Reduced sensitivity of commercial Spike-specific antibody assays after primary infection with the SARS-CoV-2 Omicron variant

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Research Article

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Abstract

The SARS-CoV-2 Omicron variant is characterized by substantial changes in the antigenic structure of the Spike (S) protein. Therefore, we analyzed whether these changes impact the sensitivity of commercial anti-SARS-CoV-2 antibody assays. Sera from 37 convalescent individuals after primary Omicron infection were tested with 20 commercial anti-SARS-CoV-2 immunoassays. As controls, we used samples from 43 individuals after primary infection with an ancestral wildtype strain. Notably, in Omicron convalescents, there was a statistically significant reduction in the sensitivity of all antibody assays containing S or its receptor-binding-domain (RBD) as antigens. Furthermore, antibody levels quantified by these assays displayed a weaker correlation with Omicron-specific neutralizing antibody titers than with those against the wildtype. In contrast, the sensitivity of nucleocapsid-protein-specific immunoassays was similar in wildtype and Omicron-infected subjects. In summary, the antigenic changes in the Omicron S lead to reduced detection rates in commercial S- and RBD-specific antibody assays, impairing their diagnostic performance.

Background

The Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron variant, which emerged in late 2021[1], displayed more than 30 mutations in the gene coding for the Spike (S) protein, leading to substantial changes in the antigenic structure, in particular in the receptor-binding domain (RBD), the main target for neutralizing antibodies (nAbs)[2, 3, 4]. As an effect of these alterations, a significant decrease in the neutralizing capability of pre-existing antibodies induced by prior infections with other variants or vaccinations was observed [3, 5, 6, 7]. In contrast, antibodies produced after primary infections with the Omicron variant were recently found to have limited neutralizing activity against earlier variants, including the wildtype and Delta variant [8, 9].

While neutralization tests (NTs) could be rapidly adapted by using clinical isolates or pseudoviruses[8, 9], most commercial antibody assays have not been modified so far[10]. However, such adaptations could be required because most of these assays had been developed before emergence of variants of concern (VOCs) and contain the S or RBD protein as target antigens derived from the ancestral wildtype (WT) isolated in Wuhan[10].

Commonly used antibody tests include enzyme-linked immunosorbent assays (ELISA), chemiluminescence immunoassays (CLIAs), and immunoblots (IBLs)[11, 12, 13, 14], often standardized by the World Health Organization (WHO) measuring binding antibody units per milliliter (BAU/ml)[15]. In addition, surrogate virus neutralization tests (sVNTs) are in use, quantifying the antibody-mediated inhibition of binding of the RBD to the angiotensin-converting enzyme 2 receptor (ACE2) as a correlate for neutralization[13, 16, 17].

Therefore, the question has arisen whether Omicron S- and Omicron RBD-specific antibodies bind less efficiently to the antigens used in these commercial antibody assays[10]. In the present study, we analyzed

serum samples from 37 non-hospitalized individuals with Omicron primary infections in a panel of 20 commercial SARS-CoV-2 antibody assays. The detection rates of the assays were compared with those obtained with a matched control cohort of 43 convalescents after WT primary infections. In addition, since the nucleocapsid protein's (NC) structure is mainly preserved in the Omicron variant[2], we included these assays as a further control. We found that detection rates of S-based assays were strongly reduced, in contrast to NC-based assays, which has important implications for the design and the possible adaptation of commercial antibody assays.

Results

Characteristics and matching of SARS-CoV-2 convalescent individuals

The study included serum samples from 37 non-hospitalized, unvaccinated convalescents (21 female, 16 male, median age: 33 years, range: 4-81) after primary infection with the Omicron variant. In all 37 individuals, a positive RT-PCR result from a nasopharyngeal swab preceded the acquisition of the respective serum sample (median interval between RT-PCR positivity and acquisition of the serum sample: 33 days, range: 16-96). The swabs were obtained during a period (January – March 2022) when the Omicron sub-lineages BA.1 or BA.2 circulated in Austria with over 98% predominance[18]. Furthermore, the samples of these individuals displayed significantly higher BA.1- or BA.2-specific titers of neutralizing antibodies (nAbs) than against a WT strain with the D614G mutation (B.1.1) and the Delta variant of concern (VOC) in live-virus NTs, as demonstrated previously (Supplementary Figure 1a)[9].

Serum samples from 43 non-hospitalized convalescents after infection with WT virus early in the pandemic (before the emergence of VOCs, February 2020 to December 2020) served as controls. RT-PCR-positivity in controls preceded the collection of the respective serum samples with a median interval of 35 days (range: 16-70). Samples from WT controls were matched to those from convalescents after Omicron infection based on the concentration of variant-specific nAbs, age, the interval between PCR diagnosis serum sampling, and the absence of hospitalization.

Supplementary Figures 1a and b show that the matched groups of convalescents after Omicron and WT infections exhibited comparable virus-specific neutralization titers (BA.1/BA.2 vs. WT titers: $p = 0.42$, two-tailed Mann-Whitney U test; Supplementary Figure 1b). In addition, there was no difference in age ($p = 0.32$; Supplementary Figure 1c) or the interval between PCR-positivity and the time point when serum samples were obtained ($p = 0.86$, two-tailed Mann-Whitney U test, respectively; Supplementary Figure 1d).

