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RESEARCH LETTER

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SARS-CoV-2 antibodies: Comparison of three high-throughput immunoassays versus the neutralization test

On 11 March 2020, the World Health Organization (WHO) declared the novel coronavirus (SARS-CoV-2) outbreak a global pandemic.[1] Currently, no specific therapy has proved to be effective against the infection. With the approval by WHO[2] of the first vaccine for emergency use against SARS-CoV-2 on 31 December 2020, it is important to understand the strength and duration of immunity after administration, which is induced by neutralizing anti-SARS-CoV-2 antibodies (nAbs).[3] The same antibodies are also present in individuals once the SARS-CoV-2 infection is resolved, at levels that depend on the duration and severity of clinical symptoms.[4] The efficacy of passive antibody therapy has been associated with the concentration of nAbs in the convalescent plasma (CP) of recovered patients.[5,6] Thus, it would be useful to immediately identify donors with high nAbs titres. The gold-standard test used to detect nAbs is the plaque reduction neutralization test (PRNT).[7] However, neutralization assays are time- and cost-consuming, as well as being limited in availability, since they require biosafety level 3 laboratories with qualified staff.[8] Several manufacturers have developed immunoassays compatible with global laboratory infrastructures for the COVID-19 emergency, enabling widespread testing of hundreds to thousands of samples per day. Recently, commercial SARS-CoV-2 antibody immunoassays have focused on the receptor-binding domain (RBD) of the spike protein (S),[9,10] which appears to be the main antigen responsible for eliciting neutralizing antibodies.[5,9,11,12] We aimed to investigate the correlation between nAbs titres identified by PRNT and the Maglumi 2019-nCoV IgG assay (Snibe, Shenzhen, China) with spike protein– and nucleocapsid (N)-based target, the Elecsys Anti-SARS-CoV-2 S assay (Roche, Basel, Switzerland) and the Maglumi SARS-CoV-2 S-RBD IgG assay, both of which target the spike protein RBD. Residual serum samples from 118 potential candidates for COVID-19 CP donation, with a confirmed negative PCR for COVID-19, were collected. The samples had been tested previously by PRNT, with the following distribution of titres: 1:20 in 3.4% of samples, 1:40 in 29.7%, 1:80 in 20.3%, 1:160 in 17.8% and 1:320 in 28.8%. The immunoassay signal values ranged from 0.45 to 2294.00 U/mL (median = 146.00 U/mL, IQR = 48.00-389.00 U/mL), from 2.10 to 791.70 AU/mL (median = 35.92 AU/mL, IQR = 21.82-92.52 AU/mL) and from 0.01 to 40.13 AU/mL (median = 4.07 AU/mL, IQR = 1.33-6.82 AU/mL) for the Elecsys Anti-SARS-CoV-2 S, Maglumi SARS-CoV-2 S-RBD IgG and Maglumi 2019-nCoV IgG assays, respectively. The overall difference in median serological test values among the titre groups was statistically significant. The reported data are shown in Figure 1. Using the predefined assay thresholds to establish whether the test results are positive or negative (0.8 U/mL for Elecsys Anti-SARS-CoV-2 S, 1 AU/mL for both Maglumi SARS-CoV-2 S-RBD IgG and Maglumi 2019-nCoV IgG assays), 21.37% of samples were below the threshold for the S/N IgG assay but were reactive for the RBD Ig assays. The simple linear regression analysis performed on the transformed data showed that there is a stronger positive association between serological test values and the PRNT for the Roche assay ($R^2 = 0.566$, $P < .0001$) than for the Maglumi RBD IgG ($R^2 = 0.372$, $P < .0001$) and the Maglumi S/N IgG assays ($R^2 = 0.149$, $P < .0001$). Moreover, the Spearman test confirmed a strong positive linear relationship between RBD Ig antibodies and neutralization titres: rs = 0.767; $P < .0001$ for the Roche total RBD Ig assay; and rs = 0.643; $P < .0001$ for the Maglumi RBD IgG assay, whereas the magnitude of the correlation between the assay targeting S-binding IgG antibodies (Maglumi S/N IgG) and neutralization activity is weaker: rs = 0.395, $P < .0001$ (Figure 2). Owing to the difficulty finding CP donors with a neutralizing titre of at least 1:160 for human passive immunization studies, a titre of 1:80 was considered to be acceptable if an alternative matched unit is not available. For this reason, we evaluated the performance of the immunoassays to detect nAbs titres ≥1:80 through receiver operating characteristic (ROC) curves (Figure 3): the area under the curve (AUC) for the Roche assay (AUC = 0.973) was greater than for the other two assays (AUC = 0.917 for Maglumi RBD IgG and AUC = 0.796 for Maglumi S/N IgG). We selected optimal cut-offs as predictors, using the maximum value of the Youden index, which maximizes the sum of sensitivity and specificity, as shown in Table 1. Compared with other studies, which show that anti-S IgG antibody titres correlate with the plaque reduction neutralization test,[13-15] we observed that the Roche total RBD Ig assay showed the best correlation

