



Aalborg Universitet

AALBORG UNIVERSITY
DENMARK

Long-term detection of SARS-CoV-2 antibodies after infection and risk of re-infection

Hønge, Bo Langhoff; Hindhede, Lotte; Kaspersen, Kathrine Agergård; Harritshøj, Lene Holm; Mikkelsen, Susan; Holm, Dorte Kinggaard; Nilsson, Anna Christine; Sækmose, Susanne Gjørup; Sørensen, Erik; Aagaard, Bitten; Hjalgrim, Henrik; Jørgensen, Charlotte Sværke; Krause, Tyra Grove; Ullum, Henrik; Pedersen, Ole Birger Vestager; Ostrowski, Sisse Rye; Erikstrup, Christian

Published in:
International Journal of Infectious Diseases

DOI (link to publication from Publisher):
[10.1016/j.ijid.2022.01.041](https://doi.org/10.1016/j.ijid.2022.01.041)

Creative Commons License
CC BY-NC-ND 4.0

Publication date:
2022

Document Version
Publisher's PDF, also known as Version of record

[Link to publication from Aalborg University](#)

Citation for published version (APA):
Hønge, B. L., Hindhede, L., Kaspersen, K. A., Harritshøj, L. H., Mikkelsen, S., Holm, D. K., Nilsson, A. C., Sækmose, S. G., Sørensen, E., Aagaard, B., Hjalgrim, H., Jørgensen, C. S., Krause, T. G., Ullum, H., Pedersen, O. B. V., Ostrowski, S. R., & Erikstrup, C. (2022). Long-term detection of SARS-CoV-2 antibodies after infection and risk of re-infection. *International Journal of Infectious Diseases*, 116, 289-292.
<https://doi.org/10.1016/j.ijid.2022.01.041>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal -



ELSEVIER

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Long-term detection of SARS-CoV-2 antibodies after infection and risk of re-infection

Bo Langhoff Hønge^{1,2,*}, Lotte Hindhede¹, Kathrine Agergård Kaspersen^{1,3},
 Lene Holm Harritshøj⁴, Susan Mikkelsen¹, Dorte Kinggaard Holm⁵,
 Anna Christine Nilsson⁵, Susanne Gjørup Sækmose⁶, Erik Sørensen⁴, Bitten Aagaard⁷,
 Henrik Hjalgrim^{8,9,10}, Charlotte Sværke Jørgensen¹¹, Tyra Grove Krause¹², Henrik Ullum¹³,
 Ole Birger Vestager Pedersen^{6,14}, Sisse Rye Ostrowski^{4,14}, Christian Erikstrup^{1,15}

¹ Department of Clinical Immunology, Aarhus University Hospital, Denmark² Department of Infectious Diseases, Aarhus University Hospital, Denmark³ Danish Big Data Centre for Environment and Health (BERTHA), Aarhus University, Roskilde, Denmark⁴ Department of Clinical Immunology, Copenhagen University Hospital, Rigshospitalet, Denmark⁵ Department of Clinical Immunology, Odense University Hospital, Denmark⁶ Department of Clinical Immunology, Zealand University Hospital, Denmark⁷ Department of Clinical Immunology, Aalborg University Hospital, Denmark⁸ Department of Epidemiology Research, Statens Serum Institut, Denmark⁹ Danish Cancer Society Research Centre, Department of Haematology, Copenhagen University Hospital, Rigshospitalet, Denmark¹⁰ Department of Clinical Medicine, Copenhagen University, Denmark¹¹ Department of Virus and Microbiological Species Diagnostics, Statens Serum Institut, Denmark¹² Infectious Disease Preparedness, Statens Serum Institut, Denmark¹³ Statens Serum Institut, Denmark¹⁴ Department of Clinical Medicine, University of Copenhagen¹⁵ Department of Clinical Medicine, Aarhus University

ARTICLE INFO

Article history:

Received 21 December 2021

Revised 15 January 2022

Accepted 17 January 2022

Keywords:

SARS-CoV-2
 seroprevalence
 Wantai
 sensitivity
 blood donors

ABSTRACT

Objectives: To evaluate long-term sensitivity for detection of total antibodies against SARS-CoV-2

Methods: From week 41, 2020, through week 26, 2021, all Danish blood donations were tested for SARS-CoV-2 antibodies with the Wantai assay. The results were linked with polymerase chain reaction (PCR) test results from the Danish Microbiological Database (MiBa).