Detection rates of commercial antibody assays in convalescents after Omicron infection

Serum samples from matched groups of convalescents after primary Omicron (n = 37) and WT (n = 43) infection were tested using a panel of 20 commercial antibody assays by seven manufacturers. Detailed

information on the antibody assays, including test principle, target antigens, measuring units, covered immunoglobulin class, and cutoff values, are provided in Supplementary Table 1.

As shown in Figure 1, we observed significantly reduced detection rates in all commercial antibody assays based on S or RBD as target antigens with samples from convalescents after primary Omicron infection as compared to the WT control group ($p < 0.05$ for all anti-S and anti-RBD antibody assays; two-tailed Fisher's exact test, Bonferroni correction for multiple testing).

In contrast, NC-based assays displayed no significant differences in the detection rates among convalescents after Omicron and WT infection ($p > 0.05$ in all assays).

Two IBLs, the SARS-CoV-2 ViraChip® IgG by Viramed and the recomLine SARS-CoV-2 IgG by Mikrogen, which integrate detection of both S-RBD and NC-specific antibodies into a single test result, were not affected by a reduced detection rate due to the unchanged sensitivity in measuring NC-specific antibodies.

The detailed results for all assays, including absolute and relative detection rates and comparative analyses, are displayed in Table 1.

Detection rates in subgroups of primarily Omicron infected convalescents

Next, we analyzed whether the detection rates of the commercial antibody assays were also reduced in the subgroup of convalescents, in whom a variant-specific PCR additionally confirmed Omicron infection. Omicron BA.1- or BA.2-specific PCR results were available for nine convalescents (BA.1: n = 4; BA.2: n = 5). As for the entire cohort (Figure 1), the detection rates were significantly reduced in almost all S- and RBD-specific immunoassays (13 of 16), while all NC-specific antibody assays displayed similar detection rates among Omicron or WT-infected groups (Supplementary Table 2).

To clarify whether the reduction in the detection rates of the assays was due to different antibody concentrations among the two groups, we calculated the diagnostic performances using only samples from Omicron and WT convalescents with NT titers $\geq$20 against the respective variant (Omicron: n = 30, WT n = 35). Indeed, the commercial antibody assays' detection rates were also significantly reduced in samples with overall high antibody concentrations (all anti-S/anti-RBD assays $p < 0.05$; all anti-NC assays $p > 0.05$; Fisher's exact test; Supplementary Table 3).

Correlation of quantitative antibody levels and variant-specific nAb titers

Finally, we analyzed the correlation between titers of variant-specific NT titers with antibody levels quantified by the commercial antibody assays. Figure 2 shows a robust correlation between RBD-ACE2 binding inhibition quantified by commercial sVNTs and the respective NT titers in convalescent samples

obtained from WT-infected subjects (r = 0.7–0.8). In contrast, a much weaker correlation was observed in sera from Omicron-infected subjects (r = 0.2–0.3), as indicated by a flattened steepness of the regression line (Figure 2).

The S- and RBD-specific ELISAs, CLIAs, and the IBLs displayed an overall reduction in the signal intensity, i.e., the regression lines in the Omicron cohort shifted downwards (Figures 3a-3d, Supplementary Figures 2 - 8). In contrast to Anti-S- and Anti-RBD-immunoassays, the signal intensities and correlations were comparable for NC-specific antibody assays among both cohorts (Figures 3e and 3f, Supplementary Figures 2e, 3c, 5d, 7d, and 9).

Methods

Samples from convalescent individuals

In total, the study included serum samples from 80 non-hospitalized convalescents after RT-PCR confirmed SARS-CoV-2 infection from nasopharyngeal swabs. In 37 of 80 (female n = 21, male n = 16, median age: 41 years, range: 4-81), SARS-CoV-2 infection was confirmed by PCR between January and March 2022, when the Omicron variant circulated with over 98 % predominance in Austria[18]. None of the subjects had been vaccinated or had had a positive SARS-CoV-2 test before. Furthermore, in 10 of the 37 individuals, a previously acquired serum sample (in December 2021) tested negative for Anti-SARS-CoV-2 antibodies (using the SARS-CoV-2 ViraChip® IgG assay, Viramed Biotech AG, Planegg, Germany). Seroconversion was thus documented in these convalescents, confirming SARS-CoV-2 primary infection. In addition, infection with Omicron BA.1 or BA.2 variants was assessed by variant-specific RT-PCR in nasopharyngeal swabs obtained from 9 of 37 subjects, using the mutation assay VirSNiP SARS-CoV-2 Spike S371L S373P (TIB MOLBIOL, Berlin, Germany), as described previously[9]. The presence of S371LS373P and S371FS373P98 indicated infection with Omicron BA.1 (n=4) and BA.2 (n=5), respectively.

Forty-three convalescents (female n = 21, male n = 22, median age: 33 years, range: 16-96) served as controls. In these individuals, SARS-CoV-2 infection was confirmed by RT-PCR from nasopharyngeal swabs taken between February 2020 and December 2020, a period when only an ancestral WT strain of the early pandemic circulated in Austria. None of the control individuals was vaccinated against SARS-CoV-2 since the vaccination was not yet available when the serum samples were obtained. Clinical information (including vaccination status, previous SARS-CoV-2 infections, documentation, date of RT-PCR positivity, and absence of hospitalization) was recorded before anonymizing samples.

