were systematic and declining for almost all the 40 inpatients in the present study, who had at least 3
258 blood samples collected within the first month. Also, for the total of 368 blood samples collected from
259 160 COVID-19 inpatients during the first month, the median [NP] declined exponentially with time,
260 and then consistently remained below the diagnostic cut-off value of 10 pg/ml for all samples
261 collected during the succeeding 6 months after infection.
262 When verifying the diagnostic performance of an antigen test by using PCR analysis of an URT swab as
263 the reference, all misclassifications (false negatives and false positives) by definition, will be ascribed
264 to the antigen test, no matter whether the test material is matching or different. Almost all rapid
265 antigen tests for COVID-19 are lateral flow immunoassays for the qualitative detection of SARS-CoV-2
266 NP in extracts of URT swabs. Possibly, more rightfully characterized as tests of individual
267 infectiousness (12)(13), their diagnostic performance is typically evaluated by comparison to the
268 outcome of PCR analysis of the same or a simultaneously collected URT swab and thereby affected by

269 the same risks of a sampling-associated false negative result as PCR. The diagnostic performance of
270 the quantitative ELISA for SARS-CoV-2 NP investigated in the present study, also relied on using PCR
271 analysis of a URT swab as reference. Despite the distinct sampling techniques and test materials of
272 these two laboratory diagnostic procedures, the analysis of blood samples by the NP ELISA highly
273 confirmed the laboratory diagnosis of SARS-CoV-2 infection based on PCR.
274 According to the observed performance data, we conclude that the SARS-CoV-2 NP ELISA is suitable
275 for laboratory diagnosis of COVID-19 when used for testing serum or plasma early after infection.
276 Towards the end of the outbreak of SARS in 2002-2004, an ELISA was developed with an analytical
277 detection limit of approximately 50 pg/ml SARS-CoV NP (14). Using a diagnostic cut-off at 100 pg/ml,
278 its diagnostic sensitivity increased from 65% 1-2 days after onset of SARS symptoms to over 95% at 3-
279 5 days after first symptoms (3). In comparison, the present ELISA for SARS-CoV-2 NP (5) has a
280 substantially improved analytical sensitivity with a detection limit of around 2 pg/ml, which in
281 combination with the assay's high resistance to irregular hemolytic reactions and potentially
282 interfering blood substances, such as rheumatoid factors (5), allows the recommended low and
283 robust diagnostic cut-off at 10 pg/ml SARS-CoV-2 NP. Though differences in shedding of NP into
284 circulation and time of symptom onset after infection may vary between SARS and COVID-19, the 10-
285 fold reduction in diagnostic cut-off contributes decisively to the very early detection of SARS-CoV-2
286 infection achieved by the novel NP ELISA investigated in the present study.
287 WHO has concluded that early laboratory diagnosis of SARS-CoV-2 infection can aid clinical
288 management and outbreak control of COVID-19, and that the standard confirmation of acute
289 infection should be based on a nucleic acid amplification test (2). However, URT swab collection

290 followed by PCR analysis is a tedious and expensive method for COVID-19 screening. In contrast, in
291 settings such as hospitals and blood banks, where blood samples are collected anyway, the SARS-CoV-
292 2 NP ELISA provides a simple and economical screening tool for COVID-19. For example, serum and
293 plasma samples prepared at hospitals for biochemical and other clinical laboratory analyses may also
294 be systematically examined by ELISA for the presence of SARS-CoV-2 NP, and thereby contribute
295 importantly to reduce the risk of nosocomial COVID-19 infection (15).
296 Our study has strengths and limitations. First, only a subset of the included participants had blood
297 samples collected within 0-1 days after their first PCR-positive URT swab, although this period is the
298 most clinically relevant for early detection of SARS-CoV-2. However, the wide range of collection of
299 blood samples after the first PCR-positive URT swab allowed us to investigate the individual progress
300 of NP concentration in blood for participants with numerous samples available. Second, we did not
301 have information on onset or duration of symptoms for in- and outpatients. Instead, the first PCR-
302 positive URT swab was used as the confirmatory test for COVID-19, although the infection with SARS-
303 CoV-2 may have started days before the PCR test was performed. Theoretically, in a setting where this
304 information was available, the false negative rate would be even lower, as participants with longer
305 duration of symptoms before blood sampling could be excluded from the main analyses. Third,
306 oropharyngeal swabs were utilized for URT sampling according to Danish national guidelines (9), and
307 though also stated by the FDA as appropriate for clinical testing, they may be less sensitive than
308 nasopharyngeal swabs (16). The strengths of the study include a large sample size and a standardized
309 and highly reproducible method for quantification of SARS-CoV-2 NP.

