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 Virological Methods 300 (2022) 114423

Contents lists available at ScienceDirect

Journal of Virological Methods

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

Protocols

Development and validation of novel kit for quantification of SARS-CoV-2 antibodies on clinical samples

Sneha Kumari [a], Anoushka Raina [a], Dinesh Chandra [b], Nikita Gupta [a], Nikki Dey [a], Amit Kumar Bhardwaj [b], Archana Anthwal [b], Vikash C. Mishra [b,*], Vimarsh Raina [a], Aseem K. Tiwari [c], Rasika Setia [d], A.S. Bhatia [e]

[a] Chimera Translational Research Fraternity Pvt Ltd, Delhi, India
[b] Chimera Transplant Research Foundation, Delhi, India
[c] Medanta The Medicity, Gurugram, Haryana, India
[d] BLK Super Speciality Hospital, Delhi, India
[e] Government Medical College, Jammu, India

ARTICLE INFO

Keywords:
SARS-CoV-2
Chimera
SOOCHAK
IgG
Clinical samples

ABSTRACT

Since the pandemic occurred due to the emergence of SARS-CoV-2, there has always been a demand for a simple and sensitive diagnostic kit for detection of SARS-Cov-2 infection. In January 2020, WHO approved the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) for detecting the presence of Covid-19 genetic material in individuals. Till date many diagnostic kits have arrived in the market for quantification of SARS-CoV-2 antibodies. In spite of being the gold standard method of Covid-19 detection, there are some drawbacks associated with RT-PCR which leads to false-negative results. Hence, in order to fulfil the need for an antibody testing kit for evaluating seroconversion and immunity acquisition in the population, an efficient, highly specific and sensitive assay, Chimera Soochak, an enzyme-linked immunoassay (ELISA) Kit has been developed. It works on the principle of detecting IgG antibodies developed specifically against the S1-RBD by employing a recombinant strain of S1-RBD produced in the HEK293 cell line. The developed kit was validated using different modes and methods to attain the utmost confidence on the samples collected from patients. The validation methodology included, validation with known samples, blind study, third-party validation, validation using WHO Reference Panel and comparison with FDA approved Surrogate virus neutralization kit. The kit was found successful in detecting IgG against the S1-RBD of SARS-CoV-2. The kit had been validated on multiple parameters. A total of 900 samples had been tested by using this kit and it has exhibited the sensitivity, specificity and accuracy for all the above-mentioned parameters.

1. Introduction

The coronavirus pandemic has caused severe implications since its outbreak in late 2019 in Wuhan, China (Ciotti et al., 2019). On March 11th 2020 coronavirus disease was declared as a pandemic by the World Health Organization (https://covid19.who.int/). As of May 2021, there have been over 168,040,871 confirmed cases leading to a death rate of 2% which caused 3,494,758 deaths globally (https://covid19.who.int/).

The disease, COVID-19, caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has led to various serious adverse effects and deaths worldwide. Its effects include severe pneumonia and other pulmonary manifestations, as well as fever, fatigue etc. The transmission rate of SARS-CoV-2 is higher than that of other viruses in the SARS (Shereen et al., 2020). Most infected people experience mild to moderate symptoms. Mild symptoms were observed in 80 % of the population however the remaining percentage of the infected people has shown severe- adverse illness including pneumonia to adverse respiratory distress syndrome and other symptoms like respiratory failure (Wu and McGoogan, 2020).

The available data suggest an incubation period of the virus in the human body is between 2 and 14 days, with an average of five days.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was approved by WHO in January 2020 for the detection of the presence of Covid-19 genetic material in individuals (World Health Organization,

* Corresponding author.
E-mail addresses: vikash.genebandhu@gmail.com, vikashbiotech01@gmail.com (V.C. Mishra).

https://doi.org/10.1016/j.jviromet.2021.114423
Received 4 July 2021; Received in revised form 16 September 2021; Accepted 8 December 2021
Available online 14 December 2021

2020). This gives an insight into the spread of the virus among individuals. This could in turn help to reduce the transmission rate by identifying and isolating the infected persons which will prevent others from catching the virus. The RT-PCR assay for the detection of Covid antigen is considered the gold standard among all the diagnostics assays.

In spite of being the gold standard method of Covid-19 detection, there are some drawbacks associated with RT-PCR which leads to false-negative results (Espejo et al., 2020). Hence the alternative diagnostics methods are the area of interest that provide the opportunity to improve the accuracy of diagnostic assays. Between all the diagnostic assays the detection of antibodies produced after viral infection can improve the diagnosis of the Covid-19. Previously the serology-based assay had been proved to be useful in epidemiological screening of disease.

