Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Immunological Methods 517 (2023) 113472

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Comparative evaluation of Luminex based assays for detection of SARS-CoV-2 antibodies in a transplantation laboratory

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ARTICLE INFO

Keywords:
COVID-19
SARS-CoV-2
Antibody testing
Luminex
Serology

ABSTRACT

Background: Detection of SARS-CoV-2 antibodies is essential in establishing the parameters of an individual's immune response to COVID-19, from both natural infection and vaccination. Despite this, there is currently limited clinical guidance or recommendations for serological methods for their measurement. Here, we evaluate and compare four Luminex-based assays for the multiplex detection of IgG SARS-CoV-2 antibodies.
Methods: The four assays tested were Magnetic Luminex Assay, MULTICOV-AB Assay, Luminex xMAP SARS-CoV-2 Multi-Antigen IgG Assay and LABScreen COVID Plus Assay. Each assay's ability to detect antibodies to SARS-CoV-2 Spike (S), Nucleocapsid (N) and Spike-Receptor Binding Domain (RBD) was evaluated using 50 test samples (25 positive, 25 negative), previously tested by a widely used ELISA technique.
Results: The MULTICOV-AB Assay had the highest clinical performance detecting antibodies to S trimer and RBD in 100% ($n = 25$) of known positive samples. Both the Magnetic Luminex Assay and LABScreen COVID Plus Assay showed significant diagnostic accuracy with sensitivities of 90% and 88% respectively. The Luminex xMAP SARS-CoV-2 Multi-Antigen IgG Assay demonstrated limited detection of antibodies to the S antigen resulting in a sensitivity of 68%.
Conclusion: Luminex-based assays provide a suitable serological method for multiplex detection of SARS-CoV-2 specific antibodies, with each assay able to detect antibodies to a minimum of 3 different SARS-CoV-2 antigens. Assay comparison identified there is moderate performance variability between manufacturers and further inter-assay variation of antibodies detected to different SARS-CoV-2 antigens.

1. Introduction

Since the first cases of a pneumonia of unknown cause were reported in Wuhan, China in December 2019, the causative agent identified to be severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread across the globe (Campbell et al., 2021). The recognition of this new highly transmissible virus and its rapid spread across the world led the World Health Organization (WHO) to declare this as a global pandemic on 11th March 2020 (Shaw et al., 2020).

Infection with SARS-CoV-2 can cause the disease known as COVID-19 and has varying clinical manifestations in patients, ranging from mild symptoms to severe and rapidly progressing disease (Wu et al., 2020). From the beginning of the pandemic, the disease in its most severe form quickly proved to be fatal in a minority of cases. Despite a relatively low fatality rate, due to the exponential case numbers the disease has accounted for >6.9 million deaths worldwide as of May 31st, 2022 (Sachs et al., 2022).

Currently, molecular testing through real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) of nasopharyngeal swabs is used for clinical diagnostic testing of COVID-19 infection (Wang et al., 2020). Whilst this is sufficient for diagnosis in the majority of cases, it does not provide an insight into how an individual's body is responding to infection.

Serological analysis is a fundamental tool for the detection of antibodies generated in response to infection (Rai et al., 2021). It provides an effective testing method for identification of previous infection and

Abbreviations: SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; MFI, Mean Fluorescent Intensity; S, Spike Antigen; N, Nucleocapsid Antigen; RBD, Spike-Receptor Binding Domain Antigen; NMI, Natural and Medical Sciences Institute; LC, Luminex Corporation; OL, One Lambda.

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https://doi.org/10.1016/j.jim.2023.113472
Received 17 June 2022; Received in revised form 31 March 2023; Accepted 11 April 2023
Available online 12 April 2023

has a wide range of clinical applications (Winter and Hegde, 2020). Epidemiologically, the use of serological assays for SARS-CoV-2 antibody detection enables accurate estimates of infection prevalence and incidence, which are vital for outbreak control strategy planning (Whitman et al., 2020). Clinically, serological assays have an important role in COVID-19 diagnosis in patients whose symptoms are highly suggestive of infection but who are testing negative by molecular methods (Xiang et al., 2020). A further critical use of serological assays is evaluating immune response post vaccination (Tang et al., 2020), with an ideal vaccine stimulating the immune system to generate neutralizing antibodies to stop viral entry into host cells (Hofman et al., 2021). The ability to evaluate immune response to vaccination is of increased importance for patients who are immunocompromised, as studies on well-established vaccines show substantial variation in production of neutralizing antibodies and duration of vaccine induced immunity (Windpessl et al., 2021).

Despite the clear need for accurate and reliable serological assays in the COVID-19 pandemic response, there was at first a lack of information regarding their intended applications and their clinical utility remained largely uncharacterized (Kopel et al., 2021; Bohn et al., 2020). In April 2021, the WHO published international standards for SARS-CoV-2 antibody testing in order to attempt harmonization of serological testing (Baldanti et al., 2022). Although several studies have been conducted to compare the clinical performance of antibody detection assays, there are limited comparisons of assays performance characteristics against specified SARS-CoV-2 antigens. A comprehensive review conducted by a Danish study compared 16 different serological assays and reported the sensitivity and specificity of the assays (Harritshøj et al., 2021). However, this study did not directly compare the performance features of each assay for a given antigen.