310 Though being a recommended subject for further investigations, we propose that automated routine
311 screening of blood samples by the NP ELISA will be a suitable procedure for early identification of
312 inpatients, who bring or acquire SARS-CoV-2 infection, while hospitalized. As indicated by the
313 projected confusion matrix (Table S2 in Supplemental Material), even for hospitals with a low
314 prevalence of COVID-19 among inpatients treated for other diseases, and a high number of routinely
315 analyzed blood samples, the observed 99.8% specificity of the SARS-CoV-2 NP ELISA will ensure a low
316 number of false positives and an acceptable PPV, and thereby lead to substantial reductions in the
317 requirement for laborious swab sampling and subsequent confirmatory PCR analysis.
318

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CONFLICTS OF INTEREST DISCLOSURES

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398

399 **TABLES**

400

401 **TABLE 1.** Diagnostic performance and clinical relevance of the SARS-CoV-2 NP ELISA, when testing the

402 first blood sample collected from each of 648 patients within the first week (Day 0-6) and the second

403 week (Day 7-13) of their PCR analyzed URT swab.

			SARS-CoV-2 RNA PCR test				
		Blood collected:	Positive		Negative	Total	Relevance: Prediction
			Inpatients	Outpatients	Outpatients	Patients	
SARS-CoV-2 NP ELISA	Positive	Day 0-6	92	35	1	128	99.2%
		Day 7-13	39			39	
	Negative	Day 0-6	7	8	461	476	96.8%
		Day 7-13	5			5	
	Total	Day 0-6	99	43	462	604	
		Day 7-13	44			44	
			Sensitivity	Sensitivity	Specificity	648	
	Performance of NP ELISA:	Day 0-6	92.9%	81.4%	99.8%		
		Day 7-13	88.6%				

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407 **TABLE 2.** Correlation between the results of SARS-CoV-2 NP ELISA analysis of serum (A) and plasma (B)
408 samples and the result of PCR analysis for SARS-CoV-2 infection according to of a URT swab collected
409 up to 2 weeks earlier.

Table 2A.		SARS-CoV-2 RNA PCR test				
Number of **serum samples**: 397 Blood sampling within: **Day 0-13**		Positive Inpatients	Positive Outpatients	Negative Outpatients	Total Patients	Relevance: Prediction
SARS-CoV-2 NP ELISA	Positive	117	13	1	131	99.2%
	Negative	14	2	250	266	94.0%
	Total	131	15	251	397	
Performance of NP ELISA: Correlation		Correlation 89.3%	Correlation 86.7%	Correlation 99.6%		

410

Table 2B.		SARS-CoV-2 RNA PCR test				
Number of **plasma samples**: 394 Blood sampling within: **Day 0-13**		Positive Inpatients	Positive Outpatients	Negative Outpatients	Total Patients	Relevance: Prediction
SARS-CoV-2 NP ELISA	Positive	130	22	0	152	100%
	Negative	20	6	216	242	89.3%
	Total	150	28	216	394	
	Performance of NP ELISA:	Correlation 86.7%	Correlation 78.6%	Correlation 100%		

