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Evaluation of a rapid semiquantitative lateral flow assay for the prediction of serum neutralizing activity against SARS-CoV-2 variants

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ABSTRACT

Background: Neutralizing antibodies (NAbs) against SARS-CoV-2 have been shown to correlate with protection against infection. Simple tools such as lateral flow assays (LFA) that can accurately measure NAbs may be useful for monitoring anti-SARS-CoV-2 immunity in the future.
Objectives: We assessed the performance of the ichroma™ COVID-19 nAb test, a rapid semiquantitative LFA, for the prediction of serum neutralizing activity against SARS-CoV-2 variants.
Study design: Serum samples were collected from COVID-19 recovered patients and vaccinated individuals. The result of the ichroma assay was provided as inhibition rate, and was compared to anti-SARS-CoV-2 IgG levels, and NAbs against Alpha, Delta and Omicron variants.
Results: A total of 90 sera from recovered unvaccinated patients and 209 sera from the vaccine cohort were included in this study. In post-infection samples, the ichroma inhbition rate was found to be correlated with IgG levels ($\rho = 0.83$), and with anti-Alpha NAbs levels ($\rho = 0.78$). In the vaccine cohort, a good correlation was also observed between the ichroma inhibition rate and IgG levels ($\rho = 0.84$), as well as NAbs against Alpha ($\rho = 0.62$), Delta ($\rho = 0.88$) and Omicron ($\rho = 0.74$). An ichroma inhbition rate of 77.2%, 90.8% and 99.6% accurately predicted neutralization against Alpha, Delta and Omicron variants respectively.
Conclusions: The ichroma™ COVID-19 nAb assay, with appropriate variant cut-offs, can be useful for the monitoring of anti-SARS-CoV-2 immunization and may provide a rapid prediction of protection, especially in individuals with significant levels of NAbs.

1. Background

Despite the worldwide effort to bring COVID-19 pandemic under control, it is unlikely that SARS-CoV-2 will be fully eradicated in the near future; currently, the main hope is to reach a circulation of the virus that is similar to other seasonal viruses, and compatible with a "pre-COVID normal life". In this scenario, the evaluation of post-infection or post-vaccination immunity will likely be part of the routine practice in the future, at least in populations with high risk of severe disease.

The assessment of humoral immunity through antibody testing can be easily performed. Anti-SARS-CoV-2 antibodies have been shown to be a protective correlate for COVID-19 vaccines [1]. In addition, the presence of antibodies at high levels has also been the scientific basis for the use of COVID-19 convalescent-phase plasma in clinical practice [2]. Therefore, several assays, from manual to fully automated methods, have been developed for the quantification of anti-SARS-CoV-2 antibody levels [3, 4]. However, beyond antibody levels, neutralizing activity is important to predict protection. Neutralizing antibodies (NAbs) are a subset of the total polyclonal response, and have been shown to strongly correlate with protection against infection [5].

The dominant target of these NAbs is the receptor binding domain (RBD) located at the tip of the S1 domain of the spike (S) protein, and that is used by SARS-CoV-2 to bind angiotensin converting enzyme 2 (ACE2) on host cells [6].

Several approaches are available for measuring NAbs. Plaque-reduction neutralization and SARS-CoV-2 microneutralization assays

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are considered the gold standard. However, working with live SARS-CoV-2 virus requires Biosafety Level 3 laboratories and highly trained staff, and the assay typically takes several days for results [7].

The main alternative to avoid the use of infectious SARS-CoV-2 virus is a pseudovirus neutralization assay that incorporates SARS-CoV-2 spike as the surface entry protein for a reporter virus, which can be performed in a BSL2/PC2 culture laboratory [8]. Other simpler and faster approaches based on ACE2-RBD interaction have been developed as enzyme-linked immunosorbent assay (ELISA), such as the surrogate virus neutralization test (sVNT) that requires no cell culture [9], or as rapid lateral flow assays (LFA) to detect anti-SARS-CoV-2 NAbs [10]. The LFA is cost-effective, easy to perform with results usually available in less than 30 min, and provides the opportunity of point-of-care diagnosis.