The antibody detection assays have become important with the progression of the pandemic since it is extremely important to evaluate the pervasiveness of antibodies against Covid-19 in the population that has already been exposed to the virus, due to infection or vaccination. Several studies have indicated that for the time duration that a person has antibodies against coronavirus, he or she is likely to not get reinfected (Harvey et al., 2021; Abu-Raddad et al., 2021). The presence of antibodies against Covid-19 also implies that the people who have recovered from the disease are good candidates for donating convalescent plasma (Casadevall and Pirofski, 2020).

The immune response generated by each protein of the coronavirus is significantly different. Coronavirus is composed of 4 major proteins. These include Spike Protein (S), Nucleocapsid Protein (N), Envelope Protein (E) and Membrane Protein (M) (Awasthi et al., 2020; Tai et al., 2020). Several studies had indicated that antibody response is generated mostly against the Nucleocapsid Protein(N) and Spike Protein(S), majorly against the Receptor Binding Domain of the S1 subunit of SARS-CoV-2 (Post et al., 2020; Lee et al., 2020).

There is adequate scientific evidence to corroborate that the Receptor Binding Domain of Spike Protein (S1 region) facilitates the entry of the virus into the human cell via binding to the ACE-2 cell receptor (Lan et al., 2020). Detection of antibodies against this region of the novel coronavirus would provide insight into the natural defence mechanism of the population and also helps to evaluate the vaccine efficacy (Sil et al., 2021).

Hence, in order to fulfil the need for an antibody testing kit for evaluating seroconversion and immunity acquisition in the population, an efficient, highly specific and sensitive assay, Chimera Soochak, an enzyme-linked immunoassay (ELISA) Kit has been developed. It detects IgG antibodies developed specifically against the S1-RBD by employing a recombinant strain of S1-RBD produced in the HEK293 cell line (UniProt: P0DTC2). The efficiency, sensitivity and specificity of the Kit have been validated on multiple parameters.

The aim of this study was to develop and validate a simple and sensitive ELISA kit “Chimera Soochak” for the detection of the immunity status of the individuals.

2. Materials and method

a. Reagents and Equipment

Recombinant S1-RBD protein of SARS-CoV-2 (UniProt: P0DTC2) was obtained from Trenzyme Life Science Services, Germany and used as the capture antigen. The S1-RBD protein was coated on MaxiSorp ™ Flat-Bottom Modules modules purchased from Thermo Fisher Scientific. The HRP conjugated secondary antibody (Goat derived anti-human IgG) was used to detect the immunocomplex (Serum IgG and S1 RBD). The TMB substrate (3,3′,5,5′-Tetramethylbenzidine) specific for the peroxidase was used as a substrate for colour development. For terminating the reaction 1 M H2SO4 (Thermo Scientific) was used and the reading was taken at 450 nm using an ELISA plate (ERBA Lisa Scan EM, Transasia)

b. Kit Preparation and Production

The ELISA kit was packaged with all the components required to successfully perform 96 tests. Components include 12 antigen (S1-RBD)-coated Flat bottom modules, Sample Diluent(phosphate-free), 10× Wash Buffer (Tris-based buffer @ pH 7.4 ± 0.2, containing CMIT and MIT as preservatives), HRP tagged goat derived anti-human IgG secondary antibodies solution stabilised with HRP-Protector™ (Ref), substrate 3,3′,5,5′- tetramethylbenzidine (TMB) solution and Stop Solution of 1 M sulphuric acid.

The antigen-coated modules were prepared by coating with 0.5 ug/mL of S1-RBD (100 µL/well) in the Coating Buffer (Candor Bioscience Gmbh). The antigen-coated modules were incubated for 16−18 h. The ELISA plate was secured using Liquid Plate Sealer® (Candor Bioscience Gmbh)

The antigen-coated strips were prepared by coating with 0.5ug/mL of S1-RBD. The coated microwells were secured using Liquid Plate Sealer® (Candor Bioscience Gmbh)[4], to pick a specific subset of IgG antibodies from human serum/plasma. Each completed ELISA plate, vacuum-sealed in an aluminium pouch with a desiccant, was packaged together with the above reagent components.

c. Preparation of samples and working solutions

Plasma/serum samples were used in this procedure and it was ensured that pooled specimens, grossly hemolyzed specimens, specimens with obvious microbial contamination, specimens with fungal growth, specimens with suspended fibrin aggregates, lipemic samples, icteric samples and samples containing sodium azide were not used for testing.

