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Evaluation of the performance of the quotient MosaiQ™ COVID-19 professional use microarray assay for the detection of antibodies to SARS CoV-2 in a clinical setting

José Vicario[a], Raquel Alenda-Asensi[a], Irene Lucea-Gallego[b], Javier Sánchez-Bonilla[c], Rebeca Saez[d], Reginald Clayton[e], David Robson[e], Janine Robb[f,*], Luisa Barea-Garcia[g]

[a] Immunology, Centro de Transfusión de Madrid, Av de la Democracia s/n, 28032, Madrid, Spain
[b] Blood donor laboratory, Centro de Transfusión de Madrid, Av de la Democracia s/n, 28032, Madrid, Spain
[c] Commercial and Strategy, Quotient, Business Park Terre Bonne, Route de Crassier 13, 1262, Eysins, Switzerland
[d] Commercial and Strategy, Quotient, Business Park Terre Bonne, Route de Crassier 13, 1262, Eysins, Switzerland
[e] Research and Development, Quotient, Allan-Robb Campus, 5 James Hamilton Way, Milton Bridge, Penicuik, EH26 0BF, United Kingdom
[f] Commercial and Strategy, Quotient, Allan-Robb Campus, 5 James Hamilton Way, Milton Bridge, Penicuik, EH26 0BF, United Kingdom
[g] Management, Centro de Transfusión de Madrid, Av de la Democracia s/n, 28032, Madrid, Spain

Introduction

As the SARS-CoV-2 pandemic progresses and public and patient health providers seek advice and guidance to inform health policy, the value of serological testing in establishing infection rates and correlates of immune protection will become elucidated. The detection of antibody responses may enable post- diagnosis management, the identification of asymptomatically infected individuals, and assessment of pandemic spread on a population or cohort basis. Serological testing may also be useful in the formulation of lock-down or exit strategies, safe return to work, assessing previous infection rates, confirmation of infection, use of convalescent (passive antibody therapy) plasma and assessment of vaccination programmes.

Fig. 1.

Antibody testing can provide valuable information to guide health-care providers and inform the management of patients. In an ELISA based method, SARS-CoV-2-specific antibodies were detectable in approximately 40% of COVID-19 patients at seven days after the onset of symptoms, and seroconversion rates exceed >90% at the comparatively early time of day 14 [1]. Recent studies demonstrated antibody testing to be more sensitive than nucleic acid detection by PCR after approximately eight days of COVID-19 illness duration [2]. In combination, both PCR and antibody tests will enable for accurate diagnosis [3] at and around the time of primary infection, with antibody detection more pertinent for the later stages of the disease where viral replication or persistence is reduced, or the virus has been cleared [4]. Furthermore, antibody testing enables the identification of individuals demonstrating a detectable adaptive immune response subsequent to infection that may prevent re-infection or reduce the severity of a re-infection [5], and also enable an assessment of epidemiological exposure and developing herd immunity in the population.

This field utility study, performed in an active hospital setting, was conceived and conducted to demonstrate the operational performance of the assay (sensitivity and specificity): the MosaiQ™ COVID-19 Antibody Microarray for testing of human serum, plasma or blood samples for the presence or absence of antibodies to SARS-CoV-2. We also report the assay sensitivity and specificity in specific cohorts over time, where samples were obtained post PCR diagnosis and time to clearance of the virus were considered.

Materials and methods

The study protocol was reviewed and approved by appropriate senior management at the test facility before commencement of the study.

Sample selection

In order to assess sensitivity, 100 samples were obtained from convalescent patients for which infection with the SARS-CoV-2 virus had been proven by PCR real time detection kits from Thermo Fisher Scientific, Roche and CerTest Biotec. This testing was conducted at health centres within Comunidad de Madrid, Spain. Samples were obtained from patients across the spectrum of disease; none of the patients had been hospitalised. Criteria for eligibility of sample inclusion was a PCR test (typically nasopharyngeal swab) positive result indicating infection. Samples where no PCR data was available were excluded.

Ethical approval: Written informed consent was obtained from all participants for apheresis procedures. COVID-19 convalescent plasma banking had been approved by the ethical committee of the Hospital Ramon y Cajal in Madrid, Spain.

* Corresponding author.
E-mail addresses: jlvicario.trans@salud.madrid.org (J. Vicario), raquel.alenda@salud.madrid.org (R. Alenda-Asensi), irene.lucea@salud.madrid.org (I. Lucea-Gallego), Javier.SanchezBonilla@quotientbd.com (J. Sánchez-Bonilla), Rebeca.Saez@quotientbd.com (R. Saez), david.robson@quotientbd.com (D. Robson), janine.robb@quotientbd.com (J. Robb), luisamaria.barea@salud.madrid.org (L. Barea-Garcia).

