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Two novel SARS-CoV-2 surrogate virus neutralization assays are suitable for assessing successful immunization with mRNA-1273

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ABSTRACT

Background: Due to large vaccination efforts with novel vaccines there is an increasing need for laboratory tests assessing successful immunizations with SARS-CoV-2 vaccines. Unfortunately classical neutralization assays are laborious, time-consuming and require an adequate biosafety level laboratory. Recently, convenient ELISA-based surrogate neutralization assays (sVNTs) for determination of neutralizing SARS-CoV-2 antibodies have been developed.
Study Design: Our study compares the two novel ELISA-based SARS-CoV-2 surrogate neutralization assays “cPass SARS-CoV-2 Surrogate Virus Neutralization Test Kit” (GenScript Biotech, USA) and the “TECO SARS-CoV-2 Neutralization Antibody Assay” (TECOmedical, Switzerland) using 93 sera drawn from health care workers (HCVs) 2–3 weeks following the second vaccination with mRNA-1273 and 40 control sera from the pre-SARS-CoV-2 era before 2019.
Results: We found a sensitivity of 100% and 91,4% and a specificity of 100% and 100% for the GenScript assay and the TECO assay, respectively. Both sVNTs show a high correlation with anti-S IgG. Moreover, both sVNTs correlate well with each other.
Conclusions: Surrogate neutralization assays based on the RBD as bait feature a high specificity and sensitivity for identifying humoral neutralizing activity in individuals vaccinated with the spike-based vaccine mRNA-1273. Although these assays appear well-suited for confirming successful vaccinations with spike-based vaccines, additional studies should compare both assays regarding other purposes such as screening COVID-recovered patients or individuals vaccinated with inactivated whole virus vaccines.

1. Introduction

In December 2019 the new coronavirus SARS-CoV-2 causing coronavirus disease 2019 (COVID-19) emerged (Zhou et al., 2020; Zhu et al., 2020) leading to an ongoing pandemic. Current vaccination efforts are aimed at stopping it. “Non-responders” or “low-responders” to vaccinations are commonly encountered in clinical medicine, *e.g.* regarding hepatitis B vaccinations (Heininger et al., 2010). Therefore assays confirming successful vaccinations against SARS-CoV-2 are of paramount importance. Traditionally *in vitro* neutralization assays are considered as gold standard of determining neutralizing antibodies against viruses. As these assays are labor-intense and require an appropriate biosafety-level laboratory, there is an increasing demand for convenient assays assessing the presence of neutralizing antibodies. Most recently, “surrogate virus neutralization assays” (sVNTs) for the determination of neutralizing antibodies have been developed and are entering the market (Tan et al., 2020) (Kohmer et al., 2021) (Perera et al., 2021) (Murray et al., 2021) (Meyer et al., 2020) (Müller et al., 2021). In this study, we compared two such novel assays with respect to their sensitivity and specificity.

2. Material and methods

Sera of 93 healthcare workers drawn 2–3 weeks following the second vaccination with the SARS-CoV-2 vaccine mRNA-1273 and –as specificity controls- sera of 40 individuals drawn in the “pre-SARS-CoV-2 era” before 2019 (stored at −20 °C since then and considered neither COVID-19-recovered nor vaccinated) were included in this study. Informed written consent was obtained from each health care worker. Sample and data acquisition were approved by the Medical Ethics Committee of the University Hospital RWTH Aachen (EK 093/20).

Two novel commercially available surrogate neutralization assays

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("cPass SARS-CoV-2 Surrogate Virus Neutralization Test Kit" (GenScript Biotech, USA) and "TECO SARS-CoV-2 Neutralization Antibody Assay" (TECOmedical, Switzerland) were compared in this study. These innovative *in vitro* assays measure the ability of sera to inhibit the interaction of recombinant ACE2 (angiotensin-converting enzyme 2, the human cell surface receptor for SARS-CoV-2) and recombinant RBD (the receptor binding domain of the spike protein of SARS-CoV-2, the ligand for ACE2) (Hoffmann et al., 2020). SARS-CoV-2 anti-Spike IgG antibodies were measured to determine the anti-S IgG antibody status ("Liaison SARS-CoV-2 S1/S2 IgG assay", DiaSorin, Italy). In analogy to a previous study (Krüttgen et al., 2021) the SARS-CoV-2 antibody status of the sera was defined as follows: A serum was regarded as "SARS-CoV-2 antibody positive" if at least two of our three assays (two sVNTs and the anti-S IgG ELISA) showed a positive test result. A serum was regarded as "SARS-CoV-2 antibody negative" when it was drawn in the "pre-SARS-CoV-2 era" before 2019.

