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Comparison of the measured values of quantitative SARS-CoV-2 spike antibody assays

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ABSTRACT

Background: The concentration of antibodies against the SARS-CoV-2 spike protein is frequently being measured for clinical and epidemiological purposes. The aim of this study was to examine whether the results of different quantitative SARS-CoV-2 spike antibody assays are comparable.

Material and methods: The Siemens SARS-CoV-2 IgG, Abbott SARS-CoV-2 IgG II Quant, Roche ElecsysT Anti-SARS-CoV-2 S, and Euroimmun Anti-SARS-CoV-2-QuantiVac assay were compared with 110 sera from patients 6-9 months after SARS-CoV-2 infection and the WHO First International SARS-CoV-2 antibody standard 20/136. The antibody values were converted into WHO binding antibody units (BAU)/ml. The diagnostic sensitivity of the assays was determined and the antibody values were compared.

Results: The diagnostic sensitivity ranged from 57.3% (Euroimmun) to 100% (Roche). The antibody concentration values of different assays correlated with Pearson coefficients of correlation between 0.729 and 0.953. The geometric mean antibody values of the Abbott, Siemens and Euroimmun assay varied by a factor of 1.1-1.2. The geometric mean antibody values of the Roche assay were 2.4-2.8 times higher than those from the other assays. The assays yielded varying results with the WHO International antibody standard.

Conclusions: The quantitative SARS-CoV-2 antibody assays from Abbott, Siemens, Roche and Euroimmun correlate strongly but differ in the antibody concentrations. Therefore, the same assay should be used when testing patients repeatedly. In addition, the name of the assay used and the manufacturer should be indicated along with the test results.

1. Introduction

Infection with and vaccination against SARS-CoV-2 induce an antibody response against the viral spike glycoprotein. It has been observed that the concentration of binding antibodies against the spike protein correlates with protection from symptomatic infection. This indicates that the concentration of spike protein binding antibodies can be used as a marker to predict the likelihood that an individual is protected from disease [1,2].

Many quantitative SARS CoV-2 antibody assays are commercially available and in clinical use. The assays differ regarding the target antigen that is being used, the immunoglobulin classes that are being measured and the technical principle. For instance, the Siemens SARS-CoV-2 IgG

(sCOVG) assay, the Abbott SARS-CoV-2 IgG II Quant assay and the Roche ElecsysT Anti-SARS-CoV-2 S test quantify antibodies against the receptor-binding domain (RBD) of the spike glycoprotein [3–5]. In comparison, the Euroimmun Anti-SARS-CoV-2-QuantiVac-ELISA (IgG) measures antibodies against the complete S1 portion of the glycoprotein [6]. The Abbott, Siemens and Euroimmun tests measure IgG antibodies whereas the Roche test measures high-affinity antibodies of any immunoglobulin type. In addition, the Abbott, Siemens and Euroimmun assays are classical antibody assays that use antigen-coated surfaces and secondary anti-human IgG antibodies, whereas the Roche assay is a bridge immunoassay that uses two antigen molecules to measure the antibody concentration. The differences between the assays suggest that the tests may yield different results when tested with the same set of sera.

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; COVID-19: coronavirus disease 2019; BAU: binding antibody units; RBD: receptor binding domain

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Several previous studies that compared quantitative SARS-CoV-2 antibody assays found that values obtained with different assays correlated well [7–13]. At the same time, some studies reported high proportional differences between the antibody assays. For instance, Kim et al. found 4.5–4.9 times higher antibody concentration values in the Abbott and the Siemens assay compared with the Roche assay [12]. It was further reported that differences between the results from the quantitative Abbott and Roche antibody assays depended on the time point of blood drawing after vaccination [21].

Therefore, the aim of this investigation was to examine the concordance of four quantitative SARS-CoV-2 antibody assays with sera from 6–7 and 9 months after SARS-CoV-2 infection. At this point after infection, the affinity of the IgG antibodies has matured and it was unlikely that the sera contained virus-specific IgM antibodies. We also tested, if the assays give similar values with the WHO Anti-SARS-CoV-2 antibody standard 20/136.

2. Study design

We performed a diagnostic study to determine the concordance of four SARS-CoV-2 spike protein antibody assays when measuring the spike-specific antibody concentration in sera from infected individuals.

