



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



# Application of the SARS-CoV-2-S1 ACE-2 receptor interaction as the basis of the fully automated assay to detect neutralizing SARS-CoV-2-S1 antibodies in blood samples

Daniel Filchtinski<sup>a,\*</sup>, Magnus Sundberg<sup>b</sup>, Heike Berthold<sup>a</sup>, Laura Steller<sup>a</sup>, José Kayser<sup>a</sup>, Sanja Holz<sup>a</sup>, Mario Hinze<sup>a</sup>, Oxana Braeutigam<sup>a</sup>, Johannes Schulte-Pelkum<sup>a,\*</sup>, Raimund Fiedler<sup>a</sup>

<sup>a</sup> Assay Design, Thermo Fisher Scientific, Freiburg, Germany

<sup>b</sup> Thermo Fisher Scientific, Uppsala, Sweden

## ARTICLE INFO

### Keywords:

SARS-CoV-2

Neutralizing antibodies

ELiA

ACE-2 receptor binding inhibition assay

## ABSTRACT

A quantitative, high throughput, fully automated diagnostic method for the detection of neutralizing anti-SARS-CoV-2 antibodies was developed on the Phadia system based on the interaction of SARS-CoV-2 S1 protein and the human ACE-2 receptor. This method was compared to the current state of the art plaque reduction neutralization test (PRNT) and a high correlation between the two methods was observed. Using a large cohort of blood samples from convalescent patients and controls the method displays very high sensitivity and specificity (99,8% and 99,99%, respectively). Neutralizing antibody titers of mRNA-1273 and BNT162b2-vaccinated persons can also be quantified with this method as well. This fully automated method provides the possibility to determine anti-SARS-CoV-2 neutralizing antibody concentrations in just 2 h.

## 1. Introduction

The SARS-CoV-2 coronavirus 2019 (COVID-19) infection leads to severe respiratory symptoms and life-threatening conditions with a high mortality rate (Zhou et al., 2020). The SARS-CoV-2 virus uses its spike protein for the cell entry, which is mediated by the binding of S1 as part of the spike proteins via its receptor binding domain (RBD) to angiotensin-converting enzyme 2 (ACE-2) receptor on the surface of the epithelial cells of the respiratory tract (Lan et al., 2020; Walls et al., 2020). This interaction can be impaired by neutralizing antibodies that bind to the RBD within its ACE-2 receptor binding interface and thus sterically hinder the binding of the two proteins. This principle was shown to be applied in assays to detect SARS-CoV-2 neutralizing antibodies in ELISA format (Taylor et al., 2021; Tan et al., 2020).

In fact, those neutralizing antibodies can be used as treatment in the early phase of infection to prevent patients at risk from developing severe symptoms (Zost et al., 2020; Jiang et al., 2020; Deb et al., 2021).

Various studies showed a strong antibody titer decrease reaching even the seronegative state 6–12 months post infection after second vaccination (Khoury et al., 2021; Wheatley et al., 2021; Doria-Rose,

2021; Padoan et al., 2022; Vicenti et al., 2021). Thus, there is an urgent need of tests quantifying the neutralization potential in samples to determine if the persons need to be revaccinated after 6–12 months to be safe from a COVID-19 reinfection. Furthermore, this assay should be able to be used as a tool for the characterization of neutralization potential of antibody preparations against wildtype and mutant viruses as well.

The state-of-the-art method of determining the neutralization potential of samples is the plaque reduction and neutralization test (PRNT). In this method, Vero E6 cells are infected with the SARS-CoV-2 coronavirus in the presence of diluted serum or plasma samples of convalescent patients or vaccinated individuals. Infection of Vero E6 cells with SARS-CoV-2 leads to the cytopathic effect (CPE), which is visible under the light microscope. The NT50 is defined as sample dilution, at which CPE is prevented by 50%. NT50-values are calculated and used as a quantitative value of samples' neutralization potential. This assay is laborious and needs long incubation times resulting in a week to get the required NT50-values. In addition, the PRNT requires a biosafety level 3 laboratory (Bewley et al., 2021).

As an alternative to the biosafety level 3 PRNT, the so called

\* Corresponding authors.

E-mail addresses: [daniel.filchtinski@thermofisher.com](mailto:daniel.filchtinski@thermofisher.com) (D. Filchtinski), [johannes.schulte-pelkum@thermofisher.com](mailto:johannes.schulte-pelkum@thermofisher.com) (J. Schulte-Pelkum).

<https://doi.org/10.1016/j.jim.2022.113258>

Received 12 October 2021; Received in revised form 11 March 2022; Accepted 11 March 2022

Available online 15 March 2022

0022-1759/© 2022 Thermo Fisher Scientific ImmunoDiagnostics Division Freiburg. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

pseudovirus-based neutralization test is available. This assay uses recombinant virus expressing SARS-CoV-2 Spike protein instead of the SARS-CoV-2 itself. The pseudo-virus-based neutralization test as a surrogate of the PRNT can be performed in biosafety level 2 laboratories, but still requires the use of live viruses and cells thus being tedious and time-consuming. In addition, those assays are hardly automatable, making it impossible to use them in the clinical routine diagnostics (Nie et al., 2020).