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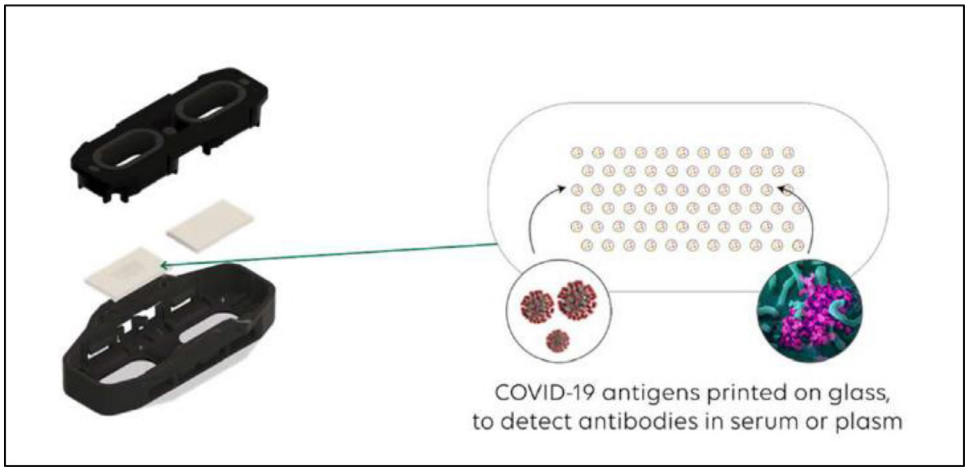

Fig. 1. MosaiQ COVID-19 micro-array.

To test the specificity of the assay, 404 EDTA plasma samples from healthy blood donors, presumed negative for SARS-CoV-2, were tested. These samples were collected and cryopreserved in 2015, and therefore the negative criterion was they were from a population cohort prior to the pandemic and consequently negative for antibodies to SARS-CoV-2.

All samples were brought to room temperature at least one hour prior to testing. Plasma and serum samples were gently mixed and centrifuged prior to testing.

Test procedures

MosaiQ Microarray Method

Testing was performed over three days. All samples were tested on the Quotient MosaiQ system as per instructions from the manufacturer. All positive sample testing was completed in one day, with all other samples tested over a further two days; sample loading was dependent on operator availability and not a limitation of the system. The time to first result on the system was 35 min, with each subsequent result being generated every 24 s, processing 125 samples per hour.

The MosaiQ Instrument was installed and validated according to manufacturers' instructions, utilising the MosaiQ COVID-19 Antibody Microarray Magazines, each of which contained 250 microarrays. The MosaiQ COVID-19 Antibody Microarray was used in combination with the MosaiQ System for the automated determination of the presence of antibodies directed to SARS-CoV-2 antigens in plasma, serum or whole blood. The MosaiQ COVID-19 Antibody Microarray enables the detection of antibodies directed to the spike protein specific to SARS-CoV-2. The proteins (antigen) are immobilised during a printing process onto single use microarrays. The assay combines antigen-antibody interactions with automated image capture and subsequent image analysis, providing a result interpretation of the reaction. Each microarray houses two panels: one comprising COVID-19 antigens and controls for the qualitative determination of human antibodies against SARS-CoV-2 and an empty panel with no printed probes. The proprietary quick-loading magazine enables high throughput screening of samples within a clinical laboratory setting.

Sample and diluent are dispensed together by the MosaiQ instrument onto the COVID-19 Antibody Microarray. An incubation step enables antibodies (if present) to bind to the printed antigens on the glass and is followed by removal of unbound antibodies using a wash buffer. This is then followed by addition of a detection reagent containing a gold-conjugated secondary antibody formulation that binds to human IgG and IgM. Excess detection reagent is removed by washing and an additional wash precedes the final stages of the assay. Finally, the addition of enhancement reagent provides a soluble silver solution, which nucleates on the gold nanoparticles and increases the diameter of the gold nanoparticle. Subsequent washing with purified water reveals a readily detectable reaction by reflection of incident light during image acquisition and analysis by the instrument.

The instrument optical system imaged and analysed each microarray and delivered a positive result (reactive) or negative result (non-reactive) accordingly.

