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Three rounds of a national external quality assessment reveal a link between disharmonic anti-SARS-CoV-2 antibody quantifications and the infection stage

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Abstract

Objectives: The WHO's standardized measuring unit, "binding antibody units per milliliter (BAU/mL)," should allow the harmonization of quantitative results by different commercial Anti-SARS-CoV-2 immunoassays. However, multiple studies demonstrate inter-assay discrepancies. The antigenic changes of the Omicron variant affect the performance of Spike-specific immunoassays. This study evaluated the variation of quantitative Anti-SARS-CoV-2-Spike antibody measurements among 46, 50, and 44 laboratories in three rounds of a national external quality assessment (EQA) prior to and after the emergence of the Omicron variant in a diagnostic near-to-real-life setting.

Methods: We analyzed results reported by the EQA participant laboratories from single and sequential samples from SARS-CoV-2 convalescent, acutely infected, and vaccinated individuals, including samples obtained after primary and breakthrough infections with the Omicron variant.

Results: The three immunoassays most commonly used by the participants displayed a low intra-assay and inter-laboratory variation with excellent reproducibility using identical samples sent to the participants in duplicates. In contrast, the inter-assay variation was very high with all samples. Notably, the ratios of BAU/mL levels quantified by different immunoassays were not equal among all samples but differed between vaccination, past, and acute infection, including primary infection with the Omicron variant. The antibody kinetics measured in vaccinated individuals strongly depended on the applied immunoassay.

Conclusions: Measured BAU/mL levels are only interchangeable among different laboratories when the same assay was used for their assessment. Highly variable ratios of BAU/mL quantifications among different immunoassays and infection stages argue against the usage of universal inter-assay conversion factors.

Keywords: antibodies; assay; BAU/mL; harmonization; reference; SARS-CoV-2; standardization.

Christoph Buchta and David Springer contributed equally to this work.

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Introduction

To harmonize quantitative measurements of antibodies against Severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), the World Health Organization (WHO) provided an International Standard (NIBSC code 20/136) as a Certified Reference Material [1]. From this panel, the standardized measuring unit of "Binding Antibody Unit (BAU) per mL" was derived, which should facilitate the calibration of antibody levels quantified with different commercial binding assays like chemoluminescence immunoassay (CLIAs) and enzyme-linked immunosorbent assays (ELISAs) [1].

Nevertheless, single analyses with reference materials and re-tested sample cohorts showed only a moderate agreement

of BAU/mL quantifications among different commercial immunoassays [2–9]. Thus, the possibility of using internal calibration protocols and conversion factors to mathematically correct inter-assay deviations has been discussed [6, 8, 10–12]. However, such conversion factors might not apply to the same extent for samples obtained at different time points after infection and vaccination [4, 9, 13].

The emergence of the Omicron variant, which harbors significant antigenic changes in its Spike protein, may further impede the standardization of Anti-SARS-CoV-2 antibody testing [14–17]. Indeed, detection rates of immunoassays with the Spike protein as the target antigen are reduced in individuals after primary Omicron infection [18]. To date, it is unknown whether and to what extent Omicron-specific antibodies may increase the variation among different immunoassays [19].

In this study, we evaluated manufacturer-dependent differences in BAU/mL quantifications prior to and during the Omicron era in a near-to-real-life setting. To this end, we analyzed data from three rounds of a national external quality assessment (EQA) scheme with 61 participating laboratories and 14 comprehensively characterized serum samples from vaccinated, convalescent, and acutely SARS-CoV-2 infected individuals, including samples obtained after primary and breakthrough infections with the Omicron variant.

We observed a low intra-assay inter-laboratory variation for the three immunoassays most commonly used by the EQA participants (Abbott SARS-CoV-2 IgG II Quant assay, Liasion SARS-CoV-2 TrimericS IgG assay, and the Roche Elecsys SARS-CoV-2 S assay). In contrast, there was a substantial inter-assay deviation between these assays. Notably, the ratios between BAU/mL levels quantified with these immunoassays varied among serum samples obtained after vaccination, past, and acute infection and were explicitly divergent after acute infection with the Omicron variant.

Materials and methods

EQA setting

Three rounds of a national EQA were conducted in August and November 2021 (pre-Omicron era) and April 2022 (Omicron era). A total of 15 vials of serum samples (three panels of five samples per round, labeled S1–S5, respectively) were dispatched to participant laboratories all over Austria by the Austrian Association for Quality Assurance and Standardization (ÖQUASTA). The three panels consisted of 14 different clinical samples. One sample was filled in duplicates in different vials (labeled S2 and S5 in the first EQA round) to assess the reproducibility of test results in duplicates (unknown to the participants).

In total, 61 laboratories participated in at least one round, of whom 45 participated in two and 32 in three EQA rounds.

Samples

All 14 samples were characterized using multiple SARS-CoV-2-specific antibody assays at the Center for Virology of the Medical University Vienna.

For the first two EQA rounds representing the pre-Omicron era (Supplementary Table S1), two samples were acquired from acutely infected individuals with recent and symptomatic SARS-CoV-2 infection 29 and 30 days after PCR diagnosis (both infections occurred in October 2020). In addition, two samples were collected from convalescent individuals after past infection, one 142 and one 346 days after PCR diagnosis (infections in March and September 2020, respectively). An additional sample was subsequently collected from one of these individuals after 527 days post PCR diagnosis, 162 days after the donor received one dose of mRNA-1273 SARS-CoV-2 vaccination (Spikevax, Moderna). Another pair of samples was derived from another donor, who had been vaccinated with two doses of BNT 162b2 (Comirnaty, Biontech Pfizer; one sample 87 days and one 238 days after the second vaccination dose). Before the samples were obtained, this individual had not been infected with the wild-type virus.

