Sanquin
Blood Supply

Performance of different antibody EIAs: results from testing serial samples spanning 10 months of follow-up

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TTID working party, subgroup Virology meeting, March 11th 2021

Blood and Beyond

Why serological testing for SARS-CoV-2 ?

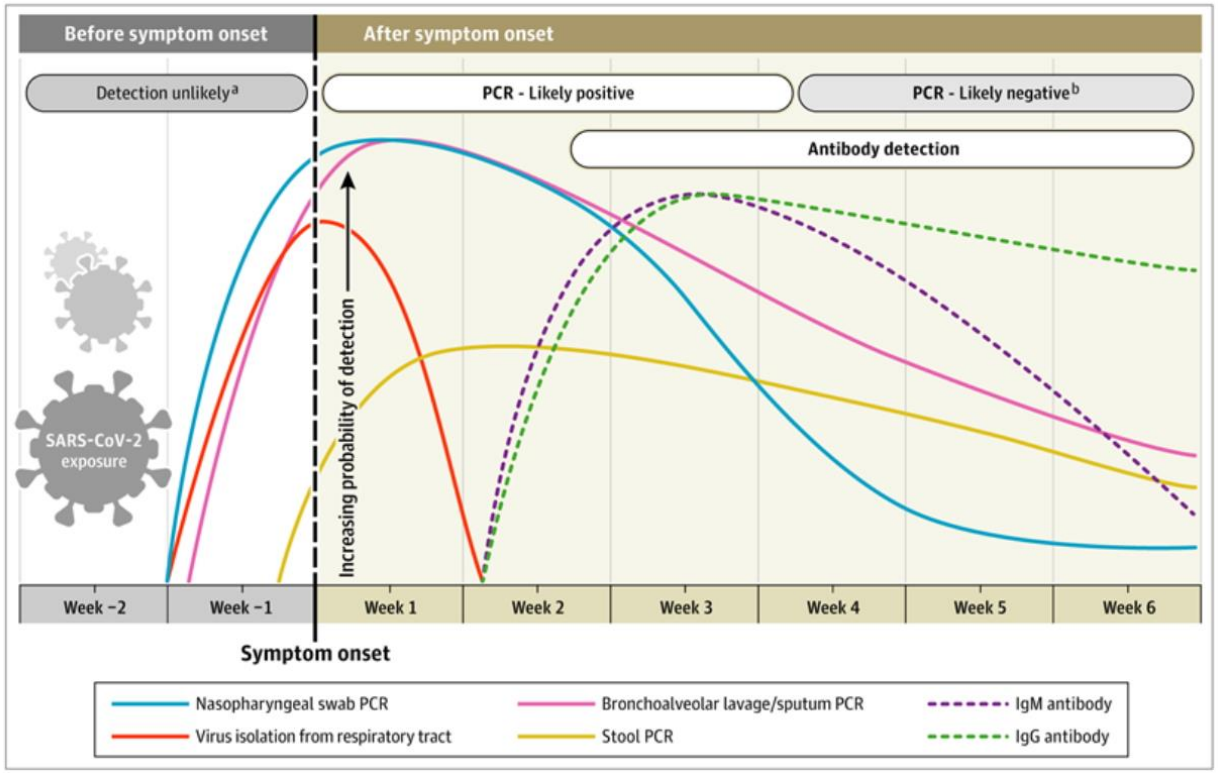
- Diagnosis (special cases, weeks after symptoms, Kawasaki disease, retrospective interpretation of symptoms)
- Monitoring of the outbreak
- Selection/qualification of donors
  - convalescent plasma (treatment)
  - IVIG (preventive, treatment)
- Durability of immune response
- Detection of reinfections
- Response to vaccination (both vaccination response and breakthrough infections, using different antigens)

Three levels of uncertainty

- Specificity
- Does ‘Ab positive’ mean immunity or protection from symptomatic disease?
- Sensitivity: how many people have no Ab response
  - Powerful local innate immunity?
  - Sterilizing cross-immunity from previous coronavirus infections?

SARS-CoV-2 testing

Figure. Estimated Variation Over Time in Diagnostic Tests for Detection of SARS-CoV-2 Infection Relative to Symptom Onset

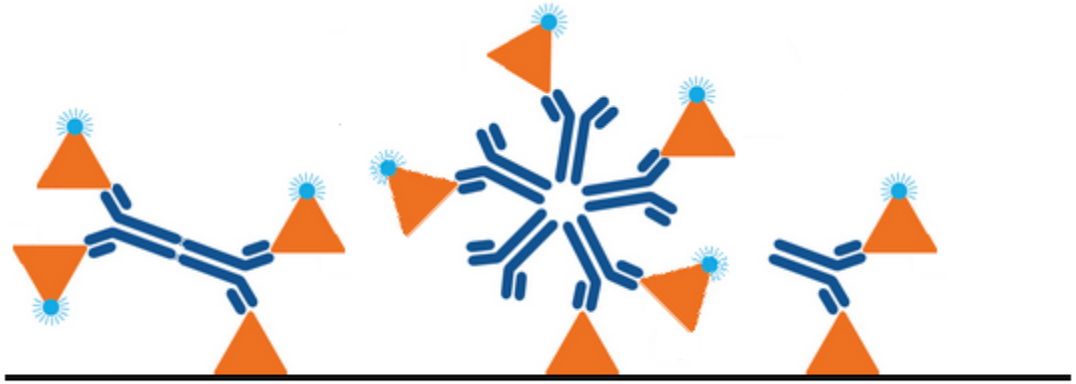


Sethuraman et al, JAMA, May 6 2020

- Diagnosis only based on PCR (or antigen) test
- Antibodies detectable ~1-2 weeks after onset
- Immune response strongly correlates with severity of symptoms

Types of serological tests

- Almost all SARS-CoV-2 ab tests use **Spike (S1, RBD)** antigen or **Nucleocapsid** antigen
- Total antibody tests ***vs*** IgA / IgM / IgG indirect EIAs
- Automated ***vs*** 96-well ***vs*** rapid tests
- Generally most sensitive: ‘double antigen sandwich’/ ‘bridging’ assay



double antigen sandwich / bridging assay

indirect ELISA

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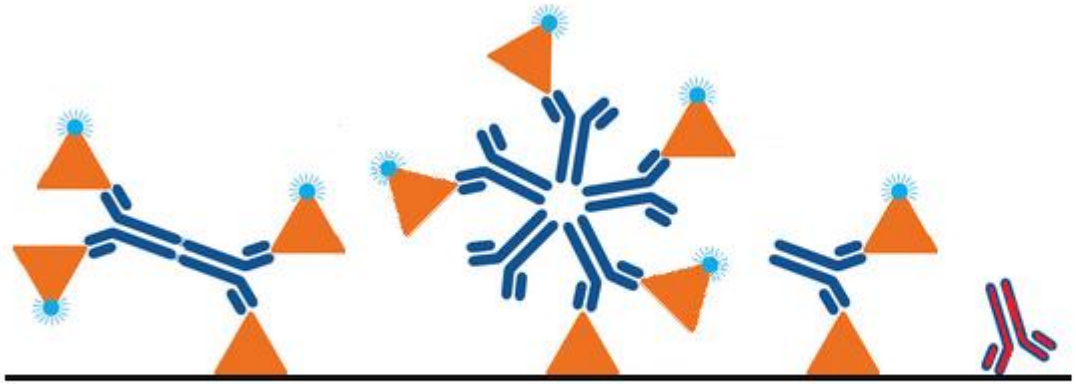
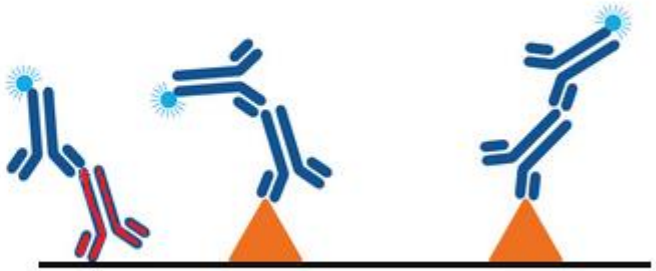


