Original Research Paper

Volume - 12 | Issue - 12 | December - 2022 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Transfusion Medicine

PERFORMANCE EVALUATION OF TWO ENZYME LINKED IMMUNOASSAYS FOR THE DETECTION OF SARS-COV-2 IGG ANTIBODIES IN WHOLE BLOOD AND PLASMA DONORS IN A REGIONAL BLOOD TRANSFUSION CENTRE

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ABSTRACT **Background** For serosurveillance of SARS-CoV-2 specific antibodies can be tested. Antibodies against SARS CoV 2 can be detected by ELISA.We compared performance of two Enzyme linked Immunoassays in the setting of blood donation including convalescent plasma donation **Methodology** Donor serum samples were collected more than 14 days post-symptom onset or post-initial positive reverse transcriptase PCR (RT-PCR).Serum samples (n=50)were selected from convalescent plasma donors who were recovered from COVID 19 confirmed by chemiluminescence assay .Negative controls(n=41) were selected from donors who never had symptoms of covid and tested seronegative in confirmatory assay **Results** A total of 50 positive convalescent and 41 negative samples were evaluated. ELISA test by Covid Kawach for SARR COV2 identified 47 /50 of the positive samples as positive and 40 /41 of the negative samples as negative resulting following diagnostic characteristics.Sensitivity was 94% and Specificity 97.5%(Table 1).Platelia Total Antibody SARS COV2 assay by M/s Biorad identified 49/50 positive samples and 39/41 negative samples as negative. Sensitivity was 97.92% and Specificity 97.56% **Conclusion** This study indicates acceptable performance of immunoglobulin class G-based serology for SARS-CoV-2-specific antibodies with Covid Kawach microlisa testing developed by J mitra& Co and Biorad Platelia Total antibody ELISA for SARS COV2 for convalescent donor testing

KEYWORDS :

INTRODUCTION

As the coronavirus disease 2019 (COVID-19) pandemic continues to affect countries worldwide, the World Health Organization (WHO) urged health authorities to rigorously test all suspected cases in order to isolate patients and interrupt the transmission chain [1]. The gold standard method for diagnosis of COVID-19 is the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genetic material with real-time PCR[2]. Although RT-PCR is the diagnostic test in early infection , the detection of antibodies is possible once an individual has been infected for atleast 7 days[3]. For serosurveillance of SARS-CoV-2 specific antibodies can be tested. Antibodies against SARS CoV 2 can be detected as early as 4-7 days in approximately 40% of COVID -19 patients, with sero conversion rates reaching > 90% by day 14[4].

Several affected individuals never display symptoms of the disease, resulting in an underestimation of disease incidence and prevalence.Therefore, detection of anti-SARS-CoV-2 IgG antibodies is one of the better approaches available in order to determine the number of affected individuals in the community; the latter is clearly crucial for decision-making to inform public health policies.

In blood banking , serology testing also has other advantages: it is easy to perform on a large scale and shall allow us to ascertain the status of the donor populations with regard to herd immunity(sero survey studies).It also facilitates the selection of best candidate donors (with highest antibody titres) for plasma exchange; & it aids in assessing the efficacy of vaccines that are in development. Due to urgency and demand in the current crisis, a large number of commercial serological testshave been developed and introduced into the global market, but often with insufficient validation on clinical samples.

Traditionally, antibody determination is performed using various techniques such as Enzyme-Linked ImmunoSorbent Assay (ELISA), chemiluminescent immunoassay (CLIA), rapid lateral flow (immunochromatographic) tests or fluorescence Immunoassays (FIA).[5,6,7]

The present study evaluated the performance of two commercial ELISA kits for detecting anti-SARS-CoV-2 IgG antibodies in samples from confirmed COVID-19 patients in this period.The sensitivity, positive predictive value,negative predictive value, positive percent agreement, and Cohen's kappa were measured for each assay using samples collected from SARS-CoV-2 seropositive blood donors.

Covid Kawach IgG Microlisa is intended for qualitative detection of igG antibodies in serum / plasma of patients with SARS CoV -2 infection. Biorad Platelia Total Antibody Kit is used to evaluate IgG+IgM in potentially infected patient.