The plasma/serum sample and controls (Both NC and PC) were diluted (1:100) with the Sample Diluent. The 10X Wash Buffer provided in the kit was diluted to 1X using Type II laboratory grade water.

d. Assay Protocol

Chimera Soochak employs an indirect ELISA method for the detection of IgG antibodies against the S1-RBD antigen of SARS-CoV-2. The principle of the assay is shown in Fig. 1.

The assay was performed in microwell strips. 100 µL of diluted plasma/serum samples, NP, PC, and Blank(s) were transferred to corresponding microwells. Strips(s) were covered with adhesive plate sealer(s), placed on a plate shaker (100 rpm) and incubated for 2 h at room temperature. After incubation, the modules were washed 3 times with 300 µL of 1x Wash Buffer. After washing, 100 µL of HRP conjugated secondary antibody (goat derived), was added to each microwell and incubated for 1 h at room temperature. In the final washing step, the unbound reagent was washed off and 100 µL of the Substrate (TMB) was added. The Substrate was incubated for 5−10 min in the dark for colour development. After this step, 50 µL of Stop Solution was added to each microwell to terminate the reaction. The optical density (OD) of samples was recorded using a plate reader at 450 nm within 10 min. The final OD was calculated using the following formula:

$$\text{Final OD} = \text{Sample OD} - \text{Blank OD}$$

The results were considered negative if the value of the Final OD was found less than or equal to 0.6 and ambiguous if the value of final OD was in the range of 0.6 to 0.8. The results were considered positive if the results of the final OD were above 0.8. With every run, both positive and negative controls were used as a quality indicator of the test.

3. Kit validation

The kit has been validated using different modes and methods to attain the utmost confidence. The validation methodology includes the following parameters:

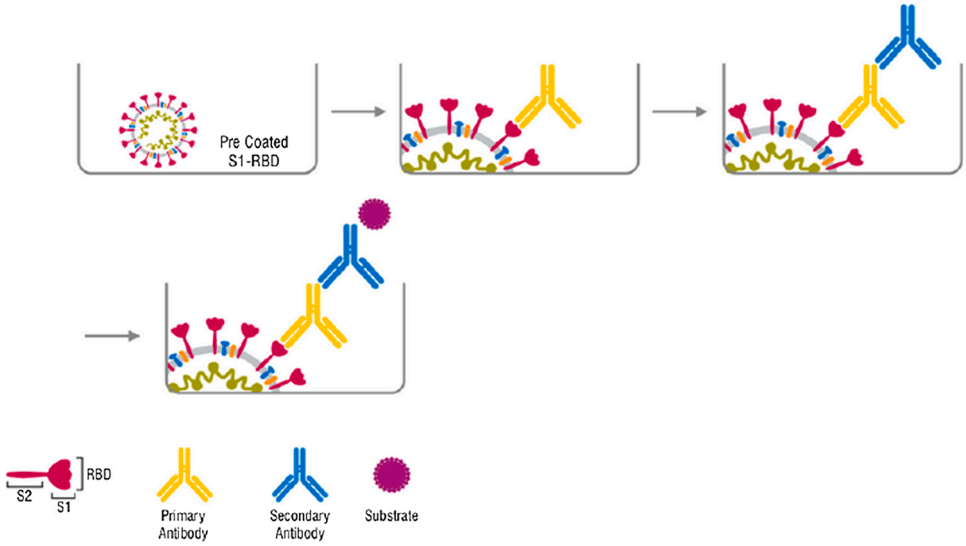

Fig. 1. Principle of Chimera Soochak.

a. Validation with known samples
b. Blind study
c. Third-party Validation
d. Validation using WHO Reference Panel
e. Comparison with FDA approved Surrogate virus neutralization kit.

a. Validation with known samples

Validation of the kit was conducted with previously tested samples. The convalescent plasma samples were considered as positive samples and samples that were collected pre-COVID-19 period as negative. A total of 163 samples were collected from three different clinical sites viz. Government Medical College, Jammu (GMCJ), Jammu and Kashmir, Medanta the Medicity (MM), Gurugram, Haryana and Jaypee Hospital (JPH), NOIDA, Uttar Pradesh. A total of 20 positive and 32 negative samples were collected from JMC, 17 positive and 13 negative samples were collected from MM and 43 positives and 38 negative samples were collected from JPH. All the samples were tested in duplicates (n = 2) to ensure correct specificity and sensitivity. Hence, a total of 326 tests were carried out. The details of the samples tested are mentioned in the Results and Calcultaion section (Section 4a). The testing was done as per the Assay Protocol, as described in Section 2d.