double antigen sandwich / bridging assay



indirect ELISA

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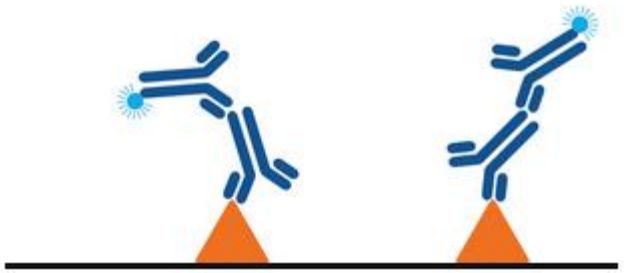
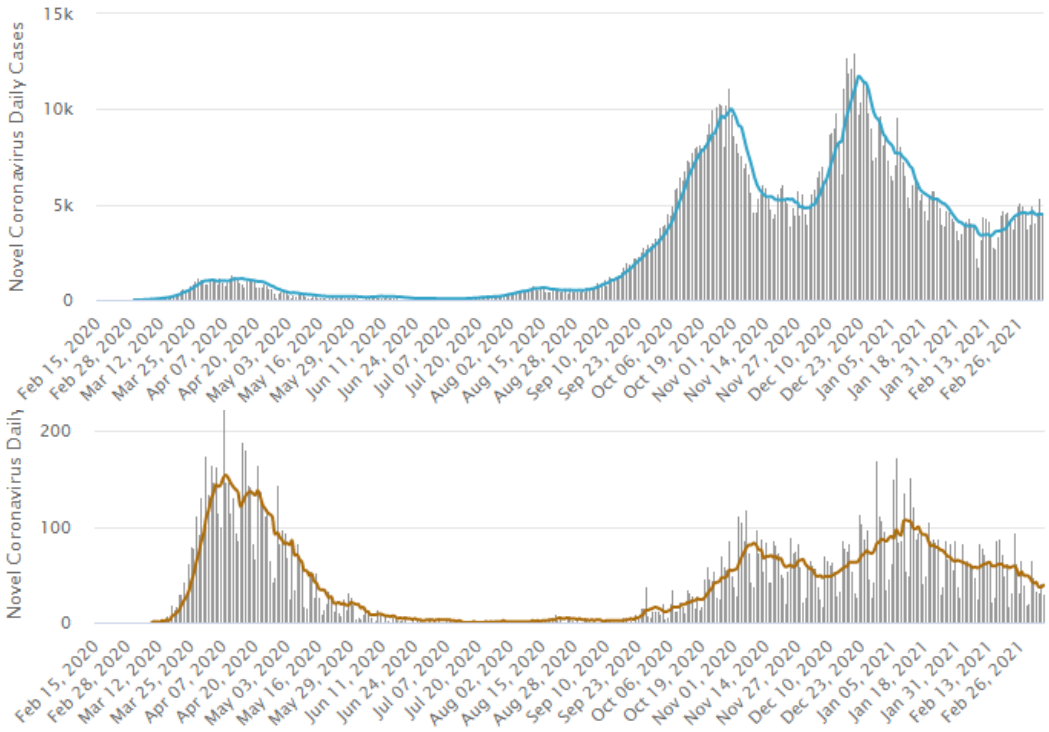
- Total antibody tests ***vs*** IgA / IgM / IgG indirect EIAs
- Automated ***vs*** 96-well
- Generally most sensitive: ‘double antigen sandwich’/ ‘bridging’ assay
- For selection of convalescent plasma donors: IgG test preferable
- Neutralization assay is golden standard (e.g. PRNT), but not possible at large scale
- IgG test, especially using Spike antigen correlates reasonably well with high neutralization titer



indirect ELISA

COVID-19 epidemic in the Netherlands



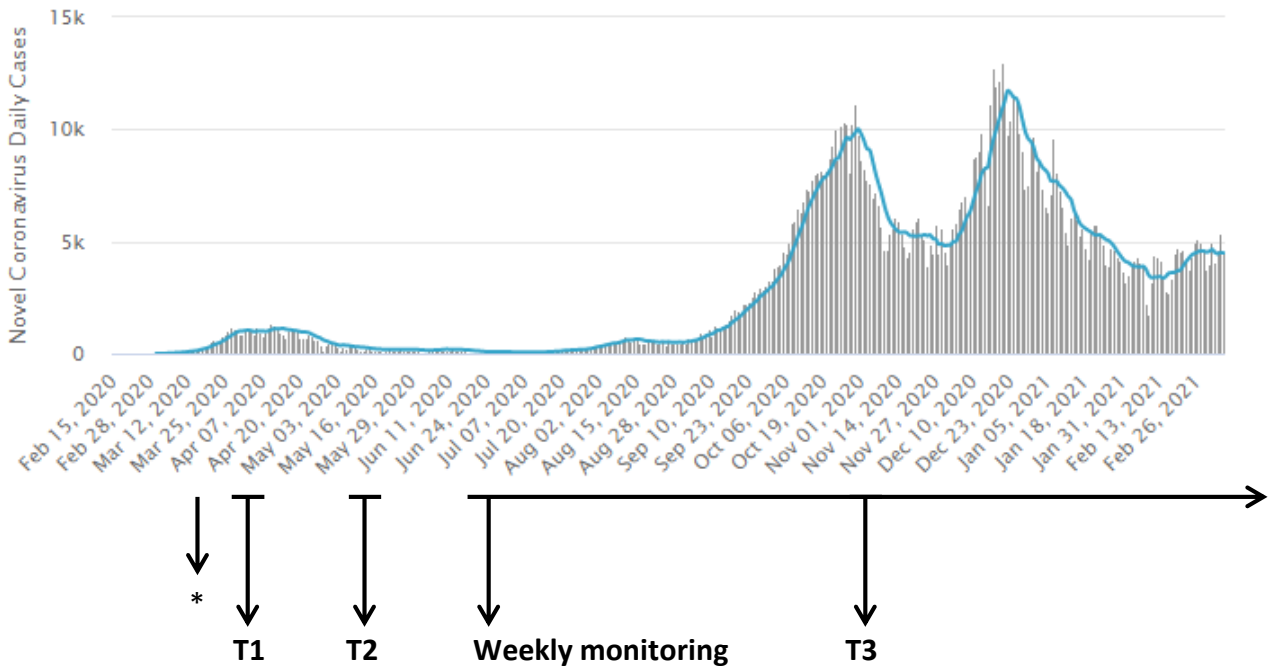
Daily Cases
(total: 1.120.075, March 07 2021)

Daily Deaths
(total: 15.833, March 07 2021)

Source:
Wordometer.info

- First case February 27th 2020
- Many cases imported after holiday in Italy, Austria; amplified by carnival
- Numer of deaths in first wave underestimated by factor ~2
- Low incidence June-Aug, measures reduced
- Second and third waves Oct-Dec 2020
- Despite more drastic measures hardly any reduction in no. of cases (UK variant)

COVID-19 research by dept. of Blood-borne Infections



***Decision to perform sero-survey, using Wantai total ab test**

T1: T1 study (testing 7361 plasma donors, April 1-10th)

T2: T2 study (testing 7154 plasma donors, May 10-20)

Weekly monitoring: screening of >2000 donations per week (selection based on age, sex and zip-code), starting June 22nd