International "binding antibody units" (BAU/mL) were calculated for the Diasorin assay as recommended by the manufacturer and correlated to the neutralization value results (% inhibition) from the sVNTs.

All assays were performed as recommended by the manufacturer. Preliminary experiments showed that all sera from vaccinees contained antibody levels exceeding the upper quantification limit of the antibody assays. Thus, all sera were prediluted by 1:20 to obtain a set of samples with antibody levels within the measurable range of the assays.

3. Results

3.1. Determination of sensitivity and specificity of the surrogate virus neutralization assays (sVNTs)

93 sera were of health care workers drawn 2–3 weeks after the second vaccination with mRNA-1273 were included in this study. Each serum was subjected in parallel to the following assays: an ELISA for determination of IgG antibodies against the spike (S) protein of SARS-CoV-2 (from DiaSorin) and two surrogate neutralization assays (from GenScript and TECO).

As described in Materials and Methods each of the 93 post-vaccination sera was confirmed as "SARS-CoV-2 antibody positive", whereas 40 sera obtained from the time before SARS-CoV-2 emerged were used as specificity controls.

Using the GenScript assay each of the 93 sera (= 100 %) drawn after vaccination yielded a positive result (cut-off >20 %) indicating the presence of neutralizing antibodies (supplementary Table 1). Using the TECO assay only 85 of 93 sera (= 91,4%) were determined positive (cut-off >20 %). False-negative results in the TECO assay were obtained mostly in samples with low anti-S IgG levels (<100 BAU/mL). None of the sera of the "pre-SARS-CoV-2 era" tested positive with any of the surrogate neutralization assays. This results in a sensitivity of 100% and 91,4% for the GenScript assay and the TECO assay, respectively. The corresponding results for the specificity are 100 % for both assays.

3.2. Correlation of the results obtained with surrogate neutralization assays and anti-S IgG ELISA

Results of both sVNTs for each of the 93 sera drawn after vaccination are displayed in Fig. 1. Data obtained with both sVNTs correlate well (r = 0,88). The trend line of this correlation can be described with y = 1,069 x–21,479. As shown in Fig. 1 the trend line does not cross the origin of the graph. This indicates that the values determined with the TECO assay tend to be lower compared to the values determined with the GenScript assay for a given serum. This difference correlates with the lower sensitivity of the TECO assay.

The correlation between anti-Spike IgG levels and the two surrogate neutralization assay are displayed in Fig. 2 (Fig. 2a: Genscript; Fig. 2b: Teco) The correlation coefficient r was calculated as 0,72 for the Genscript assay and 0,76 for the Teco assay indicating that the neutralization capacity of sera of individuals vaccinated with mRNA-1273 correlates well with anti-S IgG levels.

4. Discussion

Non-responders or low-responders to vaccinations are commonly encountered in clinical medicine. Thus, the recent large-scale use of novel vaccines against SARS-CoV-2 has triggered demand for convenient laboratory tests confirming successful immunizations. To bypass work-intense and time-consuming cell-culture based virus neutralization assays, surrogate virus neutralization assays have been developed and are entering use in clinical laboratories. We compared two novel ELISAs which are based on the concept that sera containing neutralizing antibodies against SARS-CoV-2 disrupt binding of the viral RBD to its receptor ACE2. Besides evaluating the recently characterized GenScript assay (Meyer et al., 2020; Müller et al., 2021; Murray et al., 2021; Taylor et al., 2021), our focus was the evaluation of the new Swiss TECO assay for which rather sparse data are available. Moreover, unlike in previous studies which used sera from COVID-recovered patients to evaluate the GenScript assay, our study characterizes the GenScript assay and TECO assay by using sera from a different kind of cohort (namely HCWs vaccinated against SARS-CoV-2 with mRNA-1273) and with a different aim (assessing the value of these new sVNTs in confirming successful

