CORESMA - COVID-19-Outbreak Response combining E-health, Serolomics, Modelling, Artificial Intelligence and Implementation Research

WP 2	**Differential serolomics to assess sero-prevalence, cross and pre-existing immunity against coronaviruses**
Deliverable D2.9	**Report**
Title of Deliverable:	**Publications Submitted**
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Deliverable 2.9 Publications Submitted

Overview

The major aim of CORESMA work package 2 was the development of a multiplex serotest towards SARS-CoV-2 and the endemic "common cold" coronaviruses 229E, OC43, NL63 and HKU1 as serological immunoassays are essential tools to investigate previous pathogen exposures within populations as part of epidemiological studies, to understand the role of antibody responses in disease progression or to develop novel therapeutics or vaccines. In the precise context of CORESMA, the developed serotest was initially intended for assessing sero-prevalence of previous infections with coronaviruses and their implications for pre-existing, cross or partial immunity against COVID-19 by testing samples from the German National Cohort (NAKO) and Nepal. However, by now, the developed test MULTICOV-AB™ has been used in a much wider study context for instance, to analyze samples from the largest German SARS-CoV-2 seroprevalence study MUSPAD or to characterize differences in SARS-CoV-2 infection and vaccination responses in adults and children. As focus switched from antibody presence to antibody function, RBDCoV-ACE2, a competitive inhibition assay that allows functional analysis of SARS-CoV-2 antibodies in a cell- and virus-free screening format was developed. While both assays were dominantly utilized in vaccination response studies since multiple COVID-19 immunizations became available, both were also continuously expanded by variant of concern and variant of interest RBDs to accommodate the fast genomic evolution of the ancestral Wuhan isolate.

Overall, the immunoassays developed within WP2 of the CORESMA project have resulted in a significant scientific impact. MULTICOV-AB and RBDCoV-ACE2 have been utilized in over 25 publications with over 1200 citations to date (as of November 2023), including 3 publications that have already been cited more than 100 times each. Results included in these publications have also been included in bulletins of national (e.g. RKI) and international (e.g. CDC, WHO) health agencies. Multiple cooperations and partnerships with clinical, basic and industrial scientists and research groups were also established, resulting in a lasting infrastructure that is still being utilized in ongoing projects. Indispensable knowledge in the field of assay development has also been gained that will be utilized indefinitely to develop the next generation of immunoassays.

In the following sections, we will highlight some of the key publications from WP2.

SARS-CoV-2 assay development

Overall, 4 serotests were developed as part of the CORESMA project, of which MULTICOV-AB (Becker et al, 2021) and RBDCoV-ACE2 (Junker et al, 2022) were the most successful.

MULTICOV-AB one of the multiplex bead-based SARS-CoV-2 serotests enables not only an in-depth quantification of the antibody response against individual antigens, but also achieved higher sensitivity and specificity compared to three broadly used commercially available IVD-certified tests from Roche, EuroImmun and Siemens (Figure 1).

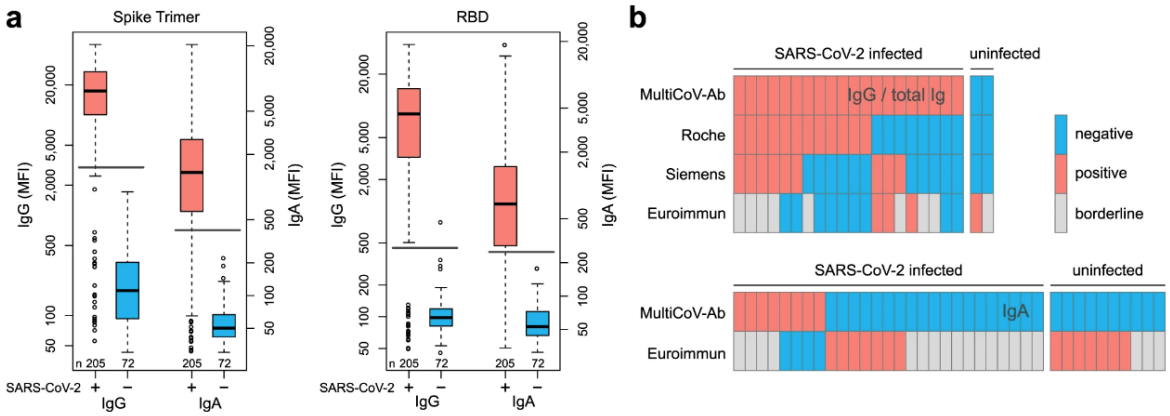

Figure 1: MULTICOV-AB assay performance for IgG and IgA based on the dual Spike and RBD cut-off (a) and increased performance compared to commercially available assays (b). Reproduced from Becker et al, 2021 - Exploring beyond clinical routine SARS-CoV-2 serology using MultiCoV-Ab to evaluate endemic coronavirus cross-reactivity. Published in Nature Communications.

RBDCoV-ACE2, a competitive inhibition assay, enables investigation into neutralizing antibodies elicited through either infection or vaccination making it particularly valuable as RBD-targeting neutralizing antibodies are considered the most promising correlate of protective immunity and were successfully used for prevention and therapy. By mimicking the interaction between ACE2 and the RBD in a multiplex format, inhibition against all variants of concern/interest could be investigated, providing not only a highly scalable, time-, cost- and material-saving alternative to classical infectious live-virus neutralization assays, but also to precisely investigate the effect of multiple immune-escaping variants of concern on protective immunity in parallel (Figure 2).

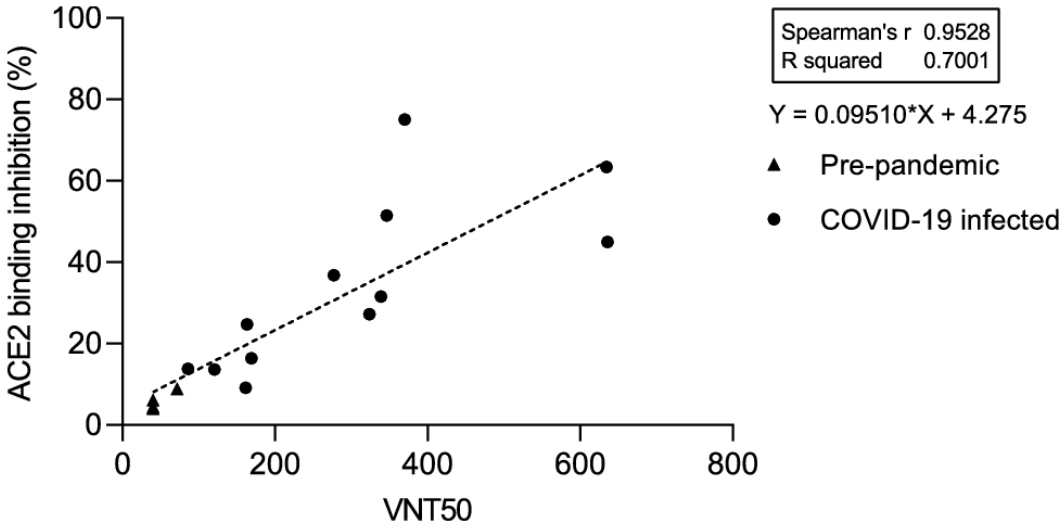

Figure 2: Correlation of RBDCoV-ACE2 with a virus neutralization assay. Reproduced from Junker et al, 2022 - COVID-19 patient serum less potently inhibits ACE2-RBD binding for various SARS-CoV-2 RBD mutants. Published in Scientific Reports.