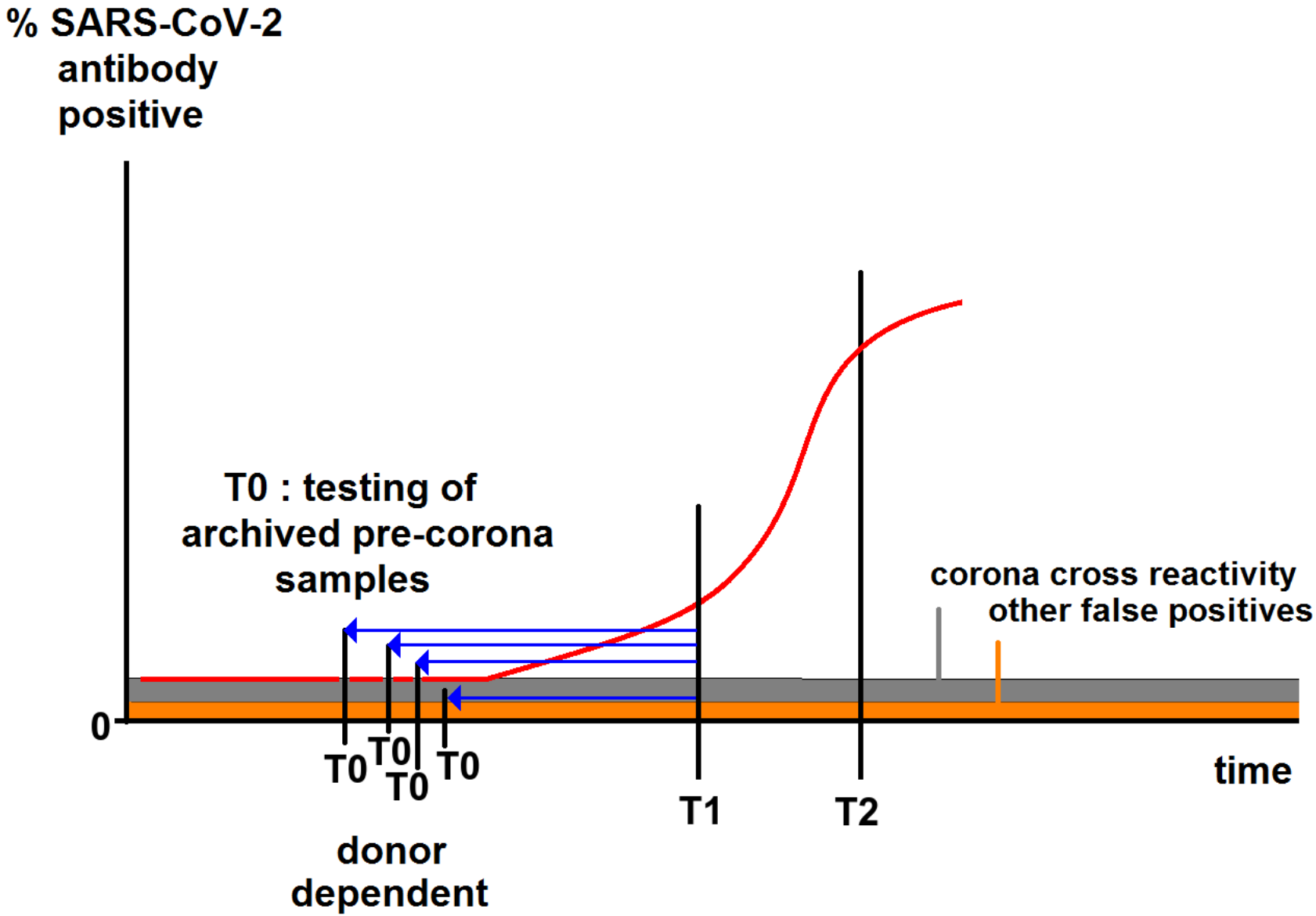
T3: T3 study (testing 8551 plasma donors, Nov 1-10)

As of March 7th 2021:

106,897 donations (from 70,022 donors) screened

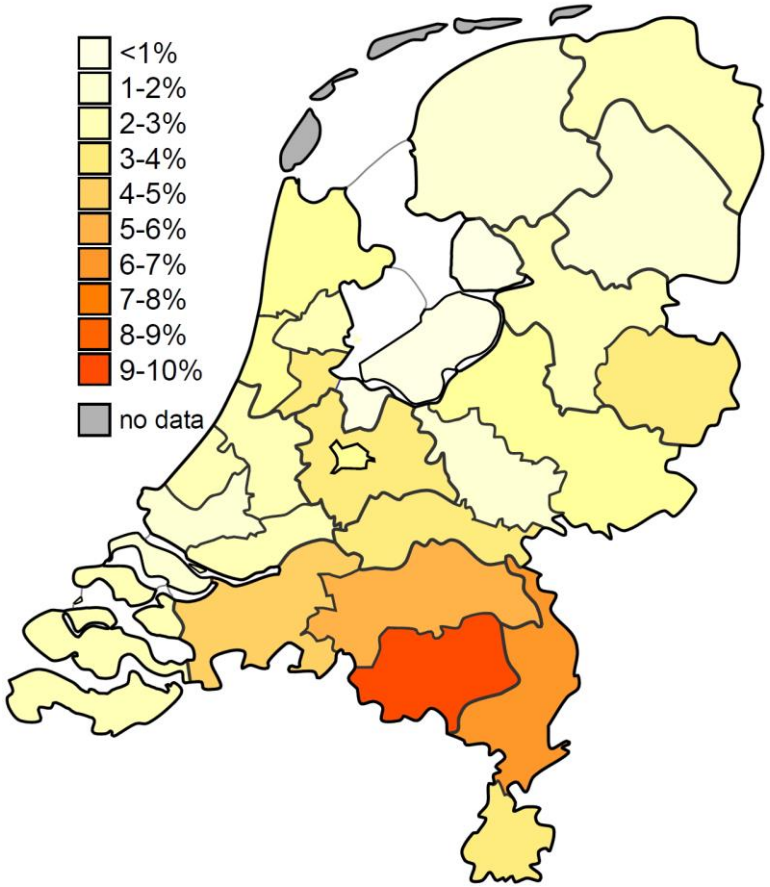
Serological follow up of 459 donors testing positive in T1 or T2 study using four additional commercial serological tests

SARS-CoV-2 antibodies in Dutch blood donors: study design



Results ‘T1’ study

- 230 / 7361 plasma donations repeat reactive (3.1%) in Wantai SARS-CoV-2 total Ab test
- 218 archive samples available: 188 seroconversions (86%)
- Thus: 14% is false positive ; specificity 99.7%
- Corrected prevalence: 2.7%