To develop an assay to identify neutralizing antibodies for the clinical routine testing, a high throughput screening system is needed. As such, the Phadia system with its fully automated instruments with different sample capacity is an optimal platform to measure neutralizing SARS-CoV-2 antibodies in the clinical laboratory environment. It utilizes a fluorescence-based solid phase enzyme immunoassay (FEIA) format with EliA microcavities, individually operated for each sample in the random-access instrument's run (Villalta et al., 2002).

Here, we present a fully automated, high throughput assay showing a high correlation to the PRNT and being able to detect neutralizing antibodies in samples of convalescent and vaccinated persons sensitively and with a high specificity.

## 2. Material and methods

### 2.1. Samples

#### 2.1.1. Correlation to PRNT

For the correlation of the ACE-2 receptor binding inhibition assay to PRNT, 37 serum and plasma samples from PCR-proven COVID-19 convalescent individuals with PRNT<sub>50</sub> values determined in the PRNT by TexCell (Évry, France) and 44 pre-pandemic blood donor samples were tested. VERO E6 cells were infected with the SARS-CoV-2, isolate: 2019-nCoV/Italy INMI 2nd P VERO E6 11.02.2020, in the presence of sample dilutions in 8 replicates in 96-well cell culture plates. The cytopathic effect (CPE) was read on 6th day post-infection. The neutralization titer 50 (NT50) corresponding to the sample dilution which prevents cells from CPE in 50% of replicates was calculated according to Spearman-Kärber formula.

#### 2.1.2. Correlation to S1 IgG

For determination of sensitivity 637 serum and plasma samples of COVID-19 convalescent persons with PCR-proven infection with 4–27 weeks post-PCR-positivity and a control cohort consisting of 158 pre-pandemic samples from different infectious diseases (Adenovirus, EBV, Enterovirus, *Haemophilus influenzae* infection, Measles, Mumps, Respiratory Syncytial Virus, Varicella Zoster Virus, HCoV-229E, HCoV-HKU1, HCoV-NL63, HCoV-OC43, Parainfluenza, Influenza, *Mycoplasma pneumoniae*, Parvo B12), as well as blood donors were used.

#### 2.1.3. Comparison of vaccinated and COVID-19-convalescent samples

For the comparison of neutralizing antibody titers of vaccinated and COVID-19 convalescent persons, serum and plasma samples of 1–3 weeks post-PCR-positivity from COVID-19 infected persons and samples of persons 1–3 weeks after second vaccination with mRNA-1273 (Moderna) (16 samples), and BNT162b2 (Pfizer) (18 samples) were used.

### 2.2. Proteins

SARS-CoV-2 Spike S1 protein, NCBI Reference Sequence: YP\_009724390.1 with amino acids 14–681, ACE-2 receptor proteins and the neutralizing antibody, clone 6G7 were obtained from Icosagen AS (Tartu, Estonia).

### 2.3. ACE-2 binding inhibition assay

There is a high degree of conformance between different Phadia

instruments, which was shown for 31 FDA-cleared assays as exemplified by the 510(k) for the EliA anti-cyclic citrullinated peptides (CCP) (Mann, 2006). As such, Phadia-100 and Phadia-250 results are assumed to be equivalent in terms of correlation and precision and the instruments were chosen only depending on the required experimental scale.

For the assay described in this paper, the fully automated, high throughput Phadia platform, in particular, Phadia-250 instrument, was used (Villalta et al., 2002). N-hydroxysuccinimide ester activated biotin was used for covalent coupling of biotin to primary amino groups (–NH<sub>2</sub>) of the SARS-CoV-2 spike protein (Icoagen AS, Tartu, Estonia). Biotinylated SARS-CoV-2 S1 protein was immobilized on streptavidin-coated microcavities at a concentration of 0,2 µg/ml in 1xPBS as buffer and Stabilcoat (Surmodics) as blocking and stabilizing agent. ACE-2 receptor (Icosagen AS, Tartu, Estonia) was coupled to β-galactosidase (Thermo Fisher Scientific, Uppsala, Sweden) using LC-SPDP (succinimidyl 6-(3(2-pyridyldithio)propionamido)hexanoate) (Thermo Scientific, Rockford, Illinois, U.S.A.) as cross-linker. The conjugate was purified on a Superose 6 column (Cytiva, Uppsala, Sweden) to remove unconjugated ACE-2 and β-galactosidase and used as assay-conjugate at a concentration of 0,425 µg/ml in EliA conjugate buffer. Microcavities and the conjugate were used in the Phadia instrument with the commercially available set of Phadia system reagents including EliA Sample Diluent, washing buffer, 4-methylumbelliferyl-β-D-galactopyranoside as substrate and stop solution.

As calibrator material, recombinant monoclonal human neutralizing antibody, whose genetic material was isolated from PBMC of a convalescent person (Clone 6G7, from Icosagen AS, Tartu, Estonia) was used in concentrations of 0–5 µg/ml. The calibration curve was calculated with a Rodbard 4-parameter fit using Excel and Graphpad.

For the correlation to PRNT and S1 IgG, samples were measured in 1:2 instrument dilution.

For other studies, dilutions were chosen to obtain a signal within the dynamic range of the calibrator curve. Concentration of neutralization antibodies in µg/ml was calculated considering the applied sample dilution.