The sensitivity of different SARS-CoV-2 to neutralizing antibodies is highly variable [11–13], and the correlation between all alternative methods and infectious virus neutralization assays need to be evaluated against each specific variant.

In this study, we assessed the performance of the ichroma™ COVID-19 nAb test (Boditech, Gang-won-do, South Korea), a rapid semi-quantitative assay, for the prediction of serum neutralizing activity against SARS-CoV-2 variants.

2. Study design

2.1. Patients and samples

Serum samples were collected either after natural infection or after vaccination. The COVID-19 recovered and unvaccinated patients were previously infected by the D614G reference virus between March and December 2020. Vaccinated individuals were enrolled in the frame of the MONITOCOV study [14]. They were aged less than 60 years, and have received BNT162b2 vaccine (Pfizer-BioNTech) including a booster dose at month 9 for some of them. Serum samples were collected at different timepoints post-vaccination.

2.2. Quantification of Anti-SARS-CoV-2 IgG antibodies

The levels of Anti-SARS-CoV-2 spike S1/S2 domain-specific immunoglobulin G (IgG) were assessed in serum samples using the LIAISON® SARS-CoV-2 TrimericS IgG assay, on the LIAISON® XL platform (Diasorin S.p.A, Saluggia, Italy). Acording to the manufacturer, the assay quantification range was between 4.81 and 2080 BAU/mL (BAU: binding antibody units), and the positivity cut-off was set to 33.8 = BAU/mL

2.3. SARS-CoV-2 neutralization assay

Neutralizing antibodies were investigated using a live virus neutralization assay. B.1.1.7 (Alpha), B.1.617.2 (Delta) and B.1.1.529 (Omicron) lineages SARS-CoV-2 strains were previously isolated from clinical specimens and propagated in Vero E6 cells. The whole-genome sequences of the viral isolates, obtained with the COVIDSeq library preparation kit (Illumina®), were submitted to GISAID (accession reference EPI_ISL_1653931, EPI_ISL_2143633 and EPI_ISL_7696,645 for Alpha, Delta and Omicron variants respectively). In brief, serial 2-fold dilutions (starting from 1:10) of the heated serum (56 °C for 30 min) were incubated for 1 h at 37 °C with a viral solution containing 100 TCID50 of SARS-CoV-2 and then added to Vero E6 cell monolayers in a 96-well plate. The cytopathic effect was recorded after 3 days, and the serum virus neutralization titer (NT50) was defined as the reciprocal value of the highest dilution that showed at least 50% protection of cells. A sample with a titer $\geq$ 20 was defined as positive. Negative results (NT50 <20) were set to 10 for statistical analyses and graphics.

2.4. The ichroma™ COVID-19 nAb assay

The test uses a competitive immunodetection method. The anti-SARS-CoV-2 NAb in the sample binds to the fluorescence-labeled (FL) SARS-CoV-2 Spike RBD antigen in detection buffer, to form the complex as sample mixture. The complex is loaded to migrate onto a nitrocellulose matrix, where covalent RBD-ACE-2-biotin complexes are immobilized on the streptavidine capture (test line), and interfere with the binding of NAbs and FL antigen. High levels of NAbs interfere more with the binding of labeled RBD to ACE-2- biotin, which results in less antigen available, and thus decreased fluorescence signal.

In this study, the test was used according to the manufacturer's recommendations. Briefly, 50 μL of serum sample was preincubated at room temperature for 5 min with 200 μL of a buffer containing fluorescence-labeled SARS-CoV-2 Spike RBD antigen. Then, 150 μL of the sample mixture was transferred to a tube containing ACE-2- biotin conjugate. After thorough homogenization, 75 μL of the new sample mixture was loaded into the sample well on the cartridge. After 15 min of incubation at room temperature, the cartridge was inserted in the ichroma™II, a fluorescence and Europium nanoparticle scanning instrument (Boditech, Gang-won-do, South Korea). The fluorescence inhibition rate was calculated by the ichroma™II instrument, and reported as percentage. A fluorescence inhibition above 30% was considered as positive by the provider.

