Letter to the Editor

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A rapid semi-quantitative test for determination of SARS-CoV-2 antibody levels

To the Editor,

Rapid and accurate measurement of SARS-CoV-2 neutralizing antibodies (nAbs) may aid in understanding the development of immunity against COVID-19 and, therefore, is an important tool in mitigating COVID-19 pandemic. The majority of current point-of-care (POCT) antibody tests developed for SARS-CoV-2 rely on lateral flow assays, but do not offer quantitative information on neutralizing antibodies (Abs) titers. To address this issue, we evaluated the diagnostic performance of a rapid SARS-CoV-2 nAb detection test called "iRapid SARS-CoV-2 Quant Neutralizing Abs" (DIESSE Diagnostica Senese S.p.A, Siena), a semi-quantitative membrane-based rapid immunoassay for the detection of IgG antibodies directed against the receptor binding domain (RBD) of SARS-CoV-2. Briefly, a recombinant SARS-CoV-2 RBD protein is conjugated with colloidal gold nanoparticles; the patient specimen is placed to the pad of the strip (S) and thereafter, two drops of buffer are added. The antibodies against SARS-CoV-2 RBD in the sample will bind to RBD protein coated to colloidal gold present in the conjugate paper. The mixture then migrates upward on the membrane

chromatographically by capillary action and reacts with the monoclonal anti-human IgG in the test line region. The results are shown as coloured lines, the colour intensity being proportional to antibodies concentration, measured in binding antibody units (BAU/mL), calculated with reference to the first international standard WHO 20/136 for anti-SARS-CoV-2. For practical purposes, Neutralizing" Ab titers are classified according to the following cutoffs: <300 BAU/mL (as negative), 450 BAU/mL, 600 BA (/mL), 800 BAU/mL, >1,000 BAU/mL (Figure 1).

The results have been compared with the "gold standard", that is the micro-neutralization assay (MN). This is currently considered the gold-standard method being the most specific and sensitive serological assay capable of evaluating and detecting functional neutralizing antibodies (nAbs). In this study, a live virus-based MN assay is presented for the quantification of SARS-CoV-2-specific nAbs in human serum samples by classical method of detection: a read-out by checking the percentage of cytopathic effect (CPE) in the cell monolayer. Briefly, serum samples were heat-inactivated for 30 min at 56 °C; two-fold serial dilutions, starting from 1:10, were then mixed with an equal volume of viral solution containing 100 TCID50 of SARS-CoV-2 virus. The serum-virus mixture was incubated 1 h at 37 °C in a humidified atmosphere with 5% CO_2. After incubation, 100 µL of each dilution mixture was added in duplicate to a cell plate containing a semi-confluent VERO E6 monolayer. The plates were then incubated for three days at 37 °C in a humidified atmosphere with 5% CO_2. After three days of incubation, the plates were analyzed with an inverted optical microscope. The highest serum dilution able to protect more than the 50% of cells from CPE was taken as the neutralization threshold. Using MN as a reference, 299 serum specimens collected (period 1–30 July, 2021) from 149 patients vaccinated for SARS-CoV2 without previous COVID-19 (negative for antibodies against SARS-CoV-2 nucleocapsid protein) and 150 subjects non-vaccinated for SARS-CoV2 with confirmed previous COVID-19 by antibodies against SARS-CoV-2 nucleocapsid protein have been investigated. In order to evaluate previously infected individuals with SARS-CoV-2, serial blood samples were tested for IgG antibodies

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IgG POSITIVE

Two coloured lines appear: one coloured line appears in the control line region (C) and another line is in the test line region (T).

The colour intensity is proportional to the antibodies concentration (BAU/ml) as follows:

>1000 BAU/mL

800 BAU/mL

600 BAU/mL

450 BAU/mL

<300 BAU/mL
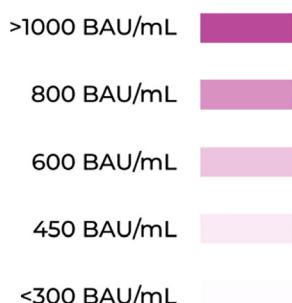
IgG NEGATIVE

Only one coloured line appears in the control line region (C). No line appears in the test line region (T).

INVALID TEST
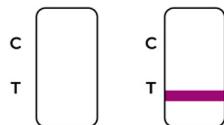
No coloured line appears at control line (C).

Figure 1: Interpretation of results.
If the specimen contains IgG antibodies against SARS-CoV-2 receptor binding domain at a concentration above limit of detection, a colored line will appear in the test line region. If there are no IgG antibodies against SARS-CoV-2 reception binding domain or to a lower concentration than limit of detection, only the control line will appear. The test will be validated only if the control line appears, otherwise the test is invalid and cannot be interpreted. The control line (C) is composed by anti-RBD antibodies able to bind the RBD protein.

against SARS-CoV-2 nucleocapsid protein using the Abbott ARCHITECT i-system (Abbott, Maidenhead, UK) immunoassay. Antibodies against viral proteins, including nucleocapsid and spike, are produced in response to SARS-CoV-2 infection [1] and probably correlate with immunity against reinfection [2]. There is also growing evidence that previously infected individuals develop greater antibody responses to SARS-CoV-2 vaccination than people who have not been infected [3–5].

Table 1 shows the diagnostic performances of the iRapid SARS-CoV-2 Quant Neutralizing Abs. Overall, the rapid assay showed a very satisfactory diagnostic

Table 1: iRapid diagnostic performances.

	MN +	MN−	Total
iRapid +	122	2	124
iRapid−	7	168	175
Total	129	170	299
SE (95% CI)	0.95 (0.89, 0.98)		
SP (95% CI)	0.99 (0.96, 1.00)		
PPV (95% CI)	0.98 (0.94, 1.00)		
NPV (95% CI)	0.96 (0.92, 0.98)		

SE, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value.

performance, with high specificity (>99%) and a very close qualitative and quantitative agreement between nAb values determined by Rapid SARS-CoV-2 Quant Neutralizing Ab and the MN assay gold standard (Weighted Kappa statistic 0.938; 95% confidence interval 0.899–0.978).

In summary, we have tested a new platform for SARS-CoV-2 antibody detection, that is faster than current POCT devices and offers a valuable semi-quantitative information. The simplicity and low cost of the assay could enable its widespread use and a range of applications, including testing in low- and middle-income settings, evaluation of the serostatus before vaccination, and post-vaccination surveillance. This test offers the opportunity to quickly assess nAbs levels as a correlate of protection, procrastinating the booster (and saving doses) which could be eventually carried out with vaccines updated on the variants of concern (VOC).

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