For the third EQA round representing the Omicron era (Supplementary Table S2), one sample (S1) was obtained from an individual with a symptomatic, primary Omicron infection 27 days post PCR diagnosis. The donor (and serological testing before the infection) confirmed that he had never been infected before and had not been vaccinated against SARS-CoV-2. The primary infection (confirmed by seroconversion) with an Omicron variant was verified by variant-specific PCR (indicating infection with the subvariant BA.2). Two additional samples (S2 and S4) were derived from donors who had been vaccinated three times with SARS-CoV-2 (three doses of BNT 162b2 in one individual and two doses of BNT 162b2 plus one dose of mRNA-1273 in the other) but acquired breakthrough infections with the Omicron variant (BA.1. and BA.2. respectively) six and three months after the third vaccination (Supplementary Table S2). The samples from these individuals were obtained 17 and 24 days after the PCR diagnosis, respectively. One additional sample (S3) was donated from a convalescent individual after a breakthrough infection with an Omicron variant (BA.1., 60 days after PCR diagnosis) after previous vaccinations with three doses of BNT 162b2 (breakthrough infection occurred one month after the third dose) and previous infection with the Delta variant (10 months before Omicron-infection).

Three serum samples, one for each EQA round, were acquired from donors in the pre-SARS-CoV-2 pandemic era and were negative control samples. The donors with acute SARS-CoV-2 infection and all donors for the third EQA round gave written consent for the study. Since the rest of the sample cohort comprised entirely anonymized samples from blood donation centers tested at the Center for Virology by routine diagnostics, the local Ethics Committee concluded that no written consent was required from these donors. The antibody testing for the study was approved by the local Ethics Committee (EK 2156/2019). The study was performed according to the Declaration of Helsinki.

Detailed information on all antibody assays used for the pretesting of the samples, including the live-virus neutralization assays, is given in Supplementary Table S3.

Sending of samples, data analysis, and reporting of results

Volumes of 500 µL of each sample were dispatched overnight at ambient temperature. The instructions provided to the participants

recommended that the samples be tested immediately or stored at 2–8 °C for no longer than six days after arrival. Importantly, participant laboratories were advised to carry out the analyses of the EQA samples as if they were routine patients' samples applying the same dilution steps as for routine diagnostics. The results were then reported to the EQA provider (ÖQUASTA) with a qualitative interpretation (positive, negative), the quantitative levels in BAU/mL (Spike-specific total Ig and/or IgG) together with the assay used (reagent and platform). Assignment of qualitative targets for samples followed *variant (a) "expert judgement"* as proposed in chapter 11.3.1 of the applicable standard ISO 13528:2015, "Statistical methods for use in proficiency testing by interlaboratory comparison" [20].

The reported results were collected, and qualitative results were judged for the EQA rounds as either correct (matching the target) or incorrect (not matching the target). Quantitative results were not assessed in this scheme but for this study. The EQA results (qualitative results, interpretations, and relevant information) were discussed in a summary report provided to the participants. In addition, the participants received reports on their performance and, if successful, a certificate.

Statistical analyses

Statistical analyses and data visualization were performed with GraphPad Prism 9.3.1. software. The levels of Spike-specific IgG antibodies that participant laboratories reported after quantification with either the Abbott SARS-CoV-2 IgG II Quant assay or the SARS-CoV-2 TrimericS IgG assay were compared using the Mann-Whitney U test. A p-value of <0.05 was considered statistically significant. Since the Roche Elecsys SARS-CoV-2 S assay quantifies Spike-specific total Ig (without differentiation of the immunoglobulin class), it was not statistically compared with the other two assays. All statistical analyses, including descriptive analyses and calculation of inter-assay ratios, were performed with the antibody levels the participants reported. In samples with a high antibody concentration, some participants did not report quantitative antibody levels but rather the exceedance of a specific threshold value. In such cases, the threshold value was illustrated in the Figures, but no statistical analyses or ratio calculations were performed.

Results

Antibody assays used by participant laboratories and qualitative results

The total number of different laboratories that reported results for at least one EQA round for SARS-CoV-2-specific antibody testing was 61, with 46 reporting for the first, 49 for the second, and 43 for the third round. The participating laboratories used 28 different antibody assays to assess Spike-specific-IgG, Spike-specific total Ig, Nucleocapsid-specific-IgG, Nucleocapsid-specific total Ig, SARS-CoV-specific IgM (with various target antigens), Spike-specific IgA antibodies and neutralizing antibodies using surrogate neutralization assays.

As shown in Supplementary Table S4, the qualitative interpretation was correct for most of the tested samples.