b. Blind study

A blinded study was conducted in collaboration with a super speciality hospital (BLK Super Speciality Hospital, Delhi). A panel of 40 blinded samples was collected from the hospital. These samples had already been tested on four similar commercially available platforms for detecting different kinds of coronavirus related antibodies. The results were compared with the results obtained using the four alternative assays.

c. Third-party validation

A government clinical site was chosen for the third-party validation of CS was done at a government clinical site to ensure its adaptability, robustness, and accuracy. In order to carry out this study, two kits (three different batches of each) were sent to Government Medical Colleges, Jammu for validation. A sample panel curated by the experts at the site was used to validate the assay. A total of 96 samples (Including controls) were tested using each kit that indicated total testing of 576 samples was done using six kits. The results are described in the results section.

d. Validation using WHO reference panel

A WHO Reference Panel(First WHO International Standard Anti-SARS-CoV-2 Immunoglobulin (Human)) was used for scientific validation of CS. The panel consisted of 5 samples with the following characterisation scale:

- Negative for SARS-CoV 2 antibodies
- Low titres of SARS-CoV 2 antibodies
- Medium titres of SARS-CoV 2 antibodies
- High titres of SARS-CoV 2 antibodies
- Low titre of anti-spike antibodies and high titres of anti-nucleocapsid antibodies.

The samples tested in the range of serial dilutions are 1:50, 1:100, 1:200, 1:400, 1:800.

e. Comparison with FDA approved Surrogate virus neutralization kit

Performance verification of CS was carried out by comparing the results obtained from CS and the FDA approved Surrogate virus neutralization kit (GenScript). A sample panel of 168 samples (84 samples each) was tested on surrogate virus assay and CS. The specificity and sensitivity of the assay performed using the CS kit was compared against the results obtained using the surrogate virus assay kit. The number of tests reported as “positive” and “negative” were recorded as endpoints.

4. Results and discussion

a. Validation with known samples

The performance validation of the Chimera Soochak was done with 163 previously tested samples which include 83 Known negatives (samples collected before 2019) and 80 known positive samples (convalescent plasma samples). All the samples were tested for the presence of IgG antibodies against SARS-CoV-2 S1-RBD antigen using the Chimera Soochak assay.

The samples were collected from three institutions in the Delhi-NCR area where they had been tested on other commercially available platforms for the presence of anti-SARS-CoV-2 antibodies. The total number of samples tested was 163.

Out of a total of 163 samples, we observed only 2 false-positive results and zero false-negative results. As shown in Table 1, there were very few false positives (https://covid19.who.int/) and no false positives.

The sensitivity and specificity of the Chimera Soochal assay were calculated based on Table 1 as follows:

Sensitivity = 81/(81 + 2) = 97.5 %

Specificity = 80/(80 + 0) = 100 %

False Negative Rate = 0

False Positive Rate = 2/(2 + 81) = 0.02 %

b. Blind Study

Total 40 blinded samples were collected from a super speciality hospital located in New Delhi. These samples had been tested on 4 separate assays (Abbott Architect SARS-CoV-2 IgG, Roche Elecsys® Anti-SARS-CoV-2 Total, Ortho-Clinical Diagnostics VITROS Anti-SARS-CoV-2 IgG (Spike) and Ortho-Clinical Diagnostics VITROS Anti-SARS-CoV-2 Total.

The results obtained for all samples from Chimera Soochak assay were compared with the results received from shared with the super-speciality hospital and were found to be concordant. with their results.

As shown in Fig. 2, the sensitivity was found to be 100 % and specificity was found to be 94.1 %.

c. Third-Party Validation Evaluation

To substantiate Chimera Soochak's adaptability and robustness, a third-party validation was carried out in collaboration with a government hospital.

The collaborator was provided with 3 different batches(192 tests each) of Chimera Soochak and was asked to perform tests on previously tested samples.

The results were concordant with a sensitivity of 100 % and a specificity of 97.4 %.