#### 2.3.1. Intra- and Inter-assay precision

To determine the intra and inter-assay precision of the ACE-2 receptor binding inhibition assay, the variability was assessed in a study evaluating 4 samples in 5 independent runs with a total of 100 replicates. The evaluation was carried out on a Phadia 100 instrument. A calibration curve was included in each run. Both intra- and inter-assay precision were characterized by calculating the mean and coefficients of variation (CV%) using Analyze-It Microsoft add-in program.

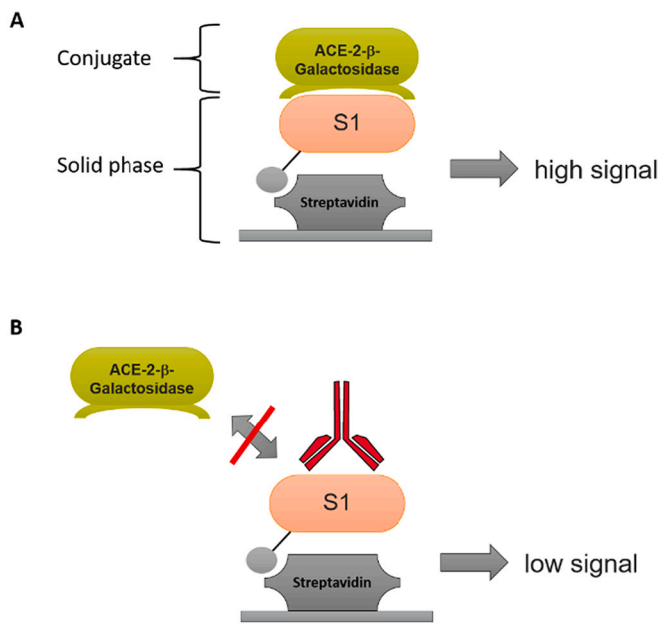
#### 2.3.2. S1 IgG FEIA

S1 IgG fluorescence enzyme immune assay (FEIA) from Thermo Scientific Sp1 IgG Test was used on fully automated Phadia instruments according to the manufacturer's instruction.

## 3. Results

### 3.1. Assay development

To detect neutralizing antibodies, the interaction between the SARS-CoV-2 Spike protein and human ACE-2 receptor was used. Biotinylated, recombinant SARS-CoV-S1 protein was immobilized onto streptavidin-coated EliA microcavities. As conjugate, recombinant human ACE-2 receptor was coupled to β-galactosidase using crosslinker technology (Fig. 1). The assay is built as a competitive assay. In absence of SARS-CoV-2 neutralizing antibodies the conjugate binds to the RBD of the immobilized SARS-CoV-S1 protein resulting in a high fluorescence signal as consequence of the cleavage of 4-methylumbelliferyl-β-D-galactopyranoside as substrate (Fig. 1 A). If RBD-binding neutralizing antibodies are present, they bind to the RBD on the solid phase at the ACE-2 receptor binding interface, thus sterically hindering the ACE-2



**Fig. 1.** Assay Principle: A: In the absence of the neutralizing antibody, ACE-2-receptor-β-galactosidase conjugate binds to the SARS-CoV-2 S1 protein on the solid phase turn the 4-methylumbelliferyl-β-D-galactosidase substrate into the fluorescent 4-methylumbelliferone generating the fluorescence signal as output. B: Neutralizing antibody binds to SARS-CoV-2 S1 protein thus inhibiting the SARS-CoV-2-ACE-2-receptor-β-galactosidase interaction.

receptor binding resulting in low assay signal (Fig. 1 B). Fig. 2A shows low signals of samples from COVID-19 convalescent persons in contrast to pre-pandemic blood donors or the sample diluent as control.

To quantify the neutralizing antibodies in samples (Fig. 2 B), a recombinant antibody at a concentration of 0–5 μg/ml could be established as calibrator.

To further characterize the ACE-2 receptor binding inhibition assay intra- and inter-assay precision was determined. The corresponding CV % values for the intra-assay precision were between 3.1 and 4.5%, and between 4.6 and 5.7% for the inter-assay precision confirming high reproducibility of the assay (Table 1).