For the quantification of Spike-specific IgG and total Ig antibodies, the participants most commonly used (A) the Abbott SARS-CoV-2 IgG II Quant assay on the Abbott Alinity or Abbott Architect platforms (Abbott Laboratories, Chicago, USA; used by 10 laboratories in the first and second EQA round, respectively and 11 laboratories in the third round), (B) the SARS-CoV-2 TrimericS IgG assay on the LIAISON platform (DiaSorin, Saluggia, Italy; used by 10 in the first, 11 laboratories in the second and nine laboratories in the third round), and (C) the Roche Elecsys SARS-CoV-2 S assay on Cobas e411, Cobas e601, or Cobas e801 platforms (Roche, Basel, Switzerland; used by 25 in the first, 29 laboratories in the second and 23 laboratories in the third round). The remaining participant laboratories used eight other immunoassays to quantify Spike-specific antibodies. Since only one or two laboratories used the same assay, these results were excluded from further analyses (Supplementary Table S4).

Samples from convalescent and acutely infected individuals (pre-Omicron)

First, we compared the BAU/mL antibody concentrations quantified by the participants' three most commonly used Anti-Spike antibody assays in the first and second rounds of the EQA (pre-Omicron era).

As shown in Figure 1, the quantitive measurements of Spike-specific antibody concentrations in the same sample from a convalescent individual filled in duplicate vials unknown to the participants (S2 and S5) were nearly identical among all participant laboratories, indicating high precision and intra-laboratory reproducibility. In addition, the intra-assay variation among laboratories was low (Supplementary Table S5). However, there was a substantial variation among the three immunoassays. Indeed, the BAU/mL levels for Spike-specific IgG antibodies quantified by the Abbott SARS-CoV-2 IgG II Quant assay and the SARS-CoV-2 TrimericS IgG assay were statistically different ($p<0.0001$; Figure 1, and Supplementary Table S5).

In samples from patients with acute SAR-CoV-2 infection, a similar pattern of a low intra-assay, inter-laboratory variation but a high inter-assay variability was observed (Figure 2, Supplementary Table S5). Notably, the SARS-CoV-2 TrimericS IgG assay yielded the highest values, and the Roche Elecsys SARS-CoV-2 S assay the lowest, although the latter not only measures IgG antibodies but total Ig (i.e., antibodies irrespective of the immunoglobulin class (Figure 2, Supplementary Table S5).

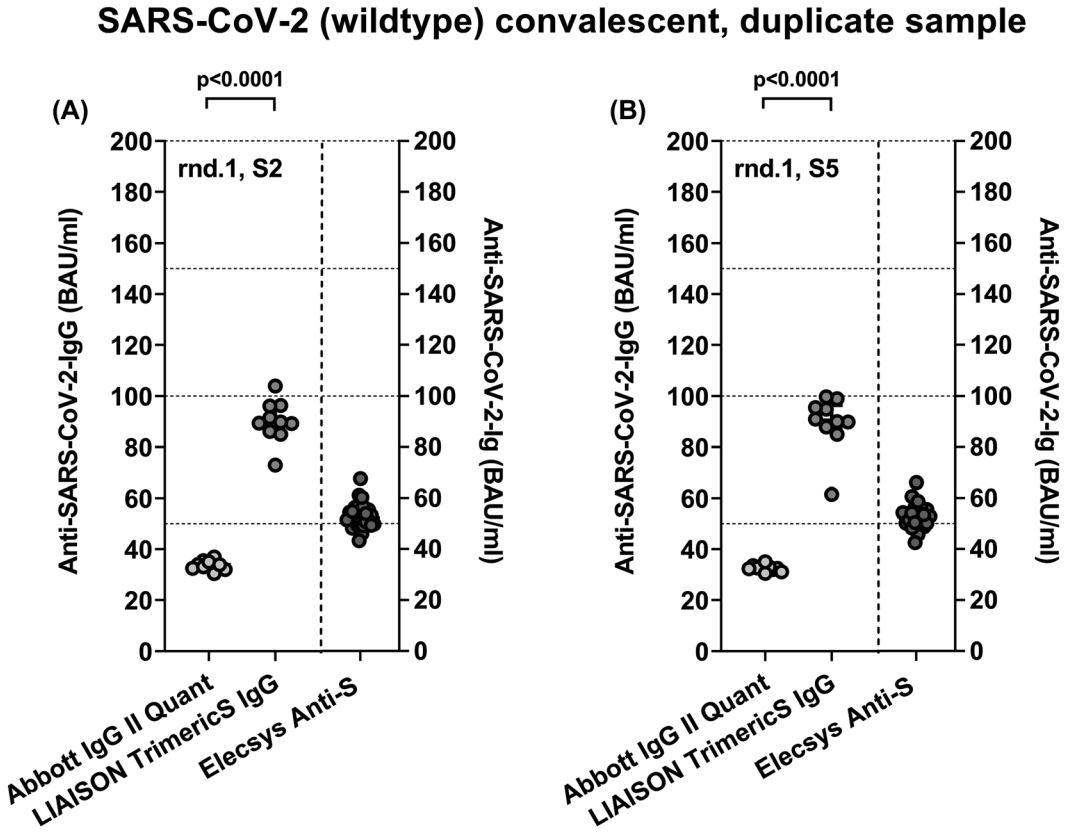

Figure 1: Anti-spike antibody levels quantified by the EQA participants in duplicate vials (A, B) of the same sample from a SARS-CoV-2 convalescent individual (142 days post-PCR-diagnosis).

Longitudinal samples from vaccinated individuals (pre-Omicron)

Then, we assessed antibody kinetics reported by the participant laboratories in the first two EQA rounds in sequential samples from the same donor after vaccination (87 and 238 after the second BNT 162b2 vaccination; Figure 3) and another convalescent individual who had been vaccinated after the infection (Figure 4).