Results: During the study period, 105,646 non-vaccinated Danish blood donors were tested for SARS-CoV-2 antibodies, and 3,806 (3.6%) had a positive PCR test before the blood donation. Among the donors with a positive PCR test, 94.2% subsequently also had a positive antibody test. The time between the positive PCR test and the antibody test was up to 15 months and there was no evidence of a decline in proportion with detectable antibodies over time. A negative serological result test was associated with a higher incidence of re-infection (Incidence Rate Ratio = 0.102 (95% confidence interval (CI): 0.039–0.262)).

Conclusion: Among healthy blood donors, 94.2% developed SARS-CoV-2 antibodies after infection, and a lack of detectable antibodies was associated with re-infection.

© 2022 The Author(s). Published by Elsevier Ltd on behalf of International Society for Infectious Diseases.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

* Corresponding author. Bo Langhoff Hønge, Department of Clinical Immunology, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, 8200 Aarhus N
 E-mail address: bohonge@gmail.com (B.L. Hønge).

Introduction

A crucial, and much debated, issue for the current vaccination strategy against SARS-CoV-2 is the duration of immunity and detectable antibodies. Reports from the initial epicenter of the pandemic in Wuhan, China, show that individuals with a previous SARS-CoV-2 infection have declining antibody titers during the first

year after infection and that male sex and younger age are associated with higher IgG antibody titers (Feng et al., 2021). Other factors such as comorbidity, immunosuppression, and genetic predisposition including blood type (Barnkob et al., 2020; Bastard et al., 2021; Cordtz et al., 2020) may influence immunity. Why some otherwise healthy individuals do not produce detectable levels of antibodies or become re-infected despite detectable antibody levels has not been thoroughly investigated.

Serological assays are used to demonstrate previous infection or vaccination response against SARS-CoV-2 and may guide health authorities in planning measures against COVID-19 spread (Jones et al., 2021). Assays detecting total immunoglobulin, that is, both IgM, IgA, and IgG, are useful to assure maximum sensitivity (SARS-CoV, n.d.). Several assay evaluations have been performed to estimate short-term sensitivity and in a comparison with 15 other serological assays, the Wantai SARS-CoV-2 Ab ELISA assay (Wantai, Beijing, China) performed the best with a sensitivity of 96.7% and a specificity of 99.5% (Harrithøj et al., 2021). However, only a few studies have explored the association between total immunoglobulin against SARS-CoV-2 and protective immunity.

We assessed the persistence of total anti-SARS-CoV-2 antibodies after real-time polymerase chain reaction (PCR) confirmed SARS-CoV-2 infection, risk factors for undetectable antibodies, and rate of re-infection among seropositive and seronegative individuals.

Methods

Participants

During the SARS-CoV-2 epidemic, more than 200,000 blood donations from Danish blood donors were screened for SARS-CoV-2 antibodies to assist the Danish health authorities in the surveillance of the pandemic. Donor data from all 5 regions in Denmark (Pedersen et al., 2012) were included in this study.

Laboratory tests

From week 41, 2020, through week 26, 2021, all Danish blood donations were tested for SARS-CoV-2 antibodies. The Wantai SARS-CoV-2 Ab ELISA assays were performed locally in each of the 5 regional blood centers. Wantai is a qualitative assay detecting antibodies against the receptor-binding domain of the SARS-CoV-2 spike protein.

Information about the SARS-CoV-2 infection was obtained from the Danish Microbiological Database (MiBa). Individuals were considered SARS-CoV-2 infected if they had a positive PCR test. Data were available from the earliest case in February 2020 until August 2021. Blood donors were given a 28-day deferral period after a positive SARS-CoV-2 test.

Statistics

The sensitivity of the Wantai assay was assessed in 3-month periods. Individuals stating that they had been vaccinated against SARS-CoV-2 were excluded from this analysis from the time of vaccination. For individuals with more than 1 donation in 1 time period, only the last donation was included in the analysis. Sensitivity with 95% confidence intervals (CIs) was calculated by bootstrapping 10^6 times. Binomial regression analysis was used to test for time-dependent loss of sensitivity, and risk factors for a negative Wantai test among PCR positives were assessed by binomial re-

gression. Donors were considered re-infected if they had 2 positive PCR tests at least 3 months apart. Poisson regression was used to calculate the rate of re-infection. The estimated number of false-positive tests among the re-infected donors was calculated by the following equation: $(1 - \text{specificity}) \times \text{total number of tests} \times \text{incidence of primary infection}$. Statistical analyses were performed using R 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria).