2.5. Statistical analysis

GraphPad Prism version 5 (GraphPad, San Diego, CA, USA) and IBM SPSS Statistics 22 (IBM Corp., Armonk, NY, USA) softwares were used for statistical analyses. Comparisons between continuous data were performed using the Man–Whitney test. Spearman's rank correlation coefficient (ρ) was used to evaluate non-linear correlation between assays. Receiver operating characteristic (ROC) analysis was performed to determine the assay cut-offs. A two-sided p-value < 0.05 indicated statistical significance.

3. Results

3.1. Performance of the ichroma™ COVID-19 nAb assay in post-infection samples

The ichroma assay was performed on post-infection serum samples collected from 90 patients, 3 months after a RT-PCR-confirmed infection. The median inhibition rate was 94.75% (range between 37.60 and 100%). Anti-SARS-CoV-2 IgG levels and anti-Alpha neutralizing activity were also determined on these samples. The median anti-SARS-CoV-2 IgG levels was 640 BAU/mL (range between 12.80 and 2080 BAU/mL). Anti-Alpha NAbs were detected in most of samples (82 out of 90), with a median NT_{50} at 80 (range between 20 and 1280). In the individuals without detectable neutralizing activity (NT_{50}<20), IgG levels ranged between 12.8 and 84.4 BAU/mL, while the ichroma inhibition rate ranged between 37.6 and 80.3%.

The ichroma inhibition rate was found to be correlated with IgG levels ($\rho = 0.83$ [95% CI: 074 – 0.89], $p< 0.0001$), and with NAbs levels ($\rho = 0.78$ [95% CI: 0.68 – 0.85], $p< 0.0001$) as shown in Fig. 1a-b.

We then assumed that samples with NAbs levels at $NT_{50}\geq 40$ ($n = 69$) could be considered as having a significant neutralizing activity. The median IgG levels in samples with $NT_{50}\geq 40$ was 851 BAU/mL versus 89 BAU/mL in those with NT_{50} <40 ($p< 0.0001$). A similar trend was observed for the ichroma inhbition rate (97% versus 67%, $p<0.0001$).

Using the same $NT_{50}\geq 40$ cut-off, the ROC analysis showed that an ichroma inhbition rate at 86.5% predicted significant serum neutralizing activity against Alpha with a sensitivity of 95.7% and a specificity of 90.5% (see Fig. 1c). The area under curve (AUC) was 0.96 [95% CI: 0.92 – 1].