Immunochromatography method

Qualitative detection of IgG/IgM antibodies against recombinant nucleocapsid SARS-CoV-2 was performed by colloidal gold immunochromatography (Genobio Pharmaceutical) according to the manufacturer's instructions. In this assay, the result was determined by the presence or absence of a test line and a control line; the presence of two lines (test and control) indicated a positive result; presence of control line but no test line was negative. A faint but visible line at the test position was interpreted as undeterminate, whereas an absence of the control line was an invalid result.

ELISA method

SARS-CoV-2 spike protein antigen was coated onto a 96-well microplate through passive adsorption. The microplate was then blocked using a commercial blocking buffer containing BSA. Samples and positive and negative controls were then added to the microplate and incubated. Following a wash step to remove any unbound material, the microplate was incubated with a secondary antibody labelled with horse radish peroxidase (HRP). The secondary antibody bound to any antibody that has attached to the microplate well. Anti-IgG and Anti-IgM secondary antibodies were used separately. Following another wash step, tetramethylbenzidine (TMB) substrate was added. Any bound secondary antibody HRP reacts with the TMB to generate a signal. The microplate was read using a standard spectrophotometer.

Quality control samples

Positive and negative control samples were provided in kit form by Quotient and were used with the MosaiQ COVID-19 Antibody Microarray assay. Acceptable control results were achieved before proceeding with testing of samples.

Data analysis - sensitivity

Sensitivity was defined as the percentage of expected positive samples reactive on the test system, samples being from patients previously diagnosed with SARS-CoV-2 infections using nucleic acid detection (RT-PCR) of SARS-CoV-2.

Table 1
Discordant sample resolution.

Sample ID	MosaiQ COVID-19 Antibody Assay result	Immunochromatography IgM	IgG	ELISA determination ELISA IgM	ELISA IgG
X	False Positive	Not tested	Not tested	Negative	Negative
Y	False Negative	Weakly reactive/ indeterminate*	Negative	Negative	Negative
Z	False Negative	Negative	Weakly reactive/indeterminate*	Negative	Negative

* Result ambiguous; elicited results at or around the limit of detection of the assay and therefore classed as weakly reactive/indeterminate.

Data analysis - specificity

Specificity was defined as the percentage of all negative samples from 2015 (pre-SARS-CoV-2) identified as non-reactive for SARS-CoV-2.

Data analysis – Confidence Interval (CI)

Confidence interval was determined using an online tool. 95% was used as the desired level of confidence.

Results

Overall results

For the determination of assay specificity, an initial panel of 404 negative samples were tested. 401 samples returned an expected negative result, and the remaining three samples were investigated for potential discordance or other factors. Two samples in the ostensibly negative cohort were excluded from the study due to sample quality issues and are therefore not reported, reducing the expected negative cohort to 402 samples. The third reported sample was considered potentially discordant following a positive result in the MosaiQ COVID-19 Antibody Microarray assay; yet this sample was obtained from a negative donor (sample collected in 2015, pre-SARS-CoV-2). Upon testing in the ELISA assay, this sample elicited a negative result and therefore it was concluded that the true serological status of the sample is negative for both IgM and IgG antibodies to SARS-CoV-2. As the sample reported as positive in the MosaiQ COVID-19 Antibody Microarray, one false positive on the MosaiQ COVID-19 assay is reported in this study.

For the determination of assay sensitivity, 94 samples from a total of 100 expected positives returned a positive result. Four samples were removed from the dataset as full investigative resolution was not performed and therefore could not be included, with the remaining two samples investigated for potential discordance (Table 1). One of the potentially discordant samples (Table 1, Sample Y) reported ambiguously for IgM in the immunochromatography method. The specificity of the Anti-IgM assay is not formally comparable to the study system and therefore this sample was further investigated by the ELISA method. The absence of both Anti-IgG and Anti-IgM in this sample was established by ELISA, indicating that this sample, although obtained from a convalescent patient testing positive for SARS-CoV-2, was truly seronegative, and most likely a sample from a non-seroconverter in the patient cohort. Similarly, Sample Z reported ambiguously for IgG in the immunochromatography method, yet this was not detected in the ELISA method. Samples Y and Z were considered truly seronegative samples. Both samples were taken more than 14 days post PCR positive diagnosis indicating that, in a manner and proportion consistent with the literature [1], these samples were obtained from patients that developed no humoral response to SARS-CoV-2 infection.

Specificity (negative percent agreement)

401/402 expected negatives returned a negative result, with one false positive reported, resulting in an overall value for the assay specificity, or Negative Percent Agreement (NPA) of 99.8%.

