



HAL
open science

Detection of SARS-CoV-2 N-antigen in blood during acute COVID-19 provides a sensitive new marker and new testing alternatives

Quentin Le Hingrat, Benoit Visseaux, Cédric Laouenan, Sarah Tubiana, Lila Bouadma, Yazdan Yazdanpanah, Xavier Duval, Charles Burdet, Houria Ichou, Florence Damond, et al.

► To cite this version:

Quentin Le Hingrat, Benoit Visseaux, Cédric Laouenan, Sarah Tubiana, Lila Bouadma, et al.. Detection of SARS-CoV-2 N-antigen in blood during acute COVID-19 provides a sensitive new marker and new testing alternatives. *Clinical Microbiology and Infection*, 2021, 27 (5), pp.789.e1-789.e5. <10.1016/j.cmi.2020.11.025>. <hal-03101905>

HAL Id: hal-03101905

<https://hal.science/hal-03101905v1>

Submitted on 19 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Detection of SARS-CoV-2 N-antigen in blood during acute COVID-19 provides a sensitive new marker and new testing alternatives

Quentin LE HINGRAT,^{1,2} Benoit VISSEAU,^{1,2*} Cédric LAOUENAN,^{1,3} Sarah TUBIANA,^{1,3} Lila BOUADMA,^{1,4} Yazdan YAZDANPANA,^{1,5} Xavier DUVAL,^{1,3} Charles BURDET,^{1,3} Houria ICHOU,² Florence DAMOND,^{1,2} Mélanie BERTINE,^{1,2} Nabil BENMALEK,² French COVID cohort management committee, CoV-CONTACT study group, Christophe CHOQUET,^{1,6} Jean-François TIMSIT,^{1,4} Jade GHOSN,^{1,5} Charlotte CHARPENTIER,^{1,2} Diane DESCAMPS,^{1,2} Nadhira HOUHOU-FIDOUH²*

1. Université de Paris, IAME, INSERM, Paris, France

2. Virology, Assistance Publique-Hôpitaux de Paris, Bichat-Claude Bernard University Hospital, Paris, France

3. Center for Clinical Investigation, Assistance Publique-Hôpitaux de Paris, Bichat-Claude Bernard University Hospital, Paris, France

4. Medical and Infectious Diseases Intensive Care Unit, Assistance Publique-Hôpitaux de Paris, Bichat-Claude Bernard University Hospital, Paris, France

5. Tropical and infectious diseases Department, Assistance Publique-Hôpitaux de Paris, Bichat-Claude Bernard University Hospital, Paris, France

6. Emergency Department, Assistance Publique-Hôpitaux de Paris, Bichat-Claude Bernard University Hospital Paris, France

* These authors contributed equally to this work

Keywords: COVID-19; SARS-CoV-2; Antigen; Antigenemia; Diagnostic; Serum; Plasma; Blood

Corresponding author: Benoit Visseaux, benoit.visseaux@aphp.fr

ABSTRACT

Objectives. Molecular assays on nasopharyngeal swabs remain the cornerstone of COVID-19 diagnostic. The high technicalities of nasopharyngeal sampling and molecular assays, as well as scarce resources of reagents, limit our testing capabilities. Several strategies failed, to date, to fully alleviate this testing process (e.g. saliva sampling or antigen testing on nasopharyngeal samples). We assessed the clinical performances of SARS-CoV-2 nucleocapsid antigen (N-antigen) ELISA detection in serum or plasma using the COVID-19 Quantigene® (AAZ, France) assay.

Methods. Performances were determined on 63 sera from 63 non-COVID patients and 227 serum samples (165 patients) from the French COVID and CoV-CONTACT cohorts with RT-PCR confirmed SARS-CoV-2 infection, including 142 serum (114 patients) obtained within 14 days after symptoms' onset.

Results. Specificity was 98.4% (95% confidence interval [CI], 95.3 to 100). Sensitivity was 79.3% overall (180/227, 95% CI, 74.0 to 84.6) and 93.0% (132/142, 95% CI, 88.7 to 97.2) within 14 days after symptoms onset. 91 included patients had a sera and nasopharyngeal swabs collected in the same 24 hours. Among those with high nasopharyngeal viral loads, i.e. Ct value below 30 and 33, only 1/50 and 4/67 tested negative for N-antigenemia, respectively. Among those with a negative nasopharyngeal RT-PCR, 8/12 presented positive N-antigenemia; the lower respiratory tract was explored for 6 of these 8 patients, showing positive RT-PCR in 5 cases.

Conclusion. This is the first evaluation of a commercially available serum N-antigen detection assay. It presents a robust specificity and sensitivity within the first 14 days after symptoms onset. This approach provides a valuable new option for COVID-19 diagnosis, only requiring a blood draw and easily scalable in all clinical laboratories.

INTRODUCTION

Molecular assays on nasopharyngeal swabs remain the cornerstone of COVID-19 diagnostic. Despite massive efforts, the high technicalities of nasopharyngeal sampling and molecular assays, as well as scarce resources of reagents, limit our testing capabilities. Several strategies failed, to date, to fully alleviate this testing process, *e.g.* saliva sampling [1,2] or antigen testing on nasopharyngeal samples [3,4]. Nucleocapsid-antigen (N-antigen) has been detected in the serum of SARS-CoV-infected patients and, recently, it has been demonstrated in SARS-CoV-2 infected patients by a single study with a global sensitivity of 41/64 patients [5,6].