This study aims to evaluate four new Luminex based serological assays for detection of SARS-CoV-2 antibodies against Spike (S), Spike-receptor binding domain (RBD) and Nucleocapsid (N) antigens, for use within a large National Health Service (NHS) transplantation laboratory. All four assays being evaluated use the Luminex platform to provide a multiplex assay for detection of IgG antibodies against different SARS-CoV-2 antigens in human blood. Two of the assays being evaluated are also able to detect the presence of antibodies to a range of endemic human coronavirus antigens (NHSBT, 2014; Tait, 2016). Luminex technology is widely used within transplantation laboratories for its high sensitivity and specificity in identification of HLA antibodies (Cravedi et al., 2020). Some studies have already reported on the use of Luminex technology in the detection of SARS-CoV-2 antibodies with consistent findings of high sensitivity and specificity (Becker et al., 2021; Turgeon et al., 2021; Dobaño et al., 2021).

This study will ascertain if translation of the Luminex transplantation testing technique provides a suitable method for completing virological antibody detection of SARS-CoV-2 antibodies. Thorough evaluation of the four assays will allow determination of suitability for implementation into clinical practice. These high sensitivity antibody assays have many potential clinical implications within solid organ transplantation laboratories. Kidney transplantation patients are known to be at increased risk of severe COVID-19 disease due to immunosuppression and the presence of comorbidities (Becker et al., 2020). As COVID antibody testing becomes a part of the pre-transplant workup at some institutions and the widespread availability of the Luminex system within transplantation laboratories, we felt it was important to compare the available Luminex based COVID antibody tests against one another. Completing SARS-CoV-2 antibody testing may provide insight into these high-risk patients COVID-19 immunity status (OneLambda, 2021). By assessing individual's COVID-19 antibody levels this may indicate both previous exposure status and provide approximation of their immune response to the virus or vaccination, which will be key information in patient risk assessments for treatment.

2. Materials and methods

This study used anonymised patient samples for test validation purposes only and thus did not require Research Ethics Committee (REC) ethical approval. This study was reviewed at the Barts Health NHS Group Clinical Services (GCS) research group meeting and was approved by the board chair of the research governance board.

2.1. Specimen collection

All samples were collected prior to December 2020 making it highly unlikely that these were from vaccinated patients as vaccines were only available within clinical trials at this point. Stored serum samples from 25 COVID-19 antibody positive patients and 25 COVID-19 antibody negative patients selected at random were provided by the Virology department. All samples were anonymised by the Virology department and given subsequent identifying numbers (1–50), these samples were used to complete all validation testing and results of this study were not used in patient management. Samples were stored at -40 °C prior to testing.

For the purpose of this study sample selection was based upon COVID-19 antibody status previously determined by the Roche Elecsys Anti-SARS-CoV-2 immunoassay by the Virology department. This is a qualitative assay that detects IgG/IgM antibodies against a recombinant protein representing the SARS-CoV-2 nucleocapsid antigen (N). Results of this test reported by virology were considered either antibody negative if the cut-off index (COI) <1.0 or antibody positive if the COI ≥ 1.0.

2.2. Luminex analysis

The principle of all testing assays evaluated in this study were based upon Luminex xMAP technology and each assay was evaluated using a Luminex 200 analyser. Luminex xMAP technology is a bead-based immunoassay based on flow cell fluorometry that provides antibody detection via micro-sphere beads that have known antigens conjugated to their surface. Equipment and reagents used are detailed in Supplementary Material A.5.

2.3. NMI 1: Magnetic Luminex assay

The Magnetic Luminex Assay for detection of antibody responses to SARS-CoV-2 and seasonal CoV antigens (Natural and Medical Sciences Institute [NMI], Germany) tests for IgG and IgA antibodies in human serum against SARS-CoV-2 and seasonal coronavirus antigens (Supplementary Material A table 1). This includes SARS-CoV-2 antigens: S, RBD, S1, S2, N and N-NTD. The assay is a Luminex multiplex assay using Luminex MagPlex magnetic beads. The assay was performed according to the manufacturer's instructions (Supplementary Material A.1). The 50 samples were tested in a 96 well flat-bottomed plate and control samples of: 2 x IgG cut-off samples, 2 x IgA cut-off samples, 1 x in-house negative control (AB29 serum) and 3 x NMI QC samples were included. Each test plate was read using the Luminex 200 analyser with settings specified in the protocol.

Samples were considered SARS-CoV-2 IgG antibody positive when their net mean fluorescence intensity (MFI) values for SARS2 S bead and SARS2 RBD bead exceeded the net MFI (NMFI) values of both the respective beads from the respective cut-off samples. The data evaluation information provided by the manufacturer's did not contain specification of how other test antigens results should be interpreted and thus were not analysed in this study. Each run was considered acceptable if the in-house negative control provided a negative result for both the SARS2 S bead and SARS2 RBD bead.

2.4. NMI 2: MULTICOV-AB assay

The MULTICOV-AB Magnetic Luminex Assay (Kit: 2021–0170) for

detection of antibody responses to SARS-CoV-2 antigens (NMI, Germany) tests for IgG antibodies in human serum against SARS-CoV-2 antigens: S Trimer, RBD, S1, S2 and N (Supplementary Material A table 2). The assay is a Luminex multiplex assay using MagPlex magnetic beads. The assay was completed as per manufacturer's protocol (Supplementary Material A.2). The 50 test samples were tested in a 96-well plate and each test plate contained the following controls in duplicate: blank (assay buffer), QC1, QC2 and QC3.