Sensitivity (Positive Percent Agreement)

94/94 expected positives returned a positive result, giving an overall value for the assay sensitivity, or Positive Percent Agreement (PPA), of 100%. An initial panel of 100 expected positives were tested and 94/100 returned a positive result; four samples were removed as resolution investigation was not performed, and following investigation, the two remaining samples were found to be non-reactive, therefore confirming the correct result of seronegativity (non-reactive) reported by the test system. The sensitivity, or positive percent agreement, was consequently 100% in this study as 94/94 positive samples were reactive.

Sensitivity grouped by time between positive RT-PCR result and sample age

Samples were grouped according to the time between the positive RT-PCR result and sample collection (Table 2). MosaiQ COVID-19 Antibody Microarray found all samples reactive at 0–7 days, 8–15 days and more than 15 days after positive PCR result, therefore showing 100% sensitivity regardless of sample age since time of presenting a positive RT-PCR result. Included in the 0–7 days group were two samples taken from patients only four days after the RT-PCR positive result was obtained.

Sensitivity grouped by time between RT-PCR positive and RT-PCR negative result

Samples were evaluated according to the time between the positive RT-PCR result and the occurrence of a negative RT-PCR result (Table 3). MosaiQ COVID-19 Antibody Microarray demonstrated 100% sensitivity for all patient samples tested, regardless of probable duration of infection with SARS-CoV-2.

Discussion

The summary performance of 100% sensitivity (Positive Percent Agreement) and 99.8% specificity (Negative Percent Agreement) represents excellent performance for an *in vitro* diagnostic serological assay. Furthermore, in the context of required levels of sensitivity and specificity required for licensure of an *in vitro* diagnostic assay in the EU and the US, this level of performance is suitable [6].

There were two samples which returned initial unexpected non-reactive results, and following a detailed investigation, were shown to be seronegative; the samples were obtained from patients that did not undergo seroconversion. In the event of non-seroconversion, an antibody negative result would be expected and the test system therefore showed the correct result as no antibodies to SARS-CoV-2 are present. This interpretation is consistent with data from studies conducted in the Wuhan and Guangdong areas in the initial outbreak; there is now significant data to show that a small percentage of patients do not seroconvert for IgM and IgG, and therefore, this result would be consistent with that lower level of unusual immune responses in the cohort [1], and is at a comparable level to that seen in the Chinese studies [2]. Of course, the true serological status of a patient must be interpreted

Table 2
Assay sensitivity grouped by time from RT-PCR positive result to sample collection.

Time from positive RT-PCR result to sample collection	Samples collected / reported positive	Sensitivity	95% CI
0–7 days	5 / 5	100%	47.8 - 100%
8–15 days	10 / 10	100%	69.2 - 100%
>15 days	79 / 79	100%	95.4 – 100%

Table 3
Assay sensitivity grouped by time after clearance of active infection (indicated by initial RT-PCR positive followed by RT-PCR negative); sample collected on day of first negative RT-PCR result.

Days between RT-PCR positive to RT-PCR negative result	Samples collected / reported positive	Sensitivity
0–5 days	18 / 18	100%
6–10 days	29 / 29	100%
11–15 days	27 / 27	100%
16–20 days	11 / 11	100%
>21 days	9 / 9	100%

based on presence of antibodies, not previous evidence of infection by PCR assay, and the removal of non-seroconverted samples from the expected positive sample cohort is therefore justified. The heterogenous immunological response across patient cohorts should be considered in establishing a test panel, and here carefully selected samples were used where infection was supported by PCR diagnosis, but the indication of IgM and IgG status still requires discordance management exercises in cases where ostensibly negative or positive samples elicit discordant results. This highlights the rigour required in establishing performance of assays to detect serological status in patients with emerging viral infections.

This study has succeeded in showing early detection of IgG and IgM antibodies to SARS-CoV-2, utilising a high throughput system. At the time of the study, a relatively small sample set was available for testing, and future studies would include larger sample sets to develop understanding of assay performance. In addition, further studies would ideally include samples from patients with new variants emerging to confirm similar performance.

In summary, we demonstrate that the Quotient MosaiQ COVID-19 Antibody Microarray test is suitable for accurate testing of blood samples for IgM and IgG antibodies to SARS-CoV-2 in an active field setting, regardless of sample age since time of presenting a positive RT-PCR result. The system demonstrated suitable sensitivity and specificity to be considered fit for its intended purpose and useful in the assessment of immunity to this virus.

Declaration of Competing Interest

Dr J. Robb, Dr D. Robson, Ms R. Saez and Mr J. Sánchez Bonilla are employees and shareholders of Quotient.

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