All samples used for this study were originally obtained for routine serological testing at the Center for Virology. Residual sample material was then anonymized and integrated into the Center of Virology's sample bank for research using a protocol approved by the local ethics committee (EK 1035/2016, EK 1513/2016). The ethics committee of the Medical University of Vienna approved the study's protocol (EK 2156/2019). Since all individuals consented that SARS-CoV-2-specific antibody testing was performed at

the Center for Virology, and only anonymized samples were retested for this study, the local ethics committee concluded that no written consent of the convalescents was required for this evaluation of commercial antibody tests (EK 2156/2019).

Live-virus neutralization test:

The NT was conducted as described previously[19, 20, 21]. In brief, the serum samples were incubated at 37° C with 50-100 TCID50 of either WT (GISAID accession number EPI_ISL_438123[19]), Delta (GISAID accession number EPI_ISL_4172121[21]), BA.1 (GISAID accession number EPI_ISL_9110894[20]), or BA.2 (GISAID accession number EPI_ISL_11110193[20]) virus strains for one hour. The mixture was then added to a monolayer of VeroE6 cells (ECACC 85020206). After 3-5 days, the NT titers were determined as the reciprocal dilution factor at which serum antibodies prevented a virus cytopathic effect (CPE). Serial dilutions ranged from 1:10 to 1:1280. NT titers ≥ 10 were considered positive.

Commercial antibody assays

The panel of evaluated antibody tests comprised 20 different commercial antibody assays. Detailed information on these assays, including the test principle, the detected immunoglobulin classes, the respective target antigens, the measuring unit, and the cutoff values, are shown in Supplementary Table 2. All assays were performed according to the manufacturer's instructions, using the protocols, dilutions, and cutoff values the manufacturers provide. Briefly, three assays, the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit by GenScript, the SARS-CoV-2-NeutraLISA by Euroimmun, and the TECO® SARS-CoV-2 Neutralization Antibody Assay by TECOmedical are sVNTs, which quantify the antibody-mediated binding-inhibition between RBD and ACE2.

SARS-CoV-2 ELISAs, CLIAs, chemiluminescent microparticle immunoassays (CMIAs), and IBLs quantify different immunoglobulin classes with various target antigens, including S, its subunits S1 and S2, RBD, and NC, as shown in Supplementary Table 2.

Statistical analyses

Data analysis was performed with GraphPad Prism 9.3.1. For each test, we recorded the number of samples that tested above the manufacturer's threshold as positive and calculated the detection rate as the percentage of positive samples of the total number of samples in the respective cohort. Detection rates of each test in the two cohorts were compared using the two-tailed Fisher's exact test, and the p-values were adjusted by Bonferroni correction for multiple testing for this analysis (Anti-S- and Anti-RBD tests, n = 16; Anti-NC assays, n = 8; and immunoblots, n = 4). The alpha level was set to 0.05. The

quantitative results of the antibody assays were plotted versus the Omicron NT -titers for the omicron cohort and against the WT NT titers for the WT cohort with a linear regression line. The correlation was assessed by calculating the Pearson correlation coefficient (r). Regression statistics are included in Figures 2 and 3 and Supplementary Figures 2-9. For these correlation analyses, the higher titer of either the BA.1- or BA.2-specific NT was used as “Omicron NT titer”.

Discussion

The antigenic changes in the S protein of the SARS-CoV-2 Omicron variant cause a significant reduction in the neutralizing activity of pre-existing antibodies induced by infections with earlier virus variants or vaccinations[5, 6, 7, 22, 23]. Furthermore, our group recently demonstrated that nABs produced upon primary infection with the Omicron variant specifically neutralize the respective BA.1 or BA.2 subtypes but lack neutralizing activity against the WT and the Delta VOC[9]. In this study, we analyzed whether the changes of the Omicron S protein affect the sensitivity of commercial antibody assays by comparing test performances in samples after primary Omicron WT infections.

Indeed, we demonstrate significantly reduced detection rates in a large panel of commercial S- and RBD-specific antibody tests in convalescent individuals after primary Omicron infection. This finding, together with a decreased correlation between nAb titers and antibody levels by S- or RBD-specific commercial assays, indicates that antibodies against the S protein of the Omicron variant bind less efficiently to the S protein of the ancestral SARS-CoV-2 wild type used as target antigen in these assays. Furthermore, we found that detection rates in Omicron-convalescents did not decrease in antibody assays containing NC as antigen, which is mainly preserved in the Omicron variant[2].

Interestingly, the three sVNTs included in our panel of antibody assays were the most significantly affected assays by the mismatch between Omicron-specific antibodies and the original RBD as the target antigen. These assays were strongly impaired in qualitatively detecting any SARS-CoV-2-specific antibodies (detection rates of 3–5% in the Omicron cohort as opposed to 70–93% in the control cohort) and their ability to correlate with neutralizing activity. Of note, not even in samples with the highest Omicron-specific NT titers, sVNTs detected any significant RBD-ACE2-binding-inhibition. These findings can be explained by the accumulation of mutations in the RBD of the Omicron variants, with most mutations occurring at the RBD binding interface to the ACE2 receptor protein[2, 3, 4]. Antibodies formed against the Omicron-specific RBD are thus less likely to inhibit the binding of the original RBD to ACE2 in the test. These findings call for additional sVNTs based on RBDs from Omicron variants. In the light of the emerging Omicron BA.4 and BA.5 sublineages with additional mutations in the RBD[24], further evaluations of the different Omicron RBDs for such assay formats are required.