Individual sample results were considered acceptable when each well bead count >35, NMFI for control bead 1 (hu-IgG bead) >15,000, and NMFI for control bead 2 (a-hu-IgG bead) > cut-off. The cut off for control bead 2 was determined using the equation: mean (mean [QC1–3] and mean [blank]). Samples were considered SARS-CoV-2 IgG antibody positive when their NMFI values for both SARS-CoV-2 Spike Trimer bead and SARS-CoV-2 RBD bead where ≥ cut-off value for each bead, calculated as the mean MFI of QC2 for both antigens separately. Only Spike Trimer, RBD and N beads results were analysed in this study. Each run was considered acceptable if: QC sample bead count >35 in a minimum 6/8 QCs and QC sample NMFI for hu-IgG bead >15,000.

2.5. Luminex corporation (LC): xMAP SARS-CoV-2 multi-antigen IgG assay

The xMAP SARS-CoV-2 Multi-Antigen IgG Assay (Luminex, Texas; United States) Research Use Only (RUO) kit version 1.00 was used. This assay tests for IgG antibodies in human serum against the SARS-CoV-2 antigens: N, RBD and S1 (Supplementary Material A table 3). The assay is a Luminex multiplex assay using Luminex MagPlex beads. The assay was completed following the manufacturer's instructions for use package insert (Supplementary Material A.3). The 50 samples were tested in a 96 well round-bottomed plate and positive and negative control samples provided in the test kit were included. The plate was then read using the Luminex 200 analyser with a provided xPONENT software protocol.

Individual sample results were considered acceptable when: IgG control bead determined as 'present' (MFI > 2500 call threshold), IgA and IgM control beads determined as 'absent' (MFI < call threshold determined by software) and background bead determined as 'passed' (MFI < 700 call threshold). Samples were considered SARS-CoV-2 IgG antibody positive when their MFI values for both Nucleocapsid bead and RBD bead were > 700 call threshold for each bead. Each run was considered acceptable when both negative and positive control samples met individual sample requirements as above and provided negative and positive SARS-CoV-2 IgG results respectively.

2.6. One Lambda (OL): LABScreen COVID Plus Assay

The LABScreen COVID Plus assay (One Lambda, California; United States) Lot 001, tests for IgG antibodies in human serum against SARS-CoV-2 and human coronavirus antigens (Supplementary Material A table 4). This includes SARS-CoV-2 antigens: S, S1, RBD, S2 and N. The assay is a Luminex multiplex assay using Luminex microsphere beads. The assay was completed following the manufacturer's instructions provided in the product insert (Supplementary Material A.4). The One Lambda (OL) test kit provided contained enough reagents to complete 20 tests, due to control requirements 18 test samples were selected of 10 positives and 8 negatives. The 10 positive samples were selected based upon the sample results generated during other assay evaluation. Testing was completed using 96 well round-bottomed plates, each containing 9 samples and a negative control sample provided by the manufacturer. Test plates were read using the Luminex 200 analyser with a provided xPONENT software protocol.

Individual sample results were considered acceptable when: bead count >50, the trimmed MFI (TMFI) for the negative control bead <1500 and ≤ half of the positive control bead TMFI, and the positive control bead TMFI >500 and ≥ 2× negative control bead TMFI. Samples were considered SARS-CoV-2 IgG antibody positive when any of the SARS-CoV-2 beads demonstrate a baseline value higher than the established cut-off in the lot specific worksheet. Where the baseline value for specific bead = (sample-specific TMFI value for bead – sample-specific TMFI for negative control bead) – (background NC serum TMFI for bead – background NC serum TMFI for negative control bead). Each run was considered acceptable if the negative control sample bead count >50 and the TMFI for each bead < specified cut-off values provided in the negative control datasheet.

2.7. Statistical analysis

Basic results interpretation was completed in Microsoft Excel for the OL assay and both NMI assays. The results of the LC assay were interpreted using xMAP SARS-CoV-2 Multi-Antigen IgG Assay Software provided by LC. Statistical analysis was undertaken using IBM SPSS. Sensitivity and specificity calculations were performed using Crosstabs descriptive statistics for each test assay to allow evaluation of diagnostic accuracy and comparisons between assay performance. Receiver Operator Characteristic (ROC) Curve analysis was performed on the NMI 2 assay's semi-quantitative results.

3. Results

3.1. Overall antibody detection

Comparison of the four xMAP Luminex assays identified they are specific for detection of SARS-CoV-2 IgG antibodies. Each assay evaluated provided a functional testing method with test run results meeting minimum acceptance criteria for interpretation. All assays detected the presence of SARS-CoV-2 IgG antibodies with a minimum of 68% sensitivity (Fig. 1).

All four assays identified true negative samples with 100% specificity (Fig. 1). The NMI 2 assay had the highest sensitivity, detecting SARS-CoV-2 antibodies in 100% of samples ($n = 25$). The LC assay had the lowest sensitivity, detecting SARS-CoV-2 antibodies in 68% of samples ($n = 17$). The NMI 1 assay and OL assays demonstrated similar levels of sensitivity of 88% and 90% respectively, with NMI 1 detecting antibodies in 22/25 samples and OL in 9/10 samples.

Each assay contained beads coated with different SARS-CoV-2 antigens to enable antibody detection. Additionally, each assay required different combinations of beads to reach a threshold level to deem a sample antibody positive or antibody negative (Fig. 2). This variation in assay construction provided further interpretation of assay performance to be conducted.