In this work, we assessed the performances of N-antigen sera detection in a large patients' population using the first commercially available assay, the COVID-19 Quantigene® (AAZ France) providing a low limit of detection at 2.98 pg/mL.

METHODS

Patients and ethics. Negative samples are composed of 50 pre-pandemic samples (collected between December 2, 2019 and January 13, 2020) and 13 pandemic samples from SARS-CoV-2 non-infected patients that tested positive for other microbial antigens (*i.e.* NS1 antigen, HBs antigen, HIV-1 p24 antigen, HKU1 coronavirus or malaria antigens). Positive samples were collected between January 25, 2020 and September 2, 2020 from study participants included in the French COVID (clinicaltrials.gov NCT04262921) and CoV-CONTACT cohorts (clinicaltrials.gov NCT04259892). We selected the first serum samples available after COVID-19 diagnosis (*cf.* Supplementary Figure 2). The following serum samples of those patients, when collected at physician discretion, were also included. They have provided written informed consent for the use of their samples for research. Ethics approval was given by the French Ethics Committee CPP-Ile-de-France 6 (ID RCB: 2020-A00256-33 and ID RCB: 2020-A00280-39) and the French National Data Protection Commission (approval #920102).

For COVID-19 patients, available sera samples were classified into different categories according to the delay since symptom onset: sera collected ≤ 14 days post-symptoms onset (142 sera from 114 patients), samples collected > 14 days post-symptoms onset (81 samples from 72 patients), samples collected from asymptomatic patients (3 sera from 3 patients), and patient without date of symptom onset (1 sera from 1 patient). Distribution of sera samples according to date of sampling and hospitalization status is detailed in Supplementary Figure 2.

N-antigen levels assessment. Prior to analysis, sera samples were stored at -80°C . N antigenemia levels were determined with a being marketed CE-IVD ELISA microplate assay, COVID-Quantigene® (AAZ), according to manufacturer recommendations. Briefly, in each well of 96-wells microplates

previously coated with anti-SARS-CoV-2 N-antibodies, 50 µL of a solution containing biotinylated anti-SARS-CoV-2 N antibodies and 50 µL of sera were added. After incubation at 37°C for 60 minutes, plates were washed 5 times with a phosphate buffer solution. Then, 100 µL of a solution containing HRP-conjugated streptavidin were added, followed by incubation for 30 minutes at 37°C. Plates were washed 5 times with the phosphate buffer solution, then 50 µL of a solution containing the substrate (3,3',5,5'-tetramethylbenzidine (TMB)) and 50 µL of a second solution containing urea were added. After 15 minutes at 37°C, the colorimetric reaction was stopped by adding 50 µL of H₂SO₄. Absorbance values were measured at 450nm, with a reference set at 630nm. In each plate, standards made of recombinant N antigens were tested, to quantify the N antigenemia levels for each patient's sample. As the purpose of this study was to assess the sensitivity of this new assay, samples with titers above 180 pg/mL were not diluted for precise quantification.

RT-PCR assays. For all patients included in this study, diagnosis of SARS-CoV-2 infection was performed in the virology department of Bichat-Claude Bernard University Hospital by RT-PCR on naso-pharyngeal swabs, as recommended. Different techniques were performed throughout the study period for nasopharyngeal samples, due to frequent shortages issues and requirements for fast turnaround time: RealStar[®] SARS-CoV-2 (Altona, Hamburg, Germany), Cobas[®] SARS-CoV-2 (Roche Diagnostics, Branchburg, NJ, USA), Simplexa[®] COVID-19 Direct (DiaSorin, Gerenzano, Italy), BioFire[®] SARS-CoV-2 (BioMerieux, Salt Lake City, UT, USA), QIAstat-Dx[®] Respiratory SARS-CoV-2 (Qiagen, Hilden, Germany) and NeumoDX[®] (QIAgen, Hilden, Germany) using the IP2 Institute Pasteur and the WHO E gene primers[7]. E gene cycle threshold (Ct) values, available for all techniques except Simplexa[®] COVID-19 Direct and BioFire[®] SARS-COV-2, were used as a proxy for viral load for 104 samples from 91 patients with paired naso-pharyngeal swabs and sera (*i.e.* collected in the same 24 hours).

For a subset of 146 samples, corresponding to 89 patients included in the French COVID-19 cohort, paired sera and plasma samples were available, allowing to determine the presence of viral RNA in plasma. Briefly, viral nucleic acids were extracted from 200 µL of plasma with the MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume (Roche Diagnostics, Branchburg, NJ, USA) and eluted in 50 µL. RT-PCR was performed on 10 µL of eluate using the RealStar[®] SARS-CoV-2 assay (Altona, Germany), according to the manufacturer recommendations. Samples with RT-PCR cycle threshold values above 40 were considered negative.

Detection of anti-SARS-CoV-2 nucleocapsid IgG. For a subset of 85 sera, corresponding to 80 patients (ICU patients: n=21, ward patients: n=36 and outpatients: n=23), we performed a chemiluminescent microparticle immunoassay detecting anti-N immunoglobulins G (Architect SARS-

CoV-2 IG Assay, Abbott). Results were reported as a signal to cut-off (S/Co) value. The positivity threshold was set to 1.4, as recommended by the manufacturer.

Data availability. A file compiling all data used in this article is available on Mendeley Data public repository (<https://data.mendeley.com/datasets/fjz6zbkxvm/1>).

RESULTS

Specificity of the COVID-19 Quantigene® was 98.4% (95% confidence interval [CI], 95.3 to 100), as N-antigenemia was negative for 62 samples out of 63 non-COVID-19 patients.