Vaccine Development

MULTICOV-AB and RBDCoV-ACE2 have been used in a variety of industrial and academic vaccine research projects, some of which are still ongoing. Both assays were technically validated for use across multiple species such as human, rat and mouse. As a result, they can be utilized across all phases of vaccine development ranging from preclinical efficacy in animal models to the clinical development phases 1-4.

As initial vaccine targets focused on developing a pan-reactive SARS-CoV-2 vaccine which could provide protection against other coronaviruses, MULTICOV-AB was used to assess whether cross-reactive endemic coronavirus B- and T-cell responses correlated, and as such, whether the SARS-CoV-2 peptides examined were stimulating a non-specific response (Figure 3). The lack of correlation enabled identification of functional T-cell epitopes which were later utilized as a basis for developing a peptide-based vaccine.

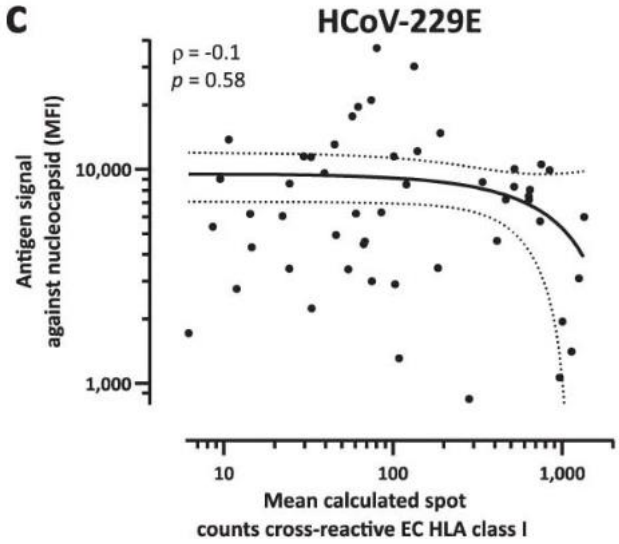

Figure 3: No correlation between antibody titers against endemic coronaviruses and the intensity of cross-reactive $CD4^+$ and $CD8^+$ T-cell responses against SARS-CoV-2. Reproduced from Nelde et al, 2021 - SARS-CoV-2-derived peptides define heterologous and COVID-19-induced T cell recognition. Published in Nature Immunology.

MULTICOV-AB was also used in the preclinical development of a recombinant modified vaccinia virus Ankara (MVA) expressing native full-length SARS-CoV-2 S protein. Antibody responses were here profiled following vaccination, leading to the identification of a predominantly S2-biased response. RBDCoV-ACE2 is currently utilized in the ongoing preclinical development of a next generation omicron-derived SARS-CoV-2 vaccine with a national vaccine developer.

Vaccine-derived immunity

Following on from the rapid development and deployment of SARS-CoV-2 vaccines at the end of 2020, MULTICOV-AB was immediately used to assess humoral responses in a cohort of

healthcare workers (Figure 4). While the multi-analyte format of MULTICOV-AB allowed the direct differentiation between responses generated by vaccination and infection (Figure 4a) the assay's sample matrix was also expanded from serum and plasma to also include saliva. Making it therefore possible to analyze mucosal immune protection at the primary site of infection (Figure 4b and c). The publication also highlighted the importance of receiving a second dose vaccination dose to boost humoral responses (Figure 4d).

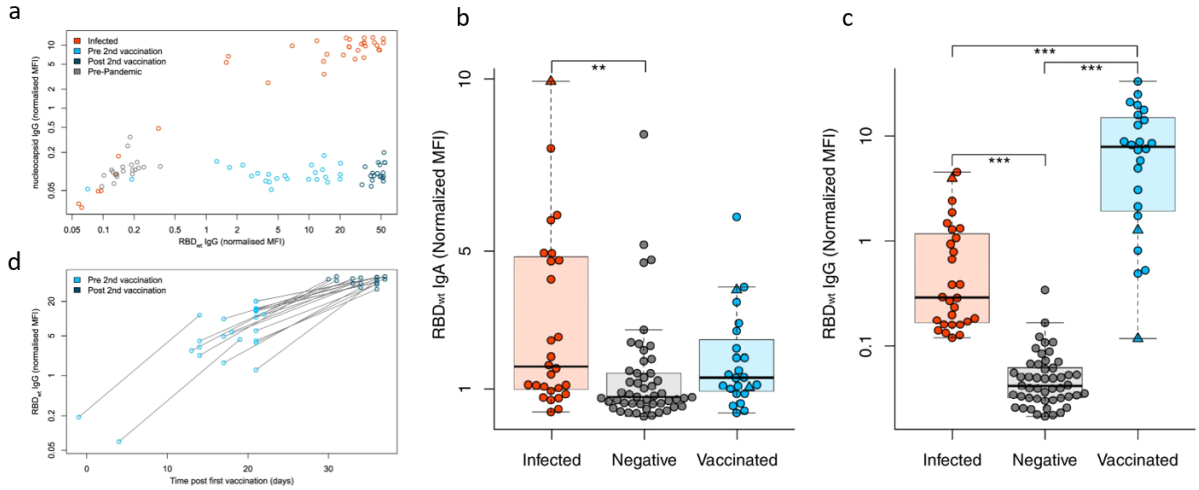

Figure 4: Dual antigen-specific detection to differentiate vaccination and infection-elicited humoral responses, to assess salivary IgG responses and to quantify the effect of the second dose on antibody titers. Reproduced from Becker et al, 2021 – Immune response to SARS-CoV-2 variants of concern in vaccinated individuals. Published in Nature Communications.

As the delta variant of concern resulted in the first wave of breakthrough infections, we utilised both serotests to assess antibody titers and ACE2 binding inhibition generated by all then in Germany approved immunizations regimens within a large population-based cohort, to comprehensively assess the duration and quality of immune response offered by the different vaccines and dosing schemes. We found that homologous mRNA-based or heterologous prime-boost vaccination produced significantly higher antibody responses than vector-based homologous vaccination (Figure 5). Performance of Ad26.CoV2S.2 manufactured by Janssen was particularly concerning with reduced titres and 91.7% of samples classified as non-responsive for ACE2 binding inhibition, suggesting that recipients required a booster mRNA vaccination, which was later recommended not only by the German STIKO.