The data from this study may not only have implications on the use of sVNTs, but also on the diagnostic application of the wide range of antibody tests using other test principles. Indeed, S- and RBD-specific ELISAs, CLIAs, and IBLs were strongly affected by a reduction of the measured signal intensity. Indeed, the overall detection rates of the commercial total antibody and IgG assays (ELISAs, CLIAs, and IBLs)

ranged from 91–100% in the WT control cohort), which is comparable to previous evaluations of these tests [11, 13, 14, 16, 25, 26, 27], as opposed to 22–57% in the Omicron cohort.

Commercial SARS-CoV-2-specific antibody assays currently face new diagnostic challenges like identification of recent or past SARS-CoV-2 infection (when RT-PCR testing was not conducted or performed too late) to explain the etiology of potential sub-acute or chronic sequelae of SARS-CoV-2 infection (e.g., Multi Inflammatory Syndrome in children[28, 29], cardiac complications such as myocarditis or pericarditis[30] or thrombo-embolic events[31]). Furthermore, an accurate serodiagnosis is also critical to elucidate a potential role of SARS-CoV-2 Omicron infection in the recent series of severe hepatitis cases in children with unknown etiology[32, 33]. Finally, while primary Omicron infections might become less prevalent given the high incidence rates recently reported worldwide[34], unvaccinated children will be continuously at risk for primary SARS-CoV-2 infections, still calling for an accurate serological diagnosis.

Since the detection rates of the evaluated NC-specific antibody assays were not significantly affected by primary Omicron infection, we thus propose to use NC-specific antibody assays for the serodiagnosis of previous SARS-CoV-2 infections in such cases. However, such testing is no complete replacement for S-specific assays, considering that the persistence and detectability of anti-NC antibodies are limited, depending on the applied assay[35].

We acknowledge that the infection with the Omicron variant was confirmed by a variant-specific PCR only in nine of the thirty-seven convalescents. However, when RT-PCR testing revealed an infection in these individuals, the Omicron variant was the dominant circulating strain in Austria (with BA.1 and BA.2 amounting to over 98% of all notified infections)[18]. Additionally, we still observed a significant loss in the sensitivity of Anti-S and Anti-RBD immunoassays when we limited the analysis to the subset of nine convalescents with PCR-confirmed Omicron infections.

Finally, this study on the performances of 20 commercial SARS-CoV-2 antibody assays in primary infection with the SARS-CoV-2 Omicron variant demonstrates that the diagnostic ability of the currently available S- and RBD-specific immunoassays, particularly of sVNTs, may be significantly impaired, calling for an adaptation of these immunoassays depending on the further course of the pandemic.

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Declarations

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Author Contributions

Thomas Perkmann, Claudia Jani, Patrick Mucher, Katja Prüger, Rodrig Marculescu, Elisabeth Reuberger, and Helmuth Haslacher established and performed laboratory analyses using commercial immunoassays. Stephan Walter Aberle and Jeremy Camp established the variant-specific RT-PCR. Josef Deutsch, Oliver Lammel ad Eva Höltl contributed materials. Elisabeth Puchhammer-Stöckl provided funding and participated in writing the manuscript. Marianne Graninger, Christian Borsodi, and Judith Helene Aberle participated in conceptualizing and writing of the manuscript. Karin Stiasny established the NT assays and participated in conceptualizing and writing the manuscript. David Springer performed data analyses, generated the figures and tables and participated in writing the manuscript. Lukas Weseslindtner supervised the work, performed the study design and participated in conceptualizing and writing the manuscript. Karin Stiasny and Lukas Weseslindtner contributed equally and share correspondence

Competing Interests statement

The authors state they do not have a commercial or other association that might pose a conflict of interest. Reagents for the immunoassays by Wantai Biological Pharmacy Ent, Beijing, China (distributor Szabo-Scandic Austria) and DiaSorin, Salluggia, Italy (distributor: DiaSorin Austria) were provided free of charge.

Data Availability Statement

The available data obtained by this study is entirely included in the Article and the Supplementary Information.

