Original Article

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Analytical Development and Optimisation of a Semi-in-House ELISA for the Detection of SARS-CoV-2 by using Spike and Nucleocapsid Protein Antigens

Microbiology Section

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ABSTRACT

Introduction: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), a betacoronavirus that emerged in late 2019, is the causative agent of Coronavirus Disease 2019 (COVID-19). The viral spike (S) and Nucleocapsid Protein (NP) are key structural components and principal targets for antigen detection. Developing reliable in-house immunoassays targeting these proteins is essential to enhance laboratory capacity and reduce dependence on commercial diagnostic kits.

Aim: To analytically develop and optimise semi-in-house direct and sandwich Enzyme Linked Immuno Sorbent Assay (ELISA) for individual and simultaneous detection of SARS-CoV-2 spike (S) and NP antigens.

Materials and Methods: This was an analytical laboratory-based study conducted between April 2025 and October 2025 at King Fahd Medical Research Centre, King Abdulaziz University, Jeddah, Saudi Arabia. For direct ELISAs, assay conditions were optimised using checkerboard titration. Recombinant SARS-CoV-2 S and/or NP antigens were used as positive controls, while MERS-CoV S and/or NP antigens were included to evaluate potential cross-reactivity. Horseradish Peroxidase (HRP)-conjugated monoclonal antibodies specific to SARS-CoV-2 S and/or NP served as detection antibodies. Optimal antibody concentrations were defined as those producing the highest positive-to-negative Optical Density (OD) ratio, including blank controls. For sandwich ELISAs, plates were coated with monoclonal anti-SARS-CoV-2 antibodies targeting S/RBD and/or NP as capture antibodies. Subsequent steps followed the optimised protocol established for the direct ELISA format. Analytical performance was evaluated by determining the assay cut-off value, Limit Of Detection (LOD), linear dynamic range, and intra- and inter-assay Coefficients of Variation (CV%). Cross-reactivity was assessed using MERS-CoV recombinant proteins and heat-inactivated virus preparations.

Results: Direct ELISAs detected S antigen at ≥6.25 ng and NP antigen at ≥3.125 ng per well whereas sandwich ELISAs improved analytical sensitivity four-fold (LOD 0.78 ng). Detection using heat-inactivated virus demonstrated analytical sensitivity down to 0.049×10^5 $TCID_{50}$/mL. Intra-assay CV ranged from 3.1-6.1% and inter-assay CV from 6.2-9.2%. No cross-reactivity with MERS-CoV was observed under tested conditions.

Conclusion: The developed semi-in-house ELISAs demonstrated acceptable analytical sensitivity and reproducibility for research applications. Clinical validation using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)-confirmed patient samples is required before diagnostic implementation.

Keywords: Antigens, Enzyme-linked immunosorbent assay, Immunoassay, Sensitivity, Specificity

INTRODUCTION

Since the emergence of COVID-19 pandemic, scientists and medical researchers have been working tirelessly to develop accurate and reliable molecular and serological assays for COVID-19 diagnosis and research [1,2]. The RT-PCR remains the gold standard for COVID-19 diagnosis as a result of its high sensitivity and specificity. RT-PCR can reliably detect viral nucleic acid. Yet, it cannot distinguish whether the detected nucleic acids are remnant viral RNA from previous infection or rather they belonged to replication-competent infectious viruses. Viral culture can confirm the presence of actively replicating viruses and can determine the contagious status of the infected individuals. However, this method requires a laboratory facility with biosafety containment level 3, which is not widely available [3,4]. Some relatively modern technologies were applied to develop nucleic acid detection of SARS-CoV-2. The CRISPR-Cas12 system has been used to develop a diagnostic test called SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) to detect SARS-CoV-2 RNA in patient samples. RNA extracted from nasopharyngeal or oropharyngeal swabs is reverse-transcribed and amplified using loop-mediated amplification (RT-LAMP). Then, utilising Cas12 that can specifically recognise particular coronavirus sequences, the detection of the virus is confirmed when the reporter molecule is cleaved [5]. A RT-LAMP reaction using specially designed primer sets has also been employed to detect the SARS-CoV-2 virus. The sensitivity and specificity of the method can be as high as RT-PCR [6].

Serological assays are also valuable tools for COVID-19 diagnosis and research. Numerous companies and research labs have developed indirect ELISA that enable detection of anti-SARS-CoV-2 IgM, IgG, and IgA in clinical samples. These assays were heavily utilised in epidemiological investigations, as well as in assessing the effectiveness of COVID-19 vaccines [7-13]. In addition, several SARS-CoV-2 antigen diagnostic assays have become commercially available, particularly after considering antigen tests as acceptable alternatives to RT-PCR. However, independent evaluation of those assays raised some concerns about the assay sensitivity which, in some instances, were as low as 53.3% [14-17].