MATERIALS AND METHODS

Study setting

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Study design

Study design was Diagnostic Test Evaluation .The performance of the ELISA for detecting anti-SARS-CoV-2 IgG antibodies was evaluated as a cross-sectional study. The performance was assessed using convalescent plasma donor samples confirmed by VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG Chemiluminiscence assay(orthoclinical diagnostics)

Sample size and sampling

Donor serum samples were collected more than 14 days post-symptom onset or post-initial positive reverse transcriptase PCR (RT-PCR).Serum samples (n=50)were selected from convalescent plasma donors who were recovered from COVID 19 .Negative controls(n=41) were selected from donors who never had symptoms of covid and tested seronegative in confirmatory assay

Study tools

ELISA kits evaluated

Covid Kawach is an ELISA TEST for detection of IgG Antibodies to COVID-19 in Human serum/plasma.Based on Indirect ELISA principle and Assay time was 130 minutes.Pre dilution of sample was required which was an additional test.Ready to use Enzyme Conjugate was used.Incubation was needed in Humidified chamber.Color coded reagent to monitor procedural steps.Shelf life :12 months at 2-8°C.Sensitivity 96.33% & Specificity 100% was claimed by manufacturer as evaluated by ICMR NIV, Pune.Pack size- 96 Test. Platelia SARS-CoV-2 Total Ab assay was developed for the detection of total anti-nucleocapsid antibodies (IgM, IgA, IgG) to SARS-CoV-2. The assay uses a recombinant SARS nucleocapsid Protein in a one-step antigen capture format assay.

Principle

IgG antibodies from serum / plasma of human will bind to the SARS CoV-2 virus whole cell antigen coated on to the Microtitre plate (ELISA wells). In the next step, anti Human IgG HRP binds to captured human IgG antibodies. Subsequently, chromogenic substrate

(TMB/H2O2) is added, the reaction is stopped by 1N H2SO4. The intensity of color / optical density is measured at 450 nm. The kit was for in- vitro use for monitoring anti-SARS CoV-2 IgG antibodies in human only.

Performance characteristics

The performance of 3 batches of CovidKawach IgG Microlisa has been validated by ICMR, NIV, Pune. Sensitivity : 96.33% Specificity : 100% was obtained .Within-run and between-run precisions have been determined by manufacturer by testing 10 replicates of five specimens : two negative and three weak covid IgG positive. The C.V.(%) of negative and weak positive values were within 10%.For Platelia Total Ab assay, 3 positive specimens and 1 negative specimen were assayed in duplicates by 2 different operators per day during 5 days. Nested ANOVA was used to estimate within run, between run, between days and total precision.The CVs obtained on the positive specimens are less than or equal to 10% for repeatability and less than or equal to 15% for intermediate precision. The diagnostic specificity obtained by manufacturer evaluation was 99.3% (168/168) with a 95% Confidence Interval of 98.3%- 99%.Sensitivity at 2-8 was 92% and 9-22 days was 100%

47 samples taken from convalescent donors which were confirmed positive by Chemiluminescence were used as gold standard positive controls and 41 COVID-19 negative control sample collections taken from the blood donors who never had any covid symptoms and were tested negative in both ELISA and CHLIA served as true negatives. Positive and negative predictive values were also calculated

Statistical analysis

Descriptive statistics were used to describe the study variables. Proportions and frequency were reported for the categorical variables.

Sensitivity (Se) and specificity (Sp) were estimated with 95% Confidence Intervals (CI), based on binomial distribution.A 5% significance level was set for all the analyses. Statistical analysis was carried out using Microsoft Excel and SPSS (version 25.0).Standard deviation (SD) was calculated for mean values and interquartile range (IQR) for median values

RESULTS

A total of 50 positive convalescent and 41 negative samples were evaluated. ELISA test by Covid Kawach identified 47 /50 of the positive samples as positive and 40 /41 of the negative samples as negative resulting following diagnostic characteristics.Sensitivity was 94% and Specificity 97.5%(Table 1).Platelia by Biorad M/s identified 49/50 positive samples and 39/41 negative samples as negative. Sensitivity was 97.92% and Specificity 97.56%

Table 1. Diagnostic Properties of ELISA Kit 1

Statistic	Value	95% CI
Sensitivity	98.00%	89.35% to 99.95%
Specificity	95.12%	83.47% to 99.40%
Positive Likelihood Ratio	20.09	5.20 to 77.67
Negative Likelihood Ratio	0.02	0.00 to 0.15
Positive Predictive Value	69.06%	36.60% to 89.62%
Negative Predictive Value	99.77%	98.40% to 99.97%
Accuracy	95.41%	88.86% to 98.69%

Table 2. Diagnostic Properties of ELISA Kit 2

Statistic	Value	95% CI
Sensitivity	97.92%	88.93% to 99.95%
Specificity	97.56%	87.14% to 99.94%
Positive Likelihood Ratio	40.15	5.79 to 278.36
Negative Likelihood Ratio	0.02	0.00 to 0.15
Positive Predictive Value	81.69%	39.15% to 96.87%
Negative Predictive Value	99.76%	98.38% to 99.97%
Accuracy	97.60%	91.88% to 99.68%