N-antigenemia sensitivity was determined on 227 sera, obtained from 165 patients included in the French COVID and CoV-CONTACT cohorts with RT-PCR confirmed SARS-CoV-2 infection. Among them, 180/227 sera tested positive, leading to a sensitivity of 79.3% (95% CI, 74.0 to 84.6). When restricting sensitivity analysis to samples collected in two first weeks after symptoms onset, 132 out of 142 samples tested positive for N-antigenemia, leading to a sensitivity of 93.0% (95% CI, 88.7 to 97.2) (Figures 1A and 1B). Patients with positive RNAemia (viremic patients) exhibited higher N-antigen sera levels (Figure 1-C). In sera collected more than 14 days after symptoms onset, N antigenemia frequently declined and was undetectable in 84.6% (11/13), 42.1% (8/19) and 32.7% (16/49) samples of outpatients, ward, and ICU patients, respectively. The lower detection in late-stage samples appears linked with the apparition of anti-N IgG (figure 1A and Supplementary Appendix 1A).

For 91 patients, 104 paired samples of sera and nasopharyngeal swabs collected in the same 24 hours were available, allowing us to compare N-antigen detection with E gene Ct values for those patients (Figure 1-D). Among patients with E gene Ct value below 30 and below 33 on their nasopharyngeal swab, only 1/50 and 4/67 tested negative for N-antigenemia, respectively (Supplementary Figure 1B). For patients with positive NP samples with Ct values ≥ 33 , only 15/25 (60%) were positive for N-antigenemia. Interestingly, 8 out of 12 patients with a negative nasopharyngeal RT-PCR presented positive N-antigenemia. The lower respiratory tract was explored for 6 of these 8 patients either the same day or in the 5 following days. RT-PCR on the lower respiratory tract sample was positive in 5 of these 6 patients.

DISCUSSION

This is the first evaluation of a commercially available SARS-CoV-2 N-antigen serum or plasma detection. This assay presented a low detection limit at 2.98 pg/mL and a sensitivity above 90% during the acute phase of the disease (*i.e.* <14 days after symptoms onset in PCR confirmed COVID-

19 patients). In the first two weeks, N-antigen negativity was associated with anti-N IgG detection (6/10) and/or low nasopharyngeal viral load in the same 24 hours (7/7, Ct value >30). This sensitivity could allow its use for COVID-19 diagnostic and is in line with RT-PCR on NP samples whose reported sensitivity rates ranged between 71 and 98%, based on negative RT-PCR tests which were positive on repeat testing [8].

Detection of viral antigens in the blood of COVID-19 patients has been recently described by Ogata and collaborators, who detected N and S1 antigens in the blood of 41 out of 64 COVID-19 patients [5]. If antigens blood circulation is not uncommon in infectious diseases, antigenemia tests usually target blood-borne pathogens, notably Dengue, CMV, HBV or HIV. In respiratory diseases, antigen circulation into non-respiratory body fluids is usually not considered, even if likely, because of the focal nature of the infection or possible pre-existence of antibodies. Antigen detection in non-respiratory fluids is still used for two respiratory bacteria: *Streptococcus pneumoniae* and *Legionella pneumophila*. Interestingly, it has also been reported in SARS-CoV-1 infection [6]. Whether the circulation of free viral antigens has an impact on disease physiopathology should be assessed in future studies.

N-antigenemia was also detectable in outpatients but the decrease seems to occur earlier in our study. Detection of viral antigens in this population was not evaluated in the study by Ogata and collaborators. Detection of N-antigenemia was higher for patients with either high NP viral loads, i.e. Ct below 30, or active replication in the lung, i.e. high Ct values (>30) in NP samples but positive RT-PCR in lower respiratory tract samples.

This innovative marker may also be of help for prognostic evaluation of patients. An association between high N-antigen levels and higher ICU admission rates has been reported by Ogata et al. [5]. In our study, we observed higher N-antigen levels in serum of viremic patients, in line with the possible association of viremia, or RNAemia, with disease severity and/or immunosuppression [9–11].

Our study presents several limitations. We included a very few number of outpatients and this population needs to be explored in larger cohorts, ideally with longitudinal samples of paucisymptomatic and asymptomatic patients. The case-control design is another limitation and the test performances could be slightly deteriorated in real life condition.

In conclusion, sensitive N-antigen detection in serum or plasma provides a valuable new marker for COVID-19 diagnosis, only requiring a blood draw, scalable in all clinical laboratories. It allows potential new developments to design rapid antigen blood test or combined ELISA assays, detecting both antigens and antibodies. It also raises new questions about the physiological mechanisms at play explaining blood circulation of this antigen and its potential correlation to disease severity.

CONFLICT OF INTEREST

Benoit Visseaux reports grants, personal fees and non-financial support from Qiagen, personal fees from BioMérieux, personal fees from Hologic, personal fees from Gilead, all outside the submitted work. Xavier Duval reports grants from Sanofi Pasteur, grants from Pfizer, all outside the submitted work. Charles Burdet reports personal fees and non-financial support from Da Volterra, personal fees from Mylan, all outside the submitted work. Jean-François Timsit reports grants and personal fees from MSD, grants and personal fees from Pfizer, grants and personal fees from beckton dickinson, personal fees from biomerieux, personal fees from Medimune, personal fees from Gilead, all outside the submitted work. Jade Ghosn reports grants and personal fees from Gilead Sciences, grants and personal fees from ViiV Healthcare, personal fees from MSD, all outside the submitted work. Charlotte Charpentier reports personal fees from ViiV Healthcare, personal fees from Gilead, personal fees from Theratechnologie, all outside the submitted work. Diane Descamps reports personal fees from ViiV Healthcare, personal fees from Gilead, all outside the submitted work. Yazdan Yazdanpanah has been a board member receiving consultancy fees from Abbvie, BMS, Gilead, MSD, J&J, Pfizer, and ViiV Healthcare, all outside the submitted work and all these activities have been stopped in the 3 past years. The other authors have nothing to disclose.