In this study, the protocols were referred to as "semi-in-house" ELISAs. This terminology indicates that while the assay design, optimisation steps, titrations, and analytical workflow were developed locally, the recombinant antigens and monoclonal antibodies were sourced from commercial suppliers. In contrast, a fully in-house assay would rely on laboratory-generated antibodies and antigens.

The semi-in-house model provides an effective balance between cost efficiency, flexibility, and accessibility.

As an effort to provide reliable serological antigen tests that provide easy-to-perform and cost-effective procedures, this study aimed to develop and optimise semi-in-house direct and sandwich-direct ELISA protocols that enable individual and simultaneous detection of SARS-CoV-2 S and NP antigens. The novelty of this study lies in the development of a novel semi-in-house ELISA incorporating both spike (S) and nucleocapsid (NP) antigens, which improves diagnostic performance by balancing assay sensitivity and specificity.

The current study aimed to analytically develop and optimise semi-in-house direct and sandwich ELISAs for individual and simultaneous detection of SARS-CoV-2 spike (S) and nucleocapsid (NP) antigens.

MATERIALS AND METHODS

This was an analytical laboratory-based study conducted from April 2025 to October 2025 at the Special Infectious Agents Unit, King Fahd Medical Research Centre, King Abdulaziz University, Jeddah, Saudi Arabia. The study focused on analytical optimisation and validation parameters and did not involve human clinical samples.

Development and Optimisation of Direct ELISAs for SARS-CoV-2 Antigen Detection

Flat-bottom 96-well microtiter plates (Immulon® 2 HB, Bloomington, MN, USA) were coated overnight at 4°C with serial dilutions (100–3.125 ng/well) of recombinant SARS-CoV-2 spike (S) protein (40589-V08B1, Sino Biological Inc., Beijing, China) or nucleocapsid (NP) protein (40588-V08B, Sino Biological Inc., Beijing, China). Phosphate-buffered saline (PBS) served as assay blank. For cross-reactivity assessment, recombinant MERS-CoV S (40071-V08B1) or NP (40068-V08B) proteins and reciprocal SARS-CoV-2 antigens were included as appropriate.

After coating, plates were washed three times with PBS containing 0.1% Tween-20 (PBST) and blocked with 5% skim milk in PBS-Tween (100 µL/well) for 1 hour at room temperature. Plates were washed again, followed by incubation for one hour at 37°C with HRP-conjugated rabbit monoclonal antibodies diluted 1:5,000–1:40,000 in blocking buffer: Anti-S monoclonal antibody (4050-D001-H) for S detection, anti-NP monoclonal antibody (40143-R040-H) for NP detection, and a combination of both antibodies for simultaneous S and NP detection. Following washing, 100 µL of TMB substrate (5120-0078, Seracare, Milford, MA, USA) was added and incubated for five minutes. The reaction was stopped with 1N HCl (100 µL/well), and OD was measured at 450 nm using an ELx800 microplate reader (Biokit, Barcelona, Spain).

Development and Optimisation of Direct-sandwich ELISAs

For sandwich ELISAs, plates were precoated overnight at 4°C with 100 µL/well of unlabeled capture antibodies diluted 1:250 in PBS: Anti-SARS-CoV-2 spike/RBD antibody (40592-MP01) for S detection, Anti-SARS-CoV-2 nucleocapsid antibody (40592-MM08) for NP detection, and a combination of both capture antibodies for simultaneous detection. After washing and blocking (as described above), antigen incubation, detection antibody application, colour development, and OD measurement were performed as described for the direct ELISA assays.

The optimised working condition in each assay was determined as the antibody concentration that offers the highest ratio of OD values for positive controls to negative controls and blank. The cut-off value for each assay was determined as the mean + 3 × SD. The LOD was defined as the lowest antigen concentration producing an optical density (OD_{450}) value consistently above the calculated cut-off across three independent experiments. Probit regression analysis was not performed as the present study focused on analytical optimisation rather than diagnostic validation.

Evaluation of the Developed Assays using Heat-Inactivated Coronaviruses

Utilising the optimised working condition, the performance of the developed assays was assessed using serial dilutions of heat-inactivated SARS-CoV-2 as a positive control, and heat-inactivated MERS-CoV as a cross-reactivity control. PBS was utilised as a blank. SARS-CoV-2 and MERS-CoV propagation were conducted in a lab facility with biosafety containment level 3. Inactivation of the viral stock was accomplished by heating at 60°C for one hour. Inactivation of the virus was confirmed by the lack of cytopathic effect in three consecutive passages of cell culture. Of note, although heat exposure may alter certain conformational epitopes, detection signals obtained post-inactivation remained above analytical cut-off values, indicating preserved immunoreactivity of targeted antigenic determinants.