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FIGURE LEGENDS

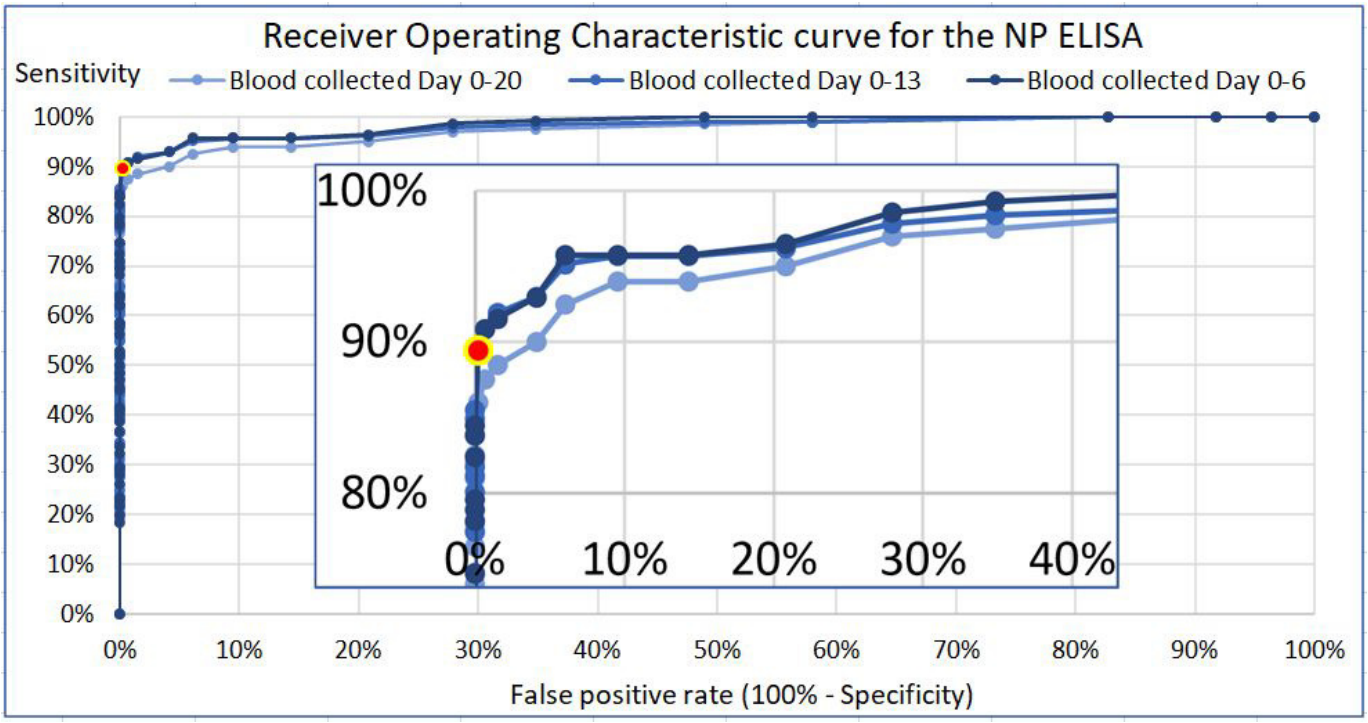
FIGURE 1. ROC curves for the SARS-CoV-2 NP ELISA according to the time gap from the first PCR-positive URT swab to first blood sampling. A zoom of the upper left corner of the curves was inserted. The area under the curve was 0.986 for the 604 blood samples collected within a time gap of 1 week (Day 0-6), 0.982 within 2 weeks (648 blood samples collected Day 0-13), and 0.975 within 3 weeks (662 blood samples collected Day 0-20). In compliance with the recommendations by the manufacturer, a diagnostic cut-off at 10 pg/ml secured a combination of very low false positive rate and high sensitivity (red point with yellow halo).