This joint effort also helped establish that the batch-to-batch variation was within an acceptable range. The inter batch coefficient of variability percentage was found to be 3.9 %.

d. *Validation using WHO Reference Panel*

A WHO Reference Panel was sourced for the scientific validation of Chimera Soochak. The panel consisted of 5 samples with the following characterisation -

- Negative for SARS-CoV 2 antibodies
- Low titres of SARS-CoV 2 antibodies
- Medium titres of SARA-CoV 2 antibodies
- High titres of SARA-CoV 2 antibodies
- Low titre of anti-Spike antibodies and high titres of anti-Nucleocapsid antibodies
- The samples were tested in serial dilutions (1:50, 1:100, 1:200, 1:400 and 1:800). The ODs of the dilutions were found to follow a parallel pattern concordant with the specification of the Reference Panel, as shown in Fig. 3. Since the assay captures S1-RBD protein of SARS-CoV 2 virus aiming for the detection of COVID-19 fighting antibodies, the sample containing Low titre of anti-Spike antibodies and high titres of anti-Nucleocapsid antibodies also confirm to the Reference panel specification.

Table 1
Validation with Pre-Tested Samples.

Clinical Sites		Known Positive	Known Negative
SITE1	**Positive**	20	1
	Negative	0	31
SITE2	**Positive**	17	0
	Negative	0	13
SITE3	**Positive**	43	1
	Negative	0	37
Total		80	83

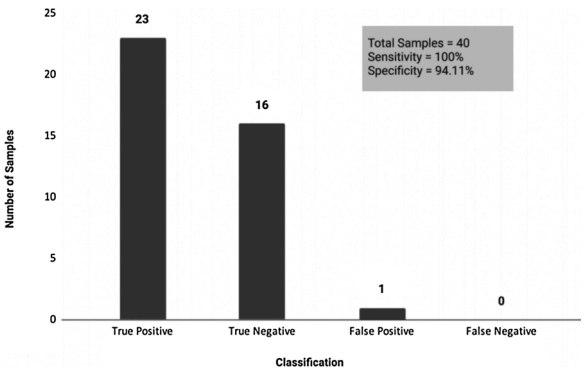

Fig. 2. Blind Study at Super Speciality Hospital.

e. *Comparison with FDA Approved Surrogate Virus Neutralization Kit:*

We have compared the results of samples tested on Chimera Soochak kit with the commercially available FDA approved surrogate virus neutralization assay that detects the virus-neutralizing antibodies in human serum/plasma. A total of 168 samples were tested parallelly on both the assays, surrogate virus neutralization assay and Chimera Soochak. The sensitivity and specificity were found to be 89.7 % and 84.44 % respectively. The details of samples are given in Table 2.

5. Stability evaluation

The stability of the kit was evaluated and the kit was found to be stable up to ten months from the date of manufacturing. A batch of samples was tested repeatedly every alternate month for validating the stability of the kit. The sample in this batch was kept at 2–8°C for ensuring the stability of the samples. The average CV of the samples was found to be 8.62 %. The results of optical density of samples tested during stability period is shown in Fig. 4.

6. Cross reactivity study

There are three highly pathogenic viruses belonging to coronavirus families. These are MERS, SARS, and SARS-CoV. It has been reported that the receptor binding region of MERS and SARS recognises different binding receptors, angiotensin-converting enzyme 2 (ACE2) and dipeptidyl peptidase 4 (DPP4) respectively. This in trun reflects the variability of the RBD region in both the kinds of viruses. Additionally,

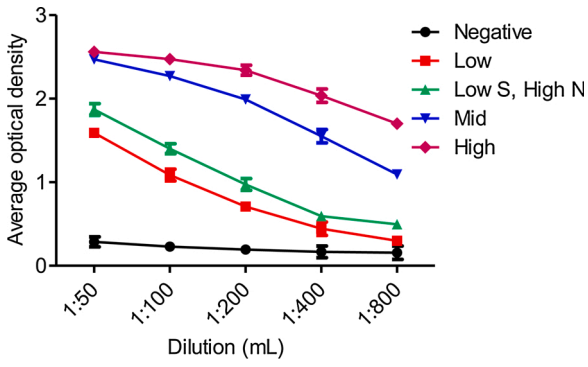

Fig. 3. Validation using WHO Reference Panel.

Table 2
Details of samples.

	Chimera Soochak	
Surrogate Virus	Positive	Negative
Positive	70	8
Negative	14	76

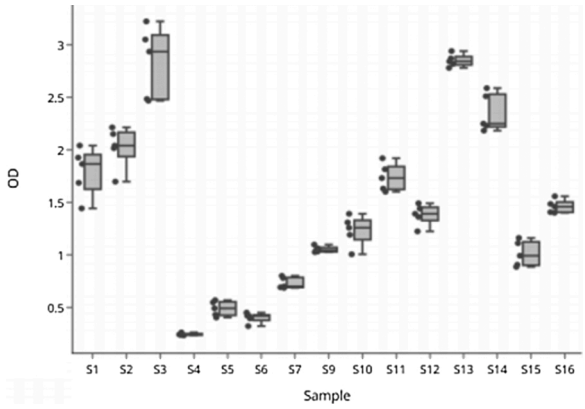

Fig. 4. Optical density of samples tested during stability period.

research has also shown that SARS-CoV-2 Receptor binding domain is not recognised by MERS polyclonal antibodies. Thus, confirming that there is no cross reactivity between the RBD regions of SARS and MERS virus.