In contrast to samples from acutely infected individuals, the Roche Elecsys SARS-CoV-2 S assay measured the highest BAU/mL levels in these samples, while the Abbott SARS-CoV-2 IgG II Quant assay measured the lowest. Thus, the degree of inter-assay variation was very high, while the intra-assay, inter-laboratory variation was low (Figure 3, Supplementary Table S5).

Although all participants correctly identified a significant decline of the Spike-specific antibody concentration in the longitudinal samples ($p<0.0001$ respectively), the extent of this decline strongly varied among the three antibody assays (median decrease: Abbott SARS-CoV-2 IgG II Quant assay: 75.53%, SARS-CoV-2 TrimericS IgG assay: 38.24%, Roche Elecsys SARS-CoV-2 S assay: 38.60%).

Furthermore, all laboratories measured a significant increase in antibody levels after vaccination in a SARS-CoV-2 convalescent individual ($p<0.0001$, respectively; Figure 4). However, due to the high antibody concentration post-vaccination, some participants did not report the exact quantitative antibody levels but only that the concentration exceeded a specific threshold value (Figures 3 and 4, Supplementary Table S5).

Of note, prior to vaccination (346 days post PCR diagnosis), the Roche Elecsys SARS-CoV-2 S assay yielded the highest BAU/mL level in the sample from the convalescent individual with long past infection (median: 964.3 BAU/mL), while the Abbott SARS-CoV-2 IgG II Quant assay measured the lowest (median: 120.0 BAU/mL).

EQA after the emergence of the Omicron variant

Next, we analyzed the antibody results from participant laboratories representing the Omicron era, including samples from an individual with a primary Omicron infection, two individuals with Omicron breakthrough

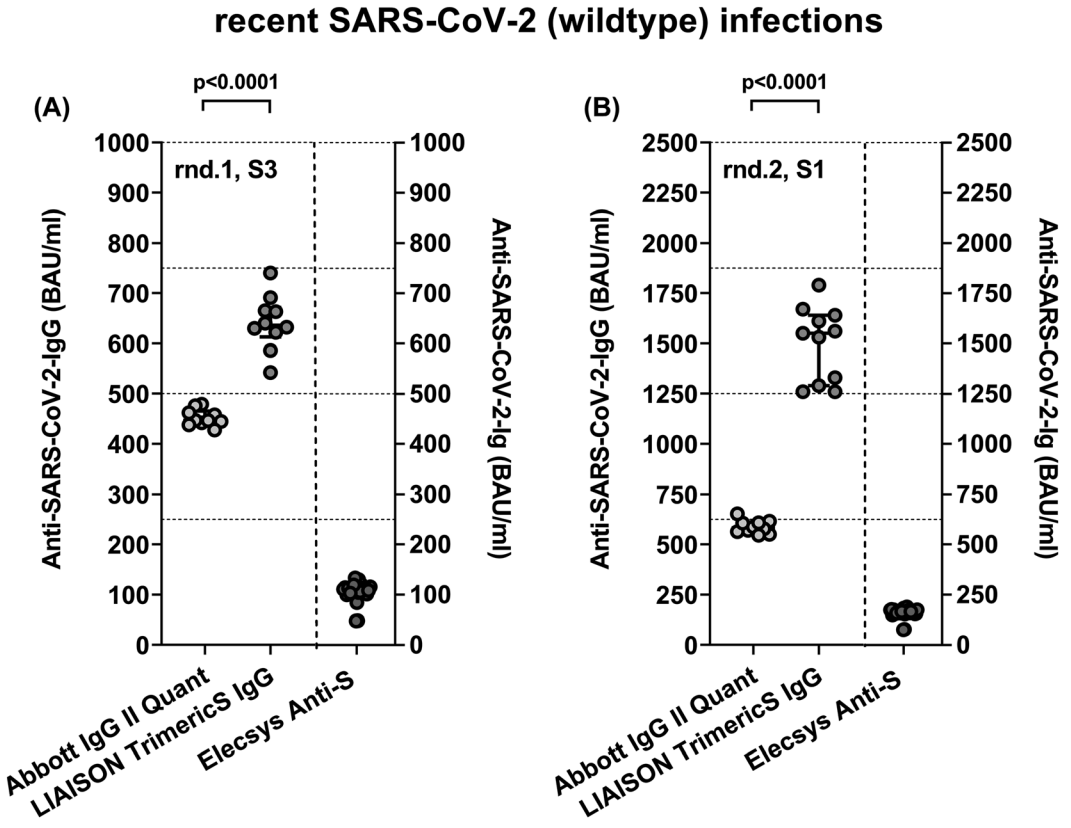

Figure 2: Anti-spike antibody levels quantified by the EQA participants in samples from two individuals after a recent (acute) and symptomatic SARS-CoV-2 infection. The samples were obtained 29 (A) and 30 (B) days post-PCR diagnosis, respectively.

infections after three vaccinations, and one individual with an Omicron infection after three vaccinations and previous infection with the Delta variant (Figure 5).

As shown in Figure 5A, the reported results from Anti-Spike antibody measurements in the sample from an individual with acute primary infection with an Omicron variant showed a similar pattern of inter-assay variation as the two other samples from individuals with acute infection. However, the discrepancy between the assays was even more substantial than in samples from acutely infected patients obtained prior to the emergence of the Omicron variant. Indeed, the highest measured concentration was measured by the SARS-CoV-2 TrimericS IgG assay (with a median BAU/mL level of 146), and the lowest by the Roche Elecsys SARS-CoV-2 S assay (with a median concentration of 6.6 BAU/mL).