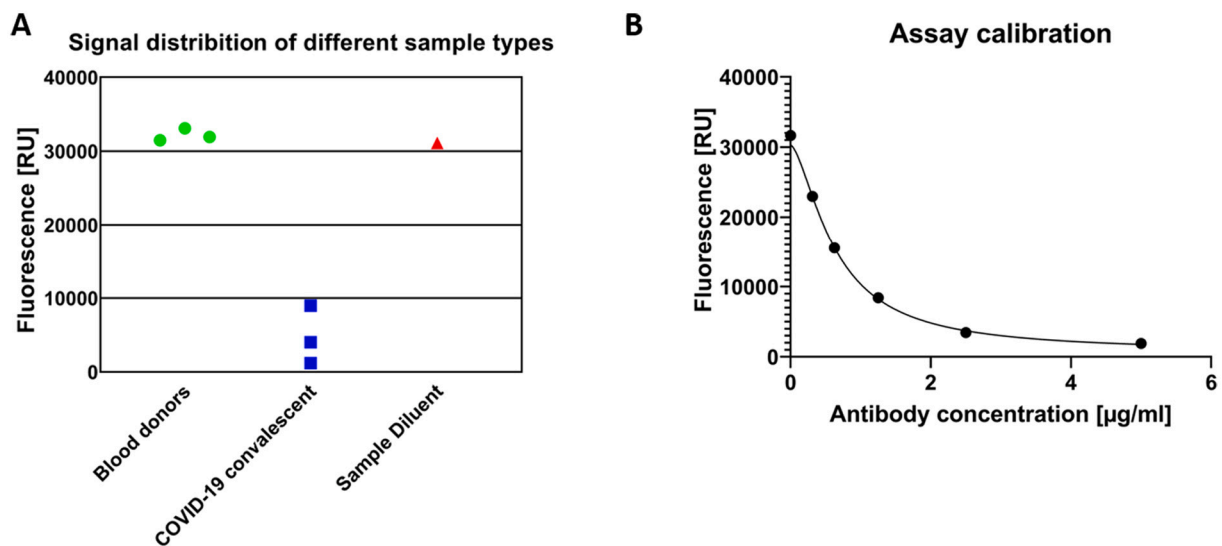
**Table 1**

Intra- and inter assay precision of the ACE-2 receptor binding inhibition assay.

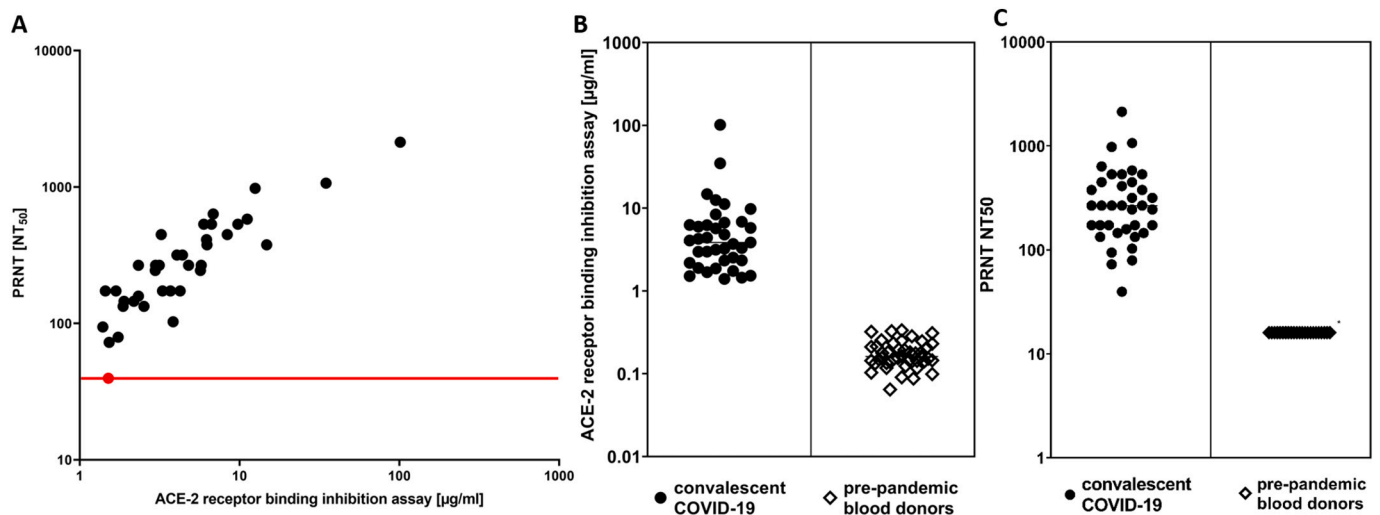
Sample No	Unit	Mean value	Coefficients of variation [%]		
			Intra-assay	Inter-assay	Total
Sample 1	μg/ml	0,32	4,5%	5,7%	7,2%
Sample 2	μg/ml	0,61	3,1%	5,4%	6,2%
Sample 3	μg/ml	1,26	3,5%	4,6%	5,8%
Sample 4	μg/ml	2,65	3,7%	4,8%	6,0%

**3.2. Correlation to Plaque reduction and neutralization assay**

The basic idea of this fully automated ACE-2 receptor binding inhibition assay is easier and faster quantification of SARS-CoV-2 neutralizing antibodies in blood samples. So far, the PRNT is recognized as the gold standard for SARS-CoV-2 neutralizing antibody detection. Therefore, it is mandatory to correlate this fully automated ACE-2 receptor binding inhibition assay to this method. For this purpose, 37 samples with the corresponding PRNT50 values from convalescent COVID-19 individuals were tested in 1:2 dilution together with pre-pandemic blood donor samples as controls in the ACE-2 receptor binding inhibition assay. A high correlation between the PRNT and ACE-2 receptor binding inhibition assay results were observed, resulting in a Pearson's R<sup>2</sup> value of 0.8389 (95% CI 0.8417 to 0.9562) (Fig. 3 A). Interestingly, there is one sample from a COVID-19 convalescent individual showing the NT50 value of 40 which exactly matches the PRNT's positivity threshold. The same sample displays a concentration of 1.5 μg/ml in ACE-2 receptor binding inhibition assay (Fig. 3 A) which is significantly higher, than the signal of the pre-pandemic blood donors (Fig. 5 B). The same pre-pandemic blood donor samples were tested in the PRNT as well. Out of 44 samples tested, 21 samples displayed cytotoxicity, different from SARS-CoV-2-induced cytopathic effect (CPE) at a 1:16 dilution (Fig. 3 C). Due to this cytotoxic effect, those samples were excluded from the specificity analysis. The remaining 23 pre-pandemic blood-donor samples displayed NT50 values of below 16 and were clearly below the positivity threshold of 40. In the ACE-2 receptor binding inhibition assay, those pre-pandemic blood donor samples showed signals significantly lower than the signals of PRNT-positive convalescent COVID-19 samples (Fig. 3 B). This suggests that the ACE-2 receptor binding inhibition assay would have similar specificity as PRNT.