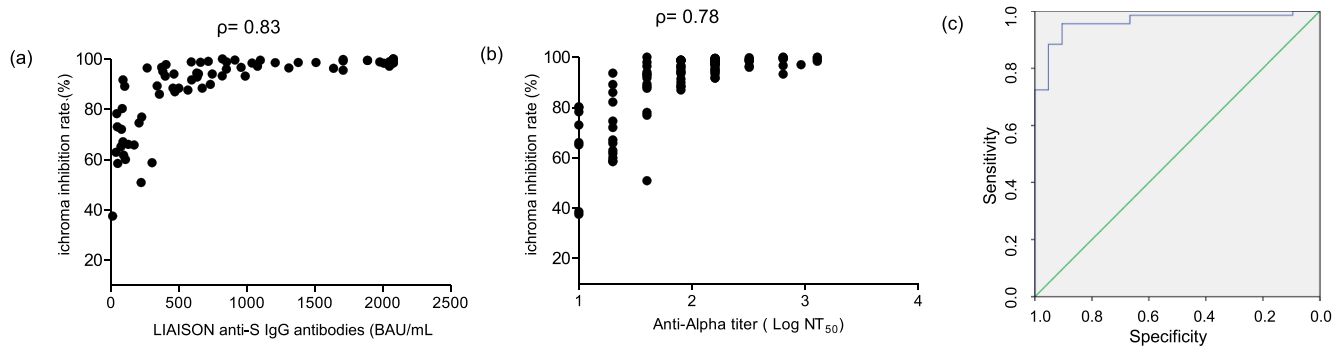

Fig. 1. Performance of the ichroma™ COVID-19 nAb assay in post-infection samples from unvaccinated individuals Sera from COVID-19 recovered patients were tested using ichroma assay, LIAISON assay and neutralization against SARS-CoV-2 Alpha variant. The correlations between ichroma result and IgG levels (a), and neutralizing antibodies levels (b) were represented. The Spearman's rank correlation coefficient (ρ) is shown. ROC analysis was performed to determine the cut-off of the ichroma inhibition rate that predict serum neutralizing activity (c).

3.2. Performance of the ichroma™ COVID-19 nAb assay in samples from vaccinees

The ichroma assay was run on a total of 209 serum samples from 110 patients, including 15 pre-vaccination samples, 99 samples at 3 months (M3) post first vaccine dose, 25 samples at M9 (before booster dose), 60 samples 1 month after booster dose, and 10 samples 3 months after booster dose. The median inhibition rate in each sample group is provided in Table 1.

The result of the ichroma assay was compared to that of other methods including IgG levels with the LIAISON®, and NAbs against the Alpha, Delta and Omicron SARS-CoV-2 variants.

Anti-SARS-CoV-2 IgG levels were determined in 191 samples. As shown in Fig. 2a, a good non-linear correlation was observed with the ichroma inhibition rate, with a Sperman ρ coefficient at 0.84 (95% CI: 0.79 – 0.87, $p< 0.0001$).

Viral neutralization assay against Alpha, Delta and Omicron variants was performed on 114, 179 and 95 serum samples respectively. The correlation between NAbs and the ichroma inhibition rate is shown on Fig. 2b-d. Overall, a moderate to strong non-linear correlation was observed between the ichroma test and the neutralization assays against Alpha, Delta and Omicron variants, with a Sperman ρ coefficient at 0.62 (95% CI: 0.49 – 0.73, $p< 0.0001$), 0.88 (95% CI: 0.85 – 0.91, $p< 0.0001$) and 0.74 (95% CI: 0.63 – 0.82, $p< 0.0001$) respectively.

Using the $NT_{50}\geq 40$ as cut-off for significant serum neutralizing activity, ROC analysis was performed to determine the optimal ichroma cut-off that could predict neutralization against each variant with the best performance (See Fig. 2e-g). An ichroma inhbition rate at 77.2% accurately predicted neutralization against Alpha variant with a sensitivity of 96.9% and specificity of 93.7%. The AUC was 0.98 [95% CI: 0.94 – 1]. Neutralization against Delta variant could be predicted by an ichroma cut-off at 90.8%, with a sensitivity of 93.3%, a specificity of 90.9%, and an AUC at 0.98 [95% CI: 0.96 – 1]. A higher inhibition rate, at 99.6% was needed to predict neutralization against Omicron variant with a sensitivity of 91.7%, a specificity of 91.5%, and an AUC at 0.97 [95% CI: 0.95 – 1].

Table 1
Results of iChroma assay in serum samples from vaccinated individuals.