3.2. Assay specific antibody-antigen results

The NMI 1 assay showed a consistent high level of sensitivity of 88% for the detection of IgG antibodies against S and RBD antigens. This assay included specified cut-off samples for S and RBD beads to provide test run specific results interpretation for these antigens, therefore only these beads results were interpreted. No false positive results were detected.

The LC assay had low test sensitivity in comparison to other evaluated assays, evaluation showed an overall test sensitivity of 68% for the detection of IgG antibodies when results were qualitatively interpreted as antibody positive or antibody negative. The analysis software interpreted the three SARS-CoV-2 antigen beads: N, RBD & S1 MFI values to a minimum threshold value. For a sample to be classified as antibody positive it was required to meet threshold value for both the N and RBD beads (Fig. 2). The LC assay included an S1 antigen which detected antibodies in 24% of positive samples, which is lower than the only other assay incorporating this antigen (OL) which detected antibodies in 40% of the samples tested.

The OL assay provided interpretation for the largest number of

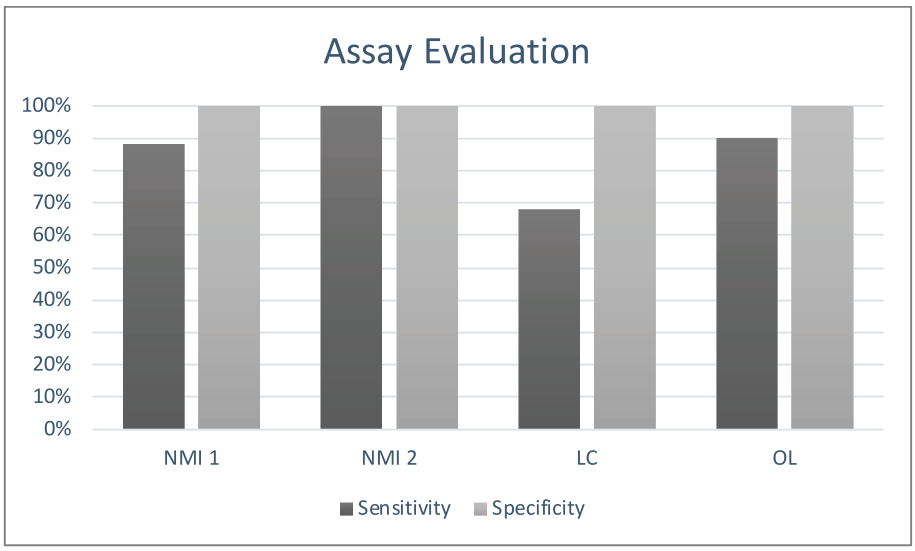

Fig. 1. Comparative sensitivity and specificity of the four different assays tested for the detection of SARS-CoV-2 IgG antibodies. Statistical analysis was carried out through comparison of each test assays qualitative results of antibody positive or antibody negative, compared to the known antibody status provided by prior virology testing using a validated test method.

different SARS-CoV-2 antigens. The OL assay was tested using a smaller cohort of 18 samples (10 positive and 8 negative) in comparison to other test assays used ($n = 50$), due to reduced reagent availability within this study. The 10 positive samples were selected based upon the sample results generated during other assay evaluation; 5 samples were selected that had given false antibody negative results when tested by LC assay ($n = 5$) and NMI 1 assay ($n = 3$), the other 5 samples selected had given antibody positive results by all three alternative assays. The OL assay showed an overall test sensitivity of 90% when results were qualitatively interpreted as antibody positive or antibody negative.

Results of the OL assay were interpreted using Microsoft Excel. The assay includes SARS-CoV-2, seasonal coronavirus, SARS and MERS antigens and cut-off values to allow interpretation are provided for all included antigens. For the purpose of this study only SARS-CoV-2 antigen results were included in analysis. To classify a sample result as antibody positive it required a minimum of 1/5 SARS-CoV-2 antigen beads to be greater than the respective bead cut-off value for. Using this interpretation, 9 of the 10 positive samples tested were classified as positive. Test samples showed different reaction patterns with the 5 SARS-CoV-2 antigens indicating different sensitivities of each antigen.

The NMI 2 assay detected antibodies against ST and RBD antigens in all known positive test samples. This assay showed the highest assay performance of those evaluated with an overall calculated sensitivity of 100% and specificity of 100% when results were qualitatively interpreted as antibody positive or antibody negative using the two required classification beads. The N antigen detected antibodies in a lower percentage of test samples, 76%, than the ST and RBD antigens. Comparatively the NMI 2 assay detected the highest percentage of samples with anti-N antibodies, with the LC assay detecting antibodies in 72% of samples and the OL assay in 50% of samples.

3.3. NMI 2: MULTICOV-AB assay

On initial investigation the NMI 2 assay demonstrated superior performance characteristics for qualitative antibody detection of SARS-CoV-2 antigens therefore, further statistical analyses were undertaken on the results. The initial results interpretation focused on the ST and RBD beads, which were analysed by assessing if a given sample's MFI value was higher or lower than a calculated cut off value provided by the manufacturer. This determined 100% assay sensitivity for both antigens. Upon further interpretation of the N bead, the NMI 2 assay showed decreased detection of antibodies against the N antigen of 76%. The interpretation of the N bead was completed using a different analysis technique compared to the ST and RBD antigens (as indicated by the manufacturer) and required calculation of normalization values for each sample for the N antigen. To calculate normalization values each sample's NMFI result was normalized to the mean of the two QC2 NMFI results. Normalization values ≥ 1 were interpreted as representative of an MFI signal above the cut-off (positive) and values <1 as below the cut-off (negative).