STATISTICAL ANALYSIS

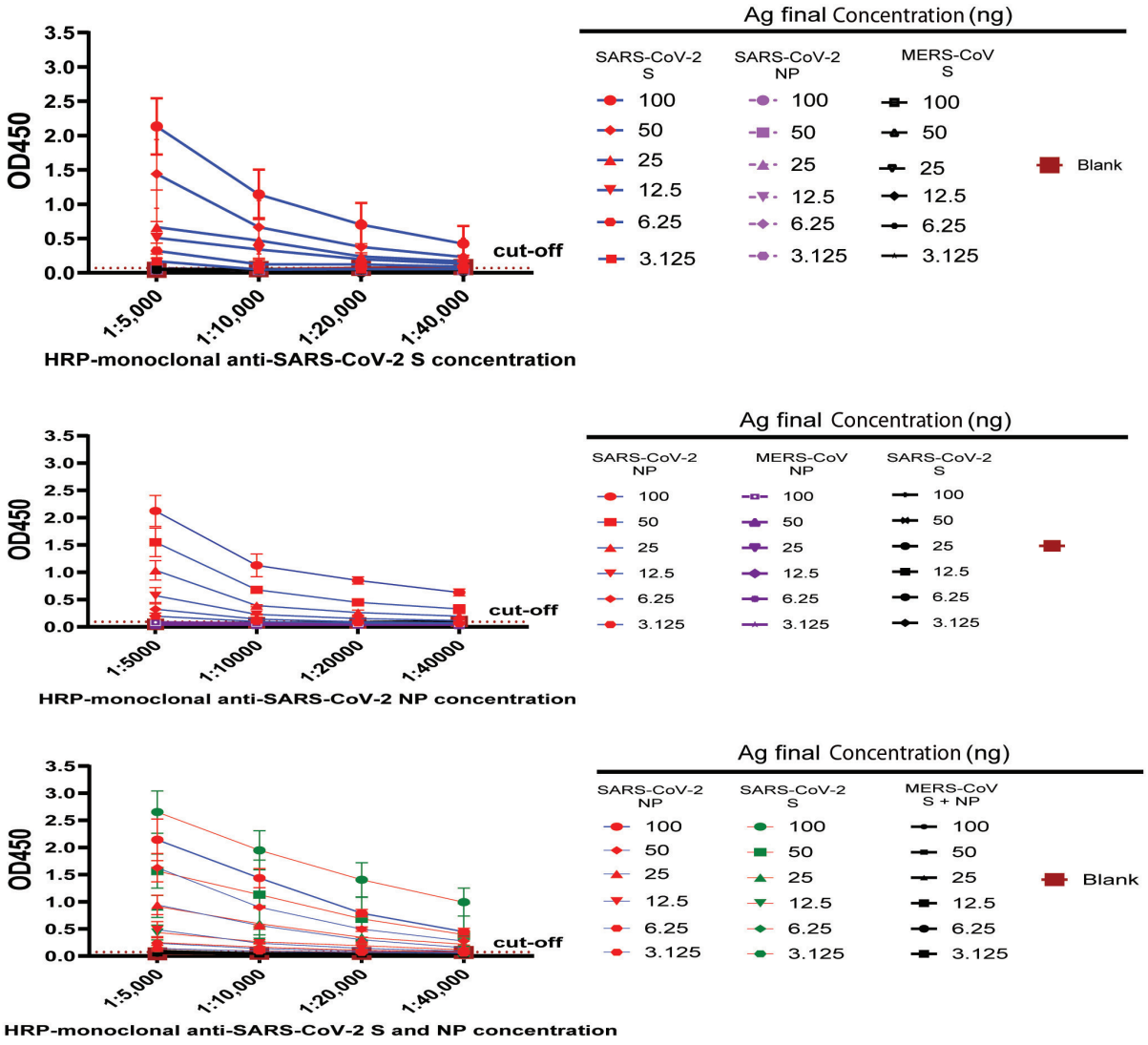
Data were expressed as mean±SD. All experiments were performed in triplicate (n=3). Intra-assay variability was assessed by calculating the CV% across triplicate wells within the same plate, while inter-assay variability was determined from results obtained from three independent experimental runs performed on different days. Data processing and graphical analyses were performed using appropriate statistical software, and results were considered reproducible when consistent across independent experiments.

RESULTS

Optimisation of direct ELISAs for SARS-CoV-2 S, NP, and combined antigen detection: For the S-based ELISA, the cut-off value was established at OD_{450} = 0.068. The assay reliably detected S antigen at concentrations between 100 and 6.25 ng per well, whereas 3.125 ng yielded values below the cut-off. For the NP-based ELISA, the cut-off value was OD_{450} = 0.093. All tested NP concentrations (100–3.125 ng per well) produced signals above the cut-off threshold. For the combined S/NP ELISA, the cut-off value was OD_{450} = 0.073, and both antigens were detectable across the full concentration range tested (100-3.125 ng per well). In all assays, blank wells and cross-reactivity controls consistently produced OD_{450} values below their respective cut-off thresholds, confirming assay specificity [Table/Fig-1].