Tables

Table 1: Detection rates of commercial antibody assays in SARS-CoV-2 wildtype (WT) and Omicron primary infections

Target Antibodies		Assay	Prin-ciple	Target Antigen(s)	WT		Omicron		Fisher
					n	%	n	%	p-value
Anti-S- / Anti-RBD antibody assay	**Surrogate Virus Neutralization Test (sVNT)**								
	IgG/A/M	**cPass** SARS-CoV-2 Neutralization Antibody Detection Kit (**GenScript**)	sVNT	RBD-ACE2 Inhibition	40/43	**93%**	2/37	**5%**	≤ 0.0001
		TECO SARS-CoV-2 Neutralization Antibody Assay (**TECOmedical**)			38/43	**88%**	2/37	**5%**	≤ 0.0001
		SARS-CoV-2-**NeutraLISA** (**Euroimmun**)			30/43	**70%**	1/37	**3%**	≤ 0.0001
	Anti-S-Total Antibody Tests								
	IgG/A/M	**WANTAI SARS-CoV-2 Ab Elisa** (**Wantai**)	ELISA	RBD	43/43	**100%**	19/37	**51%**	≤ 0.0001
		Elecsys Anti-SARS-CoV-2 **S** (**Roche**)	ECLIA		42/43	**98%**	21/37	**57%**	0.0003
	Anti-S-IgG / Anti-RBD-IgG								
	IgG	**SARS-CoV-2 IgG II Quant-Assay** (**Abbott**)	CMIA	S	43/43	**100%**	16/36	**44%**	≤ 0.0001
		SARS-CoV-2 **Virachip IgG** (**Viramed**)	MA	S1+S2+RBD +RBDd	42/43	**98%**	18/37	**49%**	≤ 0.0001
		LIAISON SARS-CoV-2 **TrimericS IgG** assay (**DiaSorin**)	CLIA	S	41/43	**95%**	14/37	**38%**	≤ 0.0001
		Anti-SARS-CoV-2-**QuantiVac**-ELISA (**Euroimmun**)	ELISA		39/43	**91%**	8/37	**22%**	≤ 0.0001
		recomLine SARS-CoV-2 IgG (**Mikrogen**)	IB	S1+RBD	39/43	**91%**	14/36	**39%**	≤ 0.0001
	Anti-S-IgM / Anti-RBD-IgM								
	IgM	**WANTAI** SARS-CoV-2 **IgM** Elisa (**Wantai**)	ELISA	RBD	37/43	**86%**	2/37	**5%**	≤ 0.0001
		SARS-CoV-2 IgM (Abbott)	CMIA	S	33/43	**77%**	3/37	**8%**	≤ 0.0001
		SARS-CoV-2 **Virachip IgM** (**Viramed**)	MA	S1+S2+RBD	27/43	**63%**	8/37	**22%**	0.0078
		LIAISON SARS-CoV-2 **IgM** (**DiaSorin**)	CLIA	RBD	26/43	**60%**	4/37	**11%**	≤ 0.0001
	Anti-S-IgA / Anti-RBD-IgA								
	IgA	**Anti-SARS-CoV-2-ELISA (IgA)** (**Euroimmun**)	ELISA	S	31/43	**72%**	6/37	**16%**	≤ 0.0001
		SARS-CoV-2 **Virachip IgA** (**Viramed**)	MA	S1+S2+RBD	20/43	**47%**	2/37	**5%**	0.0009
Anti-NC-antibody assay	**Anti-NC-Antibody Assays (IgG, IgA, IgM)**								
	IgG/A/M	**Elecsys** Anti-SARS-CoV-2 **N** (**Roche**)	ECLIA	NC	39/43	**91%**	35/37	**95%**	0.6809
	IgG	**SARS-CoV-2 IgG (Abbott)**	CMIA		38/43	**88%**	31/37	**84%**	0.7464
		recomLine SARS-CoV-2 IgG (**Mikrogen**)	IB		38/43	**88%**	32/36	**89%**	> 0.999
		Anti-SARS-CoV-2-NCP-ELISA (**IgG**) (**Euroimmun**)	ELISA		35/43	**81%**	31/37	**84%**	> 0.999
		SARS-CoV-2 **Virachip IgG** (**Viramed**)	MA		29/43	**67%**	31/37	**84%**	0.1223
	IgM	SARS-CoV-2 **Virachip IgM** (**Viramed**)			16/43	**37%**	16/37	**43%**	0.6504
		Anti-SARS-CoV-2-NCP-ELISA (**IgM**) (**Euroimmun**)	ELISA		5/43	**11%**	2/37	**5%**	0.4416
	IgA	SARS-CoV-2 **Virachip IgA** (**Viramed**)	MA		11/43	**26%**	5/37	**14%**	0.2629
Mixed	**Overall interpretation (Immunoblot, Microarray)**								
	IgG	SARS-CoV-2 **Virachip IgG** (**Viramed**)	MA	S1+S2+RBD+ RBDd+NC	42/43	**98%**	32/37	**87%**	0.0904
	IgM	SARS-CoV-2 **Virachip IgM** (**Viramed**)		S1+S2+RBD+ NC	24/43	**56%**	6/37	**16%**	0.0118
	IgA	SARS-CoV-2 **Virachip IgA** (**Viramed**)			19/43	**44%**	2/37	**5%**	0.0022
	IgG	**recomLine** SARS-CoV-2 **IgG**	IB	S1+RBD+NC	41/43	**95%**	33/36	**92%**	>

		(Mikrogen)							0.999

N number of positive samples/ number of samples tested, % percent of positive samples, Fisher Fisher's exact test (two-tailed, alpha level = 0.05, all significant p-values Bonferroni-adjusted for multiple testing assuming 28 tests), sVNT SARS-CoV-2 Surrogate Virus Neutralization Tests, ELISA enzyme-linked immunosorbent assay; CLIA chemiluminescence immunoassay; CMIA chemiluminescence micro particle assay; ECLIA electro-chemo luminescence immunoassay, IB immunoblot, S spike, S1/S2 subunit 1/2 of spike, RBD receptor-binding-domain; RBDd receptor binding domain of the Delta variant, NC nucleocapsid, MA: microarray. WT: n = 43; Omicron: n = 37; except for recomLine SARS-CoV-2 IgG (Mikrogen) and SARS-CoV-2 IgG II Quant-Assay (Abbott) where Omicron n = 36 (one sample not technically valid and no sample material left respectively). Significant p-values ($p < 0.05$): bold, underlined. The immunoblots (Virachip IgG / IgA / IgM and the recomLine IgG) are listed three times as a separate analysis of the anti-S/RBD-, anti-NC- and the overall-detection rate was calculated.