2.1. Serum samples and WHO antibody standard

Venous blood samples ($N = 110$) were obtained from 55 adults 6–7 and 9 months after infection with SARS-CoV-2 documented by RT-PCR. The participants were asymptomatic or had mild to moderate symptoms. None of the participants were hospitalized and none had been vaccinated before blood drawing [14]. Sera were prepared by centrifugation and stored at $-20\text{ }^{\circ}\text{C}$ until testing. Study subjects provided informed consent to participating in the study. The study was approved by the Ethics Commission at the Medical Faculty at the University of Leipzig (ethical vote 352/20-ek). The First WHO International Standard for anti-SARS-CoV-2 immunoglobulin, NIBSC code no. 20/136, was obtained from the National Institute of Biological Standards and Controls, Potters Bar, Hertfordshire, United Kingdom. It was developed by pooling plasma of SARS-CoV-2 convalescent patients and contains SARS-CoV-2-specific antibodies at a concentration of 1000 binding antibody units (BAU)/ml [15,16].

2.2. Quantitative SARS-CoV-2 spike antibody assays

The following four quantitative SARS-CoV-2 glycoprotein antibody assays were examined: Abbott SARS-CoV-2 IgG II Quant, Siemens Atellica IM SARS-CoV-2 IgG (sCOVG), Roche ElecsysT Anti-SARS-CoV-2-S and Euroimmun Anti-SARS-CoV-2-QuantiVac-ELISA (IgG) assay. The Abbott assay was performed with the ARCHITECT i2000SR system, the Roche assay was performed with an automated cobas e601 analyzer and the Siemens assay was performed with the Atellica IM analyzer. The Euroimmun ELISA was performed manually according to the instructions of the manufacturer and the ELISA plates were read with a Sunrise microplate reader (Tecan Switzerland AG). Serum samples were applied to the assays as recommended by the assay manufacturers. The companies provided the following conversion factors to calculate WHO BAU/ml: 1 BAU/ml corresponds to 0.142 Abbott AU/ml, 21.8 Siemens U/ml, 1.0 Roche U/ml and 3.2 Euroimmun RE/ml [7,10].

The WHO antibody standard 20/136 was prediluted 1:50 and 1:100 in antibody stabilizer solution (Candor Bioscience Antibody Stabilizer) before testing with the Abbott, Siemens and Roche assay. The standard was not tested in the Euroimmun assay. The BAU/ml values obtained were multiplied by the dilution factors and averaged. For the Siemens assay, only the WHO standard at 1:50 dilution was used, because at higher dilution the BAU values were below the linear range of the assay.

2.3. Data analysis

Equivocal results were counted as negative. Maximum and minimum antibody concentrations were calculated with all data points. Comparison of values, correlation and regression analyses were performed with positive values only. Pearson coefficients of correlation r were calculated with log-transformed values to determine the degree of correlation of the assays. The 95% confidence interval of the Pearson coefficient of correlation was determined via Fisher transformation and calculation of logarithmic upper and lower limits. Two-tailed Wilcoxon signed rank tests with BAU/ml values were performed to compare the antibody concentrations values. Passing-Bablok regression analysis was performed to calculate the proportional errors. The Statistical tests were performed with SPSS, the XLSTAT plugin for Microsoft Excel, MedCalc and Social Science Statistics software [17–20].

3. Results

3.1. Percentage of positive results and antibody concentration values

The SARS-CoV-2 antibody assays from Abbott, Siemens and Roche recognized 106 (96.4%), 76 (69.1%) and 110 (100%) of the 110 serum samples. The Euroimmun ELISA identified 63 sera (57.3%). The assays used different proprietary measurement scales. To compare the results, the measurement values were converted into WHO BAU/ml using the conversion factors provided by the manufacturers. After conversion, the lower limits of detection of the tests ranged from 7.1 BAU/ml (Abbott assay) to 35.2 BAU/ml (Euroimmun ELISA). Depending on the assay, the antibody values of the sera ranged from below the detection limits to 408, 441, 466 and 1964 BAU/ml in the Abbott, Siemens, Euroimmun and Roche assay, respectively (Table 1).