Due to the variation in clinical interpretation technique used to analysis results for the N antigen and the decreased percentage of antibodies detected, further statistical analyze of the N antigen results was conducted (Fig. 3). As the N antigen demonstrated a lower sensitivity the non-parametric Mann-Whitney *U* test was conducted to ensure there was a significant difference between the calculated normalization values of the two groups of samples, negative test samples and positive test samples. This test was completed due to the normalization values being analysed as ratio data. The result of this analysis was a p value of <0.001, indicating that there is a significant difference in the normalization values of each group.

To assess the cut-off threshold value used to determine sample positivity when interpreting the N antigen using normalization values, Receiver-Operator Characteristic (ROC) curve analysis was conducted. The potential alternative cut-off values were analysed to assess performance implications associated with adjusting the cut-off value (Fig. 4). ROC curve analysis showed that using the positive cut-off for normalization values of ≥ 1 had an Area Under the Curve (AUC) value of 0.967 indicating outstanding diagnostic accuracy. This manufacturer specified cut-off value provides 76% sensitivity and 100% specificity. The coordinates of the curve table showed that there is no single cut-off value that would allow separation of true positive and true negative results. The data was used to identify potential alternative positive cut-off values; findings showed a value of 0.595 was the lowest cut-off value that maintained 100% specificity with the highest level of test sensitivity. The AUC values indicated that a cut-off value of either 0.195 or 0.370 have the maximal AUC value of 0.806. Using the cut off value of 0.195 would provide 96% sensitivity and 84% specificity whereas the 0.370 cut off value would provide 84% sensitivity and 96% specificity.

A - NMI

SARS-CoV-2 Antigens Tested	S	RBD	S1	S2	N	N NTD
Results analysed	X	X	NI	NI	NI	NI
Value required for results interpretation	X	X	-	-	-	-
Percentage of positive test samples meeting positive threshold	88%	88%	-	-	-	-

B - LC

SARS-CoV-2 Antigens Tested	N	RBD	S1
Results analysed	X	X	X
Value required for results interpretation	X	X	-
Percentage of positive test samples meeting positive threshold	72%	72%	24%

C - OL

SARS-CoV-2 Antigens Tested	S	S1	RBD	S2	N
Results analysed	X	X	X	X	X
Value required for results interpretation	Any one bead				
Percentage of positive test samples meeting positive threshold	90%	40%	60%	90%	50%

D – NMI 2

SARS-CoV-2 Antigens Tested	ST	RBD	S1	S2	N
Results analysed	X	X	NI	NI	X
Value required for results interpretation	X	X	-	-	-
Percentage of positive test samples meeting positive threshold	100%	100%			76%

S = Spike, RBD = Receptor Binding Domain, S1 = Spike Subunit 1, S2 = Spike Subunit 2, N = Nucleocapsid, N NTD = Nucleocapsid N-terminal Domain, ST = Spike Trimer, NI = Not Interpreted.

Fig. 2. Comparison of the specific SARS-CoV-2 antigen results of the four different assays tested. For the detection of SARS-CoV-2 IgG antibodies. (A) Table showing data for NMI 1 assay: this assay had consistent antibody detection for both the antigens interpreted. The NMI 1 assay included multiple SARS-CoV-2 antigens that were tested but not analysed due to lack of manufacturer indicated cut off values. (B) Table showing data for LC assay: this assay had the least number of SARS-CoV-2 antigens included and showed large variation in detection of antibodies against different antigens. (C) Table showing data for OL assay: this assay had the least restrictive results interpretation criteria requiring antibodies to be detected to a minimum of one out of five antigens to classify a sample result as positive. (D) Table showing data for NMI 2 assay: this assay showed 100% level of detection against the ST and RBD antigens with lower percentage detection against the N antigen. This demonstrates the highest detection of RBD amongst the 4 assays.

4. Discussion

Accurate detection of antibodies against SARS-CoV-2 is an essential feature of the global recovery from the COVID-19 pandemic (Maple and Sikora, 2021). Despite this, there is currently limited standardisation of SARS-CoV-2 antibody testing with many different testing methodologies currently in use with varying ranges of clinical performance (Kopel et al., 2021; Liu and Rusling, 2021). Luminex technology has been shown to be a more sensitive technique for antibody detection than the equivalent ELISA assay within the scope of anti-HLA antibody detection for organ transplantation (Minucci et al., 2011). For this reason, this study aimed to demonstrate the use of the Luminex platform within the field of SARS-CoV-2 antibody testing by evaluating four different SARS-CoV-2 antibody assays. Evaluation of each assay's clinical performance provides direct comparison of each assay's ability to detect antibodies against specified antigens of S, N and RBD, which can be used to determine the best clinical suitability for each assay depending on its performance.