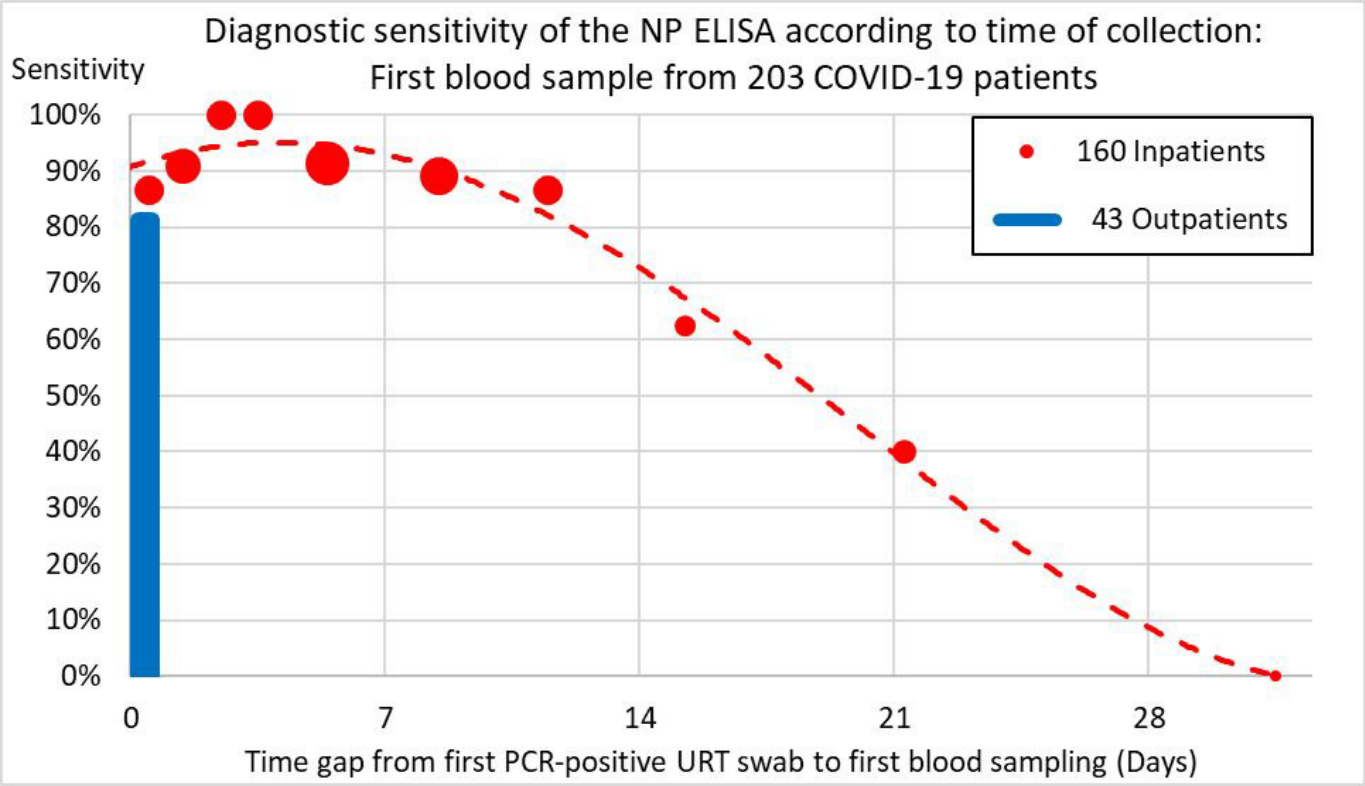
FIGURE 2. Variation in the diagnostic sensitivity of the SARS-CoV-2 NP ELISA according to the time gap from the first PCR-positive URT swab to first blood sampling. The blood sample was collected from each of 160 COVID-19 inpatients within 5 weeks after their confirmatory PCR-positive URT swab (●), and from each of 43 COVID-19 outpatients simultaneously with their PCR-positive URT swab (■). The data point area is proportional to the number of inpatients contributing to the data point.

