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Comparison of two commercial surrogate ELISAs to detect a neutralising antibody response to SARS-CoV-2

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ARTICLE INFO

Keywords:
COVID-19
SARS-CoV-2
Antibodies
Neutralising ELISA

ABSTRACT

Introduction: Reliable methods for the detection of SARS-CoV-2 neutralising antibodies (NAbs) are essential for the evaluation of vaccine candidates and for the selection of convalescent plasma donors. Virus neutralisation tests (NTs) are the gold standard for the detection and quantification of NAbs, but they are complex and require BSL3 facilities. In contrast, surrogate enzyme-linked immunosorbent assays (sELISA) offer the possibility of high-throughput testing under standard laboratory safety conditions. In this study, we investigated two commercial sELISA kits (GenScript, AdipoGen) designed for the detection of SARS-CoV-2 NAbs.

Methods: 276 plasma samples were screened using commercial IgG-ELISA and NAbs titres were determined by micro-neutralisation test (micro-NT). In addition, all samples were tested in both sELISA. Sensitivity and specificity for both sELISA were determined in comparison to the micro-NT results.

Results: 57 % of the samples were SARS-CoV-2 NAb positive in micro-NT, while 43 % tested negative. Comparison with micro-NT results showed a sensitivity of 98.2 % and a specificity of 69.5 % for the GenScript ELISA. The AdipoGen ELISA had a sensitivity of 83.5 % and a specificity of 97.8 %. False negative results were obtained mainly on samples with low NAbs titres.

Conclusion: Both sELISA were able to qualitatively detect NAbs in plasma samples. Sensitivity and specificity differed between sELISA with GenScript superior in sensitivity and AdipoGen superior in specificity. Both sELISA were unable to quantify NAbs, thus neither of them can completely replace conventional NTs. However, in a two-step diagnostic algorithm, AdipoGen could potentially replace NT as a subsequent confirmatory test due to its high specificity but only in settings where no exact NAbs quantification is needed.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) first appeared in China at the end of 2019 and was subsequently identified as the causative agent of a new respiratory disease later known as coronavirus disease 2019 (COVID-19). Symptoms can range from mild and flu-like symptoms to severe and fatal lung disease (Gandhi et al., 2020; Li et al., 2020; Zhu et al., 2020). Despite the immediate introduction of infection control measures, SARS-CoV-2 spread worldwide and soon became not only an urgent medical challenge but also a serious socioeconomic burden (Ali and Alharbi, 2020). Government interventions to slow down the spread of the virus were quickly implemented and disrupted the daily lives of billions of people. Almost nine months after the start of the SARS-CoV-2 pandemic, the scientific community and policy-makers around the world have shifted their focus from diagnosing acute COVID-19 infections to serology and how it can be used to ease the constraints of daily life (Tan et al., 2020). Antibody detection tests such as enzyme-linked immunosorbent assays (ELISAs) are widely used to estimate the prevalence and incidence of SARS-CoV-2 and dozens of companies now offer a variety of such immunoassays (Website Global Progress on COVID-19 Serology-Based Testing. In: Johns Hopkins Center for Health Security, 2020). They can also help to determine case fatality rates more accurately and facilitate the search for natural reservoirs and intermediate hosts (Petherick, 2020). However, they lack the ability to verify neutralisation, which is why they cannot distinguish between non-neutralising antibodies (Abs) and NAbs. However, since

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https://doi.org/10.1016/j.jviromet.2021.114122
Received 13 December 2020; Received in revised form 14 February 2021; Accepted 2 March 2021
Available online 8 March 2021
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virus neutralisation plays a key role in the development of a (long-term) protective immune response, the ability to detect NABs in patient samples is crucial (Jiang et al., 2020). Therefore, serological tests to detect NABs against SARS-CoV-2 are an important aid in determining herd immunity and humoral protection, assessing vaccine efficacy during long-awaited clinical trials, and selecting convalescent plasma for intensive care treatment.