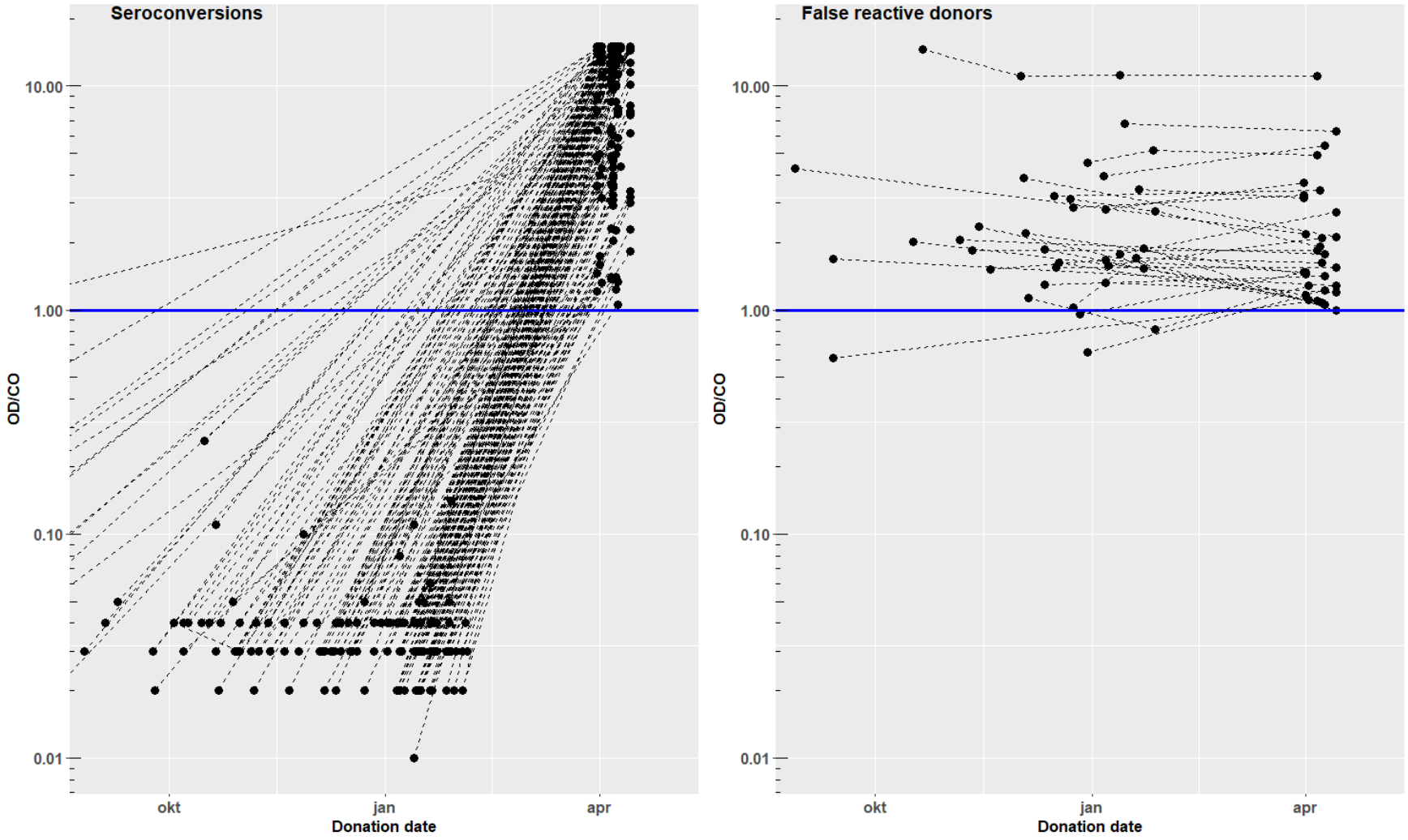


Positive predictive value of the test:

Seroprevalence	PPV
<2 %	72%
2-4 %	88%
>4 %	99%

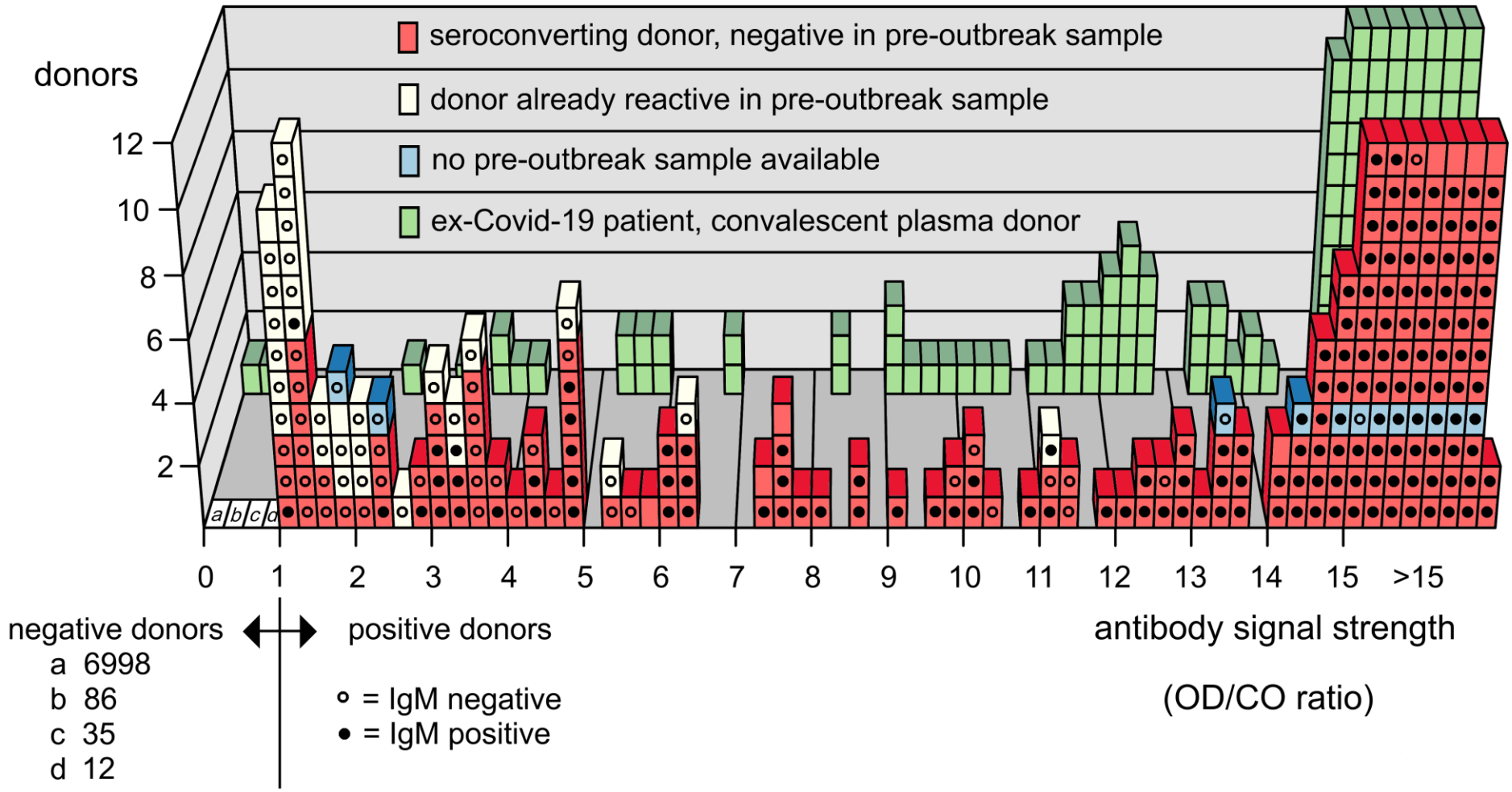
Slot et al, Nature communications, 11:5744

Results ‘T1’: seroconversions



Slot et al, Nature communications, 11:5744

Results ‘T1’: stronger signals in convalescent plasma donors



Slot et al, Nature communications, 11:5744

T2: seroprevelance study combinded with donor questionnaire

- All donors who donated between May 11 and May 18 invited for online questionaire
- Data complete for 3676 donors. 239 tested positive (6.5%)
- 38% reported no symptoms or minor symptoms
- Many symptoms correlated significantly with presence of antibodies
- **48% of positive donors did not suspect having had COVID-19**