**Fig. 2.** A: Signal distribution of different samples types, exemplified by 3 blood donor samples, 3 samples of COVID-19 convalescent persons and sample diluent as control. B: Assay calibration using a recombinant antibody from a COVID-19 convalescent person and a 4-Parameter-Fit for curve fit.



**Fig. 3.** A: Correlation between antibody binding inhibition assay on Phadia platform and PRNT. The correlation displays the pearson’s R2 value of 0.8389 (95% CI 0.8417 to 0.9562). The red data point indicates low reactive serum with the NT50 value of 40 and red line indicates the cut-off of the PRNT B: Concentration distribution in antibody binding inhibition assay measured for convalescent sera and pre-pandemic blood-donors. C: Distribution of NT50 values in the PRNT as measured for convalescent sera and pre-pandemic blood donors. Asterisk indicates, that all NT50 values for pre-pandemic blood donors were below 16. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

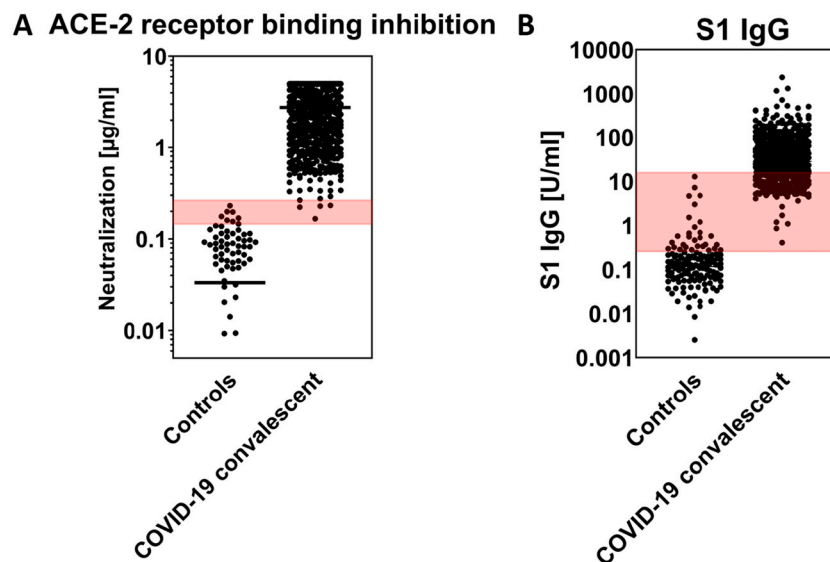
**3.3. Determination of specificity and comparison of sensitivity to the Phadia S1 IgG assay in convalescent COVID-19 samples**

After having shown a good correlation between the ACE-2 receptor binding inhibition assay and PRNT, we aimed to determine the specificity and to compare the sensitivity of the ACE-2 binding inhibition assay to the Phadia S1 IgG assay in convalescent COVID-19 samples. Therefore, a cohort of 637 samples of COVID-19 convalescent patients with proven PCR-positivity was tested. Pre-pandemic-sourced blood samples of respiratory and other infectious disease-patients, as well as blood donors served as controls. The cohort also contained samples from common cold corona viruses infected individuals: HCoV-229E/Corona, HCoV-HKU1, HCoV-NL63, HCoV-OC43. All sera were tested for SARS-CoV-2 IgG antibodies using the FEIA-based SARS-CoV-2 S1 IgG assay from Thermo Fisher Scientific (Mylemans et al., 2021) on the fully automated Phadia platform.

In the ACE-2 binding inhibition assay, the concentrations of samples from COVID-19 convalescent individuals in a dilution of 1:2 ranged between 0.2 and 5.0 µg/ml with 167 samples above the measuring range. The concentrations of control samples measured at the same dilution ranged between 0 and 0.2 µg/ml (Fig. 4 A). The mean of signals for COVID-19 convalescent samples was  $2.8 \pm 1.7$  µg/ml and for control samples  $0.03 \pm 0.05$  µg/ml, respectively, suggesting a good discriminatory ability between the two sample types. In fact, there was only a small signal overlap between the control samples and samples of COVID-19 convalescent persons. This leads to only seven samples out of 637 which could not be discriminated from the controls.

To assure a high specificity of 99.99% (95% CI = 97.6% to 99.9%) the threshold positivity value of 0.3 µg/ml was set. With this cut-off value a sensitivity of 98.9% (95% CI = 97.7% to 99.5%) was determined for this sample cohort.

In contrast to the ACE-2 receptor binding inhibition assay, the S1 IgG



**Fig. 4.** Concentration distribution of samples from convalescent COVID-19 and disease controls measured with ACE-2 receptor binding inhibition assay and S1 IgG assay. The concentration overlap between samples of COVID-19 convalescent persons and controls samples is marked red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



assay displays pronounced signal overlap between control samples and the samples of COVID-19 convalescent persons (Fig. 4 B). Using the manufacturer's cut-off (10 U/ml) a sensitivity of 86,2% (95% CI = 83.3 to 88.6% and a specificity of 99,4% (95% CI = 96.5% to 99.9) was determined for this sample cohort.