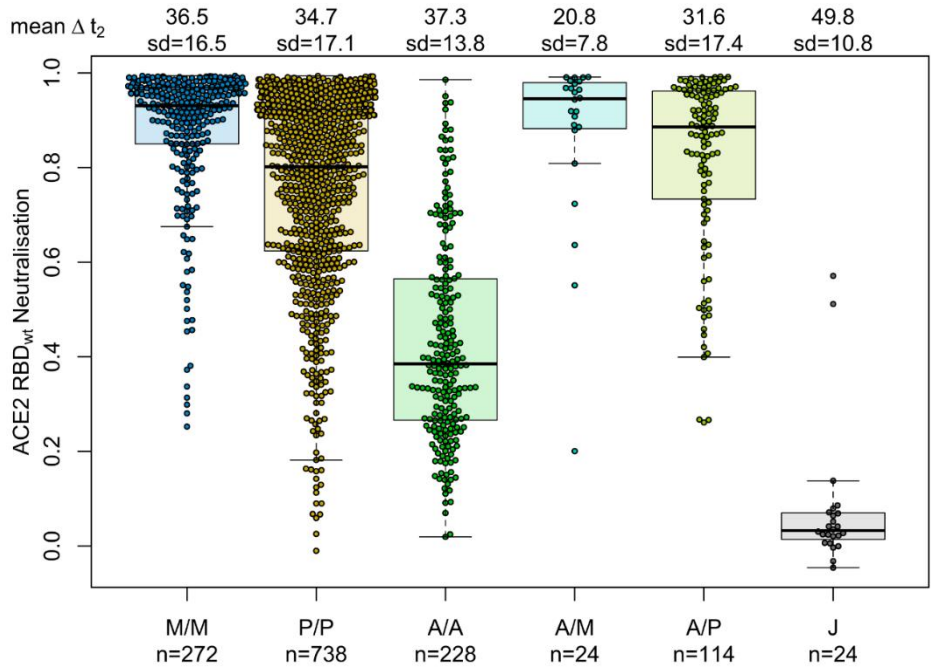

Figure 5: Different SARS-CoV-2 vaccination schemes result in distinct humoral responses. M-Moderna, P-Pfizer, A-AstraZeneca, J-Janssen. Reproduced from Dulovic et al, Comparative Magnitude and Persistence of Humoral SARS-CoV-2 Vaccination Responses in the Adult Population in Germany. Published in Frontiers in Immunology.

While mRNA vaccination induced a higher ratio of RBD- and S1-targeting antibodies, vector-based vaccines resulted in an increased proportion of S2-targeting antibodies providing an interesting molecular link towards the different clinical efficiencies seen by those vaccines (Figure 6). Previously infected individuals had a robust immune response once vaccinated, regardless of which vaccine they received, while overall, both titres and ACE2 binding inhibition peaked approximately 28 days post-second vaccination and then decreased.

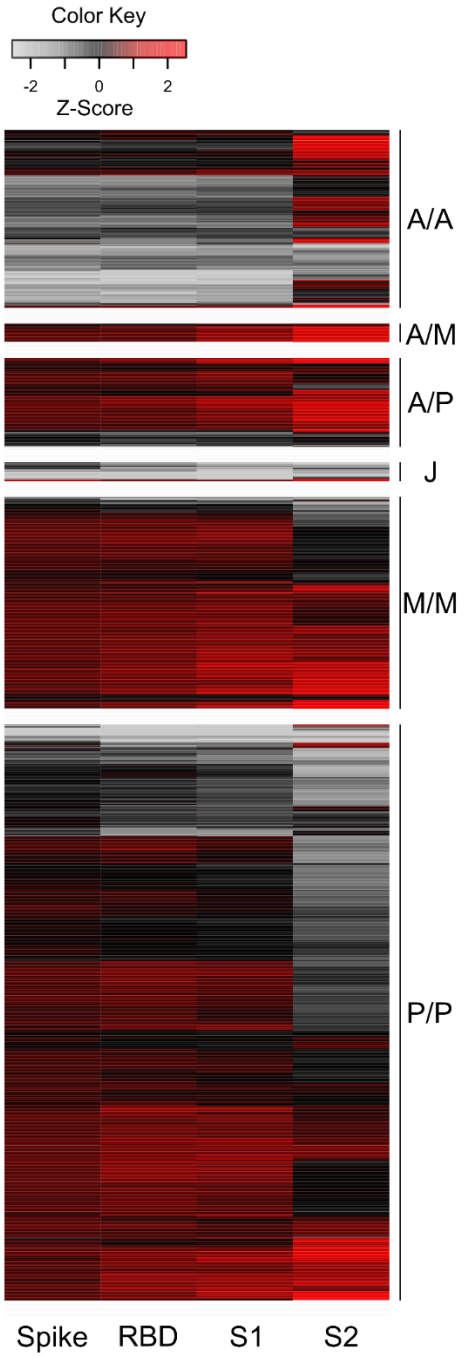

Figure 6: Humoral immune response after mRNA vaccination is skewed towards increased RBD and S1 titres, while vector-based vaccination results in increased S2 antibody levels. Reproduced from Dulovic et al, Comparative Magnitude and Persistence of Humoral SARS-CoV-2 Vaccination Responses in the Adult Population in Germany. Published in Frontiers in Immunology.

We also investigated vaccine-elicited responses in various at-risk cohorts for severe COVID19 disease. Initially, we found that patients on maintenance haemodialysis exhibited detectable but variable cellular and humoral immune responses against SARS-CoV-2 and variants of concern after a two-dose regimen of BNT162b2. Although vaccination-induced immunoglobulins were detectable in saliva and plasma, both anti-SARS-CoV-2 IgG and

neutralization efficacy was reduced compared to a vaccinated non-dialysed control population (Figure 7a-e). Similarly, T-cell mediated interferon γ release after stimulation with SARS-CoV-2 spike peptides was significantly diminished.