The current gold standard for detection and also quantification of functional NABs in blood samples are virus neutralisation tests (Okba et al., n.d.). Several variants of the neutralisation test have been developed in the past: In the Plaque Reduction Neutralisation Test (PRNT), the virus plaques are counted and compared with the initial concentration of the virus to determine the percentage reduction in total viral infectivity. In this way, PRNT endpoint titres can be calculated for each serum sample at each selected percentage reduction of viral activity (typically 50 % or 90 %). A disadvantage of PRNT is that it is labour-intensive and not easily adaptable for high throughput, which makes it difficult to use for large-scale surveillance and vaccine trials (Grigorov et al., 2011). The micro-NT is another variation whose informative value corresponds to the results of the PRNT90, but which is more suitable for the processing of large sample quantities due to the reduced amount of work needed. In this assay, the individual plaques are not counted, but the absolute virus growth in any well is measured.

However, all virus neutralisation tests depend on work with infectious viruses and for SARS-CoV-2 can therefore only be performed in a BSL3 laboratory environment, which greatly limits the number of laboratories that can perform them (GeurtsvanKessel et al., 2020; Hoehl and Ciesek, 2020). Previously published SARS-CoV-2 pseudovirus-based NTs can be performed under BSL2 conditions, but also require the cultivation of infectious virus particles in cell cultures. There is therefore no significant time advantage compared to classical NTs (Nie et al., 2020; Yang et al., 2020). Other published alternatives include the use of the genetically modified fluorescent SARS-CoV-2 virus, which slightly reduces the time required to perform neutralisation tests, but still requires the use of BSL3 facilities (Muruato et al., 2020).

Given the current scale and pace of the pandemic, diagnostic laboratories everywhere are already finding it difficult to provide timely test results. As the demand for NABs titres is expected to increase in the future, it is important to find faster, more scalable and automated high-throughput alternatives to traditional NT.

In this study we investigated two commercially available surrogate enzyme immunoassortant assays (sELISA) for the specific detection of SARS-CoV-2 NABs in human blood samples: The SARS-CoV-2 Neutralising Antibodies Detection Kit (AdipoGen Life Sciences, Liestal, Switzerland) and the cPass™ SARS-CoV-2 Neutralisation Antibody Detection Kit (GenScript Biotech, NJ, USA).

We tested both kits side by side and in direct comparison with the micro-NT to determine both sensitivity and specificity of the two sELISAs and to test their potential as an alternative to conventional NT.

2. Material and methods

2.1. Samples

A total of 276 human plasma samples were tested for SARS-CoV-2 specific NABs. Of these plasma samples, 230 had previously tested positive for SARS-CoV-2 Abs in a commercial IgG ELISA (Euroimmun, Lübeck, Germany), which was used as pre-screening. A further 46 SARS-CoV-2 Abs negative plasma samples collected before the occurrence of SARS-CoV-2 (mid to late 2018) were used as negative controls. All samples were heat-inactivated (30 min, 56 °C) prior to analysis. The study was carried out in-line with “The Code of Ethics of the World Medical Association (Declaration of Helsinki)”. The use of plasma samples complied with the guidelines of the Central Ethics Committee of the German Medical Association (Dtsch Arztebl 2003; 100(23): A-1632). In accordance with these guidelines, the anonymised use of residual material from the samples sent to our laboratory for diagnostic purposes is permissible, provided that the patients have not decided against this procedure. Samples from patients who had decided against this procedure were excluded from the analyses.