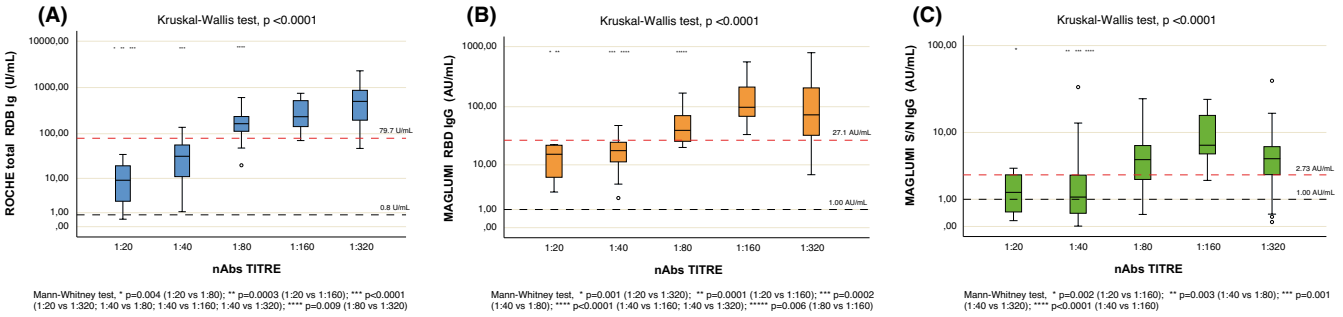

FIGURE 1 Distributions of CP donor sample immunoassay scores on a logarithmic scale within 5 nAbs titre groups, using Roche total RBD Ig Roche (A), Maglumi RBD IgG (B) or Maglumi S/N IgG (C) assays. The black dotted line represents the positivity manufacturer's threshold. The red dotted line represents the optimal cut-off to identify nAbs titre ≥1:80. nAbs, neutralizing antibodies; RBD, receptor-binding domain of spike protein; S, spike protein; N, nucleocapsid protein; CP, convalescent plasma

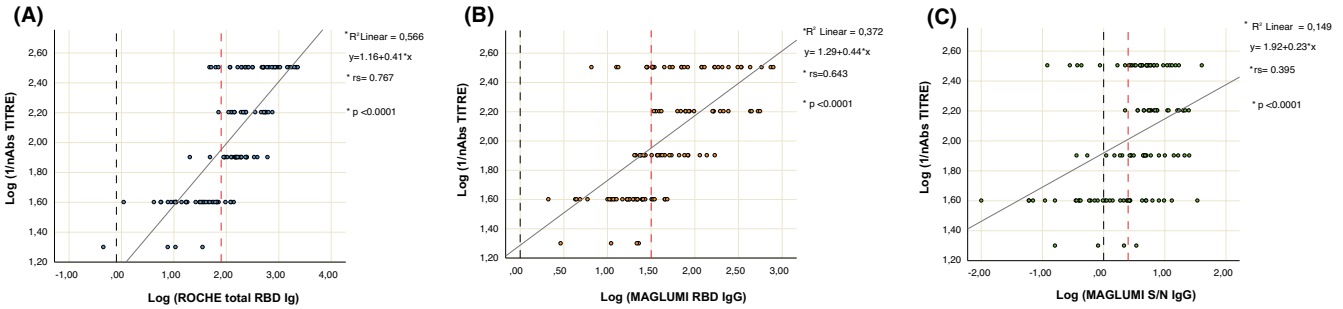

FIGURE 2 Linear regression of nAbs titres versus serological assay values and the Spearman correlation test for Roche total RBD Ig (A), Maglumi RBD IgG (B) or Maglumi S/N IgG (C) assays. The black dotted line represents the positivity manufacturer's threshold. The red dotted line represents the optimal cut-off to identify nAbs titre ≥1:80. R^2 = goodness of fit; rs = Spearman's correlation coefficient; nAbs, neutralizing antibodies; RBD, receptor-binding domain of spike protein; S, spike protein; N, nucleocapsid protein