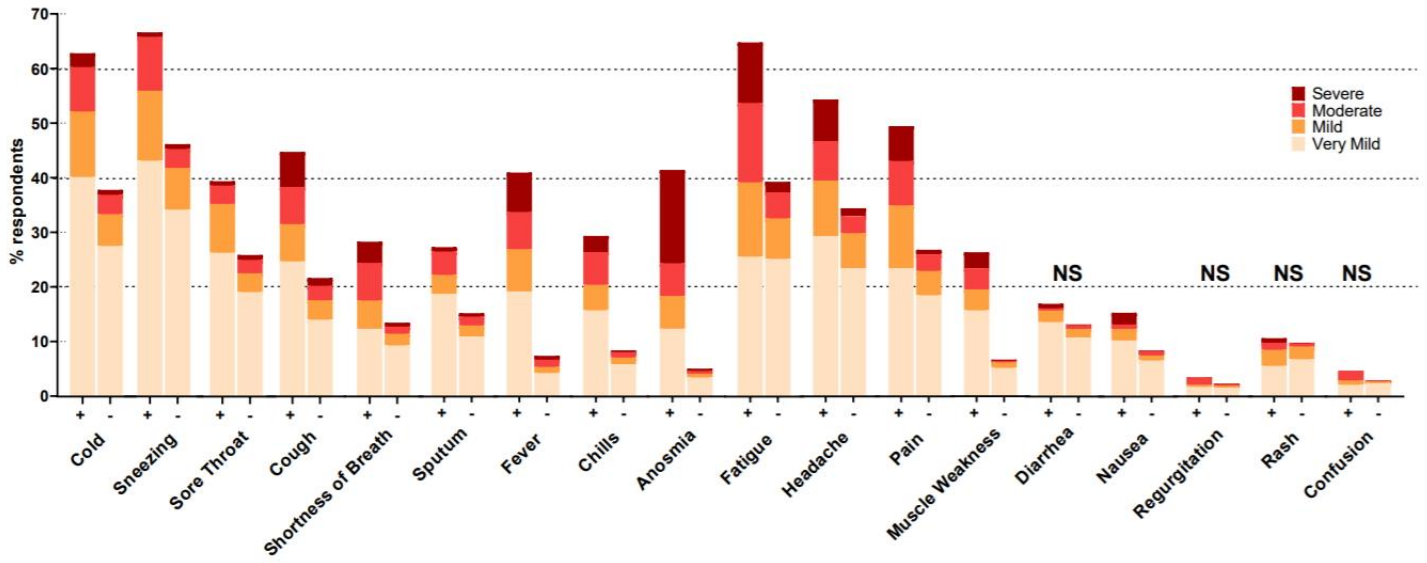
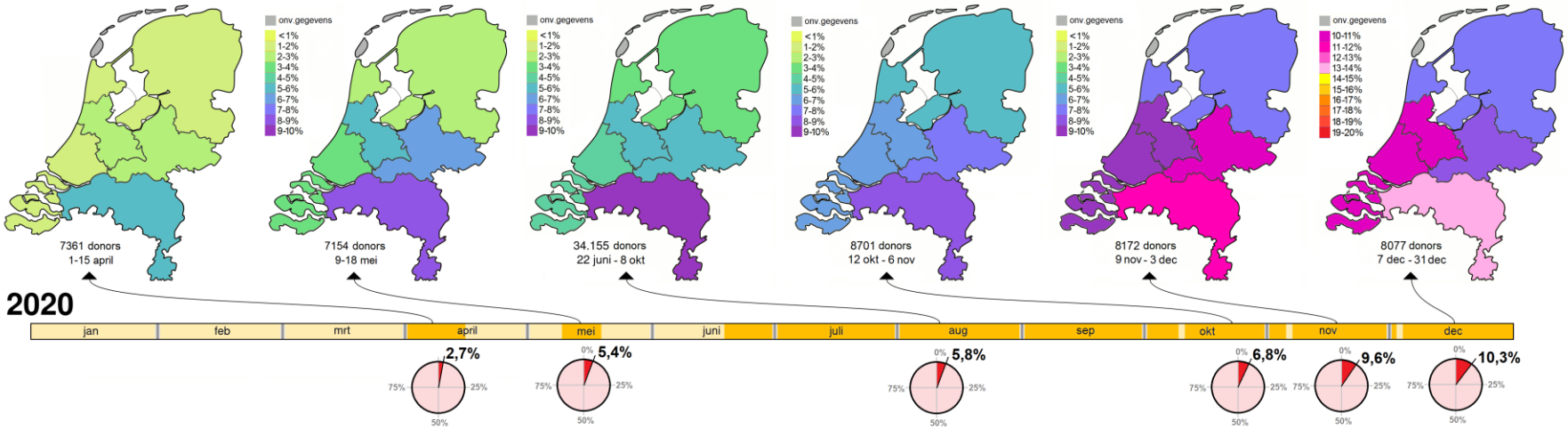


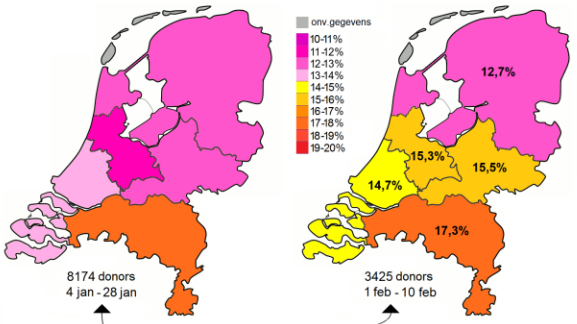
Figure 1. Percentages of individuals that reported very mild to severe symptoms by antibody status: positive (+) or negative (-). Age and sex-adjusted logistic regression models indicate higher prevalence in antibody positive versus –negative individuals for all symptoms ($p<0.001$, Table 2), except where indicated as not significant (NS). Anosmia refers to anosmia and/or dysgeusia, pain refers to muscle and/or joint pain.

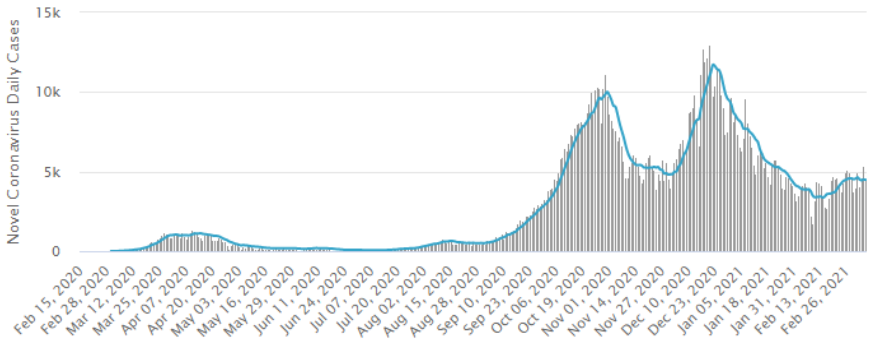
Van der Hurk et al, Cell Reports Medicine 2, 100222

Results ‘T2’ and later studies: overview

% donors met antistoffen tegen SARS-CoV-2.







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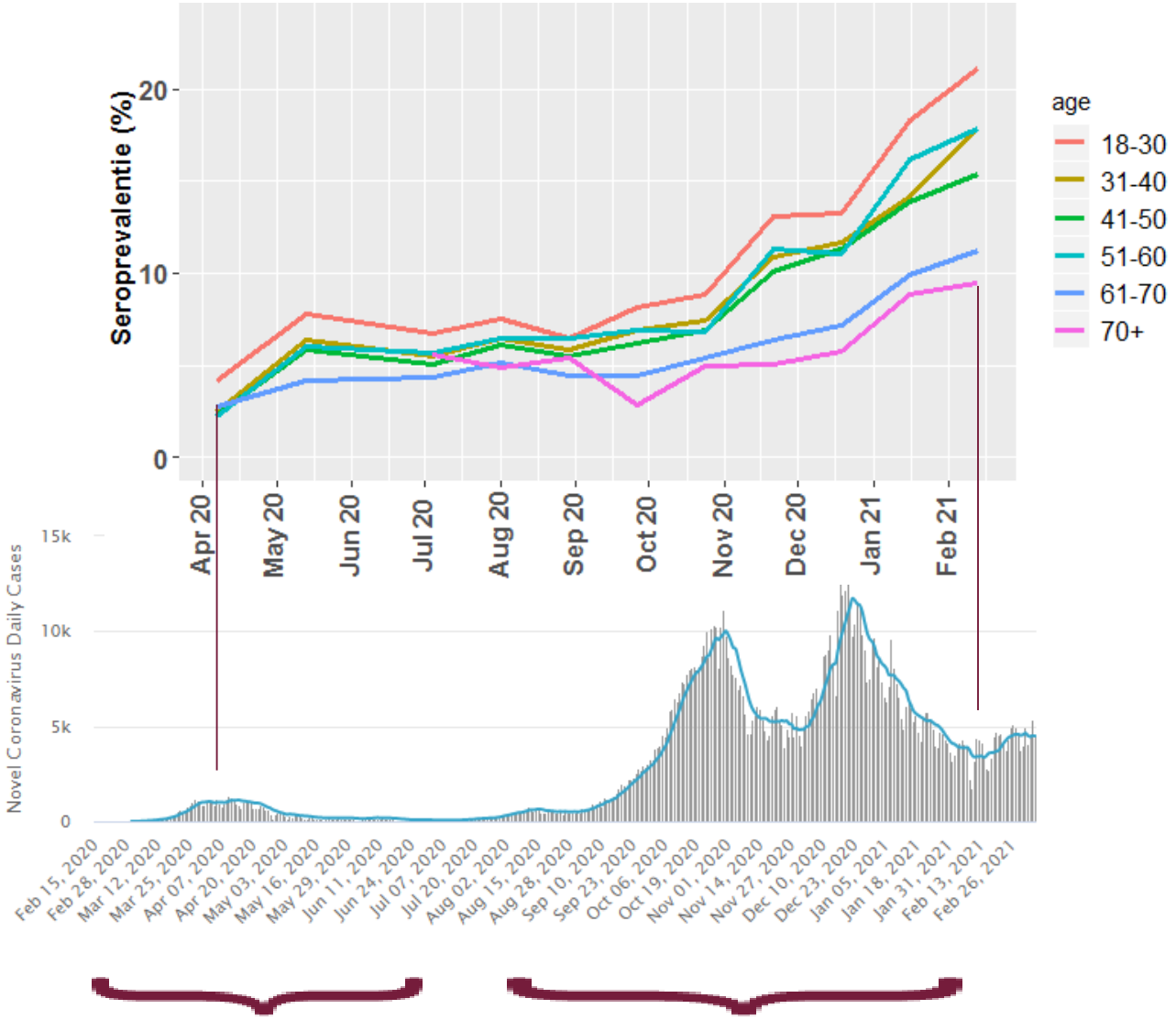