2.2. Micro-neutralisation test (micro-NT)

Micro-NT analysis of plasma samples was performed as described before (Haselmann et al., 2020). However, because plasma samples were used instead of serum samples, the original protocol was adjusted to achieve the necessary tolerance to calcium required for testing plasma instead of serum samples. Most notably, cell attachment was significantly impaired by plasma samples. Thus, the micro-NT was performed on a confluent cell monolayer (Vero E6) while the remaining protocol was not changed. In brief, SARS-CoV-2 (strain MUC IMB-1) was cultured in Vero E6 cells. Virus stocks (50 TCID/50 μl) were prepared and stored at −80 °C until further use. All micro-NTs were performed in 96-well culture plates (Greiner bio-one, Frickenhausen, Germany) on confluent cell monolayers. Plasma samples were diluted in Minimal Essential Medium (MEM, plus Non-Essential Amino Acids Solution and Antibiotic-Antimycotic Solution; all Invitrogen, ThermoFisher Scientific, Darmstadt, Germany) beginning with a ratio of 1:5 to a maximum of 1:80. A known positive and known negative plasma sample were used as controls together with a mock control and a virus back-titration on each plate.

Virus was pre-incubated with diluted plasma samples in duplicate for one hour at 37 °C before the plasma-virus suspension was added to the wells. After an incubation period of 72 h at 37 °C (5 % CO2), the supernatants were discarded and the 96-well plates were fixed in 13 % formalin/PBS, stained with crystal violet (0.1 %) and titres were determined. The NABs titre corresponded to the reciprocal of the highest virus dilution that showed complete inhibition of CPE. The result was considered invalid if the variation between duplicates was greater than one titre value. In the case of only a simple titre differences between duplicates, the lower titre was evaluated. Samples were classified as either "NT negative" (titre < 1:5) or "NT positive" (titre ≥1:5), with the highest possible titre being ≥1:80.

2.3. Surrogate enzyme linked immunosorbent assays (sELISA)

Two commercially available sELISA kits were used to determine the presence or absence of SARS-CoV-2 specific NABs: The SARS-CoV-2 Neutralisation Antibody Detection Kit (AdipoGen Life Sciences, Liestal, Switzerland) and the cPass™ SARS-CoV-2 Neutralisation Antibody Detection Kit (GenScript Biotech, NJ, USA). Both tests use the competitive inhibition of the protein-protein interaction between a recombinant NABs-CoV-2 RBD protein and recombinant human ACE2 receptor (hACE2) to measure the specific neutralising effect of antibodies in the patient sample. However, GenScript uses hACE2 coated plates for capture and HRP-conjugated SARS-CoV-2 RBD for detection while Adipogen provides RBD-coated plates and uses HRP-conjugated hACE2 for detection. Both sELISAs were performed in strict accordance with the manufacturer’s instructions. All samples were tested in duplicate and the mean value of both measurements was used to calculate the relative inhibition. The samples were classified as either "positive" (inhibition ≥20 %) or "negative" (inhibition <20 %) as suggested by the manufacturers.

3. Results

3.1. Detection of SARS-CoV-2 neutralising antibodies by NT

First, all samples were examined in the micro-NT. During the first experiment, only those plasma samples that showed a NABs titre equal to or higher than 1:10 were initially considered positive, while all samples with a lower titre (<1:10) were considered negative.

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Those initial results showed that 140 samples (50.7 %) were positive to SARS-CoV-2 specific NAbs, while 136 samples (49.3 %) were negative, including all negative control research samples from 2018. When comparing these results with the results of both sELISA, we noticed a high number of apparently false-positive results for both the GenScript (n = 53) and the AdipoGen (n = 16), resulting in rather low specificities (GenScript: 61 %; AdipoGen: 88 %) (Fig. 1).

After considering that enzymatic immunoassays are known to be generally more sensitive than NTs, we decided to re-titrate all 136 NT-negative samples from 1:5.

After re-titration, a total of 158 (57.2 %) were positive, while 118 samples (42.8 %) still showed no neutralising effect in the NT even at a dilution of 1:5. Of the positive samples, 12 % (n = 19) had a titre of 1:5, 46.2 % (n = 73) had a titre of 1:10 and 21.5 % (n = 34) had a titre of 1:20. 10.1 % each had a titre of 1:40 (n = 16) and ≥1:80 (n = 16) (Fig. 3).

This means that of the 230 plasma samples that initially tested positive in the IgG Euroimmun ELISA, only 68.7 % (n = 158) were positive (≥1:5) in the NT. In contrast, all 46 plasma samples from 2018 had a titre of <1:5, confirming the specificity even at titres lower than 1:10.