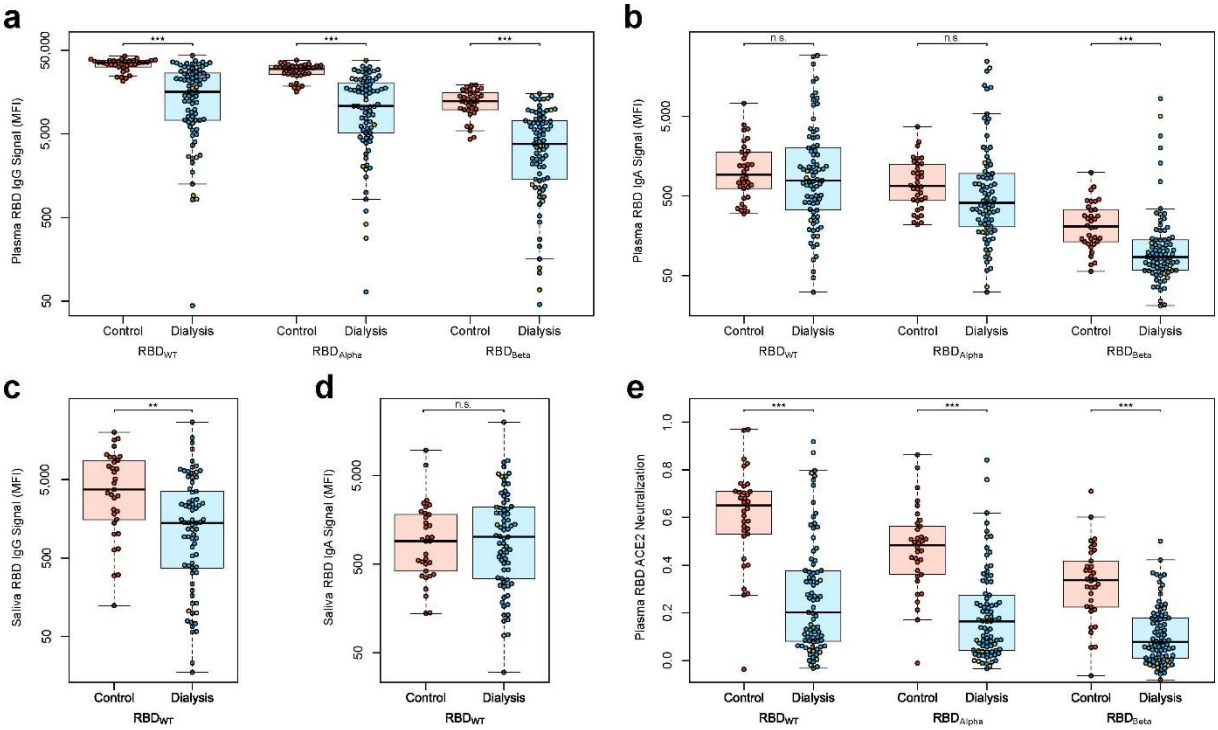

Figure 7: Humoral immune response in haemodialysed individuals after vaccination with Pfizer BNT162b2. Reproduced from Strengert et al, 2021 - Cellular and humoral immunogenicity of a SARS-CoV-2 mRNA vaccine in patients on haemodialysis. Published in Lancet Ebiomedicine.

We then followed the same cohort, identifying in July 2021 that a significant decrease in vaccine-elicited immunity was occurring months after the second vaccination. Of particular concern was that some dialysis patients exhibited no detectable B-or T-cell responses at all anymore. Critically however, even the control group consisting of healthcare workers had a decrease of approx. 70% in antibody titer and inhibitory capacity (Figure 8).

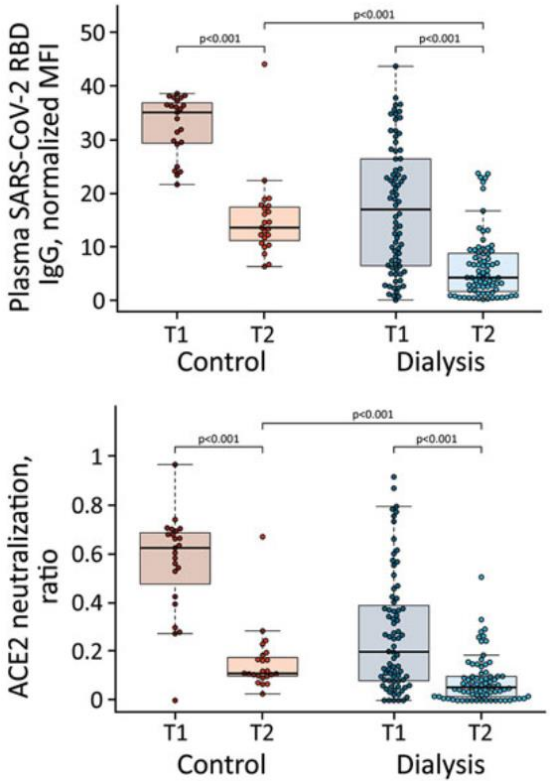

Figure 8: Diminishing immune responses post second vaccination in both dialysis and control cohorts. Reproduced from Dulovic et al, 2022 - Diminishing Immune Responses against Variants of Concern in Dialysis Patients 4 Months after SARS-CoV-2 mRNA Vaccination. Published in Emerging Infectious Diseases.

With booster vaccinations for a third, fourth or now even a fifth dose, we continued to monitor this cohort of dialysis patients by analysing both their humoral and cellular immune responses. After triple BNT162b2 vaccination, anti-RBD B.1 IgG and ACE2 binding inhibition reached peak levels in dialysis patients, but remained inferior compared to controls (Figure 9). Whilst we detected B.1-specific ACE2 binding inhibition in 84% of dialysis patients after three BNT162b2 doses, binding inhibition towards the then circulating BA.1 Omicron variant was only detectable in 38% of samples and declining to 16% before the fourth vaccination. Reassuringly, following a fourth dose, humoral immunity against all SARS-CoV-2 variants tested was strongly augmented with 80% of dialysis patients having Omicron-specific ACE2 binding inhibition (Figure 10). Modest declines in T-cell responses in dialysis patients and controls after the second vaccination were restored by the third BNT162b2 dose and significantly increased by the fourth vaccination.

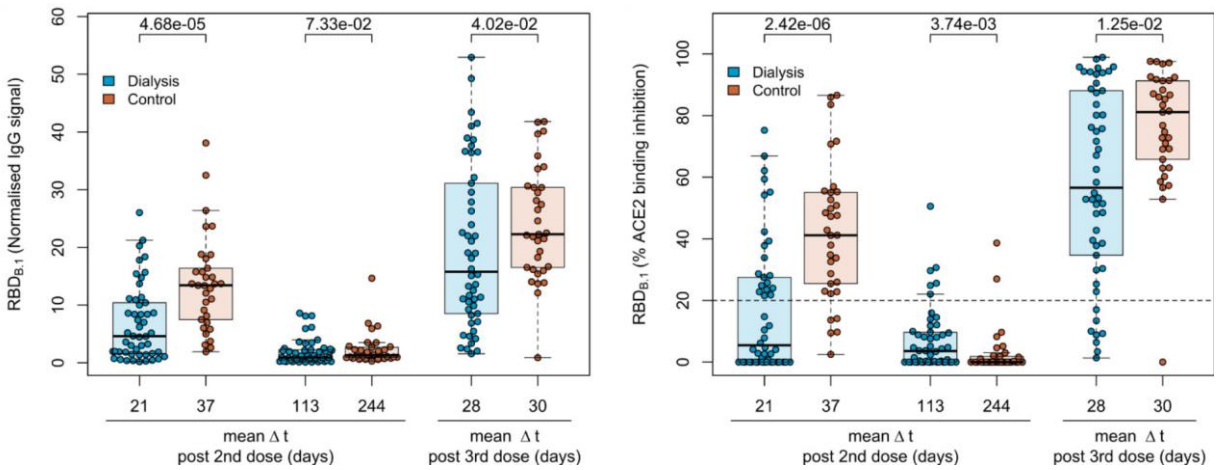

Figure 9: Humoral immune response in haemodialysis patients after a triple vaccination with BNT162b2. Reproduced from Becker et al, 2022 - Longitudinal cellular and humoral immune responses after triple BNT162b2 and fourth full-dose mRNA-1273 vaccination in haemodialysis patients. Published in Frontiers in Immunology.