This study demonstrated that all evaluated Luminex based assays were able to detect SARS-CoV-2 antibodies against the S, N and RBD proteins in post-infection serum samples. Qualitative interpretation of sample positivity or negativity for the presence of SARS-CoV-2 antibodies was performed for each assay with all but one assay reaching a test sensitivity of 88%. This indicates that Luminex technology is a suitable testing technique for SARS-CoV-2 antibody detection, however in this instance the assays provided a mildly decreased sensitivity than the control ELISA. This is in keeping with recent studies that have identified the use of individual Luminex assays can have comparable performance as compared to commercial ELISA assays (Becker et al., 2021; Hoffman et al., 2021). However, to our knowledge, this is the first study providing a direct comparison of Luminex based assays for the detection of SARS-CoV-2 antibodies.

This study directly compared four assays which identified that the NMI 2: MULTICOV-AB assay had the best performance characteristics with 100% sensitivity and specificity for detection of overall SARS-CoV-2 antibody positivity. These initial findings indicated that the NMI 2 assay may be most suitable for laboratory implementation and therefore was selected for further testing. The varying sensitivities observed by the other assays showed that a small percentage of false negative results were being found. However, both the OL LABScreen COVID Plus Assay and the NMI 1 Magnetic Luminex Assay, demonstrated significant diagnostic accuracy of detection of SARS-CoV-2 antibodies with

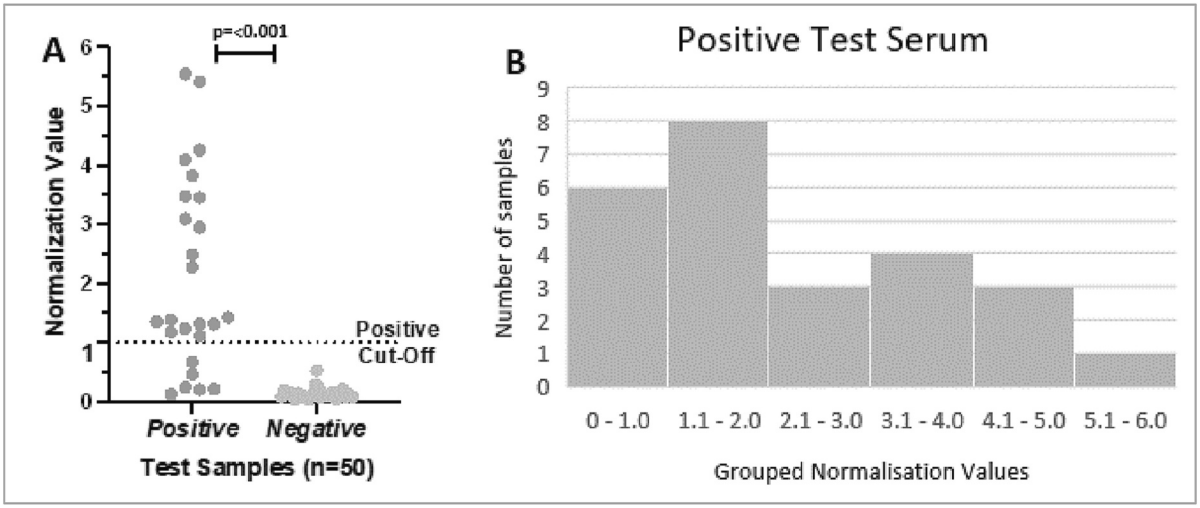

Fig. 3. Statistical analysis of NMI 2 (MULTICOV-AB) assay test samples calculated normalization value results for SARS-CoV-2 Nucleocapsid bead. (A) Scatterplot showing visualisation of the calculated normalization value for the positive (n = 25) and negative (n = 25) test samples, created using GraphPad Prism. Mann-Whitney statistical analysis performed in SPSS on the calculated normalization values, p value is shown. (B) Histogram showing the distribution of the normalization value results for the positive test serum samples, created using Microsoft Excel.