In the samples from individuals after breakthrough infection with an Omicron variant after three vaccinations, the measured concentration of Spike-specific antibodies was very high among all participant laboratories (Figures 5B–D). However, analogously to the other post-vaccination samples, not all participant laboratories reported quantitive BAU/mL levels, but rather that the concentration exceeded a specific threshold.

Ratios of quantified BAU/mL levels among the immunoassays

Finally, we evaluated whether mathematical conversion factors between the immunoassays would facilitate correcting the observed inter-assay deviations. Therefore, we calculated the ratios between the median antibody concentration measured by the three immunoassays in each sample (Supplementary Table S5). These ratios were then compared for the infection/vaccination status of the respective donors and the interval since infection/vaccination (Figure 6).

As shown in Figure 6, the ratios of the median antibody levels between the Abbott SARS-CoV-2 IgG II Quant assay and the SARS-CoV-2 TrimericS IgG assay were not explicitly linked with the infection or vaccination status or the interval since infection/vaccination (since they were similarly distributed). In contrast, the ratios between the Roche Elecsys SARS-CoV-2 S assay and the Abbott SARS-CoV-2 IgG II Quant assay or the SARS-CoV-2 TrimericS IgG assay were lower in samples from recently infected individuals than in those from individuals with past infection or vaccination with or without prior infection.

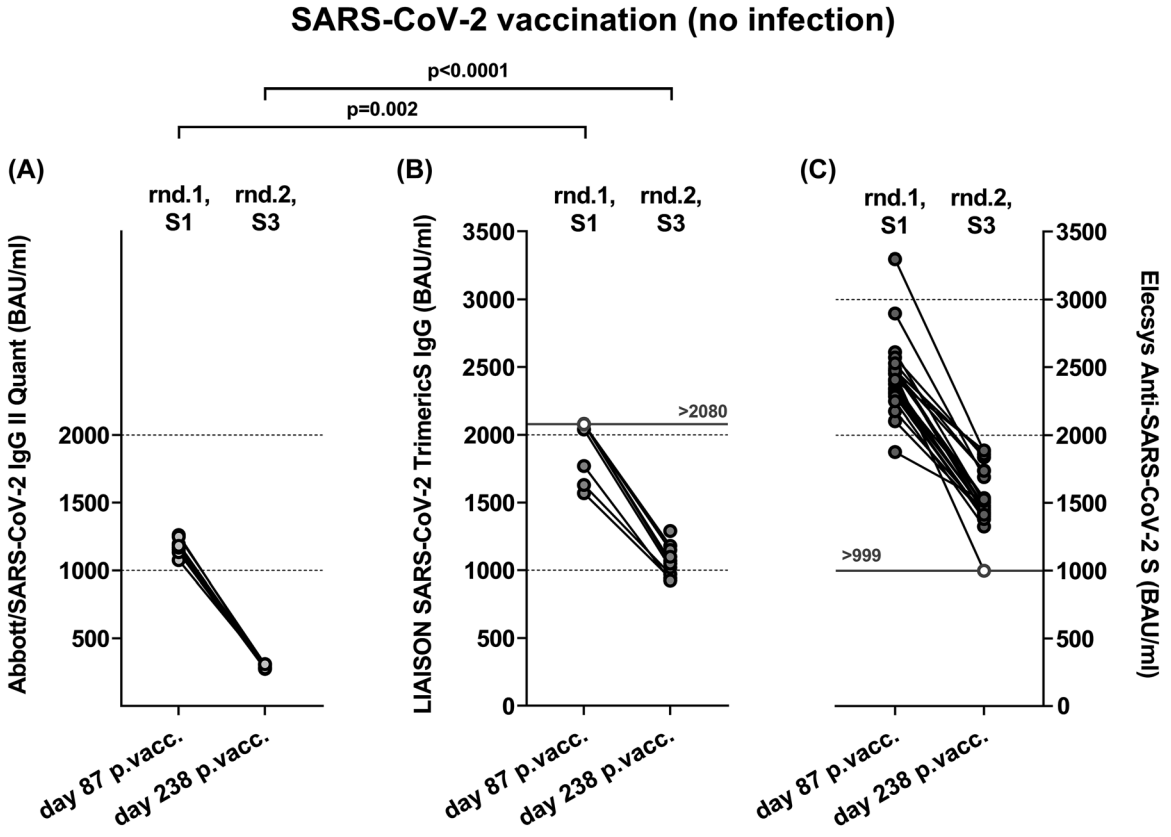

Figure 3: Anti-spike antibody levels quantified by the EQA participants in consecutive samples from the same individual after two doses of BNT 162b2 vaccination. Spike-specific IgG antibody levels reported by participant laboratories using the Abbott SARS-CoV-2 IgG II Quant assay (A) and the Liasion SARS-CoV-2 TrimericS IgG assay (B). Spike-specific total Ig antibody levels quantified by EQA participants using the Roche Elecsys SARS-CoV-2 S assay (C). The samples were obtained from the same donor 87 and 238 days after the second vaccination. Hollow gray circles with gray lines indicate that participants reported exceedance of a specific threshold value rather than a quantitive level.