~5% of infections notified

~50-60% of infections notified

Comparison between tests / follow-up of positive donors

- >1650 samples from later donations from 459 T1/T2 positive donors were tested using the
  - Wantai SARS-CoV-2 total ab test (S1 RBD antigen)
  - Wantai IgM test (S1 RBD antigen)
  - Euroimmun IgA test (S1 antigen)
  - Euroimmun IgG test (S1 antigen)
  - Abbott Architect SARS-CoV2 IgG test (Nucleocapsid antigen)

- T2 study: no archive samples tested; false positive results not removed

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Euroimmun tests:

- Among the first tests on the market
- Specificity ~97% in pre-outbreak specimens
- Especially IgA: cross reactivity with other coronaviruses
- Sensitivity high, but limited seroconversion in patients with mild symptoms
- Little known about subclinical cases

Okba et al, Emerging infectious diseases, doi: 10.3201/eid2607.200841 (2020)

Abbott Architect test:

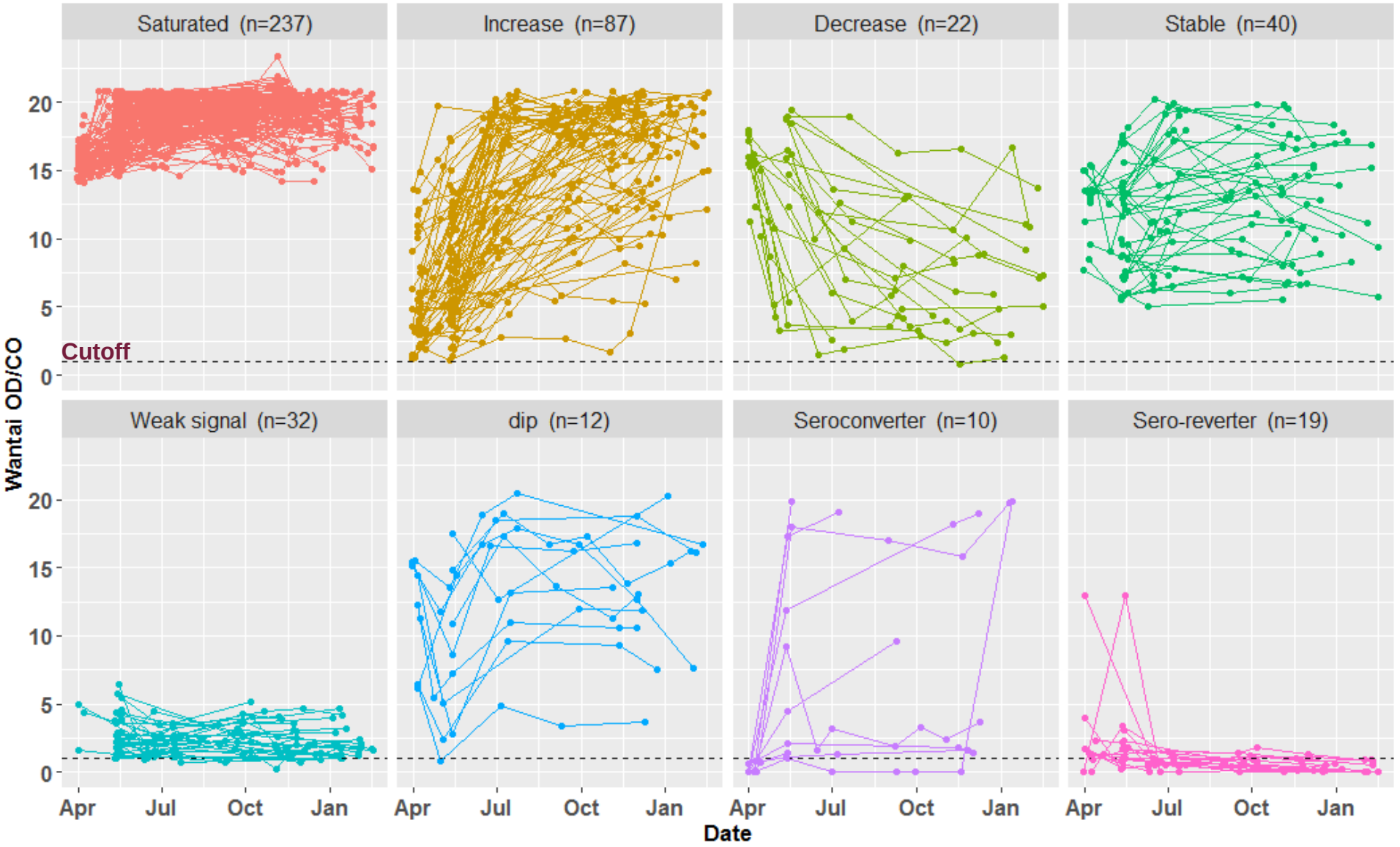
- Specificity 99.9% (n=1020)
- No cross reactivity with other coronaviruses
- Sensitivity 100% at day 17 after symptom onset
- No subclinical cases tested
- Rapid waning of antibody titers

Bryan et al J. Clin. Microbiol. Doi: 10.1128/JCM.00941-20 (2020)

Buss et al, Science, 10.1126/science.abe9728 (2020)

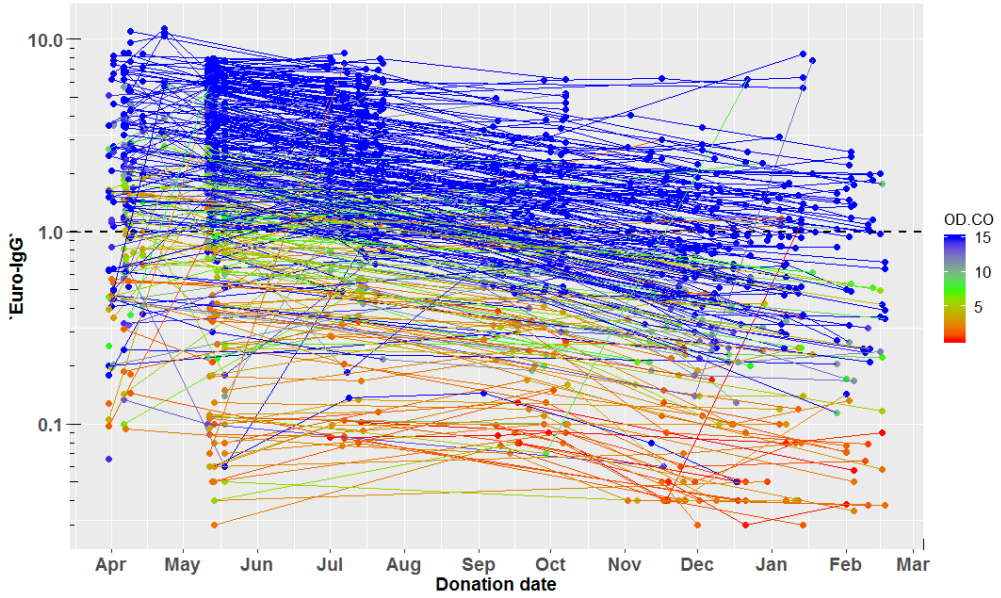
Follow-up of positive donors

- Follow up in 459 SARS-CoV-2 ab positive donors (median follow up 210 days, range 28-322 days)
- Responses were grouped in several categories