Abbreviations

Ab: antibody, ACE2: angiotensin-converting enzyme 2 receptor, BAU/ml: binding antibody unit per milliliter, CLIA: chemoluminescence immunoassay, CMIA: chemoluminescence microparticle immunoassay, CPE: cytopathic effect, ECACC: European Collection of Authenticated Cell Cultures, ECLIA: electro-chemiluminescence immunoassays, ELISA: enzyme-linked immunosorbent assay, IBL: immunoblot, Ig: immunoglobulin, nAbs: neutralizing antibodies, NC: nucleocapsid protein, NT: Neutralization test, S: Spike protein, S1: subunit 1 of the spike protein, S2: subunit 2 of the spike protein, PCR: polymerase chain reaction, SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2, sVNT: surrogate virus neutralization test, TCID50: tissue culture infection dose 50, RBD: receptor-binding domain, RT-PCR: real time polymerase chain reaction, VOC: variant of concern, WHO: World Health Organization, WT: wildtype

Figures

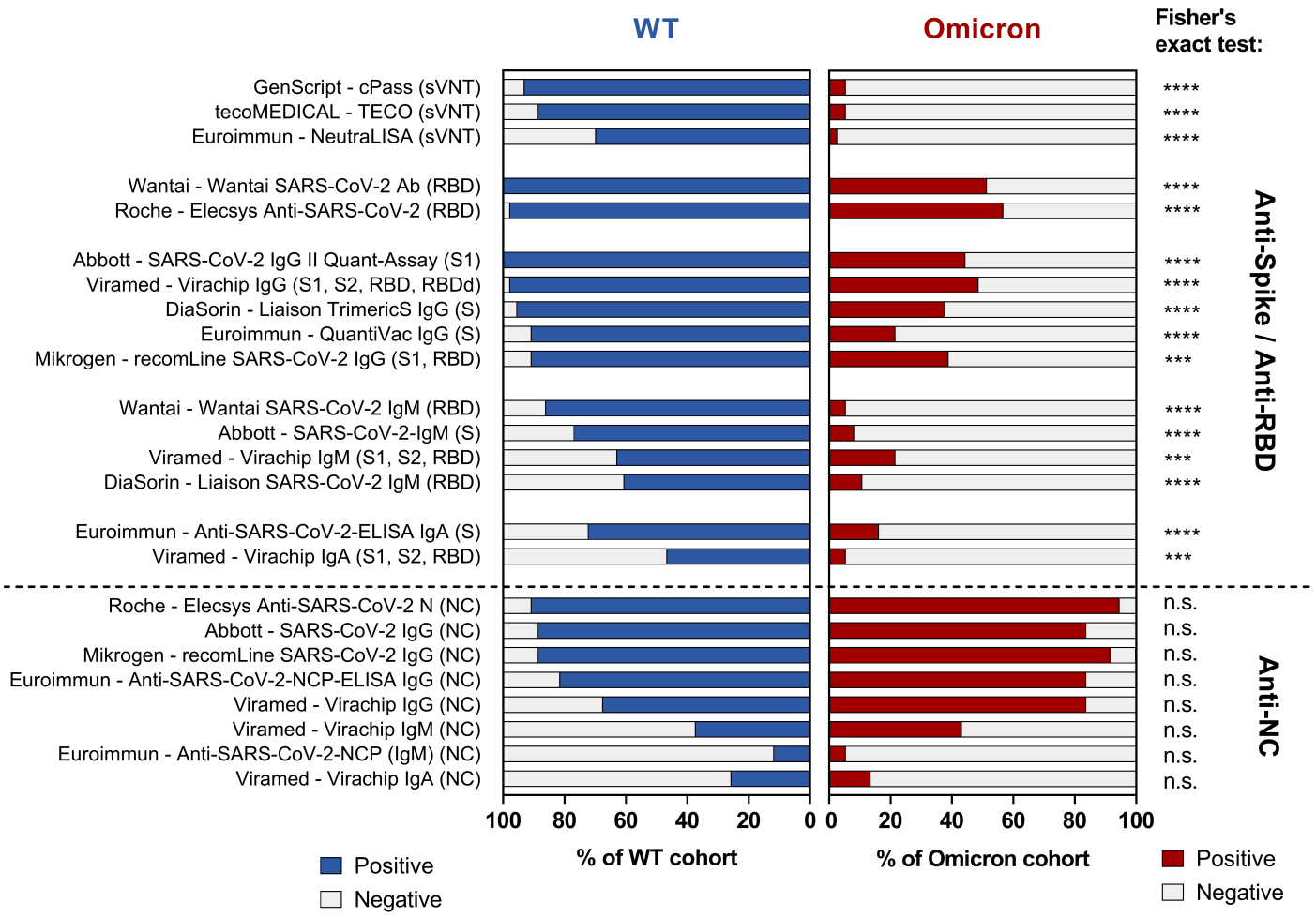

Figure 1

Detection rates of anti-spike (S)-, anti-Receptor-binding-domain (RBD)- and anti-nucleocapsid (NC) antibody assays in convalescents after primary infection with SARS-CoV-2 wildtype (WT) and the Omicron variant