Ethics

Consenting blood donors were tested for SARS-CoV-2 antibodies and informed about the result. The study was approved by the Regional Scientific Ethical Committee in Region Zealand, Denmark (SJ-740). Also, the study was approved by the Danish Data Protection Agency (P-2019-99). According to Danish law, approval from a national ethical committee is not necessary when using administrative register data without individual contacts.

Results

Sensitivity of total antibody detection after SARS-CoV-2 infection

During the study period, 105,646 non-vaccinated Danish blood donors were tested for SARS-CoV-2 antibodies, and 3,806 (3.6%) had a positive PCR test before blood donation (Table 1). Among the donors with a positive PCR test, 3,587 subsequently also had a positive antibody test (overall sensitivity 94.2%). We found no correlation between positive antibody tests and days since infection (risk ratio = 1.00 [95% CI 1.00–1.00], $P = 0.10$), and there was no difference in antibody detection between the first and the second waves of COVID-19 in Denmark ($p < 0.01$). In univariable analysis, neither sex, age, or comorbidity, nor blood type was associated with a positive test for total antibodies after SARS-CoV-2 infection (Table 1). Of the 1,218 donors with 2 or more antibody tests, 9 donors seroconverted from negative to positive, and 6 seroconverted from positive to negative.

SARS-CoV-2 re-infection and serology

Among individuals with a positive PCR test before blood donation, 21 (0.6%) had a second positive PCR test least 3 months after the initial PCR test indicating re-infection, and 18 donors also had an antibody test result in between the 2 PCR tests. Among these individuals, 11 (61.1%) had detectable antibodies and the remaining 7 had no detectable SARS-CoV-2 antibodies (negative antibody test result). The 7 individuals with a negative antibody test only donated once in the study period and were therefore only tested for antibodies once. The average time between infections was 171.9 days, and the average time between the last antibody test and the second positive PCR test was 65.7 days. The 7 individuals with negative antibody tests were compared with the 11 individuals with a positive antibody test according to the characteristics listed in Table 1. There were no differences between the groups (exact data not shown owing to the confidentiality of small group sizes). The rate of documented re-infection was 440/100,000 person-years for the seropositive group and 4,332/100,000 person-years for the seronegative group; the Incidence Rate Ratio (IRR) was 0.102 (95% CI: 0.039–0.262). In comparison, the rate of primary infection was 4,379/100,000 person-years for the seronegative group (95% CI: 4,211–4,554). With an estimated PCR test specificity of 99.98% (Hansen et al., 2021) and an incidence rate of

Table 1

Legend: Donor characteristics, antibody test sensitivity, and potential associations between a positive PCR test and negative SARS-CoV-2 serology among 3,806 donors with a positive PCR test from week 41, 2020, through week 26, 2021.

	Total antibody n (%)	Association with positive Wantai test positive	Risk Ratio (95% CI)	p-value ^a
Months since positive PCR test				
1–3	2,249	2,109 (93.8%)	-	-
4–6	1,883	1,783 (94.7%)	-	-
7–9	715	675 (94.4%)	-	-
>9	177	168 (94.9%)	-	-
Calendar periods^c				
First wave	170	164 (96.5%)	-	-
Second wave	263	254 (96.6%)	-	-
Donor characteristics				
In total	3,806	3,587 (94.2%)	-	-
Sex				
Male	1,694 (44.5%)	1,598 (94.3%)	ref	-
Female	2,112 (55.5%)	1,989 (94.2%)	1.00 (0.98–1.01)	0.84
Age, years				
17–35	1,824 (47.9%)	1,728 (94.7%)	ref	-
36–50	1,090 (28.6%)	1,015 (93.1%)	0.98 (0.96–1.00)	0.08
51–60	673 (17.7%)	636 (94.5%)	1.00 (0.98–1.02)	0.82
61–69	219 (5.8%)	208 (95%)	1.00 (0.97–1.04)	0.88
Charlson Comorbidity Index^b				
0	3,664 (96.3%)	3,454 (94.3%)	ref	-
>0	142 (3.7%)	133 (93.7%)	0.99 (0.95–1.04)	0.77
Blood type				
O	1,640 (43.1%)	1,540 (93.9%)	ref	-
B	401 (10.5%)	382 (95.3%)	1.01 (0.99–1.04)	0.26
A	1,545 (40.6%)	1,456 (94.2%)	1.00 (0.99–1.02)	0.69
AB	169 (4.4%)	161 (95.3%)	1.01 (0.98–1.05)	0.43