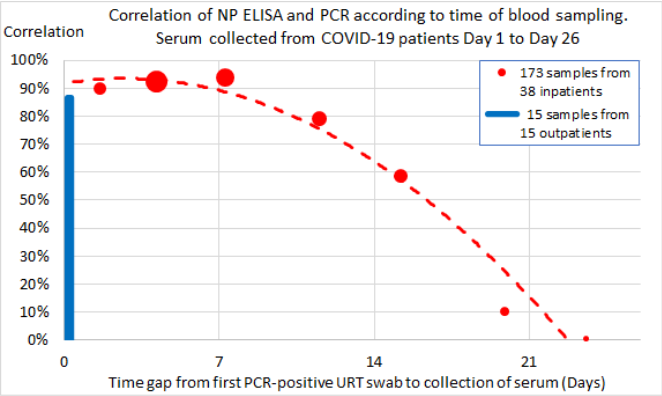
429 **FIGURE 3**. Correlation between the results of SARS-CoV-2 NP ELISA and PCR analysis according to the
430 time gap from the confirmatory PCR-positive URT swab to collection of serum (A) or plasma (B). The
431 368 blood samples were collected from 160 COVID-19 inpatients within 5 weeks after their
432 confirmatory PCR-positive URT swab (●), and 43 blood samples were collected from COVID-19
433 outpatients simultaneously with their PCR-positive URT swab (■). Each inpatient data point area is
434 proportional to the number of blood samples contributing to the data point.
435
436 **FIGURE 4**. The median [NP] declined exponentially over time for 368 blood samples collected from
437 160 COVID-19 inpatients within 5 weeks after their confirmatory PCR-positive URT swab (●). The
438 median [NP] of blood samples collected at Day 0-1 for 32 COVID-19 inpatients (1,045 pg/ml) was 36