Optimisation and comparison between direct and sandwich-direct elisa for individual and simultaneous detection of SARS-CoV-2 S and NP antigens: A direct side-by-side comparison of direct ELISA and sandwich ELISA were performed to enable a reliable comparison. The same protocol described above was followed (development and optimisation of direct ELISAs for SARS-CoV-2 antigen detection) to perform direct ELISA. Taking into consideration the data shown above on direct ELISA [Table/Fig-1], it was important to use a lower antigen concentration to determine whether sandwich protocols enhanced the ability of the developed assays to detect lower concentrations of viral antigens. Therefore, viral antigens ranging from 25 ng to 0.78 ng per well were utilised. The cut-off values of S-based, NP-based and combined S and NP sandwich ELISAs were 0.08, 0.06, and 0.056, respectively. The developed semi-in-house sandwich-direct ELISAs substantially enhanced our ability to detect low concentrations of viral antigen without noticeable cross-reactivity [Table/Fig-2].

Evaluation of the direct and sandwich-direct ELISAs using heat inactivated viruses: The performance of the direct and sandwich-direct ELISAs was evaluated utilising heat inactivated SARS-CoV-2 (Ct value=11) as a positive control and heat-inactivated MERS-CoV (Ct value=12) as a cross-reactivity control. PBS was used as a blank. In direct ELISA, heat-inactivated viruses were serially diluted (factor of 2) and added. This step was followed by washing, blocking, and washing. HRP-labelled anti-S and anti-NP



[Table/Fig-1]: Optimisation of direct ELISA using SARS-CoV-2 recombinant antigens.
Top: SARS-CoV-2 spike (S) recombinant protein (Section 2.1). Middle: SARS-CoV-2 nucleocapsid (NP) recombinant protein (Section 2.2). Bottom: Combined SARS-CoV-2 S and NP recombinant proteins (Section 2.3). The effect of secondary antibody dilution on OD_{450} values at varying antigen concentrations was shown. Red dashed lines indicate the assay cut-off values: 0.068 (top), 0.093 (middle), and 0.073 (bottom). Data were presented as mean ± SD of three independent experiments (n = 3). Error bars represent SD.

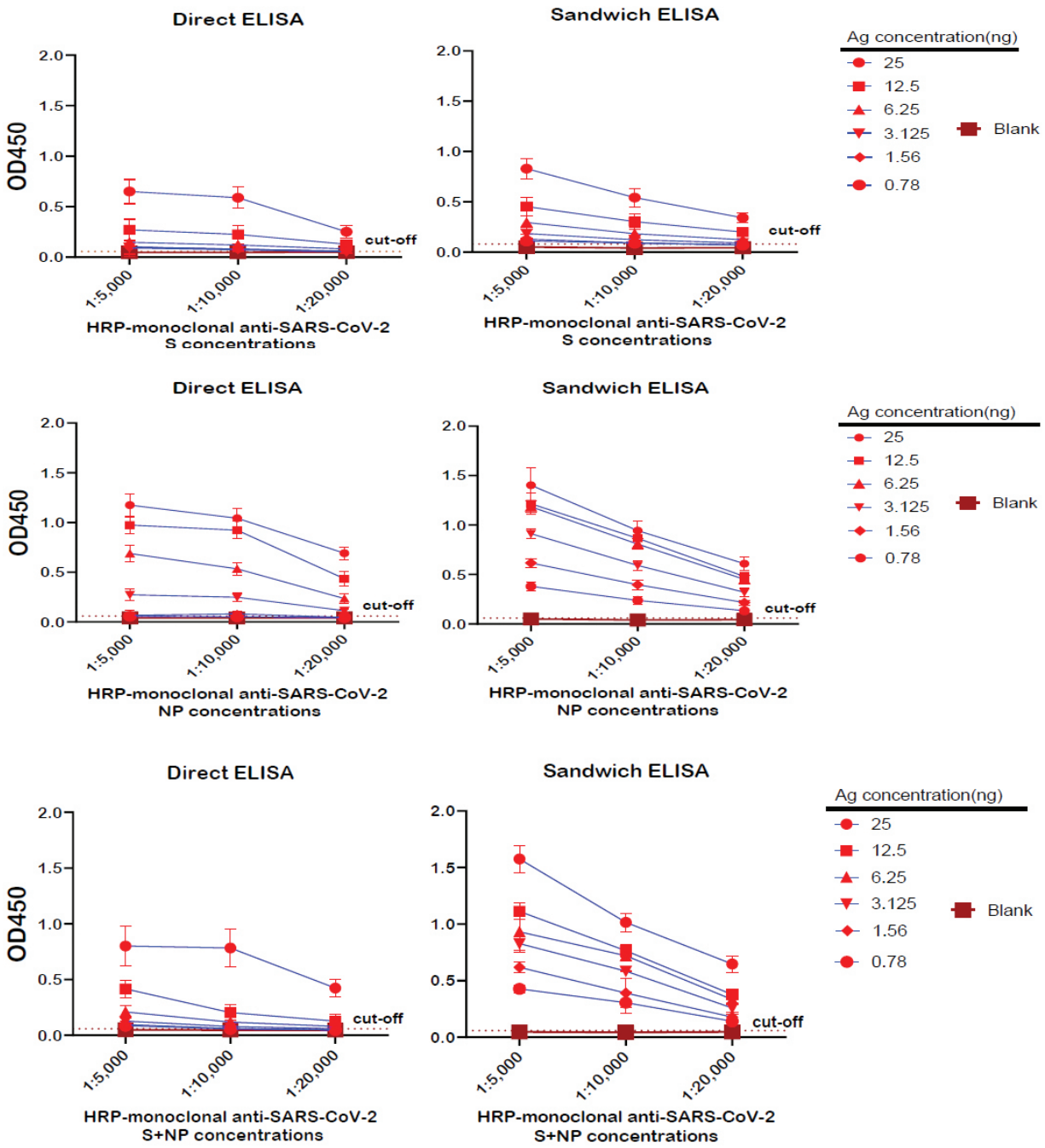
monoclonal antibodies individually and combined were added at a dilution of 1:5000. TMB was added for colour development before stopping the reaction by adding 1N HCL. The OD was read at 450 nm (OD450). In sandwich-direct ELISA, the plates were precoated with unlabelled monoclonal anti-SARS-CoV-2 S and NP antibodies, individually and combined, as capturing antibodies. This was followed by a washing step. Then, the same protocol described for the direct ELISA was followed. The developed assays were able to detect heat-inactivated SARS-CoV-2 virus down to a TCID50 of 0.395×10^5/mL for the direct assay and a TCID50 of 0.049×10^5/mL for the sandwich assay but not heat-inactivated MERS-CoV up to a TCID50 of 100×10^5/mL [Table/Fig-3].