3.2. Detection of NAbs with the SARS-CoV-2 neutralising antibodies detection kit (AdipoGen)

The test was easy to perform and took about 2.5 h from start to finish.

Of the 158 samples reactive in the micro-NT, 131 also tested positive for AdipoGen, resulting in a sensitivity of 82.9 %. No false negative results were obtained on samples with a titre of 1:40 or a titre equal to or greater than 1:80. Of the 118 NT-negative samples, 116 were also negative in AdipoGen-sELISA, resulting in a specificity of 98.3 % (Table 1), and the inhibition values of the negative NT results showed a low variance in inhibition values. In contrast, the scatter of inhibition values of positive NT results was much higher (Fig. 1A).

3.3. Detection of NAbs by cPass™ SARS-CoV-2 neutralisation antibody detection kit (GenScript)

The test was easy to perform and took about 1.5 h from start to finish.

Of the 158 samples of NAbs confirmed with NT, 156 also tested positive with GenScript, resulting in a sensitivity of 98.7 %. No false negative results were obtained on samples with a titre of 1:5, 1:20, 1:40 and equal to or greater than 1:80. The specificity was 69.5 % with 36 false positive results (Table 1) and a high spread of inhibition values within the negative NT results (Fig. 1B).

3.4. Micro-NT vs. sELISA

While the micro-NT is able to quantify the amount of NAbs in a patient sample by titration, both sELISAs use relative inhibition [%] as readout. When comparing NT titres with relative inhibition, no clear correlation could be found in either sELISA. Simple linear regression confirmed this observation (AdipoGen: R² 0.515, Y = 1.012x + 12.19; GenScript: R² 0.435, Y = 1.109x + 30.76) (Fig. 3). Thus, no conclusions can be drawn from inhibition values about neutralising titres.

In general, the higher the micro-NT titre, the less likely false negative sELISA results were obtained. No false negative sELISA results were observed in any of the examined plasma samples with a NT titre of >1:40 (Figs. 2 and 3). Overall, the relative inhibitions determined by

### Table 1

| Plasma samples | Adipogen | GenScript |
|----------------|----------|-----------|
|                | Positive | Negative | Positive | Negative |
| Total          | 276      | 133       | 143      | 158      | 64        |
| NT reactive    | 158      | 131       | 27       | 156      | 2         |
| NT negative    | 118      | 2         | 116      | 2        | 82        |
| Sensitivity [%]| 82.9     | 98.7      |
| Specificity [%]| 98.3     | 69.5      |

Fig. 1. Distribution of inhibition values determined by AdipoGen (A) and GenScript (B) within negative (≤1:5 (colored) and ≤1:10 (grey)) and positive NT results. (A) The AdipoGen ELISA is highly specific and gives almost no false positive results, while being less sensitive as indicated by a wide distribution of inhibition values of positive NT samples. (B) The GenScript ELISA is highly sensitive with very few false negative results, while it is much less specific with a wide range of inhibition values of negative NT samples. Specificities of both ELISAs benefited from the re-titration of initially negative NT samples as indicated by the original inhibition values (≤1:10) in grey.
The importance of acquired immunity and neutralising antibodies against SARS-CoV-2 has become a frequently discussed topic not only in the scientific community but also in the public debate among the general public and policy makers. Although the link between the detection of NAbs and the presence of protective immunity has not yet been finally established, NAbs are already of great interest in confirming the efficacy of potential vaccines and in the selection of convalescent plasma donors for antibody therapy. In this study we investigated two commercially available sELISA kits specifically designed to detect SARS-CoV-2 NAbs and compared their results with NAbs titres obtained by NT.

The data presented here initially confirmed that NAbs are not detectable in all patients who test positive for SARS-CoV-2 Abs in conventional antibody ELISA. We examined 230 samples that had previously tested positive for Abs in a commercial IgG ELISA (Euroimmun, Lübeck, Germany). However, only 68.7 % (158/230) of these IgG positive samples were also positive in the NT. This result is in line with a study published by Jääskeläinen et al. (Jääskeläinen et al., 2020) but differs from the results of a recently published study by Geurts van Kessel et al., in which the specificity of the Euroimmun IgG ELISA was reported to be 97–100 % (Geurts van Kessel et al., 2020). In this study, assay performance was also determined by comparing the test result with NAbs titres. However, only 68.7 % (158/230) of these IgG positive samples were also positive in the NT. This result is in line with a study published by Jääskeläinen et al. (Jääskeläinen et al., 2020) but differs from the results of a recently published study by Geurts van Kessel et al., in which the specificity of the Euroimmun IgG ELISA was reported to be 97–100 % (Geurts van Kessel et al., 2020). In this study, assay performance was also determined by comparing the test result with NAbs titres. However, titles were measured by PRNT50 and were considered positive already at PRNT > 20. However, the use of such low cut-off values leads to a considerable increase in the variability of the results and to a significant reduction in the specificity of the PRNT. Stricter PRNT90 or micro-neutralisation test titres are much more specific by reducing background serum cross reactivities. They are therefore more suitable for evaluating test systems to be used in epidemiological studies, for diagnostic purposes or for selecting...
therapeutic convalescent plasma donors.

We were also able to show that both sELISA are easy to perform and have a significant time advantage over NT: GenScript took the least time from start to finish (1.5 h), followed by AdipoGen (2.5 h), while the micro-NT took 72 h (plus the time for cell culture preparation). Furthermore, no BSL3 laboratory is required for both sELISA. In terms of assay performance (i.e. sensitivity and specificity), we found that the GenScript sELISA has a sensitivity of 98.7 %. This was higher than the sensitivity determined for the AdipoGen sELISA (82.9 %), which gave a higher number of false negative results (27/158). However, the high sensitivity of the GenScript sELISA correlated with a lower specificity of only 69.5 %, which resulted in a large number of false positive results (36/118). These results are in contrast to a recent study by Meyer et al., which reports a specificity of 99.2 % for GenScript sELISA (Meyer et al., 2020). This discrepancy might be explained by the differences in samples: In our study, we tested all plasma samples in both sELISA, including all samples, which, based on the micro-NT results, showed a false positive result in the IgG screening ELISA. This false IgG positivity might have contributed to the high number of false positive results of the GenScript ELISA. However, since the use of pre-screening assays is a common practice in laboratories to reduce sample load, the evaluation of such critical samples is important. Compared to the GenScript sELISA, the AdipoGen sELISA had a much higher specificity of 98.3 % with only two false positive results. Interestingly, both were just above the cut-off (23 % and 28 % respectively).

The differences in specificity and even more in sensitivity were somewhat surprising as both tests use the same principle to detect NAbs (i.e. the inhibition of RBD-hACE2 interaction by NAbs). Nevertheless, the different orientation of the proteins during coating and detection might contribute to the differences in the results. The preincubation of plasma with soluble RBD (GenScript) instead of immobilized RBD (AdipoGen) seems to be more sensitive. At the same time, this interaction of soluble RBD and ACE-2 directly in plasma might be more prone to deliver false positive results due to different factors and components present in plasma possibly causing steric hindrance. In contrast, the AdipoGen sELISA performs a washing step between plasma-RBD interaction and RBD-hACE2 interaction eliminating these factors, which might contribute to its higher specificity.

In general, false negative sELISA results might be attributed to the fact that the micro-NT is able to detect neutralisation irrespective of specific epitopes. Both sELISA on the other hand are only able to detect NAbs that function by blocking the interaction between the hACE2 receptor and the viral RBD, which certainly is a general limitation of ELISA-based approaches. However, the human ACE2 receptor is considered to be the major receptor for the cell entry for SARS-CoV-2 (Scialo et al., 2020) and it has been shown that the vast majority of NAbs against SARS-CoV-2 target the RBD (Burton and Walker, 2020). Thus, while both sELISA cannot fully detect all NAbs, they can certainly detect this vast majority. Also, many of the vaccine candidates currently under development rely on the expression of RBD as the target antigen, making the (sole) detection of RBD-specific NAbs important for vaccine testing in trials (Krammer, 2020).

The fact that no conclusion about titre levels could be drawn from inhibition values is a clear disadvantage of both ELISAs. Especially for the selection of convalescent plasma for therapeutic purposes, an exact titre determination is crucial (Bradfute et al., n.d.; Harvala et al., 2020). However, sensitivity generally improved with higher titres and reached 100 % with titres ≥1:40. At the same time, a slight increase of the cutoff value from 20 % to 30 % resulted in a specificity of 100 % for the AdipoGen sELISA. Thus, the AdipoGen achieved both a sensitivity and a specificity of 100 % for samples with micro-NT titres ≥1:40 (Fig. 2). This could indeed be used in situations, where samples need to be evaluated for high titres (≥1:40), rather than exact titres such as screening strategies for the selection of convalescent plasma.

Our results show that due to their lower sensitivity (AdipoGen) or specificity (GenScript) and the lack of correlation of inhibition values with neutralising titres, neither of the two sELISA assays can currently fully replace the virus neutralisation test. However, because NTs are such sophisticated tests, many laboratories use a two-step diagnostic algorithm to reduce the number of samples. Very often, a fast and easy to perform IgG ELISA with high sensitivity is used as a screening test. Only IgG-positive samples are then analyzed using NT.

In this context, the AdipoGen sELISA could serve as a useful replacement. The slightly lower sensitivity (82.9 %) could be compensated by the high sensitivity of the screening ELISA, while the high specificity (98.3 %) would ensure very few false-positive results. Additionally, with an adjustment of the cut-off value to 30 %, the specificity can be improved to 100 %. However, such a test strategy is not applicable to questions where absolute quantification of virus neutralisation is required, as is the case with large-scale screening studies on sero-prevalence with emphasis on NAbs and humoral immunity. sELISA could potentially be a useful tool in the initial clinical evaluation studies of spike- or RBD-based vaccine candidates. However, the GenScript sELISA is not suitable in this context due to its low specificity. Instead, its high sensitivity might make it a useful alternative to classical antibody screening ELISAs.

In summary, our study shows that both SARS CoV-2 sELISA were able to qualitatively detect NAbs in human plasma samples. Sensitivity and specificity differed between the two sELISA, with GenScript superior in sensitivity and AdipoGen superior in specificity. Combined with the fact that both sELISA are not capable of quantifying NAbs, these results suggest that neither of them can completely replace conventional NT. However, in a two-step diagnostic algorithm where samples are pre-screened by conventional sensitive IgG ELISA, the AdipoGen sELISA could potentially replace the NT as a subsequent confirmatory test due to its high specificity for all questions that do not require quantification of NAbs.

Author statement

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Heiner von Buttlar: Methodology, Validation, Writing - Review & Editing.
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Funding

This research was funded by the Medical Biodefence Research Programme of the Bundeswehr Medical Service.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Ali, I., Alharbi, O.M.L., 2020. COVID-19: disease, management, treatment, and social impact. Sci. Total Environ. 728, 138861 https://doi.org/10.1016/j.scitotenv.2020.138861.
Bradfute, S.B., Hurwitz, I., Yingling, A.V., Ye, C., Cheng, Q., Noonan, T.P., Raval, J.S., Sosa, N.R., Mertz, G.J., Perkins, D.J., Harkins, M.S., 2020. SARS-CoV-2 neutralizing antibody titers in convalescent plasma and recipients in New Mexico: an open treatment study in COVID-19 patients. n.d. J. Infect. Dis. https://doi.org/10.1093/infdis/jiaa505.
Burton, D.R., Walker, L.M., 2020. Rational vaccine design in the time of COVID-19. Cell Host Microbe 27, 695–698. https://doi.org/10.1016/j.chom.2020.04.022.