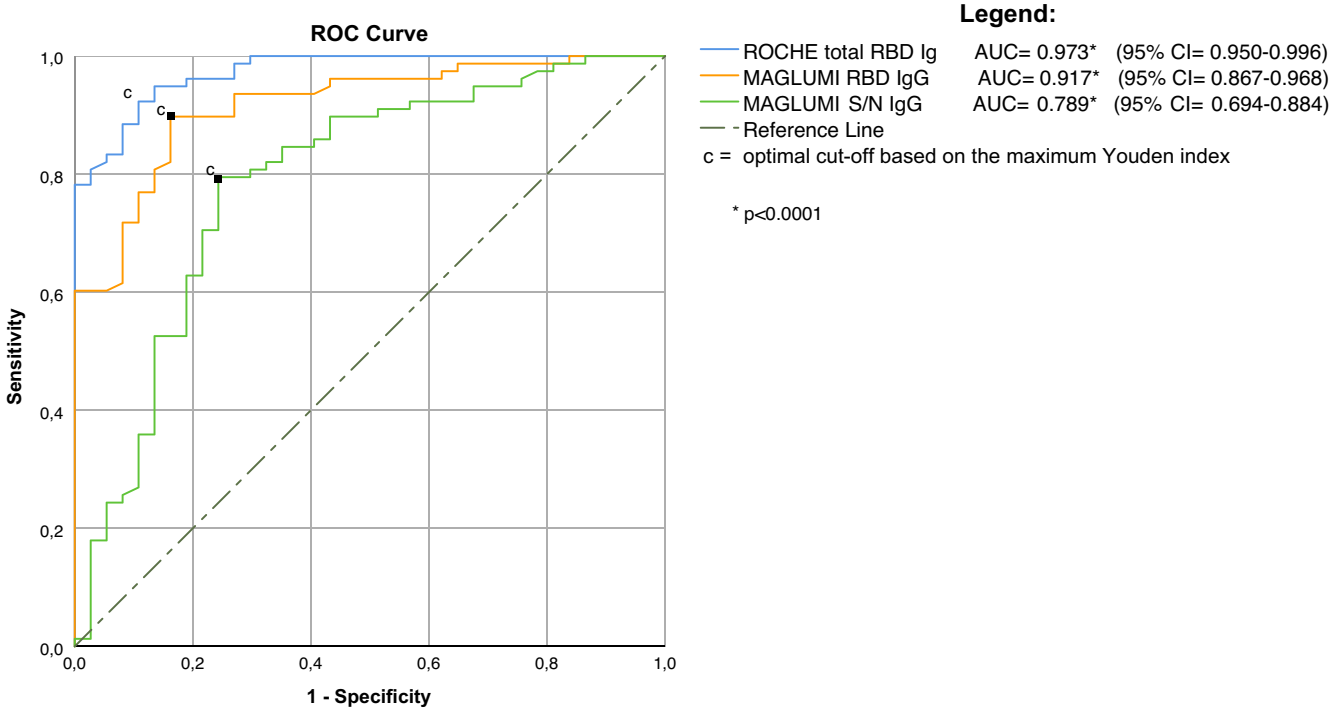

FIGURE 3 Receiver operating characteristic (ROC) curves for Roche total RBD Ig, Maglumi RBD IgG or Maglumi S/N IgG assays to detect nAbs titre ≥1:80. RBD, receptor-binding domain of spike protein; S, spike protein; N, nucleocapsid protein; nAbs, neutralizing antibodies; AUC, area under the curve; CI, confidence interval

TABLE 1 Sensitivity and specificity for the manufacturers and optimal cut-off for each immunoassay

	nAb TITRE ≥1:80					
	Manufacturer's threshold			Optimal cut-off[a]		
Immunoassay	Signal value	Sensitivity	Specificity	Signal value	Sensitivity	Specificity
ROCHE total RBD Ig	0.8 U/mL	1	0.270	79.70 U/mL	0.923	0.892
MAGLUMI RBD IgG	1 AU/mL	1	0	27.10 AU/mL	0.897	0.838
MAGLUMI S/N IgG	1 AU/mL	0.910	0.462	2.73 AU/mL	0.795	0.757

Abbreviations: N, nucleocapsid protein; nAbs, neutralizing antibodies; RBD, receptor-binding domain of spike protein; S, spike protein.

[a]Based on the maximum Youden index.

and performance for identifying nAbs titres ≥1:80, which is consistent with the fact that the RBD antigen is a key target for these antibodies.[5,9,11,12] In conclusion, we characterized the performance of three commercially available anti-SARS-CoV-2 Ig immunoassays. In particular, anti-RBD Ig assays could serve as useful surrogates for nAbs testing, as they are suitable for identifying highly neutralizing plasma samples with low biosafety requirements compared with other methods. These assays are also promising public health tools for monitoring seroconversion in the post-vaccinated population, since they can perform fast and high-throughput tests while keeping up the vaccination rate.

CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding this submission.

AUTHOR CONTRIBUTIONS

AO and FDS designed the study. RL, AS and RC wrote the manuscript and interpreted data. RB recruited the patients and collected the samples. RG, RC, RL and AS performed the assays to detect anti-SARS-CoV-2 antibodies. RL analysed data. AS and RB performed the literature search. ARM performed a linguistic review of the manuscript. All authors read and approved the final manuscript.

KEYWORDS

COVID-19, immunoassay, neutralizing antibodies, PRNT, RBD, SARS-CoV-2

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REFERENCES

1. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020. Accessed February 10, 2021
2. WHO issues its first emergency use validation for a COVID-19 vaccine and emphasizes need for equitable global access. https://www.who.int/news/item/31-12-2020-who-issues-its-first-emergency-use-validation-for-a-covid-19-vaccine-and-emphasizes-need-for-equitable-global-access. Accessed February 10, 2021.
3. Widge AT, Rouphael NG, Jackson LA, et al. Durability of responses after SARS-CoV-2 mRNA-1273 vaccination. *N Engl J Med*. 2021;384(1):80-82. https://doi.org/10.1056/nejmc2032195
4. Bošnjak B, Stein SC, Willenzon S, et al. Low serum neutralizing anti-SARS-CoV-2 S antibody levels in mildly affected COVID-19 convalescent patients revealed by two different detection methods. *Cell Mol Immunol*. Published online. 2020;18(4):936-944. https://doi.org/10.1038/s41423-020-00573-9
5. Yi C, Sun X, Ye J, et al. Key residues of the receptor binding motif in the spike protein of SARS-CoV-2 that interact with ACE2 and neutralizing antibodies. *Cell Mol Immunol*. 2020;17:621-630. https://doi.org/10.1038/s41423-020-0458-z
6. Mendrone-Junior A, Dinardo CL, Ferreira SC, et al. Correlation between SARS-COV-2 antibody screening by immunoassay and

neutralizing antibody testing. *Transfusion. Published online*. 2021;61:1181-1190. https://doi.org/10.1111/trf.16268
7. Jääskeläinen AJ, Kuivanen S, Kekäläinen E, et al. Performance of six SARS-CoV-2 immunoassays in comparison with microneutralisation. *J Clin Virol*. 2020;129:104512. https://doi.org/10.1016/j.jcv.2020.104512
8. von Rhein C, Scholz T, Henss L, et al. Comparison of potency assays to assess SARS-CoV-2 neutralizing antibody capacity in COVID-19 convalescent plasma. *J Virol Methods*. 2021;288:114031. https://doi.org/10.1016/j.jviromet.2020.114031
9. Premkumar L, Segovia-Chumbez B, Jadi R, et al. The receptor-binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients. *Sci Immunol*. 2020;5(48):eabc8413. https://doi.org/10.1126/SCIIMMUNOL.ABC8413
10. Schaffner A, Risch L, Aeschbacher S, et al. Characterization of a pan-immunoglobulin assay quantifying antibodies directed against the receptor binding domain of the SARS-CoV-2 S1-subunit of the spike protein: a population-based study. *J Clin Med*. 2020;9(12):3989. https://doi.org/10.3390/jcm9123989
11. Luchsinger LL, Ransegnola BP, Jin DK, et al. Serological assays estimate highly variable SARS-CoV-2 neutralizing antibody activity in recovered COVID-19 patients. *J Clin Microbiol*. 2020;58(12). https://doi.org/10.1128/JCM.02005-20
12. Ju B, Zhang QI, Ge J, et al. Human neutralizing antibodies elicited by SARS-CoV-2 infection. *Nature*. 2020;584(7819):115-119. https://doi.org/10.1038/s41586-020-2380-z
13. Boonyaratanakornkit J, Morishima C, Selke S, et al. Clinical, laboratory, and temporal predictors of neutralizing antibodies against SARS-CoV-2 among COVID-19 convalescent plasma donor candidates. *J Clin Invest*. 2021;131(3). https://doi.org/10.1172/jci144930
14. Patel EU, Bloch EM, Clarke W, et al. Comparative performance of five commercially available serologic assays to detect antibodies to SARS-CoV-2 and identify individuals with high neutralizing titers. *J Clin Microbiol*. 2021;59(2). https://doi.org/10.1128/JCM.02257-20
15. Wendel S, Kutner JM, Machado R, et al. Screening for SARS-CoV-2 antibodies in convalescent plasma in Brazil: Preliminary lessons from a voluntary convalescent donor program. *Transfusion*. 2020;60(12):2938-2951. https://doi.org/10.1111/trf.16065

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