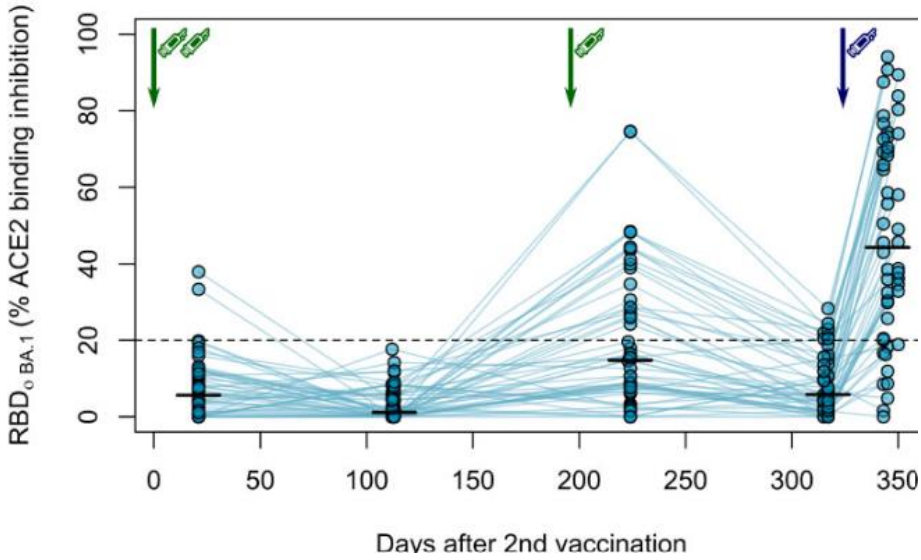

Figure 10: A fourth dose elicited ACE2 binding responses towards omicron in the majority of dialysis patients. Reproduced from Becker et al, 2022 - Longitudinal cellular and humoral immune responses after triple BNT162b2 and fourth full-dose mRNA-1273 vaccination in haemodialysis patients. Published in Frontiers in Immunology.

We also evaluated the effect of anti-TNF medications on vaccine elicited immunity in patients with inflammatory bowel disease. Because we kept to continuously expanding our assays, we could directly evaluated antibody responses against the then novel BQ1.1. and XBB.1.5 variants of concern, in addition to the then dominant BA.5. Anti-TNF medications resulted in significant reductions in ACE2 binding inhibition, compared to controls (Figure 11), although breakthrough infections within this cohort did increase the response.

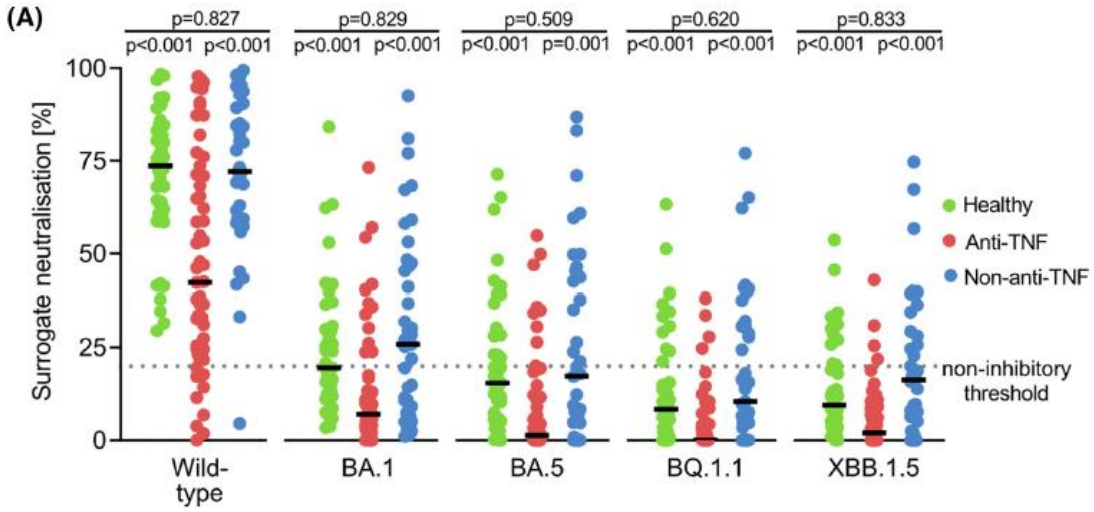

Figure 11: Functional immunity based on surrogate neutralisation against BQ.1.1 and XBB.1.5 is impaired in anti-TNF-treated patients with IBD. Reproduced from Woelfel et al, 2023 – STAR SIGN study: Evaluation of COVID-19 vaccine efficacy against the SARS-CoV-2 variants BQ.1.1 and XBB.1.5 in patients with inflammatory bowel disease. Published in Alimentary Pharmacology and Therapeutics.

Variants of Concern

At the same time as the initial vaccine rollout across Europe, SARS-CoV-2 variants were detected following increased sequencing efforts. After a mutated variant had been identified in Mink in Denmark, two variants named UK or Kent and South Africa later designated as alpha and beta respectively were considered the first variants of concern. We further developed the MULTICOV-AB assay to include RBDs of these variants, allowing direct comparison of the effect of their mutations on antibody binding in both vaccinated and infected individuals (Figure 12). While the alpha variant had minimal impact on antibody binding, there was a significant reduction seen for the beta variant, indicating it was capable of immune evasion in individuals previously infected with the ancestral Wuhan isolate or with vaccinated with unmodified first generation vaccine.

These difference in antibody binding was confirmed with a significant reduction in neutralizing antibody potential with both a classic virus neutralization assay and an early version of RBDCoV-ACE2 in the same manuscript. With the continuous emergence of new variants of interest and concern, we continually kept adapting both assays to include antigens of these variants. Given the large differences in circulating variants, our ability to measure multiple variants simultaneously with RBDCoV-ACE2 became invaluable. In particular, this assay allowed us to track how individual/cumulative mutations altered neutralizing antibody ability (Figure 13).