3.2. Correlation of antibody values

The Pearson coefficient of correlation was calculated to determine the degree of correlation between the assay results. The assays correlated with r -values between 0.729 and 0.953. The Abbott and the Siemens assays correlated most strongly with a coefficient of correlation of 0.953. The Siemens and the Roche assay showed a correlation of $r = 0.729$. The coefficients of correlation for the other assay pairs were between 0.788 and 0.829 (Table 2).

3.3. Difference and proportional relation of antibody values from different assays

The assays gave varying BAU/ml values for the same serum samples. The differences were statistically significant in the Wilcoxon signed rank test ($p \leq 0.001$) except for the Siemens and the Euroimmun assay that showed similar values ($p = 0.242$). The geometric mean antibody values of the Siemens and the Euroimmun assay were 1.21 and 1.17 times higher than the Abbott values. The geometric mean antibody values of the Roche assay were 2.4 (Roche/Siemens) and 2.8 (Roche/Abbott, Roche/Euroimmun) times higher than those of the other assays (Fig. 1).

Pairwise Passing-Bablok regression analysis was performed to determine the proportional relationship of the antibody values from each assay pair indicated by the slope of the regression curve and the systematic differences indicated by the intercept. The Siemens assay showed a 1.44 (95% CI 1.33–1.57) times proportional increase and a constant error of -18.2 (95% confidence interval (CI) -10.1 to -26.6) BAU/ml compared with the Abbott assay. The Euroimmun assay showed a 1.26 (95% CI 1.12–1.47) times proportional increase in comparison with the Abbott assay. The values were not systematically different, because the 95% confidence interval of the intercept included “0” (95% CI -22.4 to 5.6). The Euroimmun and the Siemens assay yielded proportionally (slope 0.871, 95% CI 0.681–1.022) and systematically (intercept 9.0, 95% CI -6.2 to 26.3) similar results. The Roche assay

Table 1
Minimal and maximal SARS-CoV-2 spike protein-specific antibody concentrations measured with four assays

Measure	Abbott AU ¹ /ml	BAU ² /ml	Siemens U/ml	BAU/ml	Roche U ³ /ml	BAU/ml	Euroimmun RE ⁴ /ml	BAU/ml
Minimum	50	7.1	1	21.8	12.0	12.0	11	35.2
Maximum	2877.2	408.6	20.3	441.5	1964.0	1964.0	145.8	466.5

¹ AU: Arbitrary unit.

² BAU: Binding antibody unit.

³ U: Unit.

⁴ RE: Relative Unit.

Table 2
Correlation of the antibody concentrations (BAU/ml) obtained with SARS-CoV-2 spike antibody assays.

Assay	Abbott No of sera	r (95% CI) ^a	Siemens No of sera	r (95% CI) ^a	Roche No of sera	r (95% CI) ^a
Siemens	76	0.953 (0.927–0.970)				
Roche	106	0.829 (0.758–0.881)	76	0.729 (0.603–0.820)		
Euroimmun	63	0.826 (0.727–0.891)	63	0.789 (0.673–0.867)	63	0.788 (0.671–0.867)