AUTHORS CONTRIBUTION

QLH, BV, CCha, DD, NHF conceptualized the study and its methodology. QLH, HI, FD, NB, MB performed the experiments. CL, ST, CB, CCho, XD, JFT, LB, JG, YY collected data and participated to the validation of the study. QLH, BV wrote the first draft. All authors reviewed and edited the final manuscript.

REFERENCES

- [1] Rao M, Rashid FA, Sabri FSAH, Jamil NN, Zain R, Hashim R, et al. Comparing nasopharyngeal swab and early morning saliva for the identification of SARS-CoV-2. *Clin Infect Dis* 2020. <https://doi.org/10.1093/cid/ciaa1156>.
- [2] Wyllie AL, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, Vijayakumar P, et al. Saliva or Nasopharyngeal Swab Specimens for Detection of SARS-CoV-2. *N Engl J Med* 2020. <https://doi.org/10.1056/NEJMc2016359>.
- [3] Flower B, Brown JC, Simmons B, Moshe M, Frise R, Penn R, et al. Clinical and laboratory evaluation of SARS-CoV-2 lateral flow assays for use in a national COVID-19 seroprevalence survey. *Thorax* 2020. <https://doi.org/10.1136/thoraxjnl-2020-215732>.
- [4] Dinnes J, Deeks JJ, Adriano A, Berhane S, Davenport C, Dittrich S, et al. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. *Cochrane Database of Systematic Reviews* 2020. <https://doi.org/10.1002/14651858.CD013705>.
- [5] Ogata AF, Maley AM, Wu C, Gilboa T, Norman M, Lazarovits R, et al. Ultra-sensitive Serial Profiling of SARS-CoV-2 Antigens and Antibodies in Plasma to Understand Disease Progression in COVID-19 Patients with Severe Disease. *Clinical Chemistry* 2020. <https://doi.org/10.1093/clinchem/hvaa213>.
- [6] Che X, Di B, Zhao G, Wang Y, Qiu L, Hao W, et al. A Patient with Asymptomatic Severe Acute Respiratory Syndrome (SARS) and Antigenemia from the 2003–2004 Community Outbreak of SARS in Guangzhou, China. *Clin Infect Dis* 2006;43:e1–5. <https://doi.org/10.1086/504943>.
- [7] Protocol: Real-time RT-PCR assays for the detection of SARS-CoV-2 Institut Pasteur, Paris n.d.
- [8] Arevalo-Rodriguez I, Buitrago-Garcia D, Simancas-Racines D, Zambrano-Achig P, Campo R del, Ciapponi A, et al. False-negative results of initial RT-PCR assays for COVID-19: a systematic review. *MedRxiv* 2020:2020.04.16.20066787. <https://doi.org/10.1101/2020.04.16.20066787>.
- [9] Veyer D, Kernéis S, Poulet G, Wack M, Robillard N, Taly V, et al. Highly sensitive quantification of plasma SARS-CoV-2 RNA sheds light on its potential clinical value. *Clin Infect Dis* 2020. <https://doi.org/10.1093/cid/ciaa1196>.
- [10] Buetti N, Patrier J, Le Hingrat Q, Loidice A, Bouadma L, Visseaux B, et al. Risk factors for SARS-CoV-2 detection in blood of critically ill patients. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* 2020. <https://doi.org/10.1093/cid/ciaa1315>.
- [11] Chen X, Zhao B, Qu Y, Chen Y, Xiong J, Feng Y, et al. Detectable serum SARS-CoV-2 viral load (RNAemia) is closely correlated with drastically elevated interleukin 6 (IL-6) level in critically ill COVID-19 patients. *Clin Infect Dis* 2020. <https://doi.org/10.1093/cid/ciaa449>.

FIGURE LEGEND

Figure 1: (A) Evolution of N-antigen sera levels in SARS-CoV-2-infected patients according to hospitalization status (N=227 serum samples from 165 patients); sequential samples are connected with a gray line, while the positivity threshold value for N-antigen (2.97 pg/mL) is indicated with a dashed red line. (B) N-antigenemia levels according to delay since symptoms onset. (C) N-antigen sera levels according to positive and negative RNAemia status (n=146 sera, N=89 patients). (D) N-antigen sera levels according to E-gene cycle threshold value of 104 nasopharyngeal swabs collected within 24 hours (N=91 patients).

FUNDING

This study has been funded in part by the REACTing (REsearch & ACTion emergING infectious diseases) consortium, by a grant from the French Ministry of Health (PHRC n°20-0424) and by the ANRS (Agence Nationale de la Recherche sur le SIDA et les hépatites virales). The study was supported by AAZ (Boulogne-Billancourt, France) in the form of free consumables and they had no role in conceptualization, design, data collection and analyses, decision to publish and manuscript preparation.