439 times higher than the median [NP] (29 pg/ml) of blood samples collected from 43 COVID-19
440 outpatients simultaneously with their PCR-positive URT swab (■). For each inpatient data point, the
441 time of blood sample collection is illustrated as mean ± SD number of days after the first PCR-positive
442 URT swab. Each inpatient data point area is proportional to the number of blood samples contributing
443 to the data point.
444
445 **FIGURE 5**. Individual dynamics in [NP] of 40 COVID-19 inpatients within 1 month from first PCR-
446 positive URT swab (total number of blood samples, n=200). A: 3-10 serum samples collected from
447 each of 23 inpatients; B: 3-4 plasma samples collected from each of 17 inpatients.
448

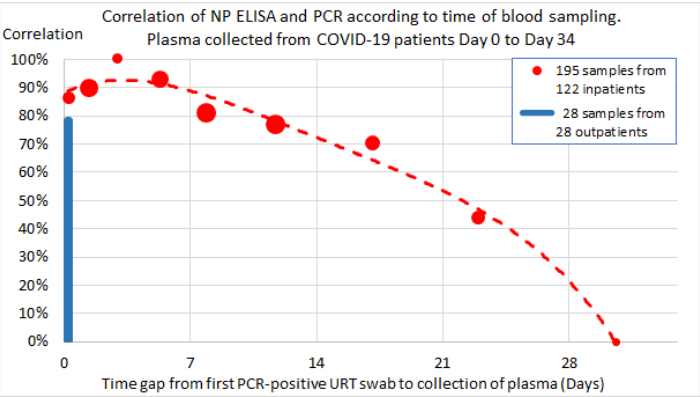
449 **FIGURE 6**. Plasma [NP] above the diagnostic cut-off value (10 pg/ml) was only observed for COVID-19

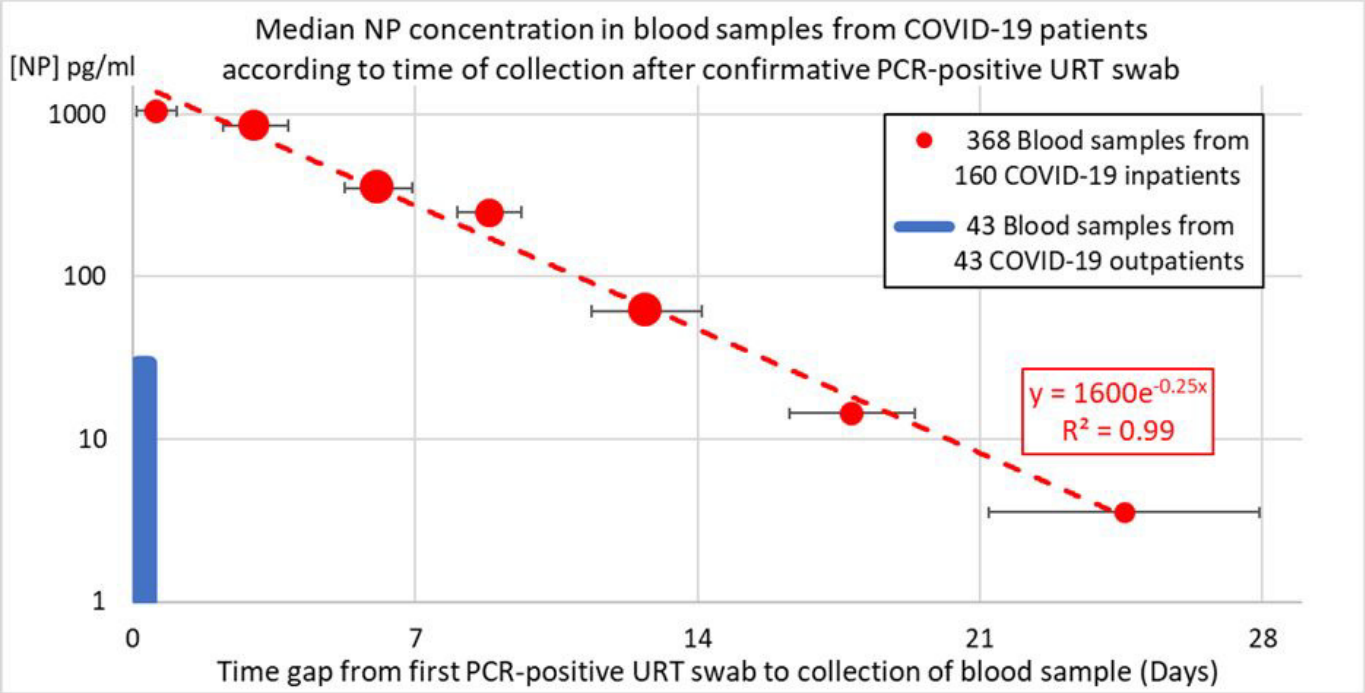
450 inpatients within the first 26 days of detection of SARS-CoV-2 infection by PCR.