However, there is partial cross reactivity between SARS-CoV and SARS-CoV-2 virus. The Spike protein from both the viruses were blasted to check for the variations and highlighted that there are differences in the RBD region of both the proteins that results in limited cross reactivity between the RBD regions of both the viruses. The results of cross reactivity studies are shown in Fig. 5.

The Spike glycoprotein sequences from SARS-CoV {Accession ID: AY541758.1} and SARS-CoV-2 {Accession ID: QHR63250.1} were blasted to look for similarities and differences between them. It was observed that there are notable differences between the sequences which might be the cause of only partial interactivity between the SARS-CoV and SARS-CoV-2 virus.

7. Discussion

The Covid pandemic has affected 171,038,461 individuals all over the world to date (https://www.worldometers.info/coronavirus/). The new mutation acquired by SARS-CoV-2 has increased the transmission rate of this virus (https://www.nature.com/articles/d41586-020-00502-). It had been declared as a pandemic by WHO on 11th March 2020. The SARS-CoV-2 is a contagious virus that spreads rapidly through inhalation and ingestion of the viral droplets produced by infected persons (https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19—11-march-2020). After entering into the host body, it causes mild symptoms like flu and cough; the severe outcome of SARS-CoV-2 infection leads to acute respiratory distress syndrome (ARDS) in some patients (Boopathi et al., 2020). The current diagnosis system for Covid-19 is varying from molecular testing to serological testing. All these assays are based on different target molecules and detection methodology (Sharma et al., 2020). Serology based assay has several advantages over molecular testing as it can be useful for large scale testing and can be performed easily at a normal laboratory setup. These testing do not require the Biosafety Level-3 facility and highly sophisticated equipment to perform the test (Loeffelholz and Tang, 2020). The role of serology-based assay in determination of convalescent plasma titer and checking vaccine efficacy has been previously identified in several studies (Alandijany et al., 2020; Grau et al., 2021; Zilla et al., 2021). Many commercial serology-based kits are available for testing the antibody against SARS-CoV-2; However, the sensitivity and specificity of these kits are uncertain (Ojeda et al., 2021; Kohmer et al., 2020). Looking at these limitations, an attempt has been made to develop a simple, specific and sensitive ELISA Kit for the detection of the IgG against S1-RBD of SARS-CoV-2. The antibodies mainly IgG produced against the S1-RBD have shown the virus-neutralizing capacity (González-Stegmaier et al., 2021). The role of S1 RBD has also been identified in the viral entry into the host cells and it is less conserved in the SARS-CoV family (Bastos et al., 2020).

We have designed this kit especially for detection of IgG against the S1-RBD of SARS-CoV-2. The kit had been validated on multiple parameters. A total of 900 samples had been tested by using this kit and it has exhibited the sensitivity, specificity and accuracy and this kit has been also validated with the WHO panel of sample and the (%) correlation was found in the results. Also, the kit has been validated against the virus neutralization assay and the result was found to be in correlation with the commercially available kit for virus neutralization (Du et al., 2009). This kit has been validated with other commercially available kits (Link for other kits information). The development of a sensitive and specific assay based on the detection of neutralizing antibodies in human serum/plasma will help to evaluate the immune status of the country and also to ensure vaccine efficacy. The Chimera Soochak Kit will provide easy-to-use and affordable kits for resource-limited