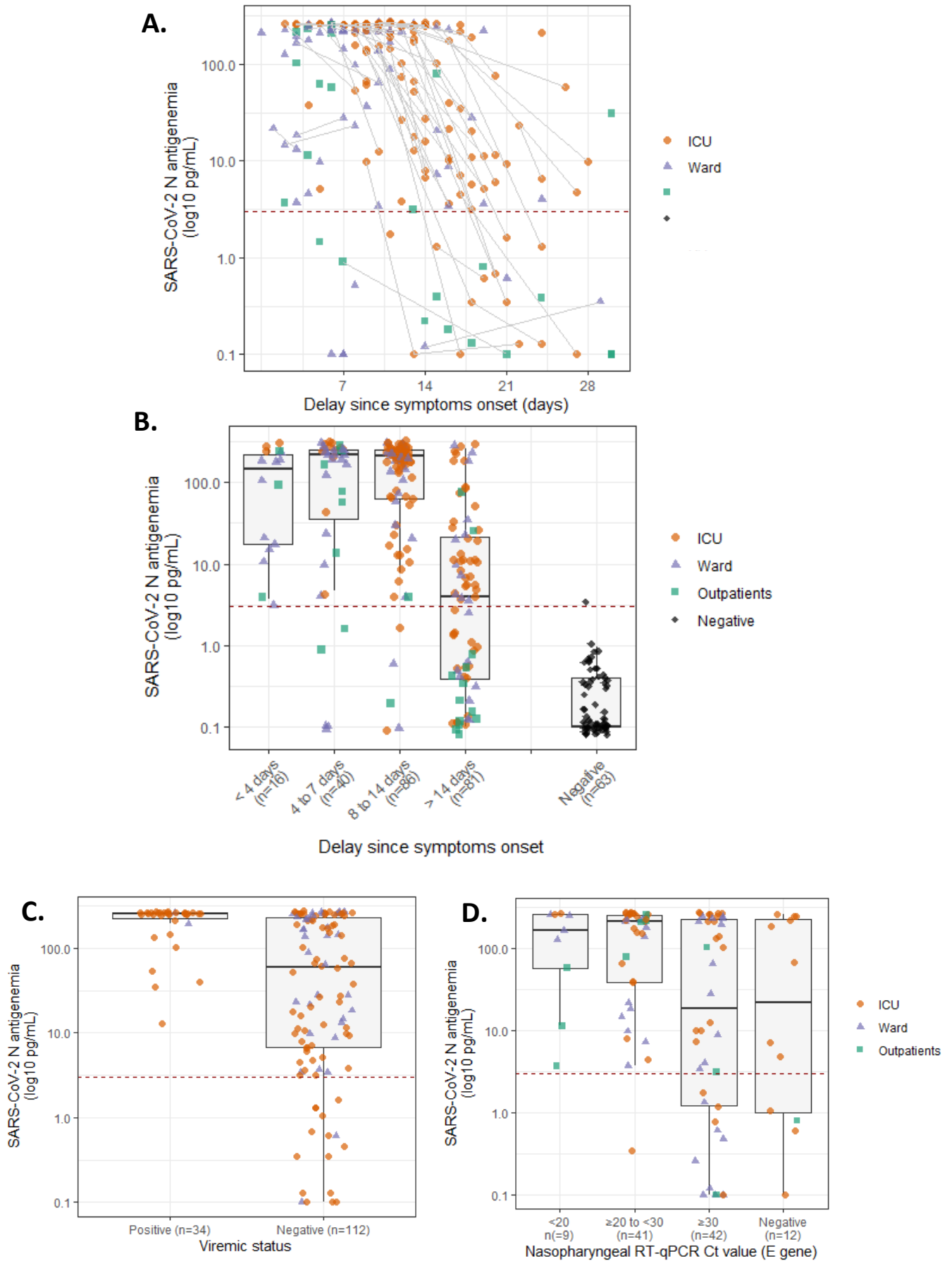
ACKNOWLEDGEMENT

The French COVID cohort was sponsored by Inserm. We wish to thank the ANRS (Agence Nationale de la Recherche sur le SIDA et les hépatites virales).