^a Pearson coefficient of correlation; CI: 95% confidence interval

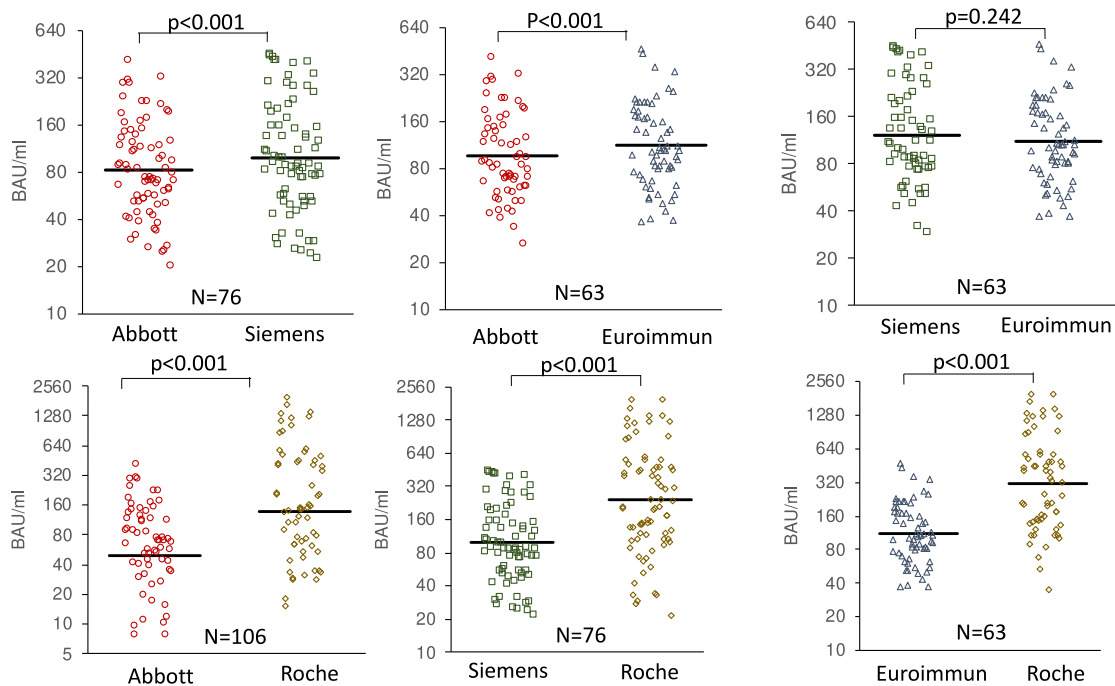


Fig. 1. Comparison of the antibody values with assay pairs. Antibody values in BAU/ml from subjects 6-9 months after infection with SARS-CoV-2 were compared with the Wilcoxon signed rank test. The bars represent the geometric mean values. N: number of sera. Abbott: Abbott SARS-CoV-2 IgG II Quant assay; Siemens: Siemens Atellica IM SARS-CoV-2 IgG (sCOVG) assay; Roche: Roche ElecsysT Anti-SARS-CoV-2-S assay; Euroimmun: Euroimmun Anti-SARS-CoV-2-QuantiVac-ELISA (IgG) assay.

showed a 4.59 (Siemens, 95% CI 3.85–5.45), 5.23 (Abbott, 95% CI 4.35–6.08), and 5.39 (Euroimmun, 95% CI 4.29–6.74) times proportional increase and systematically higher values compared with the other assays (Fig. 2 and Suppl. Table 1).

3.4. Comparison using the WHO SARS-CoV-2 antibody standard

The assays were also compared with the WHO first international SARS-CoV-2 antibody standard 20/136 which, by definition, contains 1000 BAU/ml. The Abbott assay showed 634 BAU/ml. the Siemens assay gave 870 BAU/ml and the Roche assay yielded 645 BAU/ml. Thus, the assays gave between 13% and 37% lower BAU/ml values than expected.

The Abbott and the Roche assay delivered similar results and the Siemens assay showed 1.37 and 1.34 times higher values than the other two assay (Fig. 3).

4. Discussion

Quantitative SARS-CoV-2 antibody assays are being used in clinical and research laboratories. The assays provide information about the concentration of virus spike-protein binding antibodies in serum samples after SARS-CoV-2 infection and vaccination. The tests are primarily being used to determine the magnitude and the course of the immune response in patients after vaccination and to evaluate the effectiveness