Score	Expect	Method	Identities	Positives	Gaps
356 bits(914)	2e-118	Compositional matrix adjust.	168/280(60%)	217/280(77%)	10/280(3%)

```
Query  120  DSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEY  179
            ++K+QS++I+NN+TNVVI+ C F+ C++PF  V    +     ++   ++ +A NCTFEY
Sbjct  1    NNKSQSVIIINNSTNVVIRACNFELCDNPFFAV----SKPMGTQTHTMIFDNAFNCTFEY  56

Query  180  VSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLP  239
            +S  F +D+  K GNFK+LREFVFKN DG+  +Y  + PI++VRDLP GF+ L+P+  LP
Sbjct  57   ISDAFSLDVSEKSGNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNTLKPIFKLP  116

Query  240  IGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVD  299
            +GINIT F+ +L    +  +P      W   AAAY+VGYL+P TF+LKY+ENGTITDAVD
Sbjct  117  LGINITNFRAIL----TAFSPAQDI--WGTSAAAYFVGYLKPTTFMLKYDENGTITDAVD  170

Query  300  CALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASV  359
            C+ +PL+E KC++KSF ++KGIYQTSNFRV P+  +VRFPNITNLCPFGEVFNAT+F SV
Sbjct  171  CSQNPLAELKCSVKSFEIDKGIYQTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSV  230

Query  360  YAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDL  399
            YAW RK+ISNCVADYSVLYNS SFSTFKCYGVS TKLNDL
Sbjct  231  YAWERKKISNCVADYSVLYNSTSFSTFKCYGVSATKLNDL  270
```

Fig. 5. The results of cross reactivity studies.

settings.

8. Conclusion

The results produced by Soochak ELISA showed it is a reliable method for the detection of antibody (IgG) against S1-RBD of SARS-CoV-2. The performance and sensitivity of this kit is comparable to the other available neutralization assay. This kit will provide a very cost-effective approach to screen the larger population to evaluate the seroprevalence, assess the vaccination efficiency and evaluate the immune status of the society.

The assay has an edge over the other comparative assays because it uses only a portion of the S protein (S1-RBD) that is involved in blocking the viral entry into the host cells, increasing the specificity, stability and accuracy of the assay, thereby detecting a specific subset of IgG antibodies that are responsible for fighting COVID-19.

The assay has multifold design advantage as well -

- The assay has been developed under the controlled settings and validated exhaustively to ensure quality.
- The assay is fixed with a stable buffer, to ensure that it can be conveniently stored at 2–8 degrees for a longer period of time.
- The assay does not require specialised manpower or infrastructure for use.

The present version of the kit only uses human plasma/ serum as the sample source, and does not support via secretory swabs. Presently, the kit has only been validated for IgG antibodies, however the kit can be used for the detection of total antibodies (including other classes of IgG), with one additional reagent in the assay. We are validating the performance of the reagent, and then it would be introduced as the subsequent version into the market.

Data availability

Data will be made available on request.
No data was used for the research described in the article.

Funding

None.

CRediT authorship contribution statement

Sneha Kumari: Methodology, Data curation, Writing - original draft. **Anoushka Raina:** Conceptualization, Validation, Supervision, Writing - review & editing. **Dinesh Chandra:** Methodology, Data curation. **Nikita Gupta:** Methodology, Data curation. **Nikki Dey:** Methodology. **Amit Kumar Bhardwaj:** Methodology. **Archana Anthwal:** Methodology. **Vikash C. Mishra:** Conceptualization, Validation, Supervision, Writing - review & editing. **Vimarsh Raina:** Conceptualization, Validation, Supervision, Writing - review & editing. **Aseem K. Tiwari:** Methodology. **Rasika Setia:** Methodology. **A.S. Bhatia:** Methodology.

Declaration of Competing Interest

This article did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Authors declare no conflict of interest. The manuscript has been approved by all the authors and it has been only submitted to this journal.