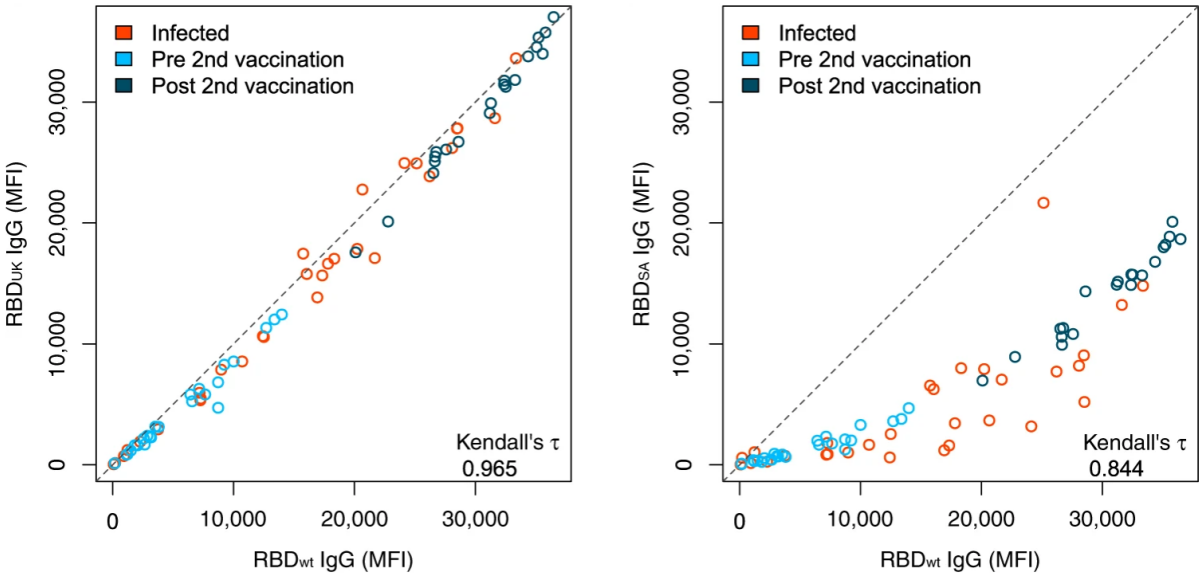

Figure 12: Effect of RBD mutations in the alpha (UK) and beta (SA) variants on antibody binding. Reproduced from Becker et al, 2021 – Immune response to SARS-CoV-2 variants of concern in vaccinated individuals. Published in Nature Communications.

As part of the RBDCoV-ACE2 assay publication, we could show how the E484 and N501 residues were particularly critical in diminishing neutralizing antibody function.

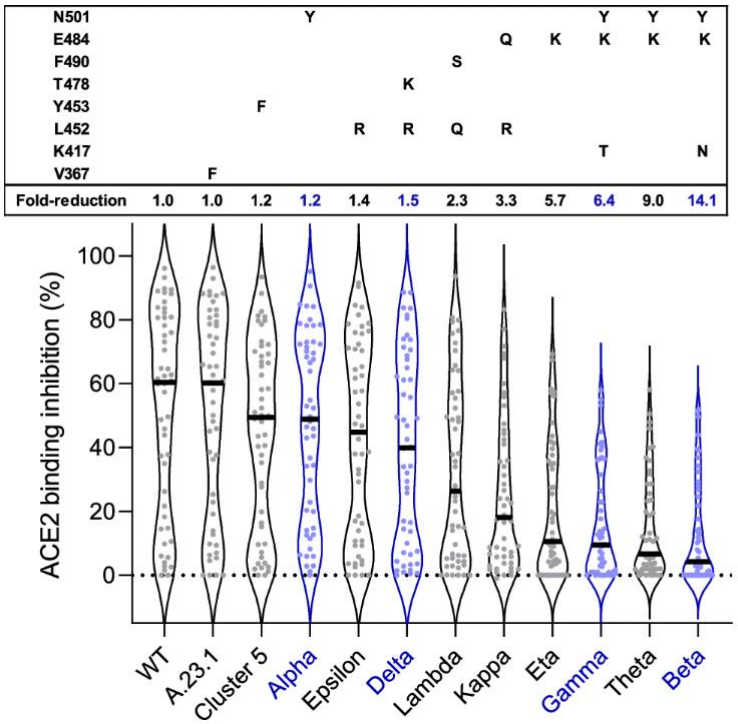

Figure 13: Neutralizing antibody function towards all then variants of concern/interest as measured by ACE2 binding inhibition in the RBDCoV-ACE2 assay. Reproduced from Junker et al, 2022 - COVID-19 patient serum less potently inhibits ACE2-RBD binding for various SARS-CoV-2 RBD mutants. Published in Scientific Reports.

Continuous adaptation of both assays also enabled us to rapidly identify the immune evasion potential of omicron, where we could show that for samples from vaccinated and infected individuals, BA.1 and BA.2 represented the largest decreases seen in both antibody binding

and ACE2 binding inhibition to date (Figure 14a and b). Critically, there was no significant difference between ACE2 binding inhibition towards BA.1/2 from samples of prepandemic origin and those from individuals who had been previously infected or vaccinated with 2 doses.