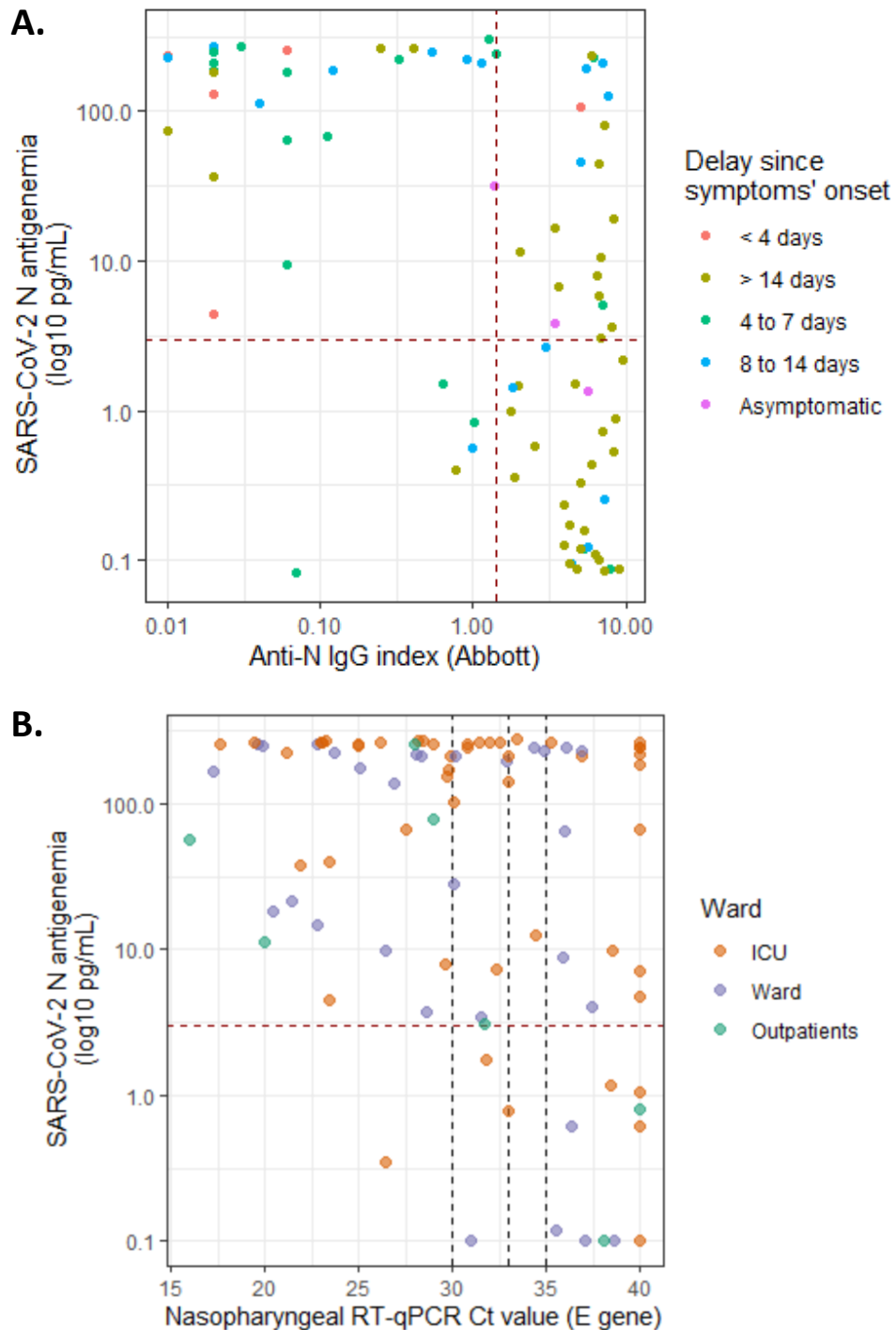
The members of the French-COVID cohort study group (by alphabetical order): Alpha DIALLO, Soizic LE MESTRE, Noémie MERCIER, Christelle PAUL, Ventzislava PETROV-SANCHEZ (ANRS, Paris); Denis MALVY (Bordeaux - SMIT); Catherine CHIROUZE (CHRU Jean Minjot, Besançon); Claire ANDREJAK (CHU Amiens); François DUBOS (CHU Lille), Patrick ROSSIGNOL (CHU Nancy); Olivier PICONE (Colombes - Louis Mourier - Gynécologie); François BOMPART (Drugs for Neglected Diseases initiative, Geneva, Switzerland); Tristan GIGANTE, Morgane GILG, Bénédicte ROSSIGNOL, Claire LEVY-MARCHAL (F-CRIN INI-CRCT, Paris); Marine BELUZE (F-CRIN Partners Platform, Paris); Jean Sébastien (HULOT (HEGP, Paris); Delphine BACHELET, Krishna BHAVSAR, Lila BOUADMA, Anissa CHAIR, Camille (COUFFIGNAL, Charlene DA SILVEIRA, Marie Pierre (DEBRAY, Diane DESCAMPS, Xavier DUVAL, Philippine ELOY, Marina ESPOSITO-FARESE, Nadia ETTALHAOUI, Nathalie GAULT, Jade GHOSN, Isabelle GORENNE, Isabelle HOFFMANN, Ouifiya KAFIF, Sabrina KALI, Antoine KHALIL, Cédric LAOUÉANAN, Samira LARIBI, Minh LE, Quentin LE HINGRAT, François-Xavier LESCURE, Jean Christophe (LUCET, France MENTRÉ, Jimmy Mullaert, Nathan PEIFFER-SMADJA, Gilles PEYTAVIN, Carine ROY, Marion SCHNEIDER, Nassima SI MOHAMMED, Lysa TAGHERSET, Coralie TARDIVON, Marie Capucine (TELLIER, Jean-François TIMSIT, Théo TRIOUX, Sarah TUBIANA, Benoit VISSEAUX (Hôpital Bichat, Paris); Noémie VANEL (hôpital la timone, Marseille); Romain BASMACI (Hôpital Louis Mourier, Colombes); François ANGOULVANT (Hôpital Necker, Paris); Florentia KAGUELIDOU, Justine PAGES (Hôpital Robert Debré, Paris); Christelle TUAL, Aurélie VEISLINGER (Inserm CIC-1414, Rennes); Sandrine COUFFIN-CARDIERGUES, Hélène ESPEROU, Ikram HOUAS, Salma JAAFOURA, Aurélie PAPADOPOULOS (Inserm sponsor, Paris), Alexandra COELHO, Alphonsine DIOUF, Alexandre HOCTIN, Marina MAMBERT (Inserm UMR 1018, Paris); Maude BOUSCAMPBET, Alexandre GAYMARD, Bruno LINA, Manuel ROSA-CALATRAVA, Olivier TERRIER (Inserm UMR 1111, Lyon); Dehbia BENKERROU, Céline DORIVAL, Amina MEZIANE, François TÉOULÉ (Inserm UMR 1136, Paris); Jérémie GUEDJ, Hervé LE NAGARD, Guillaume LINGAS, Nadège NEANT (Inserm UMR 1137, Paris); Laurent ABEL (Inserm UMR 1163, Paris); Mathilde DESVALLÉE, Coralie KHAN (Inserm UMR 1219, Bordeaux); Dominique DEPLANQUE (Lille Calmette - SMIT); Yazdan YAZDANPANAH (Paris - Bichat - SMIT); Sylvie BEHILILL, Vincent ENOUF, Hugo MOUQUET, Sylvie VAN DER WERF (Pasteur Institute, Paris); Minerva CERVANTES-GONZALEZ, Eric d'ORTENZIO, Oriane PUÉCHAL, Caroline SEMAILLE (REACTing, Paris); Marion NORET (RENARCI, Annecy), Manuel ETIENNE (Rouen - SMIT); Yves LEVY, Aurélie WIEDEMANN (Vaccine Research Institute (VRI), Inserm UMR 955, Créteil, France).