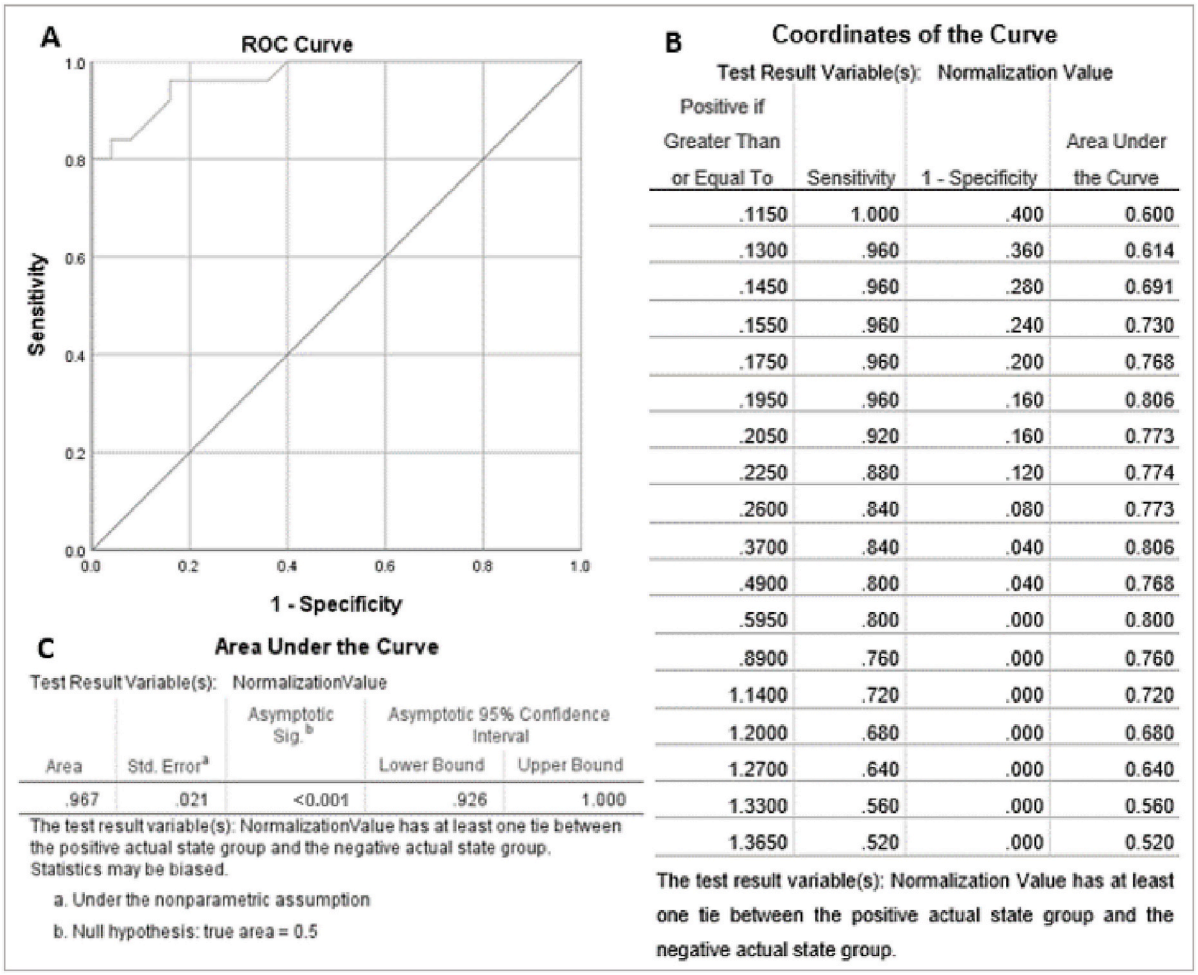

Fig. 4. ROC curve analysis of NMI 2 (MULTICOV-AB) assay normalization value results for SARS-CoV-2 Nucleocapsid bead; created using SPSS. (A) ROC Curve showing visualisation of sensitivity and 1-specificity analysis of normalization value data for classification of sample antibody positivity or negativity. (B) Coordinates of the Curve table showing a range of positive cut-off values and their relative sensitivity, 1-specificity and Area Under the Curve values. Cut-off values were included in the table if the value corresponded to a sensitivity of 50–100%. (C) Area Under the Curve data for ROC curve analysis including; area, standard error and significance values shown.

sensitivities of 90% and 88% respectively and would also be candidates for laboratory implementation. The LC assay had the lowest sensitivity only detecting SARS-CoV-2 antibodies in 68% of samples indicating it would require further evaluation prior to clinical use. This result was directly discussed with the manufacturers which concluded that decreased sensitivity may be due to manual testing technique interference within our laboratory.

These results demonstrate that the assays evaluated can accurately detect antibodies to multiple SARS-CoV-2 antigens in a sample within a single test. Research has suggested that the use of highly specific multiplex bead-based assays could provide a suitable alternative to single antibody detection serological assays (Mariën et al., 2021). This highlights a significant advantage of Luminex testing as it enables detection of multiple SARS-CoV-2 antibody specificities simultaneously, compared to standard ELISA techniques that analyze test antigens individually. This multiplexing technique could provide improved testing efficacy if antibody status to multiple SARS-CoV-2 antigens is required, as this would allow one test to be completed rather than multiple, which would also contribute to reducing laboratory workload. Importantly, none of the assays evaluated provided false positive results with all assays demonstrating 100% test specificity. This potentially indicates low cross reactivity of antibodies against endemic human CoVs to SARS-CoV-2 specific antigens. This opposes some studies which have reported cross-reactivity between antibodies against SARS-CoV-2 N protein and less pathogenic viruses (Ernst et al., 2020). This confirms that Luminex based assays have comparable capability to commercial ELISA assays which generally report a testing specificity of $\geq$99% (Harritshøj et al., 2021).

Importantly, though initial analysis demonstrated the high sensitivity of the NMI 2 MULTICOV-AB assay, this was determined by interpreting overall sample positivity using the ST and RBD results. As the control ELISA used to select positive samples for this study did so through detection of antibodies against the N protein, the NMI 2 assay was further evaluated to identify if it provided comparable results for this antigen specificity. Notably, this assay detected antibodies against the N protein to a sensitivity of 76% when interpreted using normalization values and a positive cut off value of $\geq$1, showing decreased antibody detection. Statistical analysis was undertaken using the Mann-Whitney *U* test showing a statistically significant difference between the test results of the negative sample group and the positive sample group. Additional ROC curve analysis demonstrated that the results interpretation employed using the positive cut-off value of $\geq$1 had an Area Under the Curve (AUC) value of 0.967 indicating outstanding diagnostic accuracy. Interestingly, ROC analysis revealed that alternative cut off values of 0.195 or 0.37 would increase test sensitivity to 96% or 84% respectively, however this would cause test specificity to decrease from 100% to 84% or 96% respectively. Therefore, this would both decrease false negative results and increase false positive results detected, potentially leading to clinical mis-interpretation of results. Evaluation of the clinical utility of the assay would be required prior to determining the optimal cut off value. Overall, these results demonstrate the decreased detection of anti-N antibodies compared to ST and RBD within these test samples.