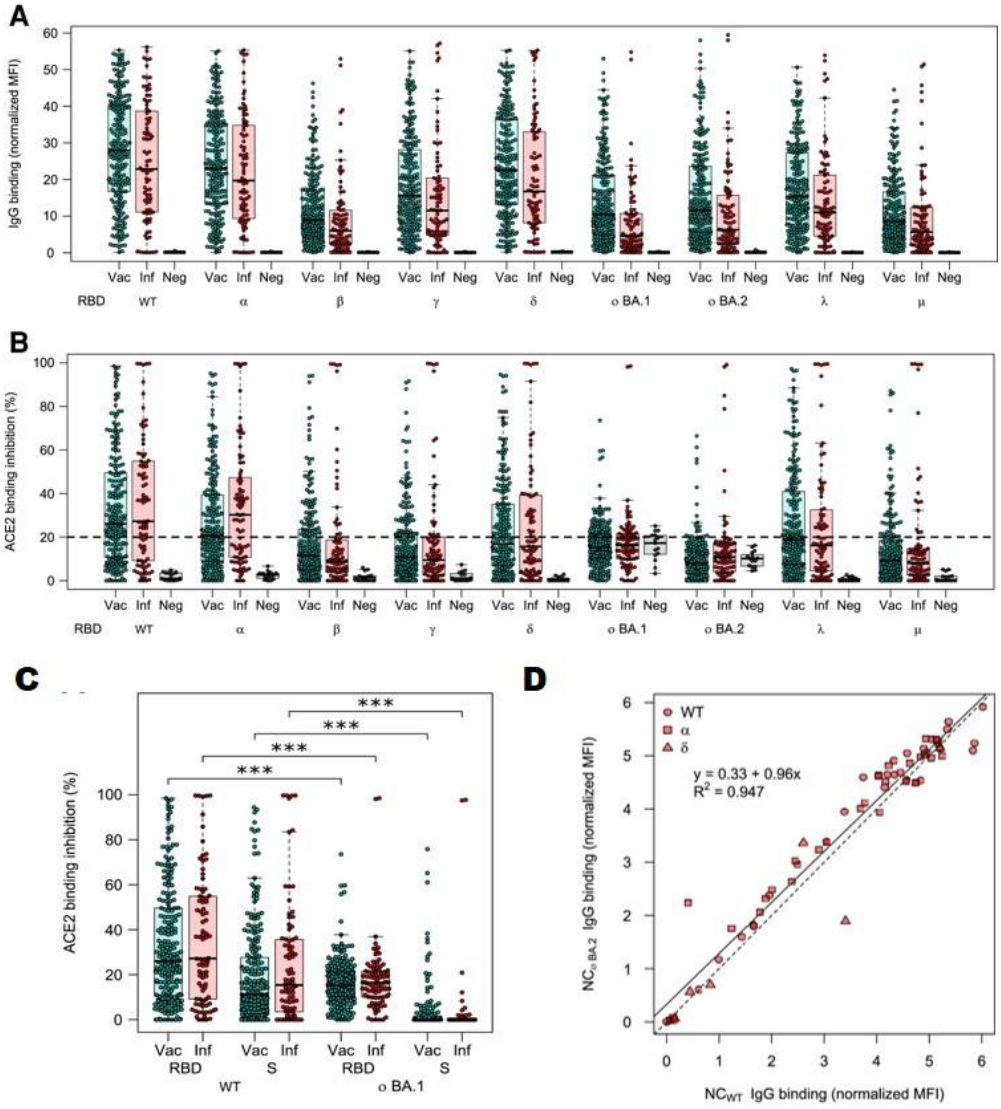

Figure 14: Comparative antibody binding (a) and ACE2 binding inhibition for all SARS-CoV-2 variants of concern and interest identified at time of publication and confirmation that immune evasion for omicron was restricted to the S and RBD (c) but not the NC (d) antigens. Reproduced from Junker et al, 2022 – Antibody binding and ACE2 binding inhibition is significantly reduced for the Omicron variant compared to all other variants of concern. Published in Clinical Infectious Diseases.

Interestingly however, reductions in humoral binding/inhibitory activity were restricted to the S and RBD (Figure 14c), where the vast majority of the novel mutations were located. By contrast, nucleocapsid antibody binding had no significant reduction for the omicron variant (Figure 14d), suggesting that while it may evade humoral control, replication might still be controlled by cellular T-cell immunity given the conservation of these epitopes.

Both assays are still continuously updated, with BA2.86 the most recent variant to be included within the assay portfolio.

Endemic Coronaviruses

Cross-reactivity and cross-protection for endemic coronaviruses is discussed in more detail in D2.6 and D2.8, which includes unpublished data generated explicitly for reporting purposes. Here, we aim to describe our published work in more detail only. Initially, we hypothesized (as others did) that seasonal endemic coronaviruses may offer some form of cross-protection towards SARS-CoV-2. As such, we included antigens from all endemic coronaviruses in the original MULTICOV-AB assay and evaluated IgG response of 1176 samples as part of assay development. The immune response towards all hCoV antigens was more dependent on coronavirus clade than on antigen choice (Figure 15a). Overall, while there was a considerable immune response to the included S1 hCoV antigens, no significant difference was seen between samples from SARS-CoV-2 infected and uninfected donors (Figure 15b).

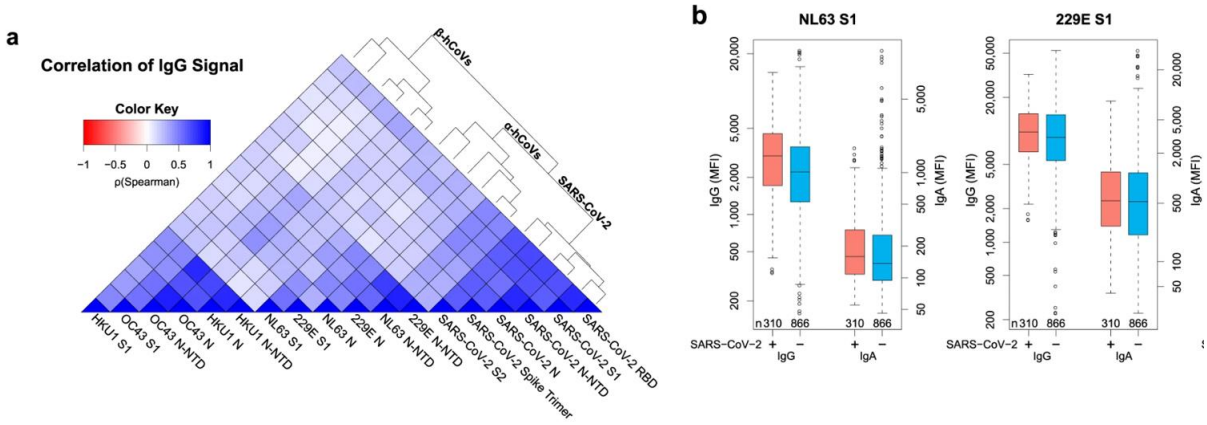

Figure 15: Endemic coronavirus antigen clustering and lack of significant difference between infected and uninfected individuals. Reproduced from Becker et al, 2021 - Exploring beyond clinical routine SARS-CoV-2 serology using MultiCoV-Ab to evaluate endemic coronavirus cross-reactivity. Published in Nature Communications.

We later evaluated hCoVs in an even larger cohort which had recruited both adults and children. What became apparent was that hCoV titers significantly increased with age as a result of increased viral exposure (Figure 16a). Within the same publication, we observed significant difference between previously SARS-CoV-2 infected and non-infected adults and children (Figure 16b), although there were significant differences between adults and children, indicating that any study into hCoVs needs to include well age-matched control groups. This became obvious in a further study that did identify significant differences in hCoV titer in samples sourced pre-pandemic and from SARS-CoV-2 infected individuals (Figure 16c), however ages of the different groups significantly varied, with pre-pandemic samples largely originating from students, while SARS-CoV-2 infected samples came from individuals with the age of 55 and older admitted to the ICU in the early months of the pandemic..