The member of the CoV-CONTACT study group. Principal investigator: Xavier DUVAL; **Steering Committee:** Charles BURDET, Xavier DUVAL, Bruno LINA, Sarah TUBIANA, Sylvie VAN DER WERF; **CoV-CONTACT Clinical Centers:** Fanny ABAD, Dominique ABRY, Loubna ALA VOINE, Jean-Sébastien ALLAIN, Karline AMIEL-TAIEB, Pierre AUDOIN, Shana AUGUSTIN, Sandrine AYALA, Hélène BANSARD, Frédérique BERTHOLON, Nolwenn BOISSEL, Elisabeth BOTELHO-NEVERS, Kévin BOUILLE, Marilou BOURGEON, Mathilde BOUTROU, Lysiane BRICK, Léa BRUNEAU, Eric CAUMES, Agnès CHABOUI, Eric CHAN THIEN, Catherine CHIROUZE, Bruno COIGNARD, Yolande COSTA, Virginie COSTENOBLE, Sylvie COUR, Claire CRACOWSKI, Jean-Luc CRACOWSKI, Dominique DEPLANQUE, Stéphane DEQUAND, Mireille DESILLE-DUGAST, Maxime DESMARETS, Maelle DETOC, Marie DEWITTE, Felix DJOSSOU, Jean-Luc ECOBICHON, Elise ELREZZI, William FAUROUS, Viviane FORTUNA, Julie FOUCHARD, Emilie GANTIER, Céline GAUTIER, Patrick GERARDIN, Sandrine GERSET, Marie GILBERT, Valérie GISSOT, Francis GUILLEMIN, Cédric HARTARD, Béatrice HAZEVIS, Didier HOCQUET, Emelija HODAJ, Emila ILIC-HABENSUS, A JEUDY, Helene JEULIN, Maty KANE, Emmanuelle KASPRZYK, John KIKOINE, Fabrice LAINE, Bruno LAVIOLLE, David LEBEAUX, Anne LECLERCQ, Eric LEDRU, Benjamin LEFEVRE, Carole LEGOAS, Amélie LEGRAND, Karine LEGRAND, Jonathan LEHACAUT, Claire LEHUR, Dalila LEMOUCHE, Quentin LEPILLER, Séverine LEPUIL, Estelle LETIENNE, Aude LUCARELLI, Jean-Christophe LUCET, Isabelle MADELINE, Adrien MAILLOT, Catherine MALAPATE, Denis MALVY, Milica MANDIC, Solène MARTY-QUINTERNET, Mohamed MEGHADECHA, Mayka MERGEAY-FABRE, Pauline MESPOULHE, Alexandre MEUNIER, Maria-Claire MIGAUD, Justina MOTIEJUNAITE, Nathalie GAY, Duc NGUYEN, Soumaya OUBBEA, Maïder PAGADOY, Adeline PARIS, Christophe PARIS, Christine PAYET, Nathan PEIFFER-SMADJA, Lucas PEREZ, Pauline PERREAU, Nathalie PIERREZ, Thierry PISTONE, Andreea POSTOLACHE, Patrick RASOAMANANA, Cécile REMINIAC, Jade REXAH, Elise ROCHE-GOUANVIC, Alexandra ROUSSEAU, Betty SCHOEMAECKER, Sandrine SIMON, Catherine SOLER, Stéphanie SOMERS, Khaly SOW, Bernard TARDY, Zaven TERZIAN, Michael THY, Anne TOURNIER, Sandrine TYRODE, Charline VAUCHY, Renaud VERDON, Pauline VERNET, Valérie VIGNALI, Nawal WAUCQUIER; **Coordination and statistical analyses:** Charles BURDET, Huong DO THI THU, Cédric LAOUÉANAN, France MENTRE, Manchon PAULINE, Sarah TUBIANA, Aline DECHANET, Sophie LETROU, Caroline QUINTIN, Wahiba FREZOULS; **Virological lab:** Quentin LE HINGRAT, Nadhira HOUHOU, Florence DAMOND, Diane DESCAMPS, Charlotte CHARPENTIER, Benoit VISSEAUX, Astrid VABRET, Bruno LINA, Maud BOUSCAMPBET, Sylvie VAN DER WERF, Sylvie BEHILLIL, Laurence GAILLANNE, Nabil BENMALEK, Mikael ATTIA, Marion BARBET, Caroline DEMERET, Thierry ROSE, Stéphane PETRES, Nicolas ESCRIOU, Marion BARBET, Stéphane PETRES, Nicolas ESCRIOU, Sophie GOYARD; **Biological center:** Ouifiya KAFIF, Valentine PIQUARD, Sarah TUBIANA; **Partners:** RECOVER, REACTING, Santé Publique France (Bruno COIGNARD, Alexandra MAILLES), Agences régionales de santé (Anne SIMONDON, Marion DREYERE, Bruno MOREL, Thiphaine VESVAL); **Sponsor:** Inserm, Karine AMAT, Douae AMMOUR, Khadija AQOURRAS, Sandrine COUFFIN-CADIERGUES, Christelle DELMAS, Vristi DESAN, Jean-Michel DOUTE, Hélène ESPEROU, Samia HENDOU, Christelle KOUAKAM, Guillaume LE MEUT, Soizic LEMESTRE, Nicolas LETURQUE, Emmanuelle MARCOUL, Solange NGUEFANG, Layidé ROUFAI; **Genetic:** Abel LAURENT, Sophie CAILLAT-ZUCMAN.

Figure 1



Supplementary Appendix 1.

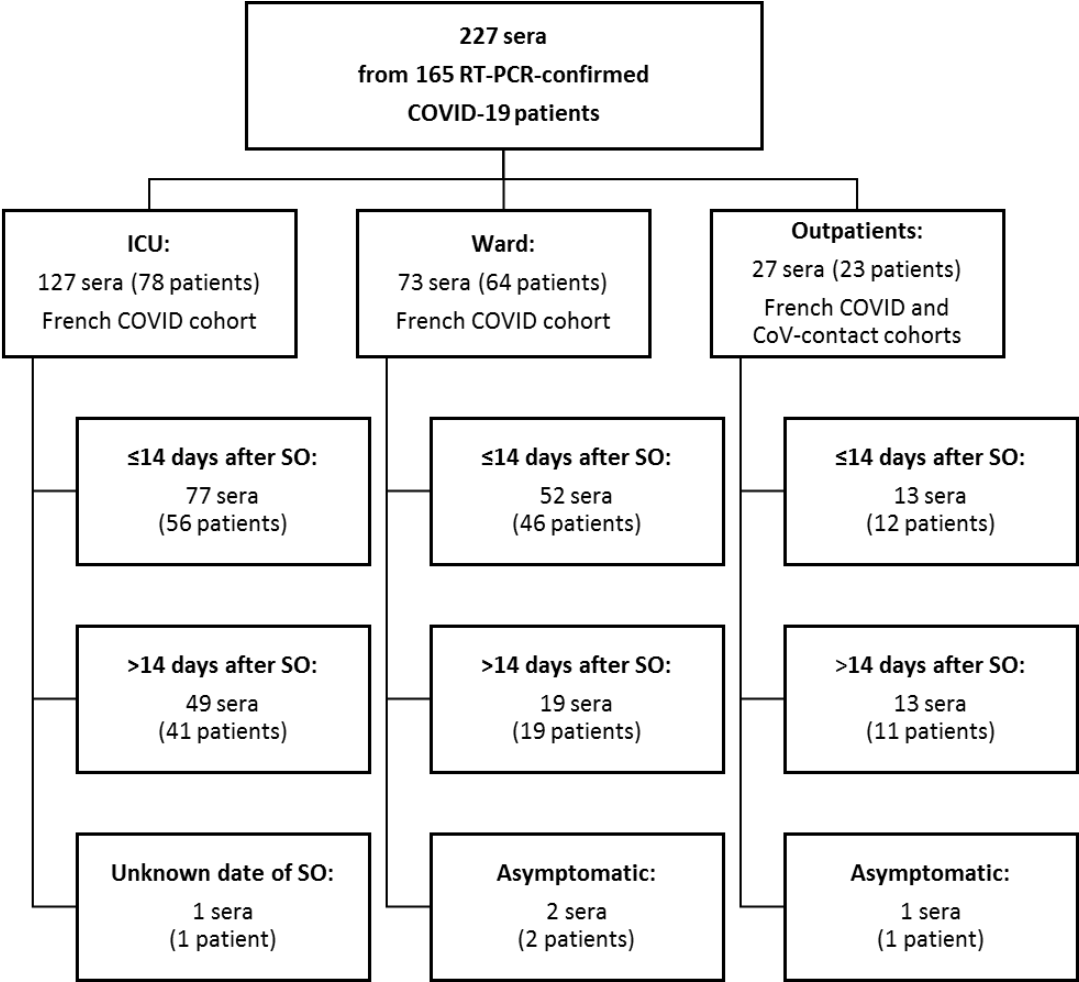


Panel (A): N-antigen sera levels according to anti-N IgG index (Abbot). The positivity threshold values for N-antigen (2.97 pg/mL) and anti-N IgG index (1.4) are indicated with dashed red lines.

Panel (B): N-antigen sera levels according to E-gene cycle threshold value of nasopharyngeal swabs collected within 24 hours. The positivity threshold value for N-antigen (2.97 pg/mL) is indicated with a dashed red line. The RT-PCR Ct values of 30, 33 and 35 are indicated with dashed black lines.

Supplementary Figure 2.

Figure legend: Flowchart of sera used in this study, according to symptoms onset and hospitalization status.



Supplementary Table 1.

Table: Contingency table for the COVID-19 Quantigene® assay.

		COVID-19 positive		COVID-19 negative
		Overall	Within 14 first days after SO	
Serum N-antigen detection	Positive	180	132	1
	Negative	47	10	62