The developed ELISA assays showed good performance and reliable results. The sandwich ELISA methods were more sensitive than the direct ELISA methods, while all assays demonstrated good reproducibility and consistent detection ability [Table/Fig-4].

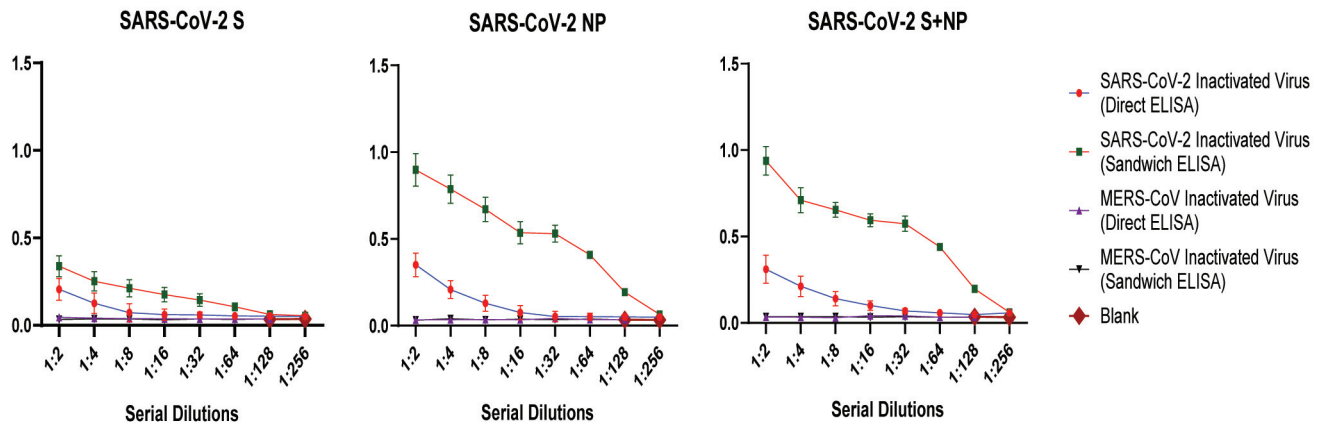
DISCUSSION

During the recent COVID-19 pandemic, one of the WHO recommendations was to enable active surveillance of newly infected cases to gain insight into disease epidemiology, guide case management, and prevent further transmission [18]. Indeed, reliable detection of viral infections is key for infection control and prevention, and it is just as important as the discovery of antivirals and the development of vaccination.