Graphical representation of the detection rates (percentage of the samples tested positive) in the anti-SARS-CoV-2 antibody assays. Blue: Control (WT) cohort (n = 43); red: Omicron cohort (n = 37), except for Mikrogen – recomLine (S1, RBD, NC) (one sample technically invalid) and Abbott – SARS-CoV-2 IgG II Quant Assay (not sufficient sample material), n = 36 respectively. Asterisks (*) indicate a significant difference in two-tailed Fisher's exact test after correction for multiple testing (alpha = 0.05). [* $p < 0.05$; ** $p < 0.01$ *** $p < 0.001$, **** $p < 0.0001$; n.s.: not significant ($p > 0.05$)]. The immunoassays are denoted as "company – test kit name (target antigen)"; The immunoblots and microarrays (recomLine IgG, Virachip IgG / IgA / IgM) are listed twice as the S/RBD- and NC-signals were analyzed separately. Detailed information on the evaluated immunoassays is provided in Supplementary Table 2.

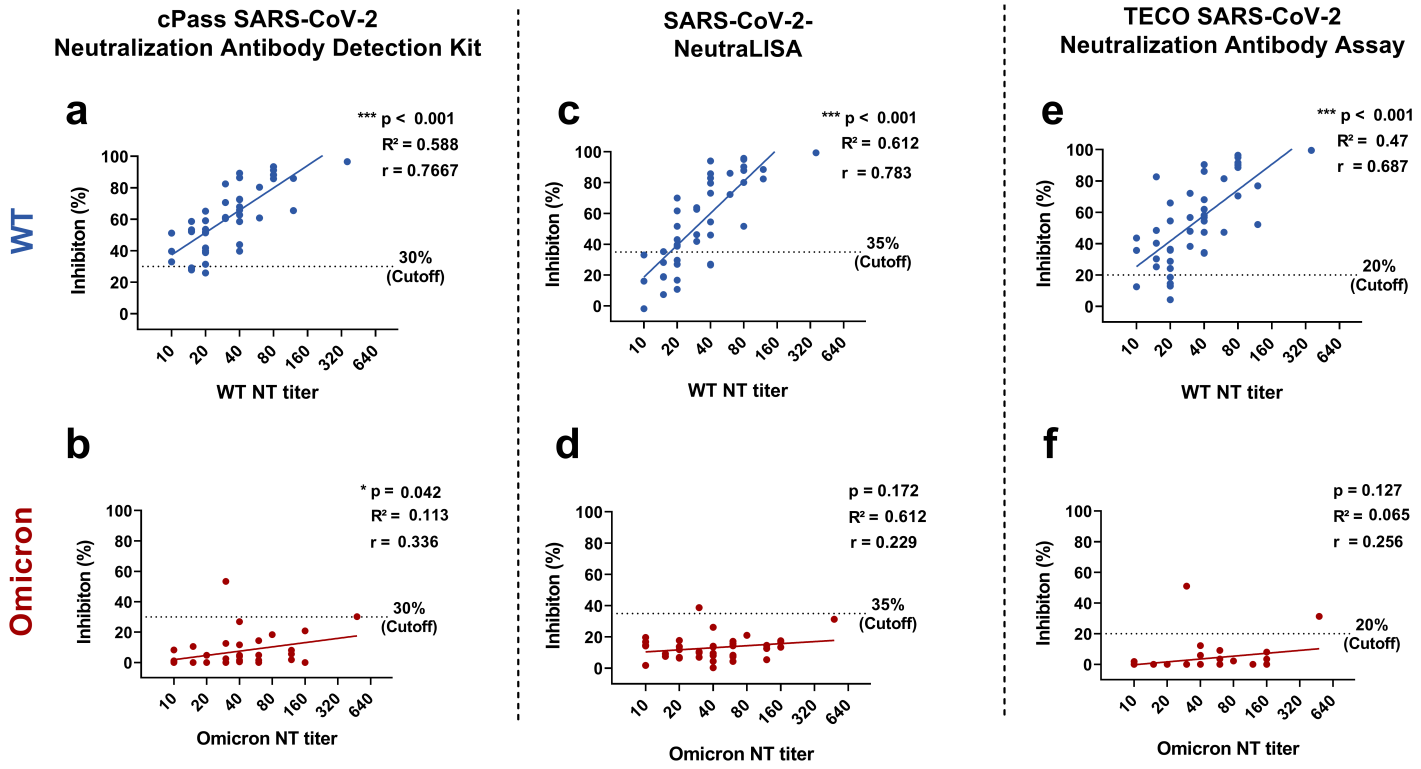

Figure 2

Correlation between antibody levels in SARS-CoV-2 Surrogate Virus Neutralization Tests (sVNTs) and variant-specific NT titers in SARS-CoV-2 wildtype (WT) and Omicron primary infections