To properly compare the two assays, sensitivity was addressed at the fixed specificity of 99%. The sensitivity at specificity of 99%, was 98.9% (95% CI = 99.1% to 100%) and 95% (95% CI = 93.0% to 96.4%) for the ACE-2 binding inhibition assay and the Phadia S1 IgG assay, respectively. This analysis suggests that the ACE-2 receptor binding inhibition assay is more specific than the S1 IgG assay. Indeed, the two highest samples from the pre-pandemic control cohort show S1 IgG signals of 12,8 and 7,2 U/ml, respectively (data not shown). One sample corresponds to a blood donor and the other one is Influenza A IgG positive. Among these two samples, one is even above the manufacturer's cut-off value and the other one is in the borderline zone. In contrast, both samples display concentrations of 0.15 and 0.09 µg/ml in the ACE-2 receptor binding inhibition assay, being thus clearly below the positivity threshold of 0.3 µg/ml.

In summary, the developed, fully automated ACE-2 receptor binding inhibition assay displays high sensitivity and specificity and can be used to quantify SARS-CoV-2 neutralizing antibodies in samples of COVID-19 convalescent persons. This assay offers even higher sensitivity than the commercially available S1 IgG used on the same Phadia platform.

### 3.4. Quantification of neutralizing antibodies in samples of vaccinated persons

So far, the ACE-2 receptor binding inhibition assay proved to be able to quantify the SARS-CoV-2 neutralizing antibodies in samples of COVID-19 convalescent persons.

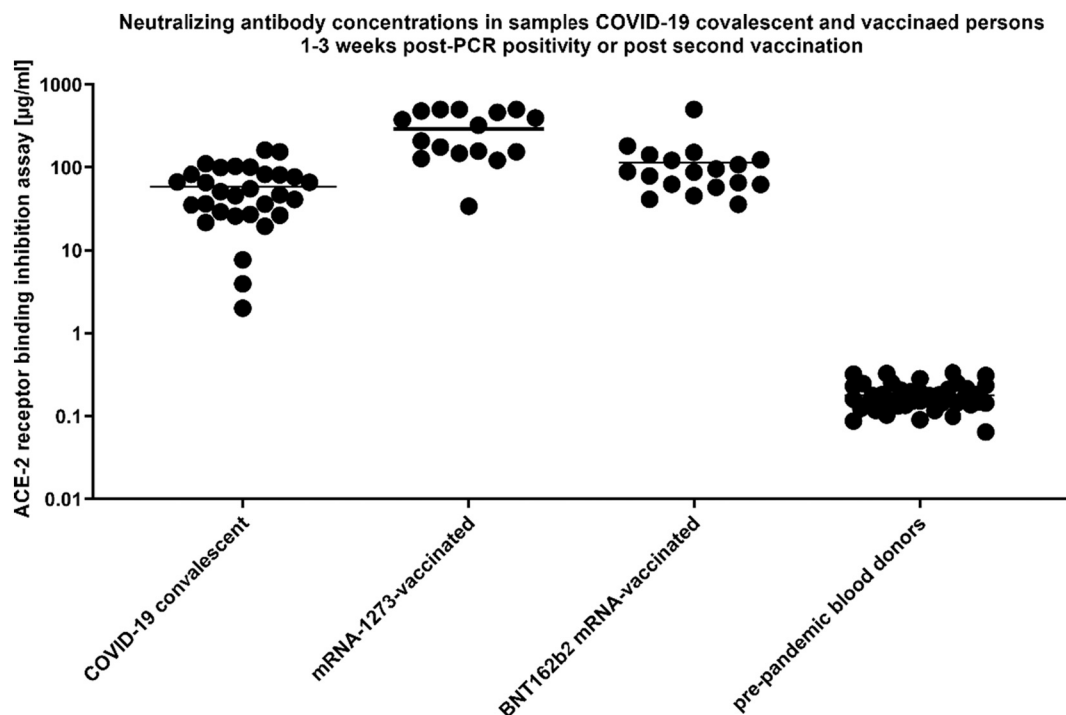
Along with worldwide SARS-CoV-2 vaccination, the need to quantify the neutralizing antibodies as a result of vaccination might become necessary. Assuming that neutralizing antibody titers from a vaccination could fade over time (Doria-Rose, 2021), it could become necessary to

determine the neutralization antibody concentration to decide about need of revaccination. This would require population wide tests for neutralizing antibodies, which cannot be fulfilled using PRNT, which can be performed with single samples only. For population-wide tests fully automated assays on scalable systems, such as ACE-2 receptor binding inhibition assay on a Phadia system, would be needed. For this purpose, we aimed to quantify neutralizing antibodies in samples of mRNA-1273-, and BNT162b2-vaccinated persons (Baden et al., 2021; Polack et al., 2020) and to compare these titers with those in samples of COVID-19 convalescent persons. As antibodies have been reliably detected 1–3 weeks following recovery from COVID-19, or after the second vaccine dose, we chose to analyze samples at these time points. Neutralizing antibody titers of 30 samples of COVID-19 convalescent persons were compared to 16 samples of mRNA-1273-vaccinated and 18 samples of BNT162b2vaccinated persons yielding a mean signal of 58.63 µg/ml (95% CI = 43,46 to 73,79 µg/ml), 291.1 µg/ml (95% CI = 202.7 to 379.4 µg/ml and 113.8 µg/ml (95% CI = 61.79 to 165.8 µg/ml) (Fig. 5) and were clearly elevated compared to signals of pre-pandemic blood donors. ANOVA shows that there is a significant difference between neutralizing antibody titers of COVID-19 convalescent and mRNA-1273-vaccinated persons (Table 2). This difference might be accounted by cohort differences, since COVID-19 convalescent persons were significantly older (mean age 69) with harsh symptoms of dyspnea and being hospitalized compared to mRNA-1273-vaccinated persons