Despite potential difficulties, being able to detect anti-N antibodies may play an important clinical role in differentiation between infection induced and vaccination induced antibody response. This is because the majority of vaccinations currently authorised for administration contain the spike protein as a viral target, either exclusively or as part of whole-virus vaccines (Forni et al., 2021). Therefore, employing Luminex multiplex assays could provide a suitable method for monitoring of post vaccination antibody production as within a single test the results would be able to indicate if the patient's antibodies are in response to vaccination alone or also recent/previous infection. This information could provide key data regarding development of vaccine induced antibody development, which is vital for understanding how vaccine response may differ between patient groups of different age and health status (Wheeler et al., 2021). Renal transplantation patients have been shown to be one of the patient groups at increased risk from COVID-19, this includes both patients awaiting transplantation and post-operative patients who are subsequently immunosuppressed (Toapanta et al., 2021). As this patient group already undergo frequent blood test monitoring for HLA antibodies, the use of Luminex SARS-CoV-2 assays could provide dual testing results without the need for further patient samples to be collected. Accurate serological testing indicating previous exposure and current immunity status may provide essential clinical information going forward allowing better risk stratification to be made for these patients. Whilst this study demonstrates that Luminex based assays have suitable clinical performance for use in post-infection antibody monitoring further research could be conducted to demonstrate an assay's capability to detect vaccination induced antibodies using a cohort of pre and post vaccination patients.

Furthermore, an assay providing results that indicate differentiation between natural infection and vaccine response also has the potential capability to allow additional research into these different types of 'immunity'. Although research has demonstrated that antibodies stimulated by natural infection can persist up to 11 months after symptom onset (Pan et al., 2021), research is yet to determine the correlation between 'natural immunity' and an individual's immunity to future SARS-CoV-2 infection (Maple and Sikora, 2021). Conversely, there is still a lack of knowledge surrounding the length of immunity generated though vaccination, however recently emerged data showed waning antibody titres at 6 months post vaccination with Pfizer-BioNTech (Levin et al., 2021). As scientific research into SARS-CoV-2 continues, attention may begin to focus more on the clinical interpretation of assay results due to the wider availability of highly sensitive commercial assays.

A limitation of this study is the limited sample size of 50 samples, this was able to provide insight into the clinical performance of the assays evaluated, however an extended study would be able to provide a more robust dataset containing a larger number of test samples. Furthermore, when evaluating the OL assay specifically, this study's limited regent supply further restricted the sample size to 18 samples. Due to this, further statistical analysis of OL assay results was not performed. Additionally, this study only incorporated post-infection patient samples as the positive control group and did not include any post-vaccination test samples. Due to the international acceleration of COVID-19 vaccination, the use of a post-vaccination sample cohort would now be available and could provide significant insight into the future of SARS-CoV-2 antibody testing. An overarching limitation to current serological assay evaluation is the lack of a gold standard test to compare the results against, due to lack of national or international guidance on antibody testing at the time of study completion. To overcome this, in this study the results were compared to a widely used commercial assay that is currently employed for patient testing within the NHS. However, the ELISA test used to determine sample positivity did so based upon the detection of anti-N antibodies, therefore only demonstrating the presence of this antibody specificity within the positive test samples. Despite these limitations, this study demonstrates significant potential for the Luminex-based assay for SARS-CoV-2 antibody testing and further research will be able to establish the potential for wider utility by testing an extended sample set.

5. Conclusion

In conclusion, this study demonstrated that Luminex bead-based assays are suitable for use in the detection of SARS-CoV-2 antibodies. Of the four antibody assays evaluated the MULTICOV-AB assay (NMI 2) demonstrated the highest overall sensitivity and specificity for the detection of SARS-CoV-2 antibodies and was comparable to that of widely used ELISA assay. Utilizing multiplex Luminex assays provides an efficient way to test for antibodies to multiple SARS-CoV-2 antigens within one test, with many potential clinical applications including

differentiation between natural immunity and vaccine response. The study found no false positive antibody results indicating low cross reactivity of antibodies against endemic human CoVs to SARS-CoV-2 specific antigens, demonstrating the highly specific nature of the assays tested. Luminex based testing is the current gold-standard method for HLA antibody detection in transplantation laboratories (Tait, 2016), by taking advantage of the established widespread use of Luminex platforms, this could provide a straightforward way to incorporate SARS-CoV-2 antibody testing for high-risk renal transplantation patients.

Author contributions

The conception and design of the study was developed by D. Kallon and A. Gupta.

The acquisition of data, data analysis and interpretation of data was carried out by A. Cox.

The drafting of the article was carried out by A. Cox, with critical revision for important intellectual content completed by M. Stevens and E. White.

Funding

This work was supported by Health Education England via the National School for Healthcare Science. Further non-grant funding was supported by the Clinical Transplantation Laboratory at the Royal London Hospital, Barts Health NHS Trust.

Declarations

AC, DK, AG & EW are employees at the Clinical Transplantation Laboratory, Barts Health NHS Trust.

Data availability

Data will be made available on request.

Acknowledgements

We thank the Virology department at the Royal London Hospital for the provision of the samples used for testing in this study. The authors thank our correspondents at NMI, LC and OL for provision of test assays reagents for evaluation and guidance for use and results interpretation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jim.2023.113472.

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