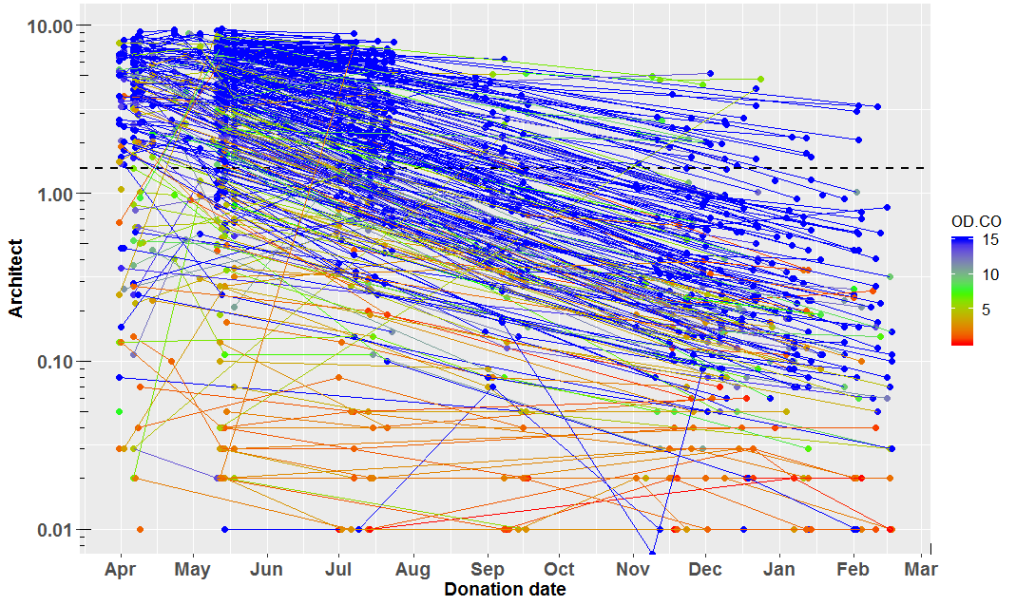


IgG responses in time

IgG response in time in the Euroimmun IgG and Abbott Architect IgG test



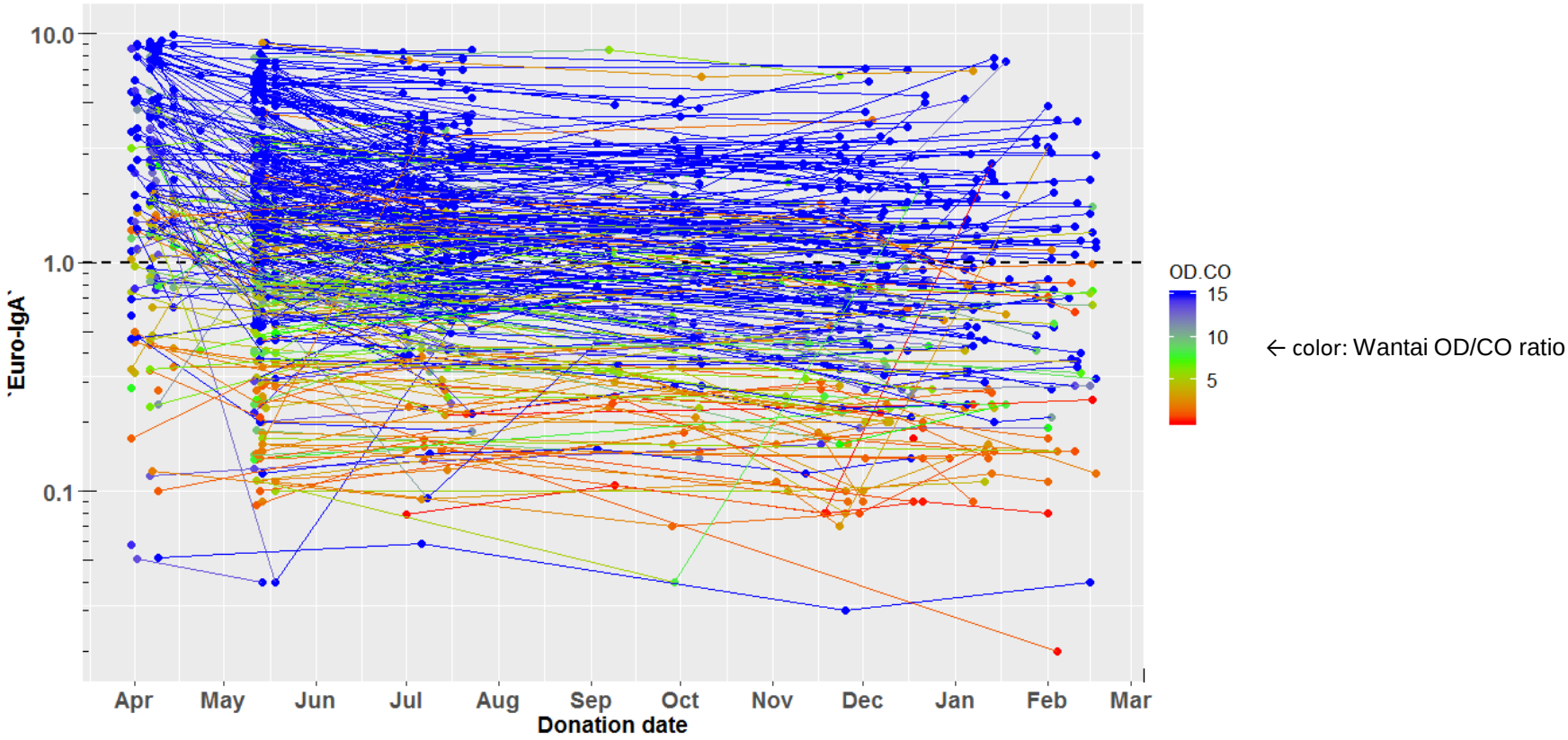
← color: Wantai OD/CO ratio



- Both IgG tests show similarly fast waning titers with
- Few saturated samples (even early on)
- (Much) stronger signals in convalescent plasma donors with PCR-proven infection