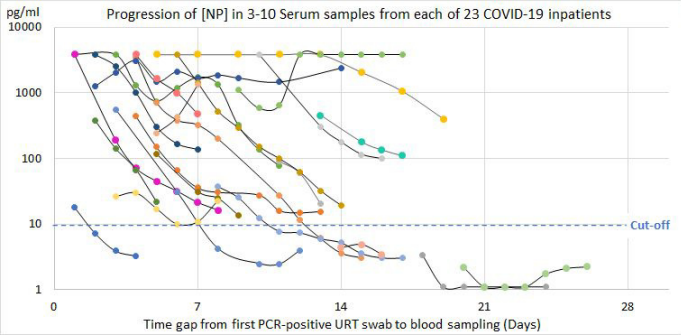


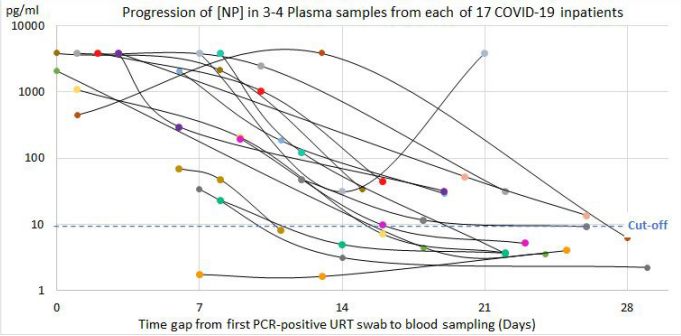


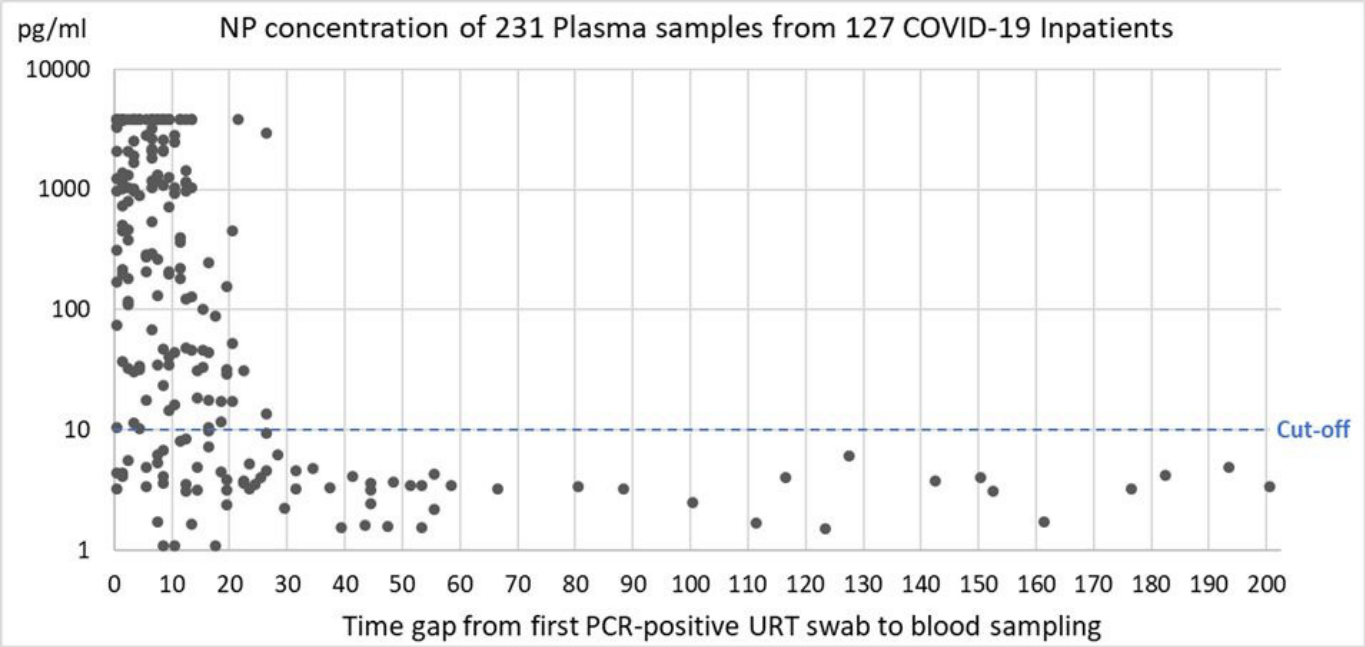












SUPPLEMENTAL MATERIAL

Detailed characteristics of patients and blood samples included in the study.

The 439 serum samples in this study were collected from 304 individuals, including 24 female COVID-19 patients (mean age ± SD: 55 ± 17 years), 29 male COVID-19 patients (62 ± 16 years), 199 non-infected females (44 ± 14 years) and 52 non-infected males (42 ± 13 years).

The 475 plasma samples in this study were collected from 366 individuals, including 73 female COVID-19 patients (mean age ± SD: 64 ± 19 years), 82 male COVID-19 patients (68 ± 18 years), 118 non-infected females (58 ± 21 years) and 93 non-infected males (62 ± 20 years).

Figure S1 shows the categorization of patients and blood samples according to 4 differentiators including PCR-based diagnosis, hospitalization, blood fractionation method, and time of collection of blood sample.

	No. of **Patients** in study 670							No. of **Samples** in study 914						
	COVID-19 208				Non-infected 462			COVID-19 447				Non-infected 467		
	Inpts. 165		Outpts. 43			Outpts. 462		Inpts. 404		Outpts. 43			Outpts. 467	
S=Serum P= Plasma	S 38	P 127	S 15	P 28		S 251	P 211	S 173	P 231	S 15	P 28		S 251	P 216
Inpts. first sample			All outpts. sampled Day 0					Inpts. all samples		All outpts. sampled Day 0				
Day 0-34	38	122						Day 0-34	173	195				
Day 0-13	33	110						Day 0-13	131	150				
Day 0-6	26	73						Day 0-6	65	84				

FIGURE S1. Schematic overview of patients and samples included in the study. Beyond the categorization of patients and blood samples according to PCR-based diagnosis, hospitalization, and blood fractionation method, the time of collection of either the inpatient's first blood sample (Patients) or any blood sample (Samples) is reported for the intervals 1 week (Day 0-6), 2 weeks (Day 0-13) and 5 weeks (Day 0-34) after the first PCR-positive URT swab.

Inverse relationship between [NP] and humoral immune response

The rapid decline in serum and plasma [NP] observed for most COVID-19 patients from approximately Day 10 after the first PCR-positive URT swab is consistent with the expected development of a humoral immune response to the SARS-CoV-2 infection.

Selected blood samples of this study were therefore also analyzed by a new prototype "SARS-CoV-2 Neutralization Antibody ELISA Kit". Very briefly, titrated serum or plasma was added to ELISA wells coated with the ACE-2 receptor binding domain of the S1 subunit of SARS-CoV-2 Spike Protein (RBD). After 15 minutes of preincubation at 37°C, ACE-2 receptor conjugated with peroxidase was added to the wells, and incubation of the mixture continued for 45 minutes at 37°C. Blood samples containing neutralizing antibodies to RBD dose-dependently reduced the absorbance of the final colorimetric reaction of the ELISA. The neutralization antibody titer was calculated at 50% of the maximal color formation and compared to the [NP] of the same blood sample (**Table S1** and **Figure S2**).

TABLE S1. Inverse relationship between the [NP] and the SARS-CoV-2 neutralizing antibody titer in the initial blood sample collected from 40 COVID-19 inpatients after confirmation by PCR of infection.

Individual [NP] dynamics	Patients	Samples	Initial sample: [NP] pg/ml			Initial sample: Neutralizing Ab titer		
			Mean ± SD	[range]	Median	Mean ± SD	[range]	Median
All samples: Over 10 pg/ml	23	122	2,287±1,714	[26-3,840]	3840	34 ± 104	[0 - 454]	0
Change with time: Over to under 10 pg/ml	13	58	911±1,344	[18-3,840]	197	43 ± 60	[0 - 195]	21
All samples: Under 10 pg/ml	4	20	2±2	[1-5]	2	298 ± 293	[13 - 700]	240

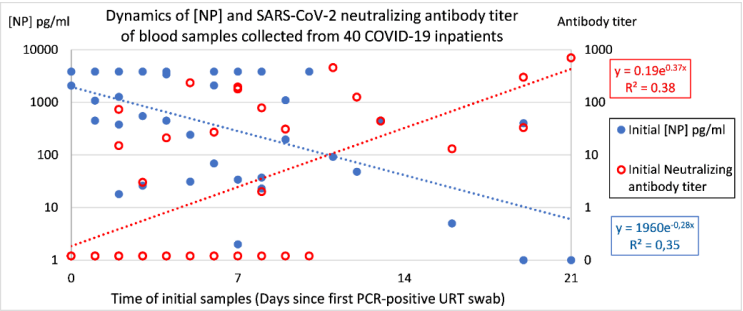


FIGURE S2. Indication of the expected inverse relationship between the [NP] and the neutralizing antibody titer of the initial serum (n=23) or plasma sample (n=17) collected from 40 COVID-19 inpatients up to 3 weeks after collection of their confirmatory PCR-positive URT swab.

Projected confusion matrix, if using the NP ELISA for nosocomial screening at a low prevalence.

Based on the estimated specificity (99.78%) and sensitivity (82.76%) of the NP ELISA for the 520 patients in the present study with a blood sample collected simultaneously with the URT swab for PCR-analysis, and on an expected rather low prevalence of 0.2% SARS-CoV-2 infection among inpatients hospitalized for more than 3 days for any other disease than COVID-19, the projected confusion matrix for analysis of 100,000 blood samples by the NP ELISA is presented in Table S2.

Table S2. Projected confusion matrix if using the NP for screening at low prevalence.

Screening for nosocomial infection **Projected** per 100,000 samples **Expected** prevalence: 0.2%		SARS-CoV-2 RNA PCR test/Swab Infected inpatients	Non-infected inpatients	Total inpatients	Clinical relevance: Predictive values	
SARS-CoV-2 NP ELISA/Blood	**Calculated** Positive	166	220	386	PPV	43.01%
	Calculated Negative	34	99,580	99,614	NPV	99.97%
	Total	200	99,800	100,000		
Performance of NP ELISA: **Given** sensitivity and specificity		Sensitivity **82.76%**	Specificity **99.78%**			

In conclusion, a preceding screening by the NP-ELISA of blood samples from 100,000 inpatients will reduce the number of required URT swabs and associated PCR-analyses from 100,000 primary analyses to 386 confirmatory tests. The screening will identify 166 of the 200 nosocomial SARS-CoV-2 infections.