Graphical description of the correlation and the linear regression of the results of the surrogate virus neutralization tests (as % RBD-ACE2-binding-inhibition) with respective quantitative titers of variant-specific NTs (Omicron, WT; both in log transformation). a) cPass vs WT NT, b) cPass vs Omicron NT, c) NeutraLISA vs WT NT, d) NeutraLISA vs Omicron NT, e) TECO vs WT NT, f) TECO vs Omicron NT. Dashed lines indicate the cutoff as recommended by the manufacturer. Blue dots: WT cohort (n = 43); red dots: Omicron cohort (n = 37). p-values, correlation coefficients r and R^2 were calculated using Pearson correlation. Asterisks (*) indicate a significant correlation. (* $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$).

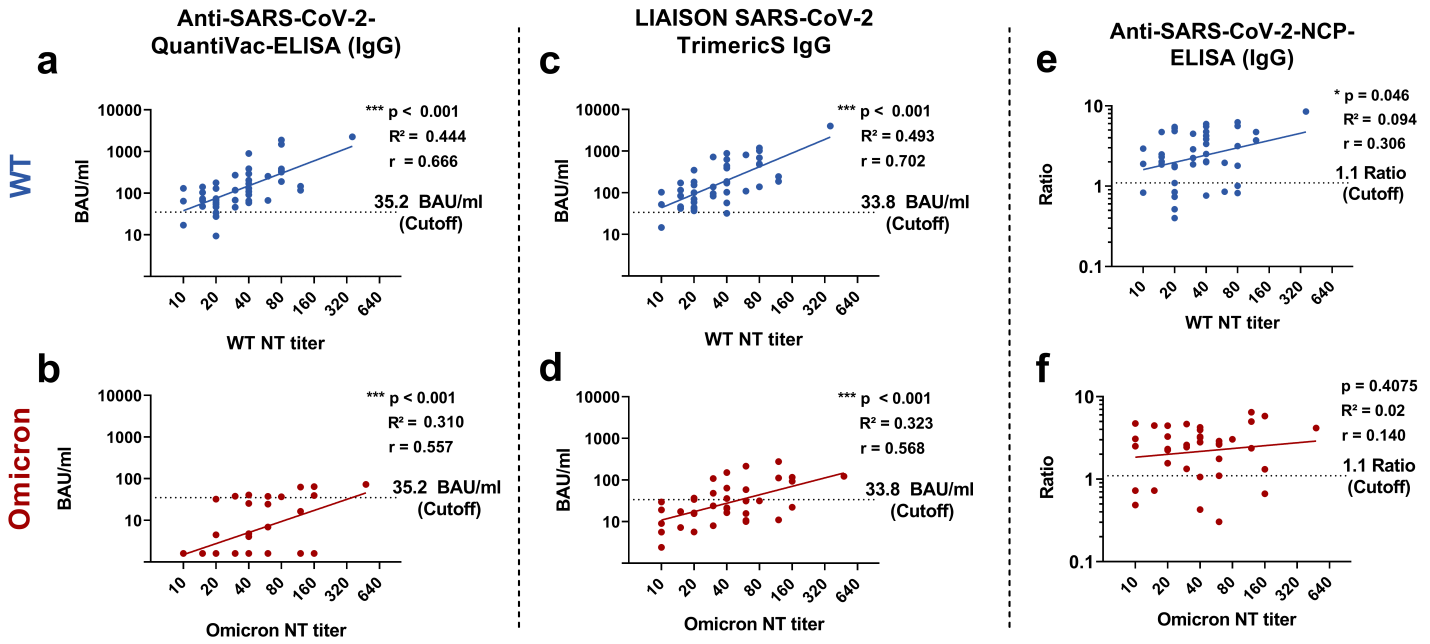

Figure 3

Correlation of anti-Spike (S)- and anti-nucleocapsid (NC) antibody levels with variant-specific NT titers in SARS-CoV-2 wildtype (WT) and Omicron primary infections

Graphical description of the correlation and the linear regression of the quantitative results by selected ELISAs and a CLIA (BAU/ml) to the respective titers of variant-specific NTs (Omicron, WT; both in log transformation). a) QuantiVac by Euroimmun: vs. WT NT b) QuantiVac by Euroimmun: vs. Omicron NT c) Liaison TrimericS IgG by DiaSorin vs WT NT d) Liaison TrimericS IgG by DiaSorin vs Omicron e) Anti-SARS-CoV-2-NCP-ELISA (IgG) by Euroimmun vs. WT NT) f) Anti-SARS-CoV-2-NCP-ELISA (IgG) by Euroimmun vs. Omicron NT) a-d: anti-S IgG assays; e-f: anti-NC IgG antibody assay. Dashed lines indicate the cutoff as recommended by the manufacturer. Blue dots: WT cohort (n = 43); red dots: Omicron cohort (n = 37). p-values, correlation coefficients r and R^2 were calculated using Pearson correlation (* $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$). Data on the correlations of the other commercial antibody assays are provided in the Supplement (Supplementary Figures 2 – 9).

Supplementary Files

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- SupplementaryInformation.docx