**Table 2**

Differences between vaccinated and COVID-19 convalescent groups.

Multiple comparison	Adjusted <i>P</i> value
COVID-19 convalescent vs. mRNA-1273-vaccinated	0,0094
COVID-19 convalescent vs. BNT162b2 mRNA-vaccinated	>0,9999
COVID-19 convalescent vs. pre-pandemic blood donors	<0,0001
mRNA-1273-vaccinated vs. BNT162b2 mRNA-vaccinated	0,5799
mRNA-1273-vaccinated vs. pre-pandemic blood donors	<0,0001
BNT162b2 mRNA-vaccinated vs. pre-pandemic blood donors	<0,0001



**Fig. 5.** Comparison of neutralization antibody concentrations in samples of COVID-19 convalescent persons, mRNA-1273- and BNT162b2-vaccinated persons 1–3 weeks post PCR positivity or post second vaccination dose. Means for the samples of COVID-19-convalescent, mRNA-1273- and BNT162b2-vaccinated persons are 58.63, µg/ml (95% CI=, 43,46 to 73,79 µg/ml) 291.1 µg/ml (95% CI = 202.7 to 379.4 µg/ml and 113.8 µg/ml (95% CI = 61.79 to 165.8 µg/ml), respectively.

with mean age of 41.

In summary, the newly developed ACE-2 receptor binding inhibition assay is able to quantify neutralizing antibody concentrations not only in samples of COVID-19 convalescent persons but also in samples of vaccinated individuals as exemplified here with samples from mRNA-1273- and BNT162b2-vaccinated persons 1–3 weeks after the second vaccination.

#### 4. Discussion

So far, assays with ACE-2 receptor immobilized on solid phases and RBD conjugated to reporter enzymes were correlated to PRNT. This assay format requires a preincubation step of sample with the enzyme conjugated RBD before this mixture can be applied to the solid phase (Taylor et al., 2021; Tan et al., 2020). This preincubation step would be problematic in the pipetting sequence of fully automated instruments, because most instruments perform the transfer of sample to the solid phase as the first pipetting step and would require either preincubation outside of the instrument or the incubation of sample-conjugate mixture directly on the solid phase. To overcome this obstacle SARS-CoV-2 S1 protein was immobilized on the solid phase and ACE-2-receptor bound to the reporter enzyme was used as conjugate. This setup fits better into the standard pipetting sequence of Phadia instruments without the need of a preincubation step outside of the instrument.

The ACE-2 receptor binding inhibition assay shows a very good agreement with PRNT in COVID-convalescent and pre-pandemic blood donor samples. In PRNT, ca. half of pre-pandemic donor samples displayed cell toxicity at 1:16 sample dilution, which interfered with the CPE analysis, and is a disadvantage of this cell-based assay in comparison to the ACE-2 receptor binding inhibition assay.

The weakness of the ACE-2-receptor binding inhibition assay is the focus on the interaction between S1 and ACE-2 receptor only. Undoubtedly, this interaction is the main mechanism for the binding of the SARS-CoV-2 to the epithelial cells. But there are other mechanisms, that might be involved in the cell entry of the virus as well. It was shown that the virus binds to the heparan sulfate on the cell surface via its RBD (Baden et al., 2021). Another neglected aspect by the ACE-2 receptor binding inhibition assay is the value of the N-terminal domain of the spike protein (Polack et al., 2020), which is a target of some neutralizing antibodies, thus playing a significant role in virus transmission (Israel et al., 2021).

Considering, that the ACE-2 receptor binding inhibition assay only focuses on the RBD ACE-2 receptor interaction, this assay displays a high correlation with PRNT. This suggests that the RBD ACE-2 receptor interaction is the crucial step of the SARS-CoV-2 infection thus mimicking the viral infection at the cellular level very well.

By comparing the ACE-2 binding inhibition assay with the Phadia S1 IgG enzyme immunoassay based on the same fully automated Phadia platform, it was found that the test even surpassed the high specificity proven by the commercial EliA SARS-CoV2 Sp1 IgG test. It seems that by focusing on the ACE2 receptor RBD interaction one would be able to reduce the impact of non-specific antibody binding to the solid phase significantly, which is a big strength of the assay (Sahin et al., 2020).

Using the samples of mRNA-1273-, and BNT162b2-vaccinated persons we could show, that the ACE-2 receptor binding inhibition assay is able to quantify neutralizing antibodies in samples of vaccinated persons as well. The concentrations are rather high, so a higher sample dilution than the one used for the measurement of COVID-19 convalescent samples is required for most of samples. It seems that the neutralizing antibody titers in samples of mRNA-1273-, and BNT162b2-vaccinated persons are higher than in the samples of COVID-19 convalescent persons having roughly the same time interval of 1–3 weeks after the last contact with either virus or vaccine as immunogen, which is in line with the current data from the literature (Israel et al., 2021; Sahin et al., 2020).