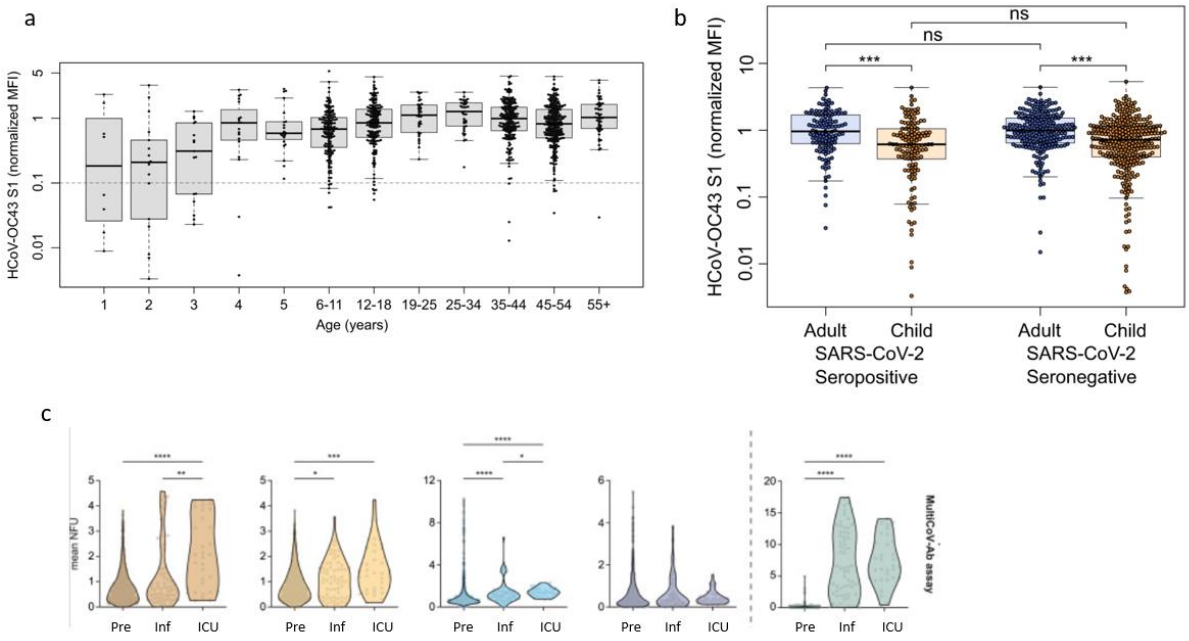

Figure 16: hCoV antibody titer increases with age (a), with no significant difference seen in titer for infected and non-infected individuals for age matched cohorts (b). However, for non-age matched cohorts, significant differences in titer between pre-pandemic and infected/ICU individuals could be found (c). (a and b) reproduced from Renk et al, 2022, Robust and durable serological response following pediatric SARS-CoV-2 infection. Published in Nature Communications. C reproduced from Wratil et al, 2021 - Evidence for increased SARS-CoV-2 susceptibility and COVID-19 severity related to pre-existing immunity to seasonal coronaviruses. Published in Cell Reports.

Publications

As of November 2023 (M45), the following publications as part of WP2 were submitted and have been accepted for publication in peer-reviewed journals:

- Becker et al, 2021 - Exploring beyond clinical routine SARS-CoV-2 serology using MultiCoV-Ab to evaluate endemic coronavirus cross-reactivity. Published in Nature Communications. https://doi.org/10.1038/s41467-021-20973-3
- Becker et al, 2021 - Immune response to SARS-CoV-2 variants of concern in vaccinated individuals. Published in Nature Communications. https://doi.org/10.1038/s41467-021-23473-6
- Wagner et al, 2021 - NeutrobodyPlex—monitoring SARS-CoV-2 neutralizing immune responses using nanobodies. Published in EMBO Reports. https://doi.org/10.15252/embr.202052325
- Nelde et al, 2021 - SARS-CoV-2-derived peptides define heterologous and COVID-19-induced T cell recognition. Published in Nature Immunology. https://doi.org/10.1038/s41590-020-00808-x
- Strengert et al, 2021 - Cellular and humoral immunogenicity of a SARS-CoV-2 mRNA vaccine in patients on haemodialysis. Published in Lancet Ebiomedicine. https://doi.org/10.1016/j.ebiom.2021.103524

- Fink et al, 2021 - Multiplexed Serum Antibody Screening Platform Using Virus Extracts from Endemic Coronaviridae and SARS-CoV-2. Published in ACS Infectious Diseases. https://doi.org/10.1021/acsinfecdis.0c00725
- Wratil et al, 2021 - Evidence for increased SARS-CoV-2 susceptibility and COVID-19 severity related to pre-existing immunity to seasonal coronaviruses. Published in Cell Reports. https://doi.org/10.1016/j.celrep.2021.110169
- Dulovic et al, 2022 - Diminishing Immune Responses against Variants of Concern in Dialysis Patients 4 Months after SARS-CoV-2 mRNA Vaccination. Published in Emerging Infectious Diseases. https://doi.org/10.3201/eid2804.211907
- Junker et al, 2022 - COVID-19 patient serum less potently inhibits ACE2-RBD binding for various SARS-CoV-2 RBD mutants. Published in Scientific Reports. https://doi.org/10.1038/s41598-022-10987-2
- Dulovic et al, 2022 - Comparative Magnitude and Persistence of Humoral SARS-CoV-2 Vaccination Responses in the Adult Population in Germany. Published in Frontiers in Immunology. https://doi.org/10.3389/fimmu.2022.828053
- Wagner et al, 2022 - Biparatopic nanobodies protect mice from lethal challenge with SARS-CoV-2 variants of concern. Published in EMBO Reports. https://doi.org/10.15252/embr.202153865
- Junker et al, 2022 - Antibody binding and ACE2 binding inhibition is significantly reduced for the Omicron variant compared to all other variants of concern. Published in Clinical Infectious Diseases. https://doi.org/10.1093/cid/ciac498
- Becker et al, 2022 - Longitudinal cellular and humoral immune responses after triple BNT162b2 and fourth full-dose mRNA-1273 vaccination in haemodialysis patients. Published in Frontiers in Immunology. https://doi.org/10.3389/fimmu.2022.1004045
- Woelfel et al, 2023 – STAR SIGN study: Evaluation of COVID-19 vaccine efficacy against the SARS-CoV-2 variants BQ.1.1 and XBB.1.5 in patients with inflammatory bowel disease. Published in Alimentary Pharmacology and Therapeutics. https://pubmed.ncbi.nlm.nih.gov/37571863/

In addition, the following manuscripts have been submitted and are under currently in peer review, while a further 4 manuscripts are still in preparation.

- Dulovic et al, Longitudinal analysis of humoral and cellular immunity in SARS-CoV-2 exposed families. Under review at Pediatrics. Preprint available at https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4332051
- Marsall et al, Development and validation of a respiratory syncytial virus multiplex immunoassay. Under review at Infection, preprint available at bioRxiv 2023.08.30.555534; https://www.biorxiv.org/content/10.1101/2023.08.30.555534v1)

Topics covered by manuscripts still in preparation include the effect of medications on breakthrough infections, immune responses to breakthrough infections and a fifth vaccination in dialysis patients, antibody diversification following breakthrough infection and the development of a multiplex immunoassay for Influenza, therefore partially completing in the proposal outlined test expansion to other respiratory pathogens.