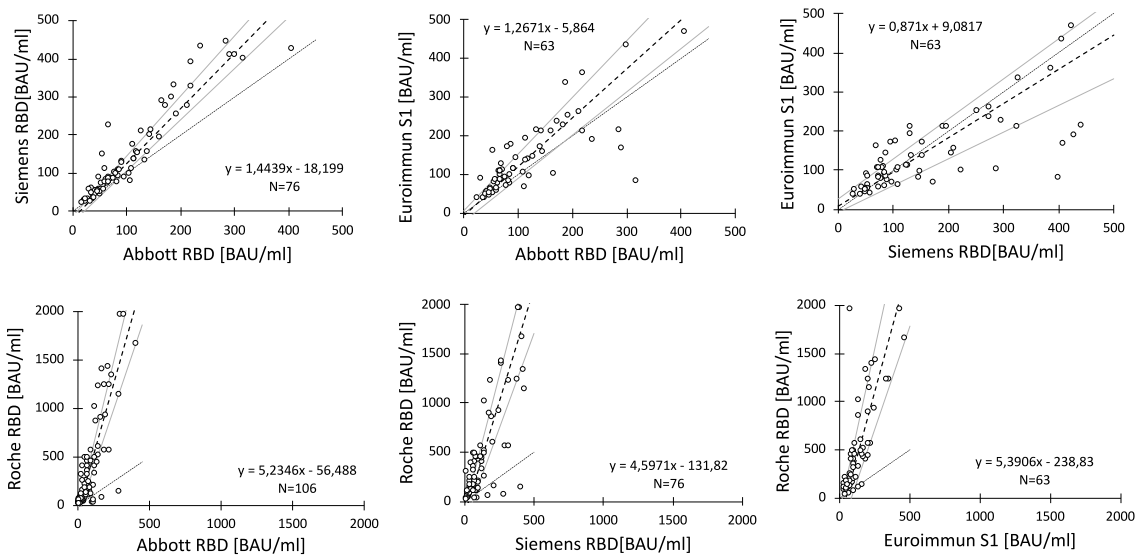


Fig. 2. Antibody concentrations in pairs of SARS-CoV-2 antibody assays. Passing-Bablok regression lines and linear equations for pairs of antibody assays. N: number of sera. Dashed lines represent the regression lines; thin lines represent the 95% confidence intervals; dotted lines represent the 45° centerline. Abbott: Abbott SARS-CoV-2 IgG II Quant assay; Siemens: Siemens Atellica IM SARS-CoV-2 IgG (sCOVG) assay; Roche: Roche ElecsysT Anti-SARS-CoV-2-S assay; Euroimmun: Euroimmun Anti-SARS-CoV-2-QuantiVac-ELISA (IgG) assay.

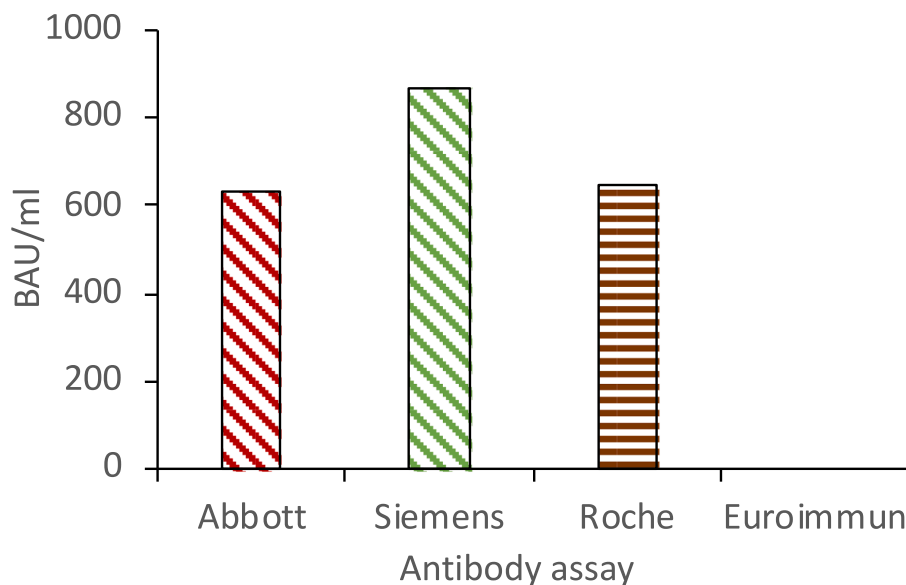


Fig. 3. Antibody concentrations of the WHO Anti-SARS-CoV-2 antibody standard 20/136. The concentration of the WHO antibody in BAU/ml obtained with the Abbott, Siemens and Roche assay; Abbott: Abbott SARS-CoV-2 IgG II Quant assay; Siemens: Siemens Atellica IM SARS-CoV-2 IgG (sCOVG) assay; Roche: Roche ElecsysT Anti-SARS-CoV-2-S assay; The Euroimmun assay was not tested.

of vaccines. The aim of the study was to determine the concordance of four of the assays. For the analysis we used sera from SARS CoV-2-infected individuals 6-7 and 9 months after infection.