IgA responses in time

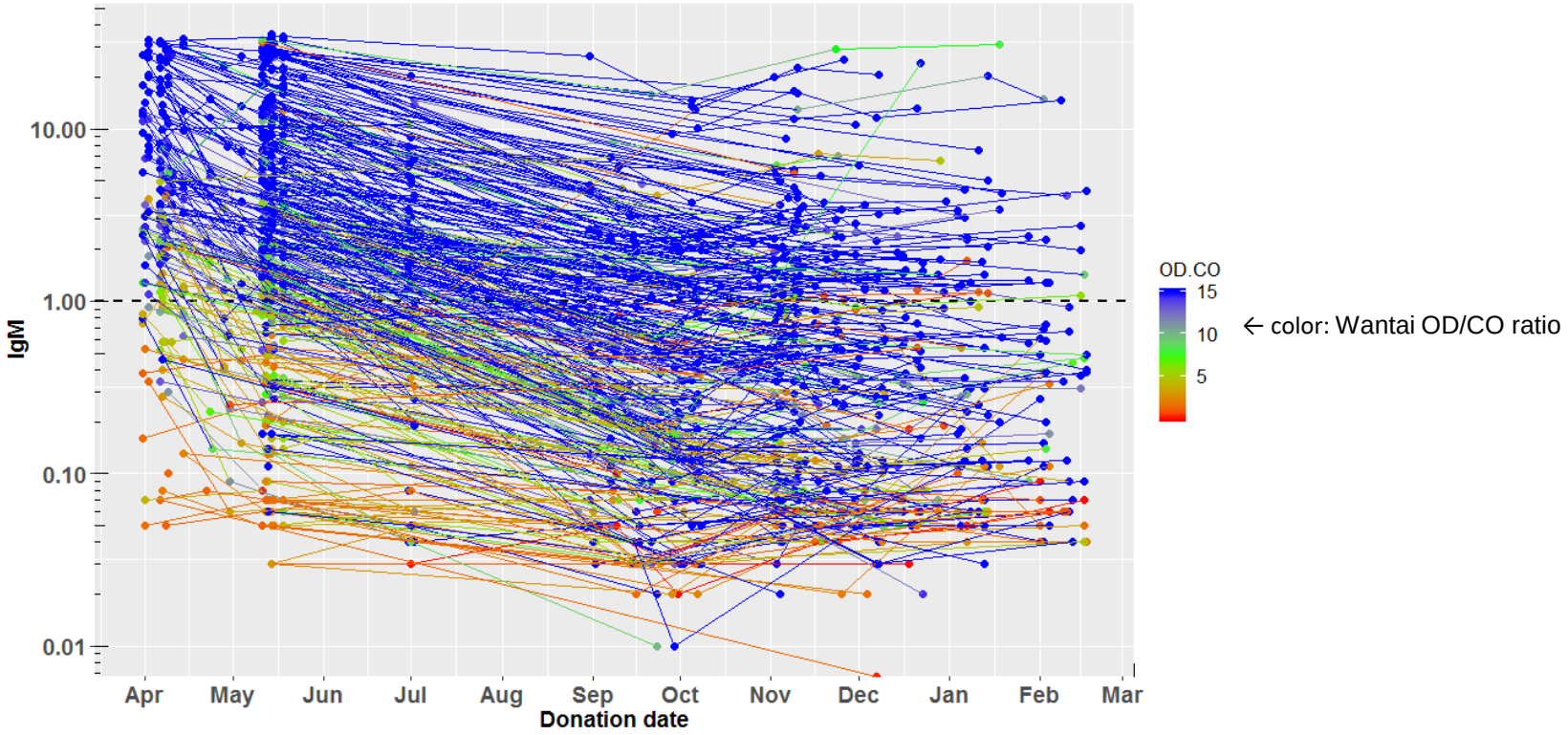
IgA response in the Euroimmun IgA test



- Overall: more variation in half-life times

IgM responses in time

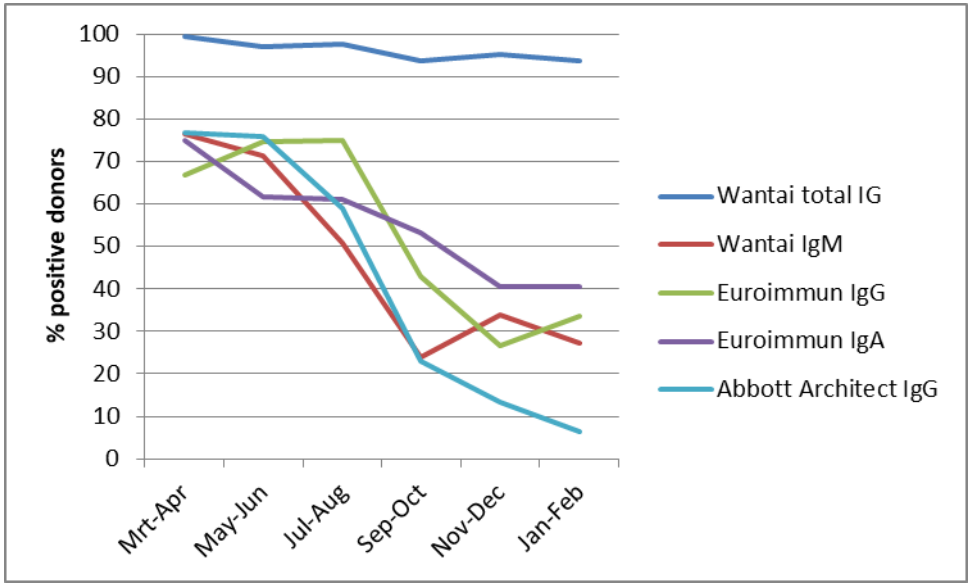
IgM response in Wantai IgM test



- Overall: titers wane, but significant percentage of donors shows sustained response

Summary

- Percentage of positive SARS-CoV-2 test results between March 2020 and February 2021 in donors who tested positive in the T1/T2 study

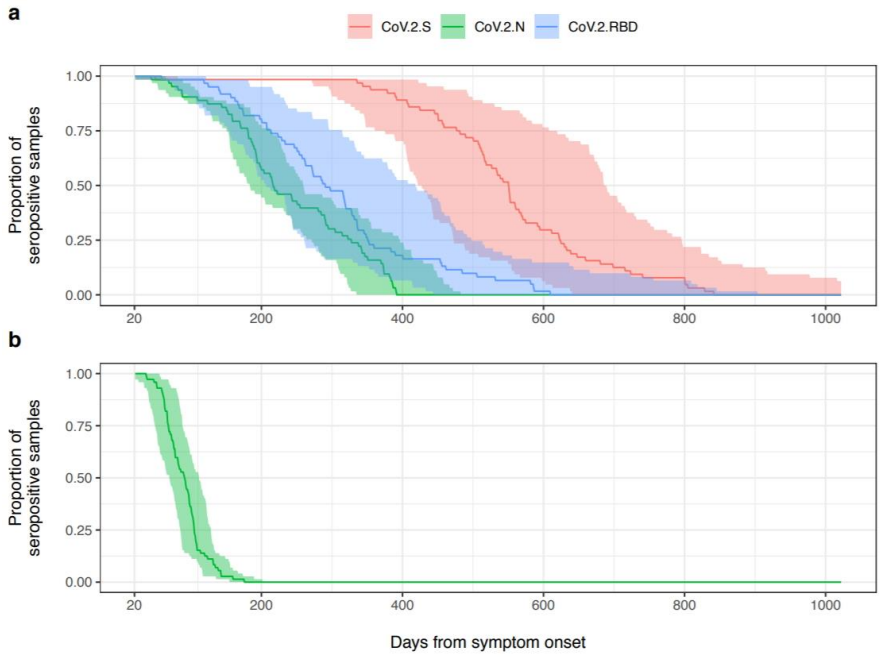


- Wantiai test superior for proving infection after longer period, probably because of format (and helped by the prolonged IgM response in many individuals)
- ~25% of the donors show no IgG response at any time
- Results may be very different for other cohorts (e.g. PCR proven infection)!

Waning of IgG responses

- Various publications point to rapid decay in IgG reponse

  Estimates:
- Half life of 36 days (Ibarrondo et al, NEJM, DOI: 10.1056/NEJMc2025179)
- Larger study: Grandjean et al (DOI:10.1101/2020.07.16.20155663), 61 patients
  - Estimated half life: Nucleocapsid 52 days, Spike / RBD: 83 days
  - The rate of decline may reduce over time
  - Extrapolated simulation **(!)** predicted the following time course:



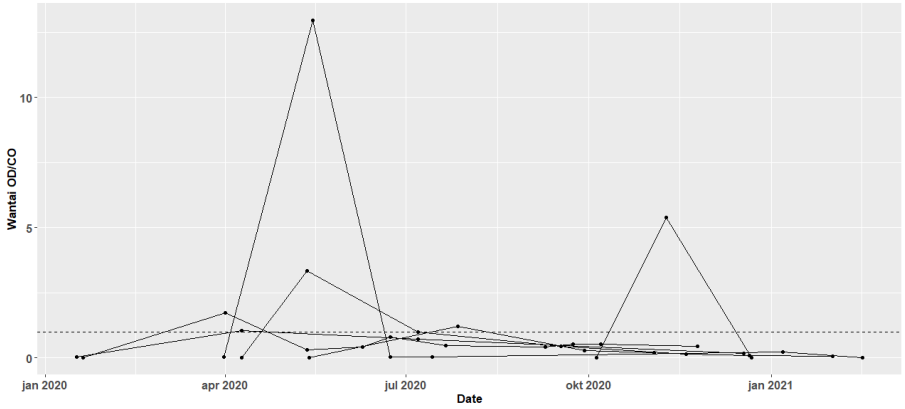
Grandjean et al
DOI:10.1101/2020.07.16.20155663

- Steenhuis et al: median half-life 62 and 59 days for anti-RBD and anti-nucleocapsid (151 donors)

  DOI:10.1101/2021.01.06.20249035

Uncertainties

- How does antibody response relate to immunity or (partial) protection?
- And: ***which*** antibody response?
- How many cases are missed even with antigen sandwich assays?
  - It's rare, but a few donors are repeat reactive in a single donation



Acknowledgements

- **Blood borne infections**
  - Michel Molier
  - Lisa Zürcher
  - Ed Slot
  - **Hans Zaaijer**

- **Donor studies**
  - Femmeke Prinsze
  - Jan Karregat
  - Eva-Maria Mertz
  - Marloes Spekman
  - Franke Quee
  - Steven Ramondt
  - Elisabeth Huis in ‘t Veld
  - **Katja van der Hurk**

- **Sanquin Natl. Screening lab**
  - Marco Koppelman
  - Johan IJlst
  - Ed Bakker