Since the emergence of SARS-CoV-2, the primary method for COVID-19 diagnosis remained RT-PCR test utilising primer/probes specifically amplifying more than one viral gene (e.g., E gene of β-coronaviruses and RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2) [19]. A downside to molecular detection of SARS-CoV-2 is the inability to distinguish between the presence of leftover viral RNA from previous infection and actively replicating virus. So, even in the case of a positive RT-PCR result, viral culture is important to determine the viral infectivity and to conclude about the patient's contagious status. SARS-CoV-2 culture and isolation, however, require laboratories that offer biosafety containment level 3 alongside specialised trained personnel. In addition, viral culture is time-consuming in comparison to other laboratory techniques. Immunoassays that enable antigen detection are acceptable alternatives as, theoretically, the detection of viral antigens suggests the presence of viral structural proteins although does not directly confirm viral infectivity. COVID-19 antigen tests have, indeed, replaced PCR in many settings (e.g., entry and exit of travellers in some countries) [3,4]. The simplicity, ease of performance, and short turnaround time of antigen tests can be key for making timely interventions. Many SARS-CoV-2 antigen detection kits are commercially available. However, independent evaluation of these kits raised controversial conclusions about their performance in terms of sensitivity and, to a lesser extent, specificity [20,21].



[Table/Fig-2]: Comparison of direct and sandwich ELISAs for SARS-CoV-2 antigen detection.
Top: Detection of SARS-CoV-2 S antigen. Middle: Detection of SARS-CoV-2 NP antigen. Bottom: Simultaneous detection of SARS-CoV-2 S and NP antigens, with combined viral antigens. The effect of secondary antibody dilution on OD_{450} values at different antigen concentrations is shown for direct (left panels) and sandwich (right panels) ELISA formats. Red dashed lines indicate the respective assay cut-off values. Data are presented as mean ± SD of three independent experiments (n = 3). Error bars represent standard deviation.



[Table/Fig-3]: Comparison between the performances of direct and sandwich ELISAs using heat-inactivated viruses.
Data of S-based (left panel), NP-based (middle panel), and S and NP-based (right panel) ELISAs are shown. The effect of virus serial dilution on OD450. Data represent mean ± SD of three independent experiments (n = 3). Error bars indicate SD.

Assay type	Cut-off (OD_{450})	LOD (ng)	LOD ($TCID_{50}$/mL)	Linear Range (ng)	Optimal Ab Dilution	Intra-assay CV%	Inter-assay CV%
S direct ELISA	0.068	6.25	0.395×10^5	6.25-100	1:5,000	5.2%	7.8%
NP direct ELISA	0.093	3.125	0.395×10^5	3.125-100	1:5,000	4.7%	8.5%
Combined direct ELISA	0.073	3.125	0.395×10^5	3.125-100	1:5,000	6.1%	9.2%
S sandwich ELISA	0.080	0.78	0.049×10^5	0.78-25	1:5,000	3.4%	6.2%
NP sandwich ELISA	0.060	0.78	0.049×10^5	0.78-25	1:5,000	3.1%	6.9%
Combined sandwich ELISA	0.056	0.78	0.049×10^5	0.78–25	1:5,000	4.2%	7.4%

[Table/Fig-4]: Summary of performance characteristics of the developed direct and sandwich ELISAs.

To address this issue and as a continuation of our effort to develop in-house and semi-in-house molecular tests and immunoassays, semi-in-house direct and sandwich-direct ELISA have been developed and optimised that enable robust individual and simultaneous detection of SARS-CoV-2 S and NP antigens without cross-reactivity with antigens from relevant coronaviruses. At the optimised working condition, the direct ELISA protocols enabled specific detection of the targeted viral antigens at a concentration of ≥ 3.125 ng. The LOD was improved at least four-fold with the developed sandwich ELISA protocols. These findings hold true when the assays were optimised to provide individual and simultaneous detection of SARS-CoV-2 S and NP. Further, when the performance of the developed assays to detect SARS-CoV-2 and MERS-CoV was evaluated, both direct and sandwich ELISAs demonstrated specific detection of SARS-CoV-2 with substantially improved performance of sandwich protocols. To relate the analytical sensitivity to clinically relevant viral loads, it is estimated that 1 $TCID^{50}$ correlates with approximately 10^4 -10^5 viral RNA copies, with NP comprising ~1-1.5% of total virion protein mass. Based on this estimate, the LOD of the sandwich ELISA (0.049×10^5 $TCID_{50}$/mL) corresponds to viral loads typically observed in upper-respiratory samples with Ct values ranging from 20-25. Therefore, the assay is expected to reliably detect moderate-to-high viral load samples, which represent the most infectious phase of SARS-CoV-2 infection.