The diagnostic sensitivity of the assays varied from 57.3 to 100%. The Roche assay recognized all sera as antibody positive. Assays with lower limits of detection showed higher diagnostic sensitivity. These assays are preferable for seroepidemiological studies based on serologic proof of previous SARS CoV-2 infection.

The antibody concentrations obtained with the four assays correlated strongly. Thus, sera with high BAU/ml values in one of the assays had also high BAU/ml values in the other assays and a serum with a low value in an assay showed a low value in the other assays, as well. This indicates that, in principle, the results from an assay can be converted to

those from another assay.

The assays delivered varying serum antibody concentration values. On the average, the Siemens and Euroimmun assay gave slightly higher values than the Abbott test. Therefore, for consistency of clinical laboratory results, repeated testing of patients should be performed with the same assay. In addition, the assay name and the manufacturer should be reported together with the test results. Alternatively, differences between the assay results can be reduced by applying the equation of the regression curve. Conversion of the results from an assay to another assay is being performed by putting the antibody values of the assay shown on the x-axis into the equation of the Passing Bablok regression line at x and solving the equation for y.

It must be added that slight differences of the antibody concentration

may be clinically or epidemiologically irrelevant. For instance, it was previously shown that an increase in protection from infection by 10% in a population was associated with a two-fold higher antibody concentration [1,2]. Thus, the quantitative difference between the antibody results obtained with the Abbott, Siemens and Euroimmun assay may be epidemiologically negligible.

The situation is more complex with the Roche assay. The assay showed considerably higher BAU/ml values than the three other tests. Several previous studies that compared the Roche antibody test with other assays found lower or similar values with the Roche assay [7,10,12]. Another study with vaccinated individuals showed that the ratio of the antibody concentration depended on the time point of blood drawing after immunization. Early after the first vaccine dose, the Roche assay gave lower values and several weeks after vaccination and after the second vaccine dose, the Roche assay delivered 5-6 times higher values than the Abbott assay [21]. Moreover, the Roche antibody assay that uses two antigen molecules to measure the antibody concentration that uses two antigen showed stable or increasing antibody concentrations in consecutive sera from the same patients whereas antibody assays that use secondary antibody for IgG detection showed declining IgG concentrations [22]. Additional studies indicated that the antibody values shown by the Roche assay depended not only on the concentration of the antibodies but on the affinity of the antibodies to the target antigen. Serum with antibodies with higher affinity led to higher measurement values [23]. Together, the data indicate that the Roche dual antigen binding assay yields different antibody values compared with assays that use secondary antibody depending on the time point after infection or vaccination and the associated increase in antibody affinity. In this study, the sera were from several months after infection at a time when antibodies have matured to high affinity. This resulted higher antibody values with the Roche assay compared with the other assays.

The assays delivered varying BAU/ml values with the International WHO SARS-CoV-2 antibody standard 20/136. The differences that were seen with the international standard were similar to those observed with the sera. For instance, the Siemens assay that gave a 1.37 times higher value than the Abbott assay with the international standard gave 1.21 times higher values than the Abbott assay with serum samples. This suggests that the assays have been graduated differently with the WHO antibody standard. It indicates that the difference between the Abbott and the Siemens assay results could be reduced by recalibration and adjusting the BAU conversion factors of the assays.

It must be mentioned at this point that the emergence of new viral variants makes the reporting and clinical interpretation of quantitative SARS-CoV-2 antibody assay results challenging. For optimal use of the antibody assays, the association of antibody concentrations in BAU/ml and protection from infection and disease needs to be re-examined with assays that measure the antibody concentrations against the spike protein of the circulating or impending viral strains. Finally, the association of antibody concentration and protection from illness needs to be better defined in order to use the values as predictive markers in individual patients.

In conclusion, the quantitative SARS-CoV-2 antibody assays from Abbott, Siemens, Roche and Euroimmun yielded correlating but varying antibody concentrations. The Roche assay results were considerably higher than those from the other assays owing to technical differences of the assays. Part of the variation between the Abbott and the Siemens assay is most likely due to different calibrations with the WHO international SARS-CoV-2 antibody standard. Therefore, if antibody concentrations are being monitored in the clinical setting, for instance, to observe the immune response in immunosuppressed patients, the same assay should be used when testing patients repeatedly and the name of the assay and the manufacturer should be specified along with the test result.

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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.

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Supplementary materials

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