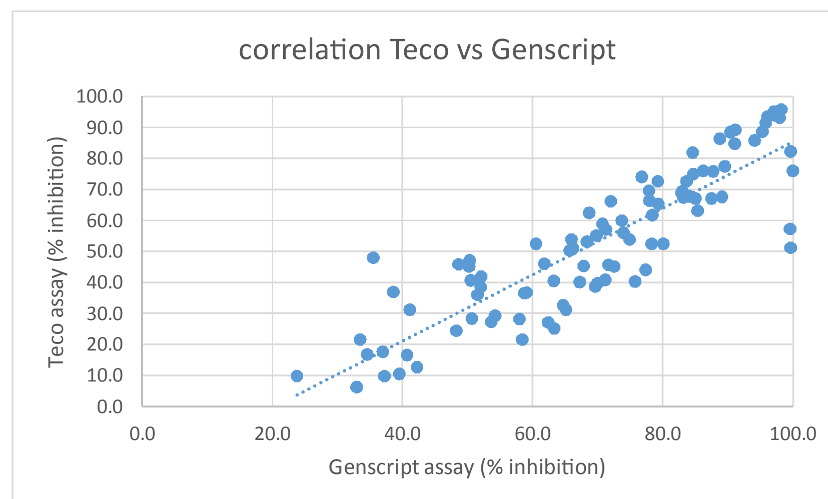
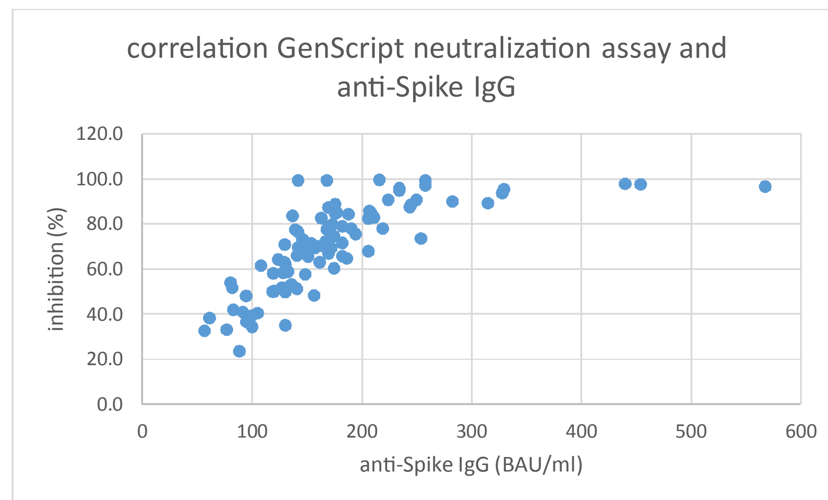


Fig. 1. Correlation of the Teco and Genescript surrogate neutralization assays.

a) GeneScript surrogate virus neutralization assay.



b) Teco surrogate virus neutralization assay.