Although a direct head-to-head comparison with commercial SARS-CoV-2 antigen ELISA kits was not performed due to limited access, it is important to contextualise the expected performance of semi-in-house assays relative to commercial systems. Commercial kits often offer standardised reagents but may suffer from high costs, limited availability, and variable sensitivity as reported in independent evaluations. Present study semi-in-house assays offer lower cost, customisable antigen/antibody combinations, and improved flexibility for local optimisation. However, future studies should include direct comparison with widely used commercial antigen ELISAs to benchmark diagnostic performance. It is also worth noting that Alkaline Phosphatase (ALP)-based ELISA formats have recently achieved higher analytical sensitivity than conventional HRP systems, particularly when coupled with fluorogenic signal-amplifying chemistries [22]. For example, ALP-driven platforms using $ARS/PyB(OH)_2$ fluorescence restoration have reported low detection limits for SARS-CoV-2 N protein, suggesting that similar ALP-based amplification strategies could further enhance the sensitivity of our semi-in-house assays.

Limitation(s)

A major limitation of this study was the absence of validation using clinical nasopharyngeal or saliva samples due to regulatory and logistical constraints. Therefore, evaluation against RT-PCR confirmed clinical specimens representing a range of viral loads and Ct values is necessary to determine diagnostic sensitivity, specificity, and predictive values. Another limitation was the use of MERS-CoV as the only cross-reactivity control; assessment against other coronaviruses such as SARS-CoV, HKU1, and 229E would provide a more comprehensive evaluation of potential non specific reactions. In addition, only one local SARS-CoV-2 strain was tested, so the ability of the assays to detect emerging variants remains unverified. Thus, further validation using clinical samples, additional cross-reactivity controls, and multiple SARS-CoV-2 variants is required before diagnostic implementation. Nevertheless, the developed assays have potential applications in COVID-19 research, such as evaluating antigen integrity and immunogenicity after viral inactivation or antiviral treatment. Moreover, the workflow can be adapted for other viral, bacterial, or toxin antigens by substituting pathogen-specific antibodies, enabling rapid assay development and expansion of local diagnostic capacity.

CONCLUSION(S)

The development of in-house and semi-in-house laboratory assays is a key factor for self-reliance, sufficiency, and sustainability in healthcare and medical research. Herein, authors have developed several semi-in-house ELISAs that enable individual and simultaneous detection of SARS-CoV-2 S and NP antigens. These assays can be utilised for research and, upon proper validation using clinical samples, for diagnostic purposes. The knowledge obtained from this study is not limited to COVID-19, as it could be applied to the development of similar tests for other infectious and non infectious diseases.

Author's contribution

Conceptualisation: TAA, EIA; Methodology: TAA, HMT, SAE, SSS, ATA; Investigation: TAA, HMT, AAF, AMH; Validation: TAA, SAE, SSS, ATA; Formal Analysis: TAA, HMT, AAF, AMH; Resources: TAA, EIA; Data Curation: TAA, HMT, AAF; Writing: Original Draft: TAA, HMT, AAF; Writing - Review and Editing: TAA, HMT, AAF, AMH, SAE, SSS, ATA, EIA; Supervision: TAA, AAF, EIA; Project Administration: TAA.

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REFERENCES

[1] Nucleic Acid Amplification Tests (NAATs) | CDC. [Internet]. [cited 2025 May 01]. Available: https://www.cdc.gov/coronavirus/2019-ncov/lab/naats.html.
[2] Guidance for Antigen Testing for SARS-CoV-2 for Healthcare Providers Testing Individuals in the Community | CDC. [Internet]. [cited 2025 May 01]. Available: https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antigen-testsguidelines.html.
[3] Puhach O, Meyer B, Eckerle I. SARS-CoV-2 viral load and shedding kinetics. Nat Rev Microbiol. 2023;21(3):147-61. Doi: 10.1038/S41579-022-00822-W.
[4] Case JB, Bailey AL, Kim AS, Chen RE, Diamond MS. Growth, detection, quantification, and inactivation of SARS-CoV-2. Virology. 2020;548:39-48. Doi: 10.1016/J.VIROL.2020.05.015.
[5] Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, et al. CRISPR-Cas12-based detection of SARS-CoV-2. Nat Biotechnol. 2020;38(7):870-74. Doi: 10.1038/S41587-020-0513-4.
[6] El-Kafrawy SA, El-Daly MM, Hassan AM, Harakeh SM, Alandijany TA, Azhar EI. Rapid and reliable detection of SARS-CoV-2 using direct RT-LAMP. Diagnostics (Basel, Switzerland). 2022;12(4). Doi: 10.3390/DIAGNOSTICS12040828.
[7] Alandijany TA, El-Kafrawy SA, Tolah AM, Sohrab SS, Faizo AA, Hassan AM, et al. Development and optimization of in-house ELISA for detection of human IgG antibody to SARS-CoV-2 full length spike protein. Pathogens. 2020;9(10):1-11. Doi: 10.3390/PATHOGENS9100803.
[8] Alandijany TA, El-Kafrawy SA, Al-Ghamdi AA, Qashqari FS, Faizo AA, Tolah AM, et al., Lack of antibodies to SARS-CoV-2 among blood donors during COVID-19 lockdown: A study from Saudi Arabia. Healthcare (Basel). 2021;9(1):51. Doi: 10.3390/HEALTHCARE9010051.
[9] Faizo AA, Alandijany TA, Abbas AT, Sohrab SS, El-Kafrawy SA, Tolah AM, *et* al. A reliable indirect ELISA protocol for detection of human antibodies directed to SARS-CoV-2 Np protein. Diagnostics (Basel). 2021;11(5):825. Doi: 10.3390/DIAGNOSTICS11050825.