Sample group	Median iChroma inhibition rate (range)
Pre-vaccination ($n = 15$)	10.0% (10.0 – 10.0)
M3 post vaccination ($n = 99$)	96.7% (71.2 – 99.9)
M9 post vaccination ($n = 25$)	73.8% (42.9 – 99.7)
M1 post booster dose ($n = 60$)	100.0% (88.8 – 100.0)
M3 post booster dose ($n = 10$)	99.9% (96.4 – 100.0)

4. Discussion

At the current stage of the pandemic, a significant proportion of the population, at least in western countries, have already developed a total or partial immune response against SARS-CoV-2, through vaccination and/or natural infection, even if questions remain on how long the immunity will last or whether individuals will be protected against new variants. Simple tools that are able to give information on the presence of NAbs, which predicts protection, could be useful in routine. In addition, it is known that NAb titers following immunization wane over time, which means that regular testing may be warranted.

In this study, we report an independent evaluation of the commercially available ichroma™ COVID-19 nAb test, which was developed for the detection of neutralizing antibodies. The design of this assay is attractive because it uses a reader and provides a semiquantitative measure of NAbs, while most of available lateral flow assays provide a qualitative ('yes/no') result.

We found a good correlation with IgG levels in serum samples from both COVID-19 recovered and vaccinated individuals. More interestingly the correlation with live virus neutralization assay using three different variants of concerns (Alpha, Delta and Omicron) was investigated. A moderate to strong non-linear relationship was observed between NAb levels determined by neutralization assay and the inhibition rate found by the ichroma™ COVID-19 nAb test. As expected, we found that higher cut-offs were needed to predict a neutralization against Delta and Omicron variants, as compared to the Alpha variant. Overall, an inhibition rate at 100% with the ichroma™ COVID-19 nAb test can accurately predict a neutralization against all tested variants.

Cell culture based assays are considered as gold standard for the investigation of NAbs; however, beyond the difficulty of routine implementation, their performance can also depend on several factors, such as the maturation state or viral titer, the cell type, and assay conditions used [10]. Tests based on ACE2-RBD interaction represent an acceptable alternative to these cell culture-based assays.

Several LFA reporting qualitative detection of NAbs have been developed [10, 15, 16]. Lake et al. described a LFA with a report of quantitative result in line density values, and observed a good agreement on 38 samples using a recombinant SARS-CoV-2 micro-neutralization assay [17]. Fulford et al. report a LFA prototype with a visual semi-quantitative readout of NAb levels, assessed against a pre-determined reference level. Using a pseudovirus micro-neutralization of 1:40 as reference, they found that an inhibition in the RBD-ACE2 test higher than 50% yielded a very good prediction [18]. More recently, Duan et al. developed a dual-detection fluorescent immunochromatographic assay (DFIA), with a built-in self-calibration process, that enables rapid quantitative detection of NAbs, and observed a good agreement with a commercial ELISA kit [19].