Discussion

Even before the WHO's reference sample panel was introduced, national and international EQAs proved suitable for assessing the diagnostic abilities and variations among different SARS-CoV-2-specific antibody assays [21, 22]. In the same near-to-real-life setting of three EQA rounds, our study indicates significant inter-laboratory manufacturer-dependent variations in the quantification of Spike-specific antibodies in standardized BAU/mL prior to and particularly during the Omicron era. Importantly, our data demonstrate that the inter-assay variations are associated with the infection/vaccination status of the samples' donors and may further be influenced by infection with serologically distinct variants such as the Omicron variant.

For many reasons, the harmonization of commercial antibody tests for quantifying SARS-CoV-2-specific antibodies remains challenging. The composition of antibodies in an individual serum sample is remarkably heterogeneous regarding the polyclonal specificity, concentration, binding avidity, neutralizing capabilities, and the composition of different immunoglobulin classes [23–27]. On the other hand, commercial antibody assays differ for the target antigen, the processing of the antigen, the covered immunoglobulin class, and the optimal concentration range [28–32]. Those factors might contribute to the inter-assay discrepancies observed by previous studies in quantifying antibody levels despite standardization with a reference sample [3–9]. Accordingly, our study confirmed substantial intra-assay discrepancies for Anti-Spike antibody measurements in a near-to-real-life setting among multiple national laboratories, although all participating laboratories used the WHO's standardized measuring unit of BAU/mL.

Since there is no clear clinical correlate of protection against SARS-CoV-2 infection, immunoassays are still mainly recommended for research studies rather than diagnostic testing [33]. However, in certain countries like Austria, there has been a strong public demand to frequently measure the individual level of SARS-CoV-2-specific immunity in the general population in diagnostic laboratories. In this context, data

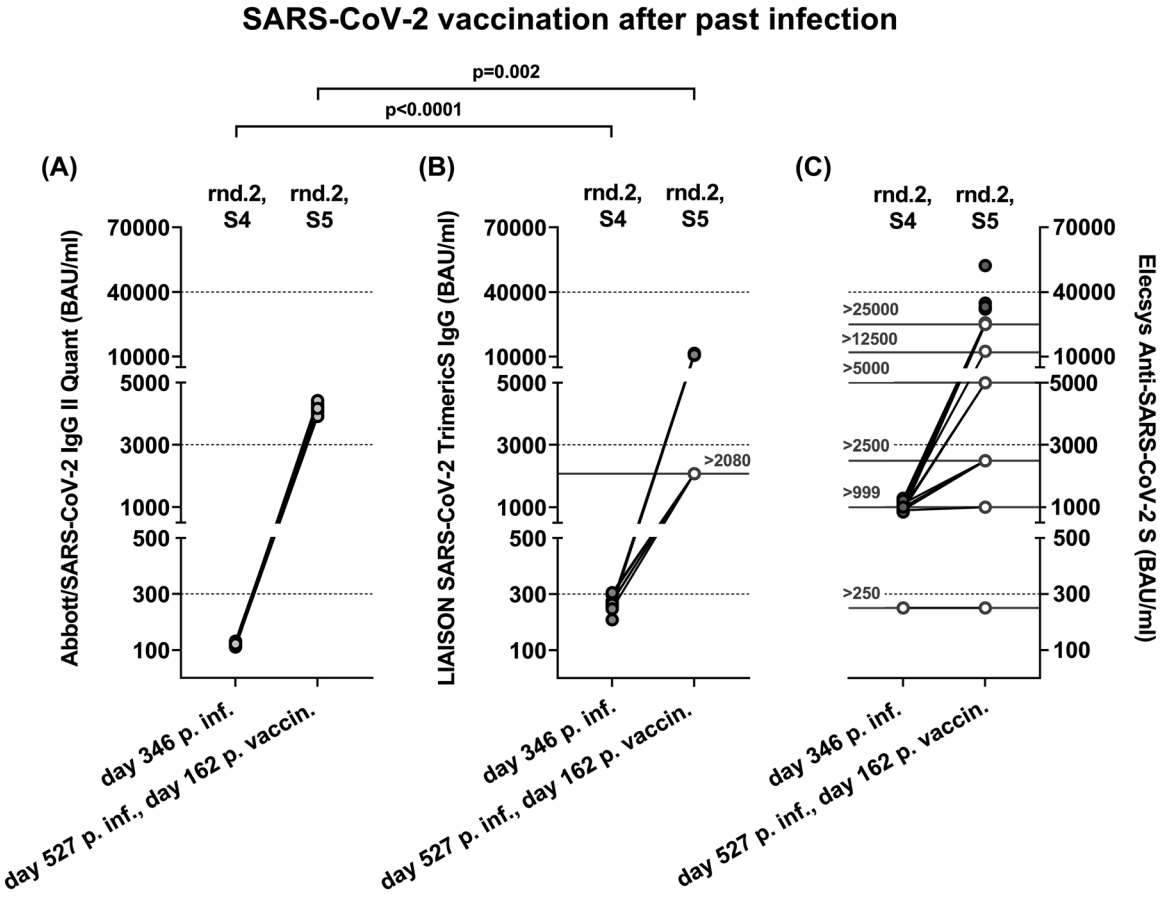

Figure 4: Anti-spike antibody levels quantified by the EQA participants in a SARS-CoV-2 convalescent individual before and after mRNA-1273 vaccination. Spike-specific IgG antibody levels quantified by EQA participants using the Abbott SARS-CoV-2 IgG II Quant assay (A) and the Liasion SARS-CoV-2 TrimericS IgG assay (B). Spike-specific total Ig antibody levels reported by participant laboratories that used the Roche Elecsys SARS-CoV-2 S assay (C). The consecutive samples were obtained from the same donor 346 and 527 days post-PCR-diagnosis, the latter 162 days after one dose of mRNA-1273 vaccination. Hollow gray circles with gray lines indicate that participants reported exceedance of a specific threshold value rather than a quantitive level.

from this and previous studies on a significant disharmony of commercial SARS-CoV-2-specific immunoassays strongly argue against a broad application of SARS-CoV-2-specific antibody tests as routine diagnostics [4, 9, 13]. At least, all these studies indicate that BAU/mL levels are only interchangeable among different laboratories and can only be compared longitudinally within an individual when the same assay is used for their assessment.

