ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Comparison Of Different Methods For Detection Of Ig G Antibodies Against The Spike (S1) Receptor-Binding Domain (RBD) Of SARS-COV-2.

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**ABSTRACT**

The aim of this study was to know, the antibody response following infection with SARS-CoV-2 virus and post vaccination using kits from two different manufacturers and on two different platforms were carried out. This study was conducted by the department of Microbiology, over a period of 4 months from 18 January to May 2021 from the samples received in Neuberg Ehrlich Laboratory. Samples from 50 vaccinated (Covishied) subjects were taken for this study. We found that the quantitative assay was more sensitive than the qualitative assay in detecting the Ig G antibodies against the spike receptor-binding domain (RBD) of SARS-CoV-2.

**Keywords:** Covishield vaccine, chemiluminescent immunoassay (CLIA), chemiluminescent microparticle immunoassay (CMIA), Plaque reduction neutralization tests (PRNT).

https://doi.org/10.33887/rjpbcs/2022.13.3.14

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ISSN: 0975-8585

## INTRODUCTION

Following infection or vaccination, the concern is to identify the presence of antibodies that could be protective for variable periods of time. A surrogate for neutralization antibodies are antibodies specific for RBD [1- 3]. To detect the presence of viral neutralising antibodies that are protective in subjects post infection and post vaccination, serological tests, that identify the antibodies specific for the Spike potein of the virus that binds to the RBD were used.

## METHODS

In this study, we compared SARS-CoV-2 IgG II Quant assay by CMIA method (quantitative) and SARS-CoV-2 Ig G assay by CLIA method (qualitative) for the determination of Ig G antibodies against the spike receptor-binding domain (RBD) of SARS-CoV-2.

50 people who had taken two doses of Covishield vaccine , developed by the Oxford-AstraZeneca and manufactured by the Serum Institute of India (SII), using a chimpanzee adenovirus – ChAdOx1 – that had been modified to enable it to carry the COVID-19 spike protein into the cells of humans were considered.

Serum samples were obtained from whole blood after centrifugation at 3500 rpm for 5 minutes. Both were automated, two-step immunoassays. The anti–SARS-CoV-2 antibody response against the spike protein was assessed at 28 days after second dose of vaccine .Results were assessed in conjunction with clinical history and presentation.

The SARS-CoV-2 Ig G II Quant assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of Ig G antibodies to SARS-CoV-2 in human serum. Sample, SARS-CoV-2 antigen coated paramagnetic micro particles, and assay diluents are combined and incubated. The Ig G antibodies to SARS-CoV-2 present in the sample bind to the SARS-CoV-2 antigen coated micro particles. The mixture is washed. Anti-human Ig G acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added. The resulting chemiluminescent reaction is measured as a relative light unit (RLU). There is a direct relationship between the amount of Ig G antibodies to SARS-CoV-2 in the sample and the RLU detected by the system optics. Titers < 50.0AU/ml are considered as Negative and ≥ 50.0 AU/ml are considered as Positive.

The SARS-CoV-2 Ig G assay is a chemiluminescent immunoassay (CLIA) intended for the qualitative detection of IgG antibodies to SARS-CoV-2 in human serum. A sample is added to a reaction vessel with buffer, and paramagnetic particles coated with recombinant SARS-CoV-2 protein specific for the receptor binding domain (RBD) of the S1 protein. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field, while unbound materials are washed away. A monoclonal anti-human Ig G alkaline phosphatase conjugate is added and the conjugate binds to the Ig G antibodies captured on the particles. A second separation and wash step remove unbound conjugate. A chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is compared to the cut-off value. Values ≤ 0.80 S/CO is Non-Reactive, > 0.80 to < 1.00 S/CO is Equivocal and ≥ 1.00 S/CO is Reactive.

## RESULTS

The results of 50 people, who had taken two doses of covishield vaccine, were tested for Ig G antibodies against the spike (S1) RBD of SARS-CoV-2 by two methodologies, SARS-CoV-2 IgG II Quant assay by CMIA which was quantitative assay and SARS-CoV-2 Ig G assay by CLIA which was qualitative assay are tabulated in Table1,2,3and 4. Results were correlated with clinical history.

Out of 50 samples, all were detected positive by SARS-CoV-2 IgG II Quant assay by CMIA. Out of 50 samples, 38 were reactive, 2 were equivocal and10 nonreactive by SARS-CoV-2 IgG assay by CLIA. Out of 10 non reactive, 2 samples were in early infection at the time of testing, which was nonreactive by SARS-CoV-2 IgG assay by CLIA.

ISSN: 0975-8585

**Table 1: Comparison of levels of Ig G antibodies against the S1 RBD of SARS-CoV-2 by quantitative and qualitative assay among who have vaccinated by 2 doses of Covishield tested 28 days after 2 dose.**

| SL NO | SARS-CoV-2 IgG II Quant assay by CMIA method (qualitative and quantitative) CUT OFF < 50.0AU/ml - Negative ≥ 50.0 AU/ml - Positive. | SARS-CoV-2 IgG assay by CLIA (qualitative) CUT OFF REACTIVE : >1 EQUIVOCAL : >0.80- <1.00 NON REACTIVE : <0.80 |
|---|---|---|
| 1 | 18794.6 | 21.67 |
| 2 | 19000 | 35.80 |
| 3 | 20543.3 | 22.33 |
| 4 | 11996.5 | 45.60 |
| 5 | 9719.3 | 14.74 |
| 6 | 7166.7 | 31.24 |
| 7 | 6542.6 | 16.23 |
| 8 | 6542.6) | 27.02 |
| 9 | 9540.6 | 15.27 |
| 10 | 12,584.5 | 24.29 |
| 11 | 10,549.3 | 19.96 |
| 12 | 5473.7 | 10.58 |
| 13 | 5473.7 | 26.72 |
| 14 | 18262.8 | 27.73 |
| 15 | 9770.7 | 27.69 |
| 16 | 13197.4 | 47.42 |
| 17 | 51890 | 60.57 |
| 18 | >80000.0 | 69.08 |
| 19 | 547.2 | 1.79 |
| 20 | