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*Published in:*
Journal of Clinical Microbiology

*DOI (link to publication from Publisher):*
10.1128/JCM.01001-21

*Publication date:*
2021

*Document Version*
Accepted author manuscript, peer reviewed version

# 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.

## INTRODUCTION

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

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

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

All patients provided written statement being part of Bio- and Genome Bank, Denmark. For participants under the age of 18 years, a parent or legal guardian provided the consent. The present methodology study was approved by the Central Denmark Region Committees on Biomedical Research Ethics (IORG-number: 0005129).

**PCR analysis**

For all in- and outpatients included in this study, the reference laboratory diagnosis of COVID-19 was performed at the involved hospitals by PCR analysis of URT swabs for the presence of unique sequences of SARS-CoV-2 RNA. All URT swabs were collected according to Danish national guidelines (9) by health professionals and taken as oropharyngeal samples. The swabs were then processed as routine samples and analyzed using a real-time reverse-transcription PCR assay. Two different PCR test kits were used, either the "Cobas® SARS-CoV-2 Test" on a Cobas 6800 system (Roche, Basel, Switzerland) or the "RealStar® SARS-CoV-2 RT-PCR Kit" (Altona, Hamburg, Germany). The result of PCR analysis was reported as positive or negative for SARS-CoV-2 genomic RNA.

**Quantification of NP in blood samples**

The quantification of NP concentration [NP] in serum or plasma was accomplished in approximately 2 hours by the "Solsten SARS-CoV-2 Antigen ELISA Kit" from Solsten Diagnostics International (Aarhus, Denmark) according to the manufacturer's guidelines. Up to 12 strips of each 8 wells precoated with antibody to SARS-CoV-2 NP were mounted in each 96-well frame. First, 50 µl of biotin-conjugated antibody was added to each well and then directly supplemented with 50 µl/well of either internal NP

calibrator, serum, or plasma. Hereafter, the wells were incubated for 1 hour at 37°C, washed, incubated with 100 µl/well of peroxidase-conjugated streptavidin for 30 minutes at 37°C, washed, and then incubated with the provided substrate for 15 minutes at 37°C before stopping the chromogenic enzyme reaction and measuring the absorbance photometrically. Standard curves based on ELISA results of the 5 internal calibrators were used for quantification of [NP] between 0 and 160 pg/ml, as defined by the manufacturer.

All samples were analyzed twice by the ELISA on different days. The first NP-quantification was done blinded for the characteristics of the individual sample except for being serum or plasma. Similarly, the second ELISA analysis was done blinded for COVID-19 status, but with insight into the [NP] determined by the first ELISA run. This allowed the appropriate dilution of samples with previously determined [NP] higher than 100 pg/ml. Serum and plasma samples, which after a 24-fold dilution, produced an ELISA absorbance value higher than the highest NP calibrator (160 pg/ml) were not further diluted for precise quantification but registered as having an [NP] of 3,840 pg/ml.

**Statistical analysis**

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

## RESULTS

### Patients and blood samples

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

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

A schematic overview of patients and samples of the study is provided in Supplemental Material (**Figure S1**).

**Diagnostic performance of the SARS-CoV-2 NP ELISA**

Based on the Receiver Operating Characteristic (ROC) curve in **Figure 1**, and prioritization of a low false positive rate, the manufacturer's recommended diagnostic cut-off value of 10 pg/ml NP was confirmed. When using this cut-off value, the specificity of the SARS-CoV-2 NP ELISA was 99.8% (95% CI: 99.4% - 100%), as 1 of 462 outpatients without COVID-19 had a false positive blood sample with an [NP] of 12 pg/ml. All 462 blood samples were collected simultaneously with a PCR-negative URT swab. The diagnostic sensitivity of the SARS-CoV-2 NP ELISA was determined at patient level by using only the [NP] measured for the first blood sample after collection of the confirmatory PCR-positive URT swab. According to results for 160 COVID-19 inpatients, the ELISA sensitivity varied with the time gap from confirmatory PCR-positive URT swab to blood sampling (**Figure 2**).

When the first blood sample was collected from COVID-19 inpatients within 1 and 2 weeks from PCR-confirmed infection, the diagnostic sensitivity of the SARS CoV-2 NP ELISA was 92.9% (n=99; 95% CI: 87.9% - 98.0%) and 91.6% (n=143; 95% CI: 87.1% - 96.2%), respectively (**Table 1**). The average [NP] (± SD) of the true ELISA-positive blood samples collected within the first 2 weeks from 131 COVID-19 inpatients was 1,734 ± 1,560 pg/ml (range: [10 - 3,840] pg/ml, median: 1,184 pg/ml). The average [NP] (± SD) of the true ELISA-positive blood samples collected at Day 0 from 35 COVID-19 outpatients was 157 ± 294 pg/ml (range: [10 - 1,377] pg/ml, median: 52 pg/ml).

According to all patients (n=520) with a blood sample collected simultaneously with the URT swab i.e., at Day 0, the PCR-defined point prevalence of COVID-19 was 11.2%: 15 PCR-positive inpatients and 505 outpatients, including 43 PCR-positive. For the SARS-CoV-2 NP ELISA, at this timepoint and prevalence, the specificity and sensitivity were 99.8% (95% CI: 99.4-100%) and 82.8% (95% CI: 73.0-92.5%), respectively, whereas the positive predictive value (PPV) and negative predictive value (NPV) were 98.0% (95% CI: 94.0-100%) and 97.9% (95% CI: 96.6-99.2%), respectively.

For COVID-19 outpatients, who had their blood sample collected at Day 0 i.e., simultaneously with the confirmatory PCR-positive URT swab, the diagnostic sensitivity of the SARS-CoV-2 NP ELISA was 81.4% (n=43; 95% CI: 69.8% - 93.0%). For the present study, where the point prevalence of COVID-19 for the analyzed group of 505 outpatients according to PCR analysis was 8.5%, the probability of having COVID-19 was 97.2% for ELISA-positive outpatients (n=36; 95% CI: 91.9% - 100.0%), and the probability of not being infected with SARS-CoV-2 was 98.3% for ELISA-negative outpatients (n=469; 95% CI: 97.1% - 99.5%).

**Serum and plasma analysis by the SARS-CoV-2 NP ELISA**

According to 368 blood samples from 160 COVID-19 inpatients, the correlation between SARS-CoV-2 ELISA and PCR confirmed SARS-CoV-2 infection varied with the time gap from confirmatory PCR-positive URT swab to blood sampling (**Figure 3**).

For 131 serum and 150 plasma samples collected from COVID-19 inpatients within 2 weeks after their confirmatory PCR-positive URT swab, the correlation was 89.3% (95% CI: 84.0% - 94.6%) and 86.7% (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).

## DISCUSSION

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

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

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

probability of not being infected with SARS-CoV-2 (n=469; 95% CI: 97.1% - 99.5%). During this early stage of infection, the SARS-CoV-2 NP ELISA thereby prove to be a very reliable predictor of COVID-19. The individual NP concentrations of COVID-19 patients varied considerably even for blood samples collected within the first week of PCR-confirmed SARS-CoV-2 infection, and probably reflected the disease severity. The [NP] in blood samples collected at Day 0 from 15 COVID-19 inpatients (median: 1,237 pg/ml, mean ± SD: 1,792 ± 1,687 pg/ml, range: [3-3,840] pg/ml) was at a substantially higher level than the [NP] in blood samples collected from 43 COVID-19 outpatients simultaneously with their PCR-positive URT swab (median: 29 pg/ml, mean ± SD: 129 ± 271 pg/ml, range: [2-1,377] pg/ml). Despite the variability in [NP] between patients and over time, the individual progressions in [NP] were systematic and declining for almost all the 40 inpatients in the present study, who had at least 3 blood samples collected within the first month. Also, for the total of 368 blood samples collected from 160 COVID-19 inpatients during the first month, the median [NP] declined exponentially with time, and then consistently remained below the diagnostic cut-off value of 10 pg/ml for all samples collected during the succeeding 6 months after infection.

When verifying the diagnostic performance of an antigen test by using PCR analysis of an URT swab as the reference, all misclassifications (false negatives and false positives) by definition, will be ascribed to the antigen test, no matter whether the test material is matching or different. Almost all rapid antigen tests for COVID-19 are lateral flow immunoassays for the qualitative detection of SARS-CoV-2 NP in extracts of URT swabs. Possibly, more rightfully characterized as tests of individual infectiousness (12)(13), their diagnostic performance is typically evaluated by comparison to the outcome of PCR analysis of the same or a simultaneously collected URT swab and thereby affected by

the same risks of a sampling-associated false negative result as PCR. The diagnostic performance of the quantitative ELISA for SARS-CoV-2 NP investigated in the present study, also relied on using PCR analysis of a URT swab as reference. Despite the distinct sampling techniques and test materials of these two laboratory diagnostic procedures, the analysis of blood samples by the NP ELISA highly confirmed the laboratory diagnosis of SARS-CoV-2 infection based on PCR.

According to the observed performance data, we conclude that the SARS-CoV-2 NP ELISA is suitable for laboratory diagnosis of COVID-19 when used for testing serum or plasma early after infection. Towards the end of the outbreak of SARS in 2002-2004, an ELISA was developed with an analytical detection limit of approximately 50 pg/ml SARS-CoV NP (14). Using a diagnostic cut-off at 100 pg/ml, its diagnostic sensitivity increased from 65% 1-2 days after onset of SARS symptoms to over 95% at 3-5 days after first symptoms (3). In comparison, the present ELISA for SARS-CoV-2 NP (5) has a substantially improved analytical sensitivity with a detection limit of around 2 pg/ml, which in combination with the assay's high resistance to irregular hemolytic reactions and potentially interfering blood substances, such as rheumatoid factors (5), allows the recommended low and robust diagnostic cut-off at 10 pg/ml SARS-CoV-2 NP. Though differences in shedding of NP into circulation and time of symptom onset after infection may vary between SARS and COVID-19, the 10-fold reduction in diagnostic cut-off contributes decisively to the very early detection of SARS-CoV-2 infection achieved by the novel NP ELISA investigated in the present study.

WHO has concluded that early laboratory diagnosis of SARS-CoV-2 infection can aid clinical management and outbreak control of COVID-19, and that the standard confirmation of acute infection should be based on a nucleic acid amplification test (2). However, URT swab collection

followed by PCR analysis is a tedious and expensive method for COVID-19 screening. In contrast, in settings such as hospitals and blood banks, where blood samples are collected anyway, the SARS-CoV-2 NP ELISA provides a simple and economical screening tool for COVID-19. For example, serum and plasma samples prepared at hospitals for biochemical and other clinical laboratory analyses may also be systematically examined by ELISA for the presence of SARS-CoV-2 NP, and thereby contribute importantly to reduce the risk of nosocomial COVID-19 infection (15).

Our study has strengths and limitations. First, only a subset of the included participants had blood samples collected within 0-1 days after their first PCR-positive URT swab, although this period is the most clinically relevant for early detection of SARS-CoV-2. However, the wide range of collection of blood samples after the first PCR-positive URT swab allowed us to investigate the individual progress of NP concentration in blood for participants with numerous samples available. Second, we did not have information on onset or duration of symptoms for in- and outpatients. Instead, the first PCR-positive URT swab was used as the confirmatory test for COVID-19, although the infection with SARS-CoV-2 may have started days before the PCR test was performed. Theoretically, in a setting where this information was available, the false negative rate would be even lower, as participants with longer duration of symptoms before blood sampling could be excluded from the main analyses. Third, oropharyngeal swabs were utilized for URT sampling according to Danish national guidelines (9), and though also stated by the FDA as appropriate for clinical testing, they may be less sensitive than nasopharyngeal swabs (16). The strengths of the study include a large sample size and a standardized and highly reproducible method for quantification of SARS-CoV-2 NP.

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

**ACKNOWLEDGEMENTS**

We thank all patients that participated in the study.

The Danish COVID-19Biobank (D19B at the Bio- and Genome Bank, Denmark) is acknowledged for biological material and for data information.

CGI Danmark is thanked for providing the IT solution (intellectual property) and competences at no cost for the biobank solution used in the Danish COVID-19Biobank.

We thank BioHit Healthcare (Hefei), Anhui Province, P.R. China for cooperation on the two ELISAs for quantification of the SARS-CoV-2 NP antigen and for measurement of titers of neutralizing antibodies to the ACE-2 receptor binding domain of the S1 subunit of SARS-CoV-2 Spike Protein, respectively.

**CONFLICTS OF INTEREST DISCLOSURES**

AP and NTF are employees of Solsten Diagnostics International, Aarhus, Denmark, which is the company providing the “Solsten SARS-CoV-2 Antigen ELISA Kit”.

**FUNDING/SUPPORT**

RFT reports a grant from Rigshospitalet Research Council.

SDN has unrestricted research grants from Novo Nordisk Foundation and Danish Council for Independent Research, Medical Sciences (FSS).

The present methodology study was co-financed by Innovation Fund Denmark and Eureka via grants 0173-00085 and 0221-00005, respectively.

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

| Table 2A. | | SARS-CoV-2 RNA PCR test | | | | |
|---|---|---|---|---|---|---|
| Number of **serum samples**: 397 | | Positive | | Negative | Total | Relevance: |
| Blood sampling within: **Day 0-13** | | Inpatients | Outpatients | Outpatients | Patients | 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% | | |

| Table 2B. | | SARS-CoV-2 RNA PCR test | | | | |
|---|---|---|---|---|---|---|
| Number of **plasma samples**: 394 | | Positive | | Negative | Total | Relevance: |
| Blood sampling within: **Day 0-13** | | Inpatients | Outpatients | Outpatients | Patients | 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% | | |

## 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.





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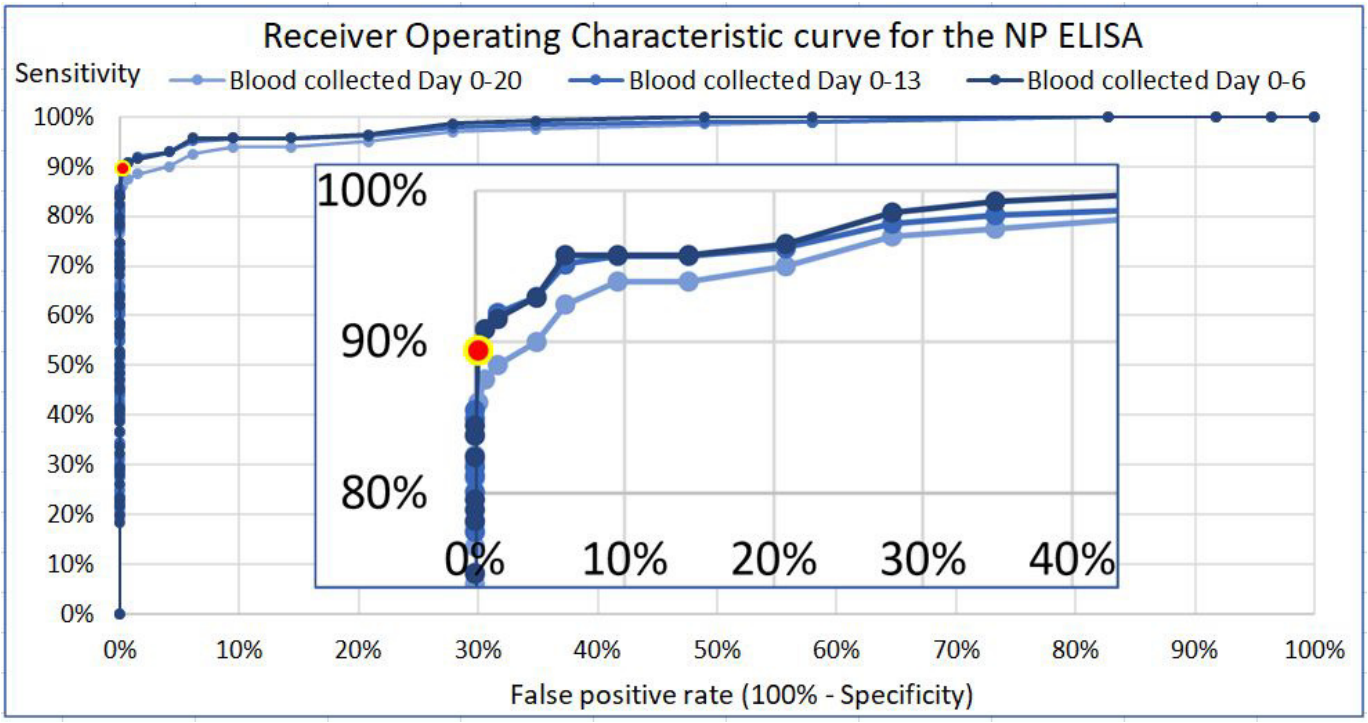
**FIGURE 3**. Correlation between the results of SARS-CoV-2 NP ELISA and PCR analysis according to the time gap from the confirmatory PCR-positive URT swab to collection of serum (A) or plasma (B). The 368 blood samples were collected from 160 COVID-19 inpatients within 5 weeks after their confirmatory PCR-positive URT swab (●), and 43 blood samples were collected from COVID-19 outpatients simultaneously with their PCR-positive URT swab (■). Each inpatient data point area is proportional to the number of blood samples contributing to the data point.

**FIGURE 4**. The median [NP] declined exponentially over time for 368 blood samples collected from 160 COVID-19 inpatients within 5 weeks after their confirmatory PCR-positive URT swab (●). The median [NP] of blood samples collected at Day 0-1 for 32 COVID-19 inpatients (1,045 pg/ml) was 36 times higher than the median [NP] (29 pg/ml) of blood samples collected from 43 COVID-19 outpatients simultaneously with their PCR-positive URT swab (■). For each inpatient data point, the time of blood sample collection is illustrated as mean ± SD number of days after the first PCR-positive URT swab. Each inpatient data point area is proportional to the number of blood samples contributing to the data point.

**FIGURE 5**. Individual dynamics in [NP] of 40 COVID-19 inpatients within 1 month from first PCR-positive URT swab (total number of blood samples, n=200). A: 3-10 serum samples collected from each of 23 inpatients; B: 3-4 plasma samples collected from each of 17 inpatients.

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.

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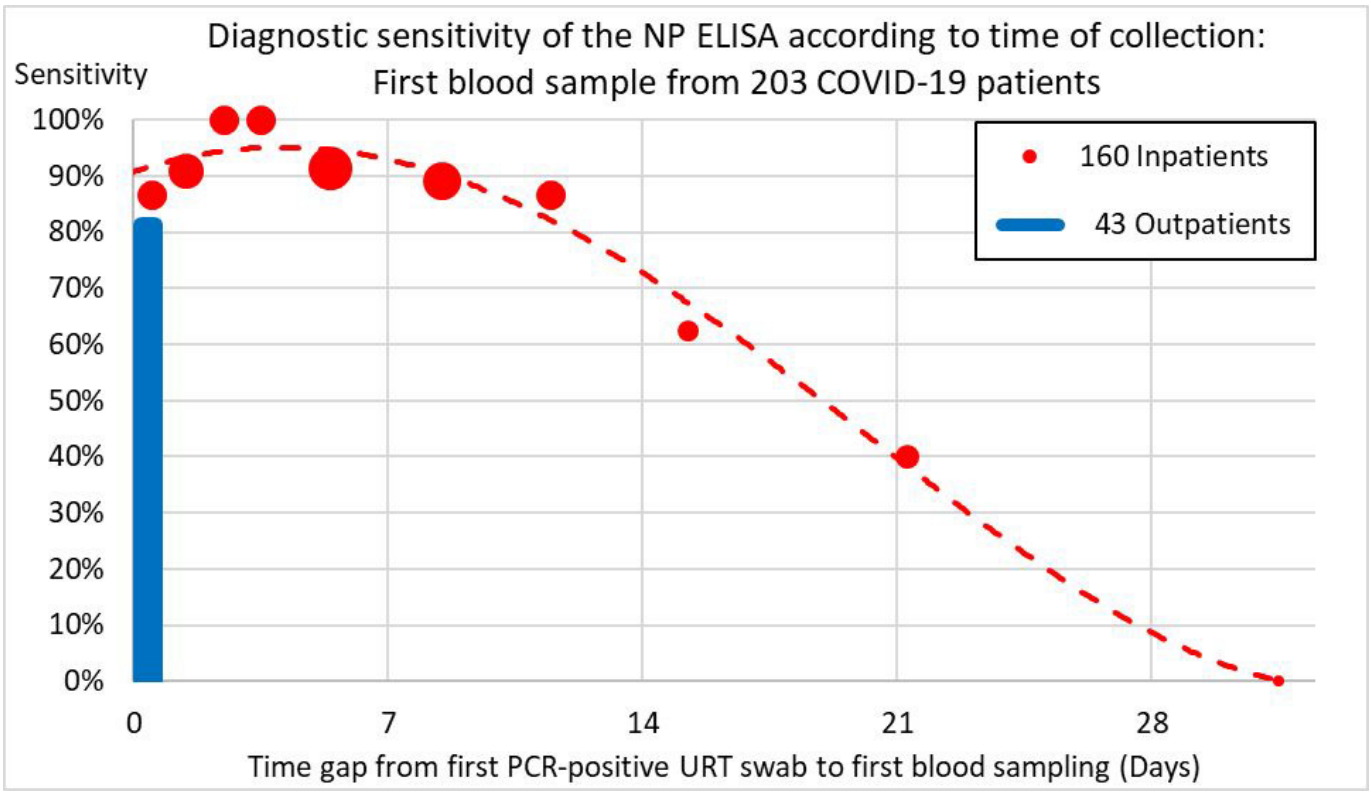




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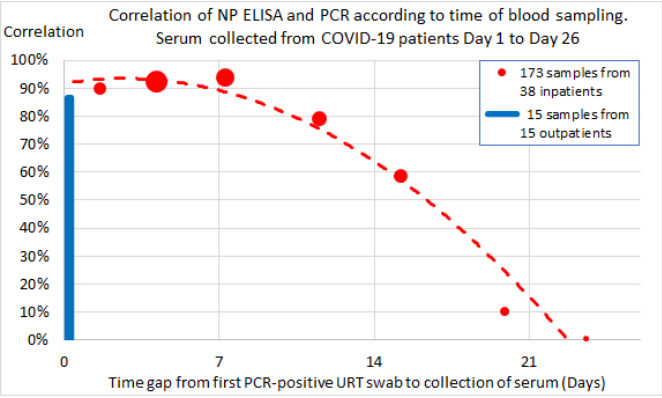


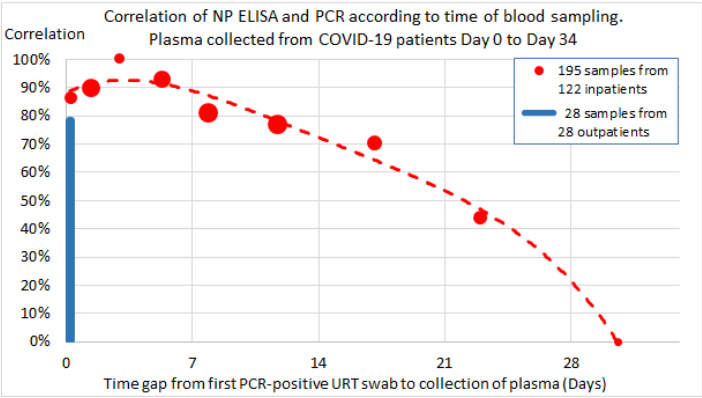

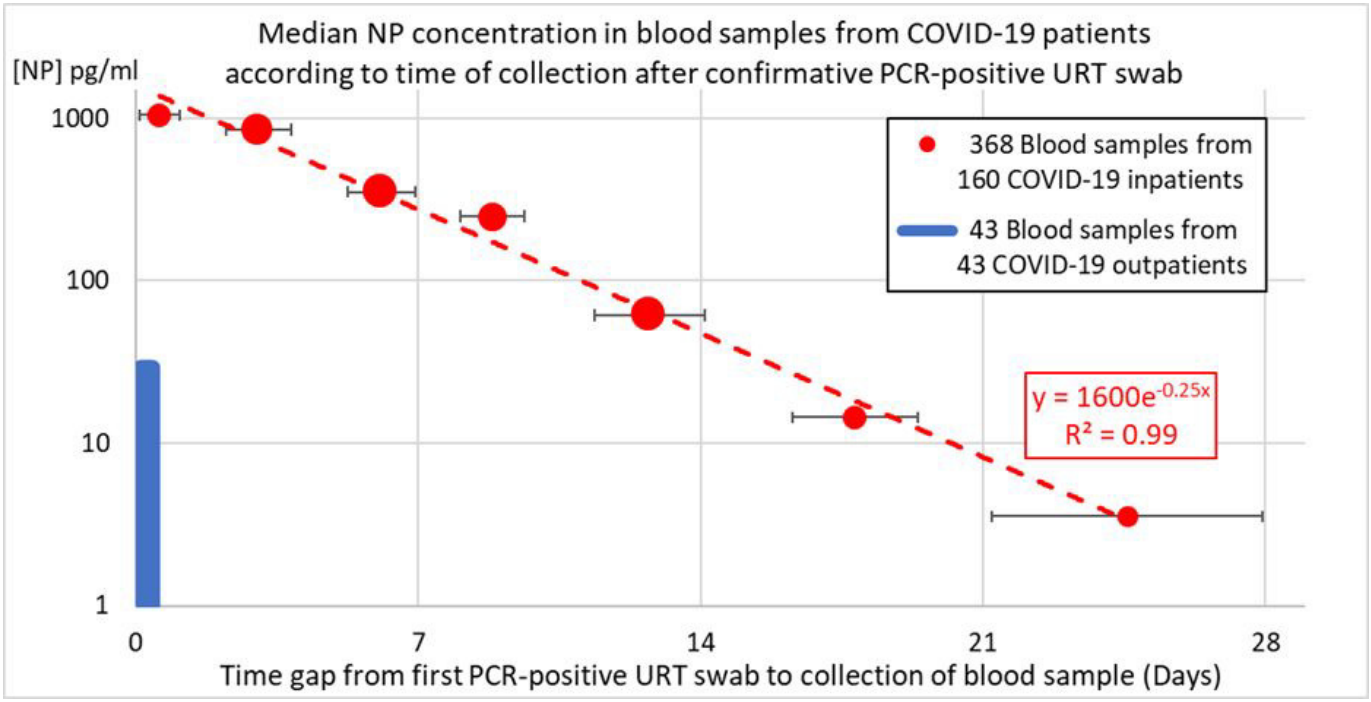

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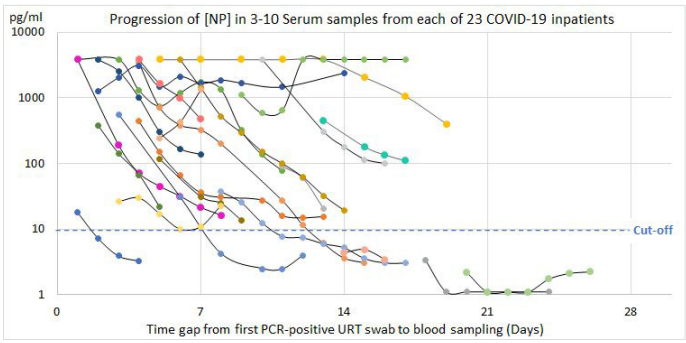


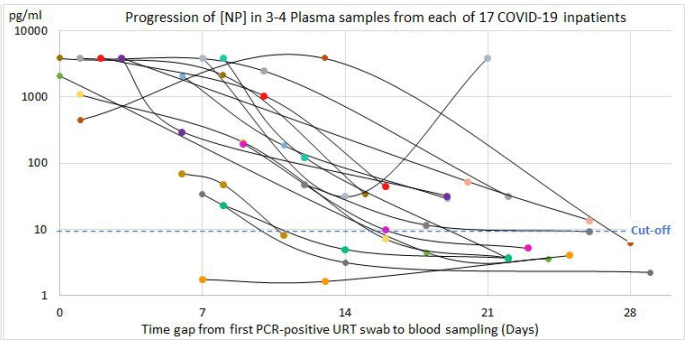

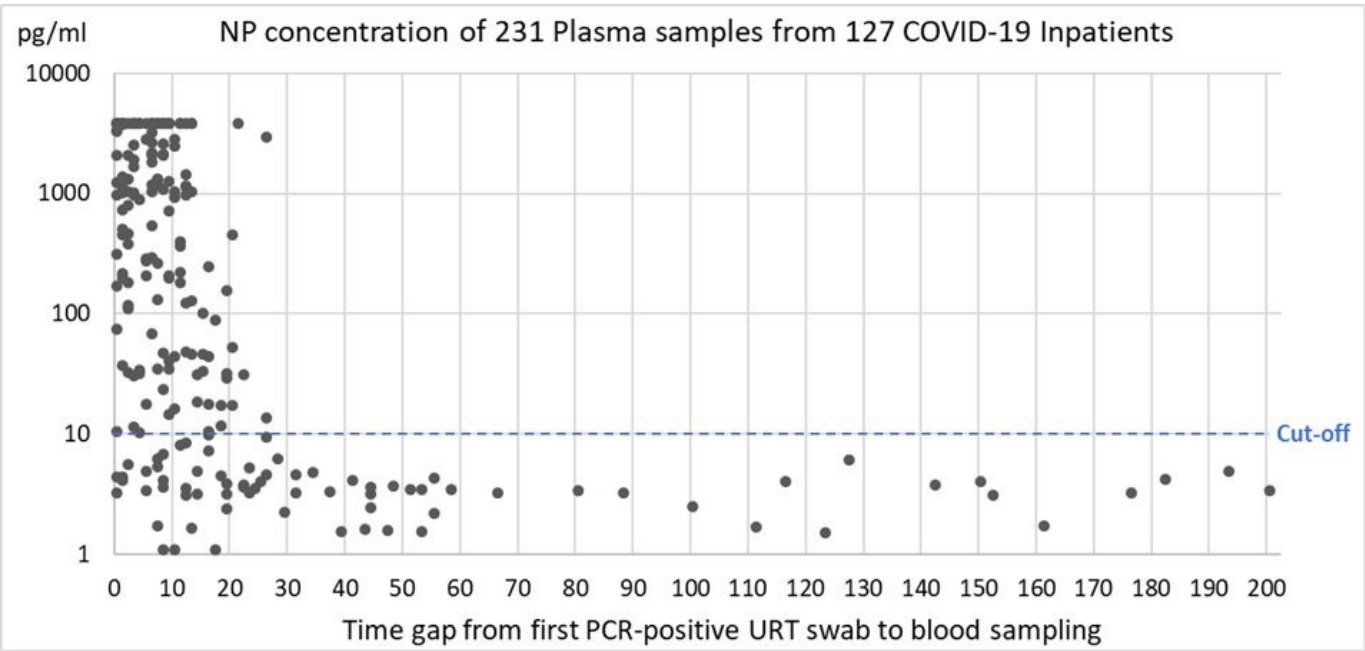

**SUPPLEMENTAL MATERIAL**

**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 | | | | | | |
|---|---|---|---|---|---|---|---|
| | COVID-19 208 | | | | Non-infected 462 | | |
| | Inpts. 165 | | Outpts. 43 | | | Outpts. 462 | |
| S=Serum P= Plasma | S | P | S | P | | S | P |
| | 38 | 127 | 15 | 28 | | 251 | 211 |

| Inpts. first sample | | | All outpts. sampled Day 0 |
|---|---|---|---|
| Day 0-34 | 38 | 122 | |
| Day 0-13 | 33 | 110 | |
| Day 0-6 | 26 | 73 | |

| | No. of **Samples** in study 914 | | | | | | |
|---|---|---|---|---|---|---|---|
| | COVID-19 447 | | | | Non-infected 467 | | |
| | Inpts. 404 | | Outpts. 43 | | | Outpts. 467 | |
| S=Serum P= Plasma | S | P | S | P | | S | P |
| | 173 | 231 | 15 | 28 | | 251 | 216 |

| Inpts. all samples | | | All outpts. sampled Day 0 |
|---|---|---|---|
| Day 0-34 | 173 | 195 | |
| Day 0-13 | 131 | 150 | |
| 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 |

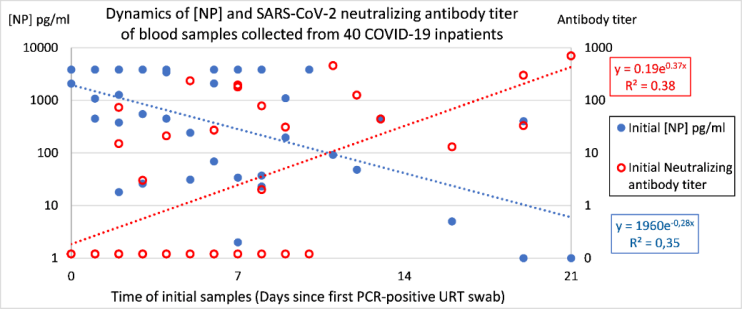


**FIGURE S2.** Indication of the expected inverse relationship between the [NP] and the neutralizing antibody titer of the initial serum (n=23) or plasma sample (n=17) collected from 40 COVID-19 inpatients up to 3 weeks after collection of their confirmatory PCR-positive URT swab.

**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<br>**Projected** per 100,000 samples<br>**Expected** prevalence: 0.2% | | SARS-CoV-2 RNA PCR test/Swab<br>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:<br>**Given** sensitivity and specificity | | Sensitivity<br>**82.76%** | Specificity<br>**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.