However, our main finding was that the assay-specific variations in Anti-SARS-CoV-2 antibody measurements strongly depended on the interval since the infection. Notably, the impact of the infection stage on individual BAU/mL levels quantified by different assays was explicitly evident in samples from acutely infected individuals within one month after the infection. Indeed, participants using the Roche Elecsys SARS-CoV-2 S assay consistently quantified significantly lower Spike-specific antibody levels in these samples than participants who used other assays.

Concordantly, a previous study showed that antibodies of low avidity from patients with acute SARS-CoV-2 infection display weaker binding to this assay's target antigen, which might have caused lower antibody levels in the measurements by EQA participants using the Elecsys SARS-CoV-2 S assay [34]. That this might be the functional cause for our observation is supported by the fact that the Roche Elecsys SARS-CoV-2 S assay, in contrast, measured the highest antibody levels in a sample from a convalescent individual (prior to vaccination) one year after the infection.

Within the scope of routine testing, a high antibody concentration in the respective sample poses another factor that might enhance the disharmony among different antibody assays and laboratories. Indeed, a previous study demonstrated that the grade of inter-assay variation significantly increases with antibody concentration [6]. Furthermore, in the near-to-real-life setting of the EQA, not all participants reported high antibody concentrations as

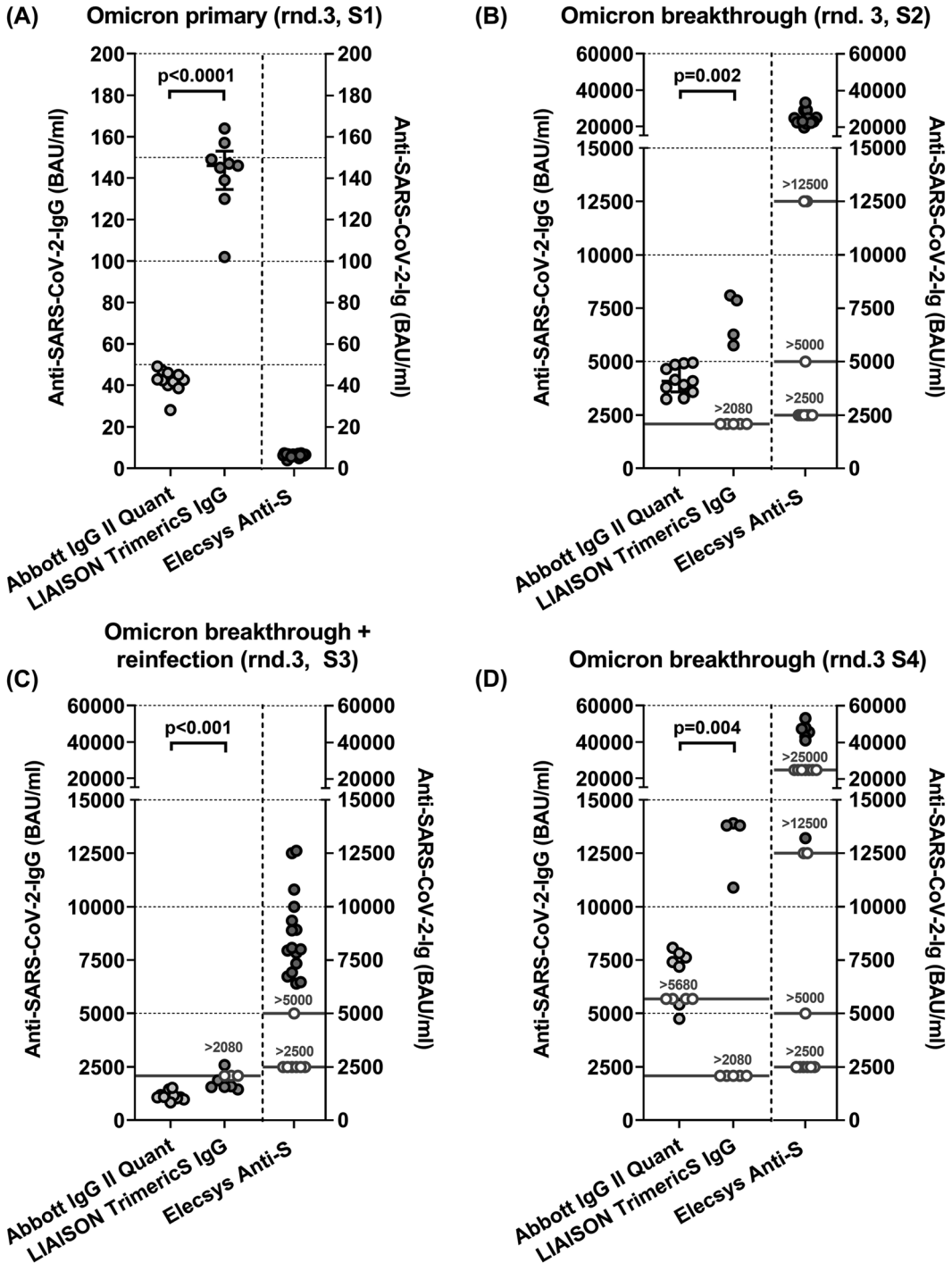

Figure 5: Anti-spike antibody levels quantified by the EQA participants in samples from four donors representative for the Omicron era. (A) Levels of spike-specific IgG and total Ig antibodies in a sample from an individual after recent primary infection with the Omicron variant (27 days post-PCR-diagnosis). (B, D) Anti-Spike antibody levels in samples from two individuals with a recent Omicron breakthrough infection after three vaccinations (sample collection 17 and 24 days after PCR diagnosis and 198 and 132 days after the third vaccination, respectively). (C) Spike-specific IgG and total Ig antibody levels in a sample from an individual with an Omicron breakthrough/reinfection after past infection with the Delta variant and three doses of BNT 162b2 vaccination (sample collection 62 days after PCR diagnosis of the Omicron-infection, 109 days after the third vaccination and 364 days after infection with the Delta variant). Hollow gray circles with gray lines indicate that participants reported exceedance of a specific threshold value rather than a quantitive level.

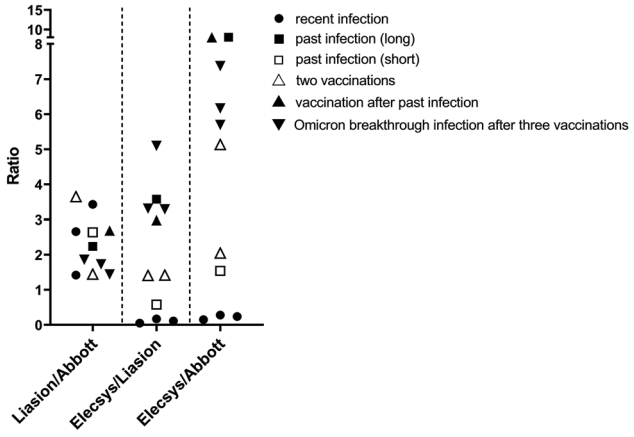

Figure 6: Ratios between the median antibody concentration quantified by the three immunoassays most commonly used by the EQA participants in each sample. Samples from individuals with recent infections were obtained within 30 days after PCR diagnosis. Samples from individuals with past infection were collected 142 (short) and 346 (long) days post-PCR-diagnosis, respectively. Samples after two vaccinations were collected from the same individual 87 and 238 days after the second vaccination. The sample obtained after vaccination of a SARS-CoV-2 convalescent was obtained 162 days after the vaccination, and samples after Omicron breakthrough infections after three vaccinations with or without previous infection with the Delta variant were collected 109, 132 and 198 days after the third vaccination, respectively).

quantitive levels, but only that the concentration exceeded a specific threshold value, further increasing the inter-laboratory variance.

Finally, with its immune-evasive properties, the Omicron variant increased the variation in the measurement of SARS-CoV-2-specific antibodies as a routine diagnostic parameter. Indeed, we recently demonstrated a reduced sensitivity of Anti-Spike antibody assays in individuals with primary infection with Omicron variants [18]. One sample from an individual with primary Omicron infection was used in the third EQA round and showed substantial inter-assay variation (Figure 5A). While participants who used the SARS-CoV-2 TrimericS IgG assay by DiaSorin measured a median BAU/mL concentration of 164 in this sample, laboratories using the Roche Elecsys SARS-CoV-2 S assay only detected a median level of 6.6 BAU/mL.

As a limitation of this study, we did not provide target concentrations for all immunoassays the participants later used in the EQA rounds and, therefore, could not assess the analytical accuracy of the participants' quantitative testing. Furthermore, due to their small number, the samples of our EQA can merely serve as representatives for different phases of the SARS-CoV-2 pandemic, e.g., we only included samples from individuals after mRNA vaccination. However, the donor samples were explicitly selected, enabling only a survey with a small to medium-sized participant number since we tried to avoid pooling samples from multiple individuals.

Nonetheless, data from this study obtained from the near-to-real-life setting of three EQA rounds show a significant variation of quantified BAU/mL levels among laboratories that use different commercial antibody assays. Since this variation depended on the interval since infection/vaccination and was particularly strong after primary Omicron infection, the widespread diagnostic use of Spike-specific antibody assays for the individual assessment of immunity should be limited. Hence, for research studies, antibody levels should only be compared among individuals when the same commercial assay was used for their quantification, with the caveat that the interval since infection/vaccination might affect results by different commercial antibody assays to varying extents.

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Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Informed consent: The donors with acute SARS-CoV-2 infection and all donors for the third EQA round gave written consent for the study. Since the rest of the sample cohort comprised entirely anonymized samples from blood donation centers tested at the Center for Virology due to routine diagnostics, the local Ethics Committee concluded that no written consent was required from these donors.

Ethical approval: Research involving human subjects complied with all relevant national regulations and institutional policies, is in accordance with the tenets of the Helsinki Declaration (as revised in 2013) and has been approved by the author's Institutional Review Board (EK 2156/2019).

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