References

Abu-Raddad, L.J., Chemaitelly, H., Coyle, P., Malek, J.A., Ahmed, A.A., Mohamoud, Y.A., et al., 2021. SARS-CoV-2 antibody-positivity protects against reinfection for at least seven months with 95% efficacy. EClinicalMedicine 35, 100861.
Alandijany, T.A., El-Kafrawy, S.A., Tolah, A.M., Sohrab, S.S., Faizo, A.A., Hassan, A.M., et al., 2020. Development and optimization of in-house ELISA for detection of human IgG antibody to SARS-CoV-2 full length spike protein. Pathogens 9 (10), 803.
Awasthi, A., Vishwas, S., Corrie, L., Kumar, R., Khursheed, R., Kaur, J., et al., 2020. OUTBREAK of novel coronavirus disease (COVID-19): antecedence and aftermath. Eur. J. Pharmacol. 884, 173381.
Bastos, M.L., Tavaziva, G., Abidi, S.K., Campbell, J.R., Haraoui, L.P., Johnston, J.C., et al., 2020. Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. BMJ 370.
Boopathi, S., Poma, A.B., Kolandaivel, P., 2020. Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment. J. Biomol. Struct. Dyn. 1–10.
Casadevall, A., Pirofski, L.A., 2020. The convalescent sera option for containing COVID-19. J. Clin. Invest. 130 (4), 1545–1548.
Ciotti, M., Angeletti, S., Minieri, M., Giovannetti, M., Benvenuto, D., Pascarella, S., Ciccozzi, M., 2019. COVID-19 outbreak: an overview. Chemotherapy 64 (5-6), 215–223.
Du, L., He, Y., Zhou, Y., Liu, S., Zheng, B.J., Jiang, S., 2009. The spike protein of SARS-CoV—a target for vaccine and therapeutic development. Nat. Rev. Microbiol. 7 (3), 226–236.
Espejo, A.P., Akgun, Y., Al Mana, A.F., Tjendra, Y., Millan, N.C., Gomez-Fernandez, C., Cray, C., 2020. Review of current advances in serologic testing for COVID-19. Am. J. Clin. Pathol. 154 (3), 293–304.
González-Stegmaier, R., Cereceda, K., Briones, J.L., Beltran-Pávez, C., Oyarzún-Arrau, A., Riquelme-Barrios, S., et al., 2021. Seroconversion and abundance of IgG antibodies against S1-RBD of SARS-CoV-2 and neutralizing activity in the chilean population. J. Immunol. Res. 2021.
Grau, R.T., Ploper, D., Avila, C.L., Pingitore, E.V., Maldonado, C., Chaves, S., et al., 2021. An In-House ELISA for SARS-CoV-2 RBD uncovers elevated immune response at higher altitudes. medRxiv.
Harvey, R.A., Rassen, J.A., Kabelac, C.A., Turenne, W., Leonard, S., Klesh, R., et al., 2021. Association of SARS-CoV-2 seropositive antibody test with risk of future infection. JAMA Intern. Med. 181 (5), 672–679.
Kohmer, N., Westhaus, S., Rühl, C., Ciesek, S., Rabenau, H.F., 2020. Clinical performance of different SARS-CoV-2 IgG antibody tests. J. Med. Virol. 92 (10), 2243–2247.
Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., et al., 2020. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 581 (7807), 215–220.
Lee, C.Y.P., Lin, R.T., Renia, L., Ng, L.F., 2020. Serological approaches for COVID-19: epidemiologic perspective on surveillance and control. Front. Immunol. 11, 879.
Loeffelholz, M.J., Tang, Y.W., 2020. Laboratory diagnosis of emerging human coronavirus infections–the state of the art. Emerg. Microbes Infect. 9 (1), 747–756.
Ojeda, D.S., Gonzalez Lopez Ledesma, M.M., Pallarés, H.M., Costa Navarro, G.S., Sanchez, L., Perazzi, B., et al., 2021. Emergency response for evaluating SARS-CoV-2 immune status, seroprevalence and convalescent plasma in Argentina. PLoS Pathog. 17 (1), e1009161.
Post, N., Eddy, D., Huntley, C., van Schalkwyk, M.C., Shrotri, M., Leeman, D., et al., 2020. Antibody response to SARS-CoV-2 infection in humans: a systematic review. PLoS One 15 (12), e0244126.
Sharma, A., Tiwari, S., Deb, M.K., Marty, J.L., 2020. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2): a global pandemic and treatment strategies. Int. J. Antimicrob. Agents 56 (2), 106054.
Shereen, M.A., Khan, S., Kazmi, A., Bashir, N., Siddique, R., 2020. COVID-19 infection: origin, transmission, and characteristics of human coronaviruses. J. Adv. Res. 24, 91–98.
Sil, B.K., Jahan, N., Haq, M.A., Oishee, M.J., Ali, T., Khandker, S.S., et al., 2021. Development and performance evaluation of a rapid in-house ELISA for retrospective serosurveillance of SARS-CoV-2. PLoS One 16 (2), e0246346.
Tai, W., He, L., Zhang, X., Pu, J., Voronin, D., Jiang, S., et al., 2020. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cell. Mol. Immunol. 17 (6), 613–620.
World Health Organization, 2020. Laboratory Testing for 2019 Novel Coronavirus (2019-nCoV) in Suspected Human Cases: Interim Guidance, 14 January 2020 (No. WHO/2019-nCoV/laboratory/2020.2). World Health Organization.
Wu, Z., McGoogan, J.M., 2020. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA 323 (13), 1239–1242.
Zilla, M., Wheeler, B.J., Keetch, C., Mitchell, G., McBreen, J., Wells, A., et al., 2021. Variable performance in 6 commercial SARS-CoV-2 antibody assays may affect convalescent plasma and seroprevalence screening. Am. J. Clin. Pathol. 155 (3), 343–353.