The data presented here are measured with the assay built with the

biotinylated SARS-CoV-2 S1 protein of the Wuhan virus strain and operated on Phadia-250 and Phadia-100 instruments. After several infection waves with the alpha, beta, delta and currently omicron variant, one might conclude, that the assay would be outdated. But, the application of biotinylated SARS-CoV-2 S1 protein on the solid phase provides the possibility to change the antigen according to current virus strain, making the assay easy to adapt to future infection waves. In addition, the application of the assay on the Phadia™ system with Phadia-200, Phadia-250 and Phadia-5000 instruments provides the unique fully automated assay of different sample scales to date.

#### Funding

Funding for this work was provided by Thermo Fisher Scientific ImmunoDiagnostics Phadia GmbH.

#### Declaration of Competing Interest

All authors are employees of Thermo Fisher Scientific ImmunoDiagnostics Phadia, GmbH.

#### Acknowledgement

The sample cohort comprised patient samples which were kindly provided by Onze-Lieve-Vrouwziekenhuis Aalst, Belgium and Centre Hospitalier Montpellier, CSL Behring and Transplant Division of Thermo Fisher Scientific. The authors thank Dr. Anja Voreck and Dr. Nadja Hoefs, Dr. Victoria Smith-Machnow for critical reading of the manuscript.

#### References

- Baden, L.R., et al., 2021. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N. Engl. J. Med.* 384 (5), 403–416.
- Bewley, K.R., et al., 2021. Quantification of SARS-CoV-2 neutralizing antibody by wild-type plaque reduction neutralization, microneutralization and pseudotyped virus neutralization assays. *Nat. Protoc.* 16 (6), 3114–3140.
- Deb, P., Molla, M.M.A., Saif-Ur-Rahman, K.M., 2021. An update to monoclonal antibody as therapeutic option against COVID-19. *Biosaf. Health* 3 (2), 87–91.
- Doria-Rose, N., et al., 2021 Jun 10. Antibody persistence through 6 months after the second dose of mRNA-1273 vaccine for Covid-19. *N. Engl. J. Med.* 384 (23), 2259–2261.
- Israel, A., et al., 2021. Large-scale study of antibody titer decay following BNT162b2 mRNA vaccine or SARS-CoV-2 infection. *medRxiv*. <https://doi.org/10.1101/2021.08.19.21262111>.
- Jiang, S., et al., 2020. Neutralizing antibodies for the treatment of COVID-19. *Nat. Biomed. Eng.* 4 (12), 1134–1139.
- Khoury, D.S., et al., 2021. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat. Med.* 27 (7), 1205–1211.
- Lan, J., et al., 2020. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* 581 (7807), 215–220.
- Mann, M.R., 2006. ELIA CCP, MODEL 14-5515-01; ELIA CCP CONTROL, MODEL 83-1009-01. In: FDA 510(k) Premarket Notification.
- Mylemans, M., et al., 2021. Diagnostic and analytical performance evaluation of ten commercial assays for detecting SARS-CoV-2 humoral immune response. *J. Immunol. Methods* 493, 113043.
- Nie, J., et al., 2020. Establishment and validation of a pseudovirus neutralization assay for SARS-CoV-2. *Emerg. Microb. Infect.* 9 (1), 680–686.
- Padoan, A., et al., 2022. Neutralizing antibody titers six months after Comirnaty vaccination: kinetics and comparison with SARS-CoV-2 immunoassays. *Clin. Chem. Lab. Med.* 60 (3), 456–463.
- Polack, F.P., et al., 2020. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N. Engl. J. Med.* 383 (27), 2603–2615.
- Sahin, U., et al., 2020. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. *Nature* 586 (7830), 594–599.
- Tan, C.W., et al., 2020. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. *Nat. Biotechnol.* 38 (9), 1073–1078.
- Taylor, S.C., et al., 2021. A new SARS-CoV-2 dual-purpose serology test: highly accurate infection tracing and neutralizing antibody response detection. *J. Clin. Microbiol.* 59 (4).
- Vicenti, I., et al., 2021. Faster decay of neutralizing antibodies in never infected than previously infected healthcare workers three months after the second BNT162b2 mRNA COVID-19 vaccine dose. *Int. J. Infect. Dis.* 112, 40–44.

- Villalta, D., et al., 2002. Evaluation of a new automated enzyme fluoroimmunoassay using recombinant plasmid dsDNA for the detection of anti-dsDNA antibodies in SLE. *J. Clin. Lab. Anal.* 16 (5), 227–232.
- Walls, A.C., et al., 2020. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell* 181 (2), 281–292 e6.
- Wheatley, A.K., et al., 2021. Evolution of immune responses to SARS-CoV-2 in mild-moderate COVID-19. *Nat. Commun.* 12 (1), 1162.
- Zhou, P., et al., 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579 (7798), 270–273.
- Zost, S.J., et al., 2020. Potently neutralizing and protective human antibodies against SARS-CoV-2. *Nature* 584 (7821), 443–449.