^a Binomial regression, univariate analyses

^b Charlson Comorbidity Index was determined as cumulated score 10 years before antibody testing

^c First wave: March to May 2020. Second Wave: November 2020 to January 2021

4,379/100,000 person-years for the seronegative group, we estimate that 1 out of the 7 “re-infected” individuals initially tested false-positive.

Discussion

In this study, we found a consistent and persistently high sensitivity of the total SARS-CoV-2 antibody assay, Wantai, up to 15 months after infection. Re-infection occurred in 0.6% of our study participants and was associated with having a negative antibody test.

The main strengths of this study are the large sample size and nationwide design using national health registers and the screening for SARS-CoV-2 antibodies in a large cohort of Danish blood donors. After donation, blood donors had access to their serological results which may have affected their behavior. Most likely, the individuals’ social behavior is affected by a positive antibody result, and they may have felt better protected against the risk of SARS-CoV-2 re-infection and engaged more in social activities compared with individuals with a negative antibody result.

It has previously been shown that individuals with COVID-19 develop detectable antibodies lasting for 1 year (Feng et al., 2021). The current total immunoglobulin assay was previously documented to be sensitive for the detection of antibodies a couple of months after infection. Long-term antibody detection of the Wantai assay has, however, only been evaluated in minor selected populations (Bal et al., 2021) and sensitivities above 95% have been reported.

A recent Danish study including all Danish citizens found that 0.65% of the population experienced re-infection with SARS-CoV-2 between the first and second waves (Hansen et al., 2021) – a re-infection rate very similar to ours (0.6%) considering a higher infection rate among the 17-70-year old population and a longer follow-up period. This indicate that our results are generalizable to the target population.

We did not identify the risk factors of a negative antibody test. This may partly be owing to a large homogeneity in a blood donor population with regard to lack of comorbidity. Also, we did not know the severity of symptoms in individuals with a positive PCR test. Among Danish healthcare workers, a negative antibody test after verified SARS-CoV-2 infection was associated with self-reported asymptomatic infection or mild infection, and with body mass index ≥ 30 (Johannesen et al., 2021). In a study by Petersen LR et al., 6.3% of previously infected individuals did not develop detectable antibodies (Petersen et al., 2021). Here, lack of antibodies was associated with asymptomatic infections, immunosuppressive medications, race/ethnicity, and obesity. In our study, we did not have information on disease severity to perform this evaluation.

Antibody response may also depend on non-individual factors such as inoculation dosage and viral burden (Walker et al., 2021). Unfortunately, we did not have information on CT-levels for the PCR tests to make such an evaluation. We found no difference in test sensitivity during the first and second waves where different SARS-CoV-2 (non-delta) variants were dominant indicating that viral strain difference had little impact on the development of detectable antibodies. We estimated the number of “false positives” in the group of re-infected donors, but this estimate contains some uncertainty.

The presence of antibodies does not necessarily equal protective immunity, and antibody titers were not known. More than 60% of the re-infections reported here occurred among individuals with detectable antibodies. In contrast, the risk of re-infection was more than 9 times higher among individuals without antibodies when compared with individuals with antibodies. Individuals with previous SARS-CoV-2 infection, but with undetectable antibodies, have approximately the same risk of re-infection as individuals without previous exposure to SARS-CoV-2 (Hansen et al., 2021).

Conflicts of Interest

The authors declare no conflicts of interest.

Funding Statement

The Central Denmark Region supported this work. The Wantai tests were acquired by The Danish Health Authority requisitioned through the Statens Serum Institut. The funders had no role in performing this study.