[10] Alandijany TA, Faizo AA. Development of serological assays and seroprevalence studies of the New Coronavirus 2019 (COVID-19): Reports from Saudi Arabia. Healthcare (Basel, Switzerland). 2021;9(12):1730. Doi: 10.3390/HEALTHCARE9121730.
[11] Ahmed WA, Dada A, Alshukairi AN, Sohrab SS, Faizo AA, Tolah AM, et al. Seroprevalence of neutralizing antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) among healthcare workers in Makkah, Saudi Arabia. J King Saud Univ Sci. 2021;33(3):101366. Doi: 10.1016/J.JKSUS.2021.101366.
[12] Faizo AA, Qashqari FS, El-Kafrawy SA, Barasheed O, Almashjary MN, Alfelali M, et al. A potential association between obesity and reduced effectiveness of COVID-19 vaccine-induced neutralizing humoral immunity. J Med Virol. 2023;95(1):e28130. Doi: 10.1002/JMV.28130.
[13] Faizo AA, Bawazir AA, Almashjary MN, Hassan AM, Qashqari FS, Barefah AS, et al. Lack of evidence on association between iron deficiency and COVID-19 vaccine-induced neutralizing humoral immunity. Vaccines (Basel). 2023;11(2):327. Doi: 10.3390/VACCINES11020327.
[14] Brümmer LE, Katzenschlager S, Gaeddert M, Erdmann C, Schmitz S, Bota M, et al. Accuracy of novel antigen rapid diagnostics for SARS-CoV-2: A living systematic review and meta-analysis. PLoS Med. 2021;18(8):e1003735. Doi: 10.1371/JOURNAL.PMED.1003735.
[15] Lisboa Bastos M, Tavaziva G, Abidi SK, Campbell JR, Haraoui LP, Johnston JC, et al. Diagnostic accuracy of serological tests for COVID-19: Systematic review and meta-analysis. BMJ. 2020;370:m2516. Doi: 10.1136/BMJ.M2516.
[16] Prince-Guerra JL, Almendares O, Nolen LD, Gunn JKL, Dale AP, Buono SA, et al. Evaluation of Abbott BinaxNOW rapid antigen test for SARS-CoV-2 infection at two community-based testing sites - Pima County, Arizona, November 3-17, 2020. MMWR Morb Mortal Wkly Rep. 2021;70(3):100-05. Doi: 10.15585/MMWR.MM7003E3.
[17] Nilles EJ, Karlson EW, Norman M, Gilboa T, Fischinger S, Atyeo C, et al. Evaluation of three commercial and two non-commercial immunoassays for the detection of prior infection to SARS-CoV-2. J Appl Lab Med. 2021;6(6):1561-70. Doi: 10.1093/JALM/JFAB072.
[18] WHO Coronavirus (COVID-19) Dashboard | WHO Coronavirus (COVID-19) Dashboard With Vaccination Data. [Internet]. [cited 2025 May 21]. Available: https://covid19.who.int/.
[19] Jakobsen KK, Jensen JS, Todsen T, Kirkby N, Lippert F, Vangsted AM, et al. Accuracy of anterior nasal swab rapid antigen tests compared with RT-PCR for massive SARS-CoV-2 screening in low prevalence population. APMIS. 2022;130(2):95-100. Doi: 10.1111/apm.13189.
[20] Scohy A, Anantharajah A, Bodéus M, Kabamba-Mukadi B, Verroken A, Rodriguez-Villalobos H. Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis. J Clin Virol. 2020;129:104455. Doi: 10.1016/J.JCV.2020.104455.
[21] Ogawa T, Fukumori T, Nishihara Y, Sekine T, Okuda N, Nishimura T, et al. Another false-positive problem for a SARS-CoV-2 antigen test in Japan. J Clin Virol. 2020;131:104612. Doi: 10.1016/J.JCV.2020.104612.
[22] Geng F, Liu X, Wei T, Wang Z, Liu J, Sha o C, et al. An alkaline phosphatase-induced immunosensor for SARS-CoV-2 N protein and cardiac troponin I based on the in situ fluorogenic self-assembly between N-heterocyclic boronic acids and alizarin red S. Sens Actuators B Chem. 2023;378:133121. Doi: 10.1016/J.SNB.2022.133121.

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