The correlation between protection against SARS-CoV-2 infection

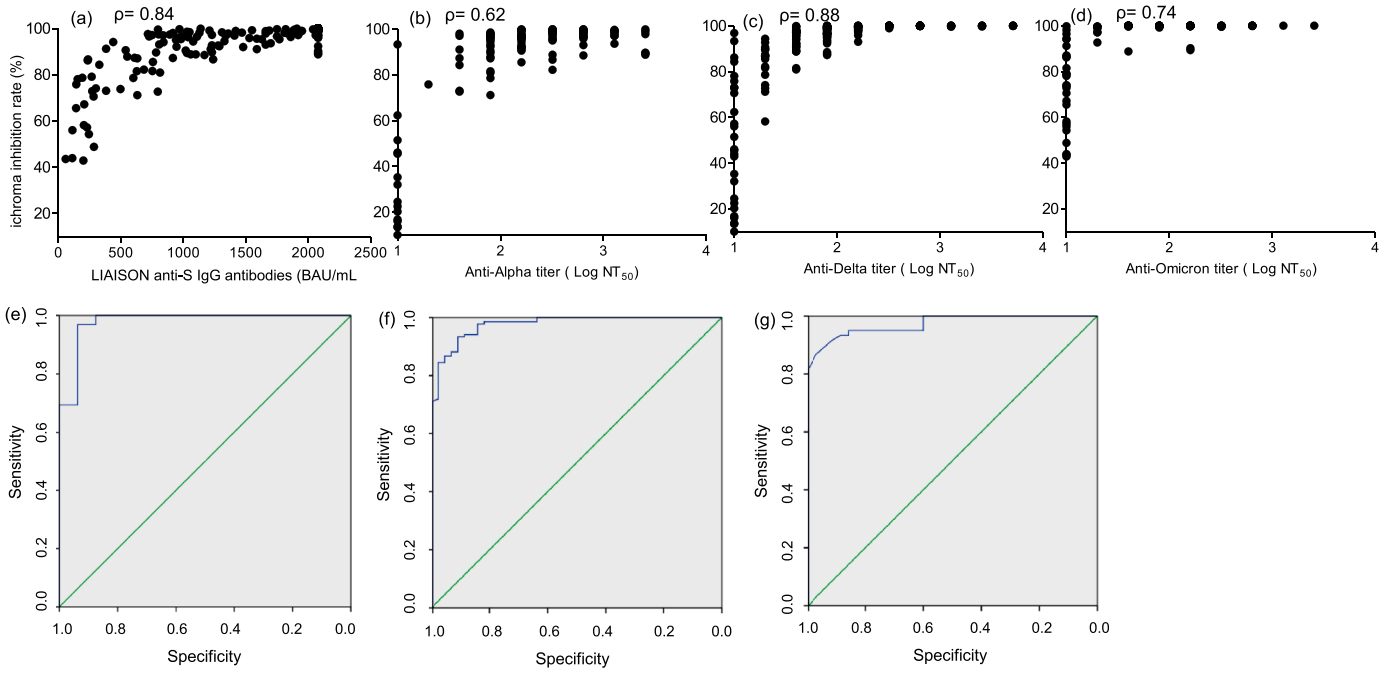

Fig. 2. Performance of the ichroma™ COVID-19 nAb assay in vaccinated individuals Sera from COVID-19 vaccinated individuals were tested using ichroma assay, LIAISON assay and neutralization against SARS-CoV-2 Alpha, Delta and Omicron variants. The correlations between ichroma result and IgG levels (a), and neutralizing antibodies levels (b-d) were represented. The Spearman's rank correlation coefficient (ρ) is shown. ROC analysis was performed to determine the cut-off of the ichroma inhibition rate that predict neutralization against each variant (e-g).

and NAb level quantified by an acceptable standard method needs to be established through large-scale clinical trials. A study in macaques involving transfer of SARS-CoV-2 NAbs from immune to naïve animals suggested that NAb titers of approximately 50 (based on pseudovirus assay) are protective against infection [20]. In the present study we set the cut-off of NAb level achieving protection to 40, which means technically 2 dilutions from a negative result. In addition this limit represents a reasonable lower limit for protection against SARS-CoV-2 [5].

The strengths of our study include the significant number of serum samples evaluated and the investigation of neutralization against three different SARS-CoV-2 variants of concern (Alpha, Delta and Omicron).

As a notable limitation, this assay has been only evaluated on serum samples as laboratory-based test and not as a point-of-care test, which could represent an interesting use. In addition, even if the evaluation of humoral immune response through NAbs is valuable, the absence or the decline of NAbs levels does not necessarily mean that individuals are fully susceptible to the infection because memory B and T cells could provide an optimal response upon exposure.

In conclusion, the ichroma™ COVID-19 nAb test, with appropriate variant cut-offs as determined in this study, can be useful for the monitoring of anti-SARS-CoV-2 immunization and can provide a rapid prediction of protection, especially in individuals with significant levels of NAbs. However, a protection against the virus cannot be ruled out in individuals with a result below the cut-off determined for each variant.

Ethics

This study was performed in accordance with the Declaration of Helsinki principles for ethical research. The MONITOCOV study was approved by the Ile-De-France V (ID-CRB 2021-A00119–32) ethics committee.

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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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