References

- Bal A, Pozzetto B, Traub-Dietatz A, Escuret V, Rabilloud M, Langlois-Jacques C, et al. Evaluation of High-Throughput SARS-CoV-2 Serological Assays in a Longitudinal Cohort of Patients with Mild COVID-19: Clinical Sensitivity, Specificity, and Association with Virus Neutralization Test. *Clin Chem* 2021;67:742–52. doi:[10.1093/clinchem/hvaa336](https://doi.org/10.1093/clinchem/hvaa336).
- Barnkob MB, Pottegård A, Størvring H, Haunstrup TM, Homburg K, Larsen R, et al. Reduced prevalence of SARS-CoV-2 infection in ABO blood group O. *Blood Adv* 2020;4:4990–3. doi:[10.1182/bloodadvances.2020002657](https://doi.org/10.1182/bloodadvances.2020002657).
- Bastard P, Gervais A, Le Voyer T, Rosain J, Philippot Q, Manry J, et al. Autoantibodies neutralizing type I IFNs are present in ~4% of uninfected individuals over 70 years old and account for ~20% of COVID-19 deaths. *Sci Immunol* 2021;6:eabl4340. doi:[10.1126/sciimmunol.abl4340](https://doi.org/10.1126/sciimmunol.abl4340).
- Cordtz R, Lindhardtsen J, Soussi BG, Vela J, Uhrenholt L, Westermann R, et al. Incidence and severeness of COVID-19 hospitalisation in patients with inflammatory rheumatic disease: a nationwide cohort study from Denmark. *Rheumatology (Oxford)* 2020;keaa897. <https://doi.org/10.1093/rheumatology/keaa897>.
- Feng C, Shi J, Fan Q, Wang Y, Huang H, Chen F, et al. Protective humoral and cellular immune responses to SARS-CoV-2 persist up to 1 year after recovery. *Nat Commun* 2021;12:4984. doi:[10.1038/s41467-021-25312-0](https://doi.org/10.1038/s41467-021-25312-0).
- Hansen CH, Michlmayr D, Gubbels SM, Mølbak K, Ethelberg S. Assessment of protection against reinfection with SARS-CoV-2 among 4 million PCR-tested individuals in Denmark in 2020: a population-level observational study. *Lancet* 2021;397:1204–12. doi:[10.1016/S0140-6736\(21\)00575-4](https://doi.org/10.1016/S0140-6736(21)00575-4).
- Harrithøj LH, Gybel-Brask M, Afzal S, Kamstrup PR, Jørgensen CS, Thomsen MK, et al. Comparison of 16 Serological SARS-CoV-2 Immunoassays in 16 Clinical Laboratories. *J Clin Microbiol* 2021;59:e02596–20. doi:[10.1128/JCM.02596-20](https://doi.org/10.1128/JCM.02596-20).
- Johannesen CK, Rezahosseini O, Gybel-Brask M, Kristensen JH, Hasselbalch RB, Pries-Heje MM, et al. Risk Factors for Being Seronegative following SARS-CoV-2 Infection in a Large Cohort of Health Care Workers in Denmark. *Microbiol Spectr* 2021:e0090421. <https://doi.org/10.1128/Spectrum.00904-21>.
- Jones JM, Stone M, Sulaeman H, Fink RV, Dave H, Levy ME, et al. Estimated US Infection- and Vaccine-Induced SARS-CoV-2 Seroprevalence Based on Blood Donations, July 2020–May 2021. *JAMA* 2021. doi:[10.1001/jama.2021.15161](https://doi.org/10.1001/jama.2021.15161).
- Pedersen OB, Erikstrup C, Kotzé SR, Sørensen E, Petersen MS, Grau K, et al. The Danish Blood Donor Study: a large, prospective cohort and biobank for medical research. *Vox Sang* 2012;102:271. doi:[10.1111/j.1423-0410.2011.01553.x](https://doi.org/10.1111/j.1423-0410.2011.01553.x).
- Petersen LR, Sami S, Vuong N, Pathela P, Weiss D, Morgenthau BM, et al. Lack of Antibodies to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in a Large Cohort of Previously Infected Persons. *Clin Infect Dis* 2021;73:e3066–73. doi:[10.1093/cid/ciaa1685](https://doi.org/10.1093/cid/ciaa1685).
- SARS-CoV W. WANTAI SARS-CoV-2 Ab ELISA n.d. <https://www.fda.gov/media/140929/download> (accessed September 23, 2021).
- Walker AS, Pritchard E, House T, Robotham JV, Birrell PJ, Bell I, et al. Ct threshold values, a proxy for viral load in community SARS-CoV-2 cases, demonstrate wide variation across populations and over time. *Elife* 2021;10:e64683. doi:[10.7554/eLife.64683](https://doi.org/10.7554/eLife.64683).