DISCUSSION

The study was conducted to provide information on performance characteristics of commercially available serological assay of immunoglobulin class G-based serology for SARS-CoV-2-specific antibodies with Covid Kawach microlisa testing developed by J mitra& Co. for donor testing. A comparative study of SARS-CoV-2 IgG assays in India evaluated independently against a strategically designated reference standard described sensitivity of Zydus-Kavach,DiaSorin CLIA and RBD ELISA as 75.7 (71.0–79.9),82.6 (78.3–86.2) and 84.7 (80.6–88.1)[8].According to Al Jighefee[9] et al, IgM assays have variable and low sensitivity, thus considered a poor marker for COVID-19 diagnosis. IgG assays can miss at least 8% of RT–PCR-positive cases.In the study by mohammed[10] et al, a sensitivity of 79% for Total Antibody assay by Platelia was reported.We had used convalescent sera as gold standard in our study which were collected from donors with good titres after around 2 weeks of infection which is the peak time for antibodies.The reason for a high sensitivity reported in our study should be viewed in this light,because this may not be equal to the actual diagnostic sensitivity.

CONCLUSION

This study indicates acceptable performance of immunoglobulin class G-based serology for SARS-CoV-2-specific antibodies with Covid Kawach microlisa testing developed by J mitra& Co and Biorad Platelia Total antibody ELISA for SARS COV2 for donor testing when tested against a batch of convalescent sera from covid positive patients. Hence these tests can be used to screen for convalescent donors in the setting of SARS COV2.

REFERENCES

1. Baloch S, Baloch MA, Zheng T, Pei X. The Coronavirus Disease 2019 (COVID-19) Pandemic. Tohoku J Exp Med. 2020 Apr;250(4):271-278
2. Teymouri M, Mollazadeh S, Mortazavi H, Naderi Ghale-Noie Z, Keyvani V, Aghababaei F, Hamblin MR, Abbaszadeh-Goudarzi G, Pourghadamyari H, Hashemian SMR, Mirzaei H. Recent advances and challenges of RT-PCR tests for the diagnosis of COVID-19. Pathol Res Pract. 2021 May;221:153443
3. Tantuoyir MM, Rezaei N. Serological tests for COVID-19: Potential opportunities. Cell Biol Int. 2021 Apr;45(4):740-748
4. Rongqing Z, Li M, Song H, Chen J, Ren W, Feng Y. Early Detection of Severe Acute Respiratory Syndrome Coronavirus 2 Antibodies as a Serologic Marker of Infection in Patients With Coronavirus Disease 2019. Clin Infect Dis. 2020 Nov 19;71(16):2066-2072
5. Horn MP, Jonsdottir HR, Brigger D, Damonti L, Suter-Riniker F. Serological testing for SARS-CoV-2 antibodies in clinical practice: A comparative diagnostic accuracy study. Allergy. 2022 Jul;77(7):2090-2103
6. Chiereghin A, Zagari RM, Galli S, Moroni A, Gabrielli L. Recent Advances in the Evaluation of Serological Assays for the Diagnosis of SARS-CoV-2 Infection and COVID-19. Front Public Health. 2021 Feb 18;8:620222
7. Adams E.R., Anand R., Andersson M.I., Auckland K., Baillie J.K., Barnes E., Bell J., Berry T., Bibi S., Carroll M., et al. Evaluation of antibody testing for SARS-Cov-2 using ELISA and lateral flow immunoassays. medRxiv. 2020 doi: 10. 1101/ 2020.04.15. 20066407
8. Al-Jighefee HT, Yassine HM, Nasrallah GK. Evaluation of Antibody Response in Symptomatic and Asymptomatic COVID-19 Patients and Diagnostic Assessment of New IgM/IgG ELISA Kits. Pathogens. 2021 Feb 3;10(2):161
9. Chaudhuri S, Thiruvengadam R, Chattopadhyay S, Mehdi F, Kshetrapal P, Shrivastava T, Desiraju BK, Batra G, Kang G, Bhatnagar S; DBT India Consortium for COVID-19 Research. Comparative evaluation of SARS-CoV-2 IgG assays in India. J Clin Virol. 2020 Oct;131:104609
10. Mohammed T, Brewer JVV, Pyatt M, Whitbourne SB, Gaziano JM, Edson C, Holodniy M; VA Million Veteran Program COVID-19 Science Initiative. Evaluation of independent self-collected blood specimens for COVID-19 antibody detection among the US veteran population. Diagn Microbiol Infect Dis. 2022 Oct;104(2):115770.