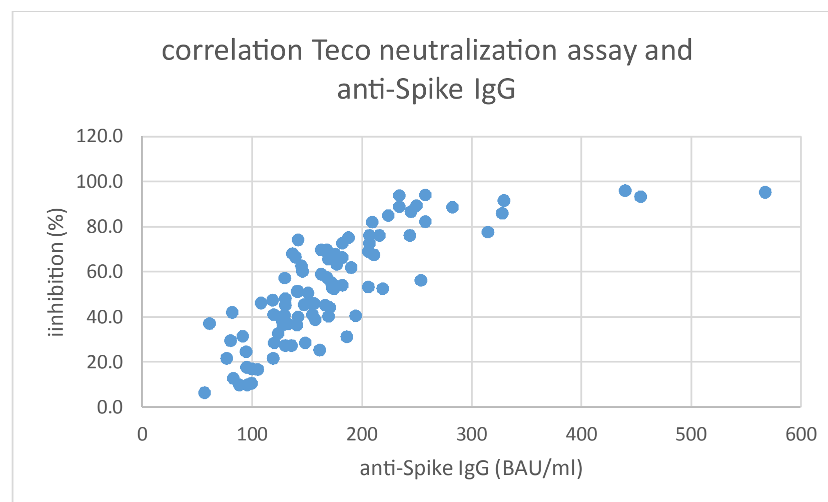


Fig. 2. Correlation of surrogate neutralization assays and anti-Spike IgG levels.

vaccinations).

We found that the TECO assay has a sensitivity/specificity of 91,4%/100% whereas the GenScript assay has a sensitivity/specificity of 100 %/100 %. Thus both assays are suitable for verifying humoral protective immunity upon vaccination with spike-based vaccines. Although both assay measure disruption of ACE2:RBD interaction by sera, the GenScript assay showed a higher sensitivity in our study. Potentially, the "reverse design" of both assays plays some role regarding the observed difference in sensitivity, as the GeneScript assay features ACE2-coated plates and soluble RBD:HRP; whereas the TECO assay features RBD-coated plates and soluble ACE2:HRP.

Both assays are based on the RBD as "bait" for neutralizing antibodies in sera. As the RBD is a part of the spike protein, one might expect a positive correlation between results from our sVNTs and anti-Spike IgG ELISA. This is indeed supported by our data as the correlation coefficient *r* for the TECO assay and anti-spike IgG was calculated to be 0,76 indicating that neutralization capacity of sera of individuals vaccinated with mRNA-1273 correlates well with their anti-S IgG levels. We also found a good correlation for the GenScript assay and anti-S IgG levels ($r = 0{,}72$).

A basic limitation of both assays as surrogate assay for humoral immunity against the SARS-CoV-2 virus is the sole use of RBD (a small fragment of full-length spike) *in lieu* of the whole SARS-CoV-2 virus. Therefore these RBD-based surrogate assays might not be well-suited to determine neutralizing responses evoked by whole SARS-CoV-2 viruses (either by natural infection or by vaccines based on inactivated viruses), as this might evoke antibodies targeting epitopes unrelated to RBD. *Per se*, such non-RBD-related immune responses cannot be detected by the RBD-based Genscript and Teco assays. Thus, the TECO and GenScript assays should preferentially be used on samples of individuals who received RBD-containing vaccines. The use of sera from COVID-recovered patients might help explain divergent sensitivities in

previous studies evaluating the GenScript assay (Meyer et al., 2020; Müller et al., 2021; Murray et al., 2021; Taylor et al., 2021).

In conclusion these two assays will be of high added value for diagnostic laboratories by greatly facilitating the 1) confirmation of successful vaccinations with RBD-based vaccines 2) assessment of immune-protection at later time points after RBD-based vaccination when immunity might wane. We envision that these two assays will play an important role in a similar way as assays measuring anti-HBs levels after HBV vaccination. Based on negative sVNTs results (perhaps confirmed by classical virus neutralization tests), those SARS-CoV-2-vaccinees (especially HCWs with high risk of exposure) who do not show serological responses in sVNTs assays should be offered additional doses of the same vaccine or perhaps additional doses with other SARS-CoV-2 vaccines. Establishing international units per milliliter (IU/mL) for SARS-CoV-2 neutralizing activity in sera would be very valuable for the purpose of comparing the results of surrogate virus neutralization assays from different manufacturers.

A limitation of our study is the restriction to mRNA-1273 vaccinees (which –however- can also be considered a strength because we examined a homogenous cohort). Further studies with differentially stratified cohorts (such as covid-recovered patients or individuals

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Author contributions

Maike Lauen: investigations.
Hanna Klingel: data analysis
Alexander Krüttgen: writing, investigations, conceptualization.
Matthias Imöhl: review, editing.
Michael Kleines: Conceptualization, investigations, writing.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jviromet.2021.114297.

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