Manuylov V et al., Arch Microbiol Immunology 2022
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Table 1: Results for the determination of the "protective" NtAb titer ≥1:160 (see the main text for discussion) obtained in the studied ELISA assays and presented in a four-field-table view. Gray cells – "true" results; blank cells – "false" results; TP – true positive; FP – false positive; FN – false negative; TN – true negative. The "sensitivity" and "specificity" of the experimental tests were calculated according to the Bayes theorem as follows:

Results in the VNA	IgG to RBD (BAU/ml)		BAU×AI index		sVNT titer	
	"positive" (≥230), n	"negative" (<230), n	"positive" (≥150), n	"negative" (<150), n	"positive" (≥1:320), n	"negative" (<1:320), n
There was a protective NtAb titer (≥1:160)	57^{TP}	8^{FN}	60^{TP}	5^{FN}	62^{TP}	3^{FN}
There was not a protective NtAb titer (<1:160)	1^{FP}	38^{TN}	1^{FP}	38^{TN}	4^{FP}	35^{TN}
Sensitivity of the experimental test	87.7±8%		92.3±6.5%		95.4±5.1%	
Specificity of the experimental test	97.4±5%		97.4±5%		89.7±9.5%	

Sensitivity = TP / (TP+FN) × 100%;
Specificity = TN / (TN+FP) × 100%.
The 95% CIs for the sensitivity and specificity values were calculated using the binomial distribution.

- The concentration of the IgG to RBD ≥ 230 BAU/ml corresponds to the NtAb titer ≥ 1:160 with 87.7±8% sensitivity and 97.4±5% specificity (hereafter, CI 95% was calculated using the binomial distribution, $p<0.05$);
- The BAU×AI index ≥ 150 has sensitivity of 92.3±6.5% and specificity of 97.4±5%;
- The sVNT titer ≥ 1:320 has sensitivity of 95.4±5.1% and specificity of 89.7±9.5%.

In other words, if a patient has IgGs in amounts greater than 230 BAU/ml, there is a 97.4±5% probability that he or she also has a supposedly protective NtAb titer of 1:160 or higher; if a patient has a BAU×AI index ≥ 150 (a measure of the concentration of high-affinity IgGs), he or she will also have a protective NtAb titer of 1:160 or more, with a probability of 97.4±5%; finally, if there is an sVNT titer ≥ 1:320 in the sample, its donor has at least a 89.7±9.5% probability of carrying a 1:160 NtAb titer.

On the other hand, if a patient actually has a protective NtAb titer of 1:160 or more, there is an 87.7±8% probability that he or she will have IgG concentrations ≥ 230 BAU/ml; a 92.3±6.5% probability that the BAU×AI index will be 150 or more; and a 95.4±5.1% probability that the sVNT titer will be 1:320 or higher.

Thus, among the methods tested, determination of the sVNT titer gives the highest sensitivity (only 4.6% of samples that have a "true" NtAb titer ≥1:160 will be recognized as false negatives), and determination of BAU/ml and the BAU×AI index recognizes the same titer with maximum specificity (only 2.6% of samples with the NtAb titer lower than a "true protective" will be misdiagnosed as positive). We believe that tests to determine the protective level of immunity should be as specific as possible, even at the expense of sensitivity, because the patient who receives a positive result should be confident in his/her protection. Therefore, we can state that the simple IgG quantification (in BAU/ml) is quite effective in assessing the levels of protective antibodies; as such, we recommend using the BAU×AI index when possible, since it has a higher sensitivity with an equally high specificity. This is the main practical result of our study.

Of course, our study had a number of limitations. We investigated only volunteers who had been vaccinated against the Wuhan variant; there were no patients in the studied group who were additionally immunized by an infection with the Delta or Omicron variants. Next, most of the participants had IgGs of rather high avidity: on average, avidity exceeded 50% in the group (see Table A-2, Supplementary A). We hypothesize (although we do not provide any evidence to support this hypothesis in this article) that testing for the BAU×AI index might be even more effective in determining the protective force of immunity in an early period of immunization, when IgGs are being produced in large amounts, but their avidity is still low (<40-50%) [15, 21].

As already noted, the obtained results cannot be directly applied to the protection force of immunity against the Omicron infection, but are still applicable regarding the Delta-like variants, which may re-emerge in the human population. In any case, the described methodology may be useful in the evaluation of arbitrary NtAb titers in routine clinical laboratories, e.g., for making decisions about preventive re-vaccination, in cases when the determined ELISA marker (BAU/ml, sVNT, etc.) has become too low to protect the patient against COVID-19 re-infection.

Declaration of Competing Interest

The authors declare no conflict of interest.

Authorship Contributions Statement

Victor Manuylov: project administration, conceptualization, writing; Inna Dolzhikova: samples collection, virus neutralization assay; Alexandra Kudryashova: ELISA, surrogate virus neutralization

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assay; Bogdan Cherepovich: IgG quantification, avidity index assay; Anna Kovyrshina: samples collection, virus neutralization assay; Anna Iliukhina: samples collection, virus neutralization assay; Olga Kharchenko: investigation, methodology; Maria Semashko: data processing, ethics; Artem Tkachuk: laboratory resources, editing; Vladimir Gushchin: investigation, writing; Olga Borisova: supervision, methodology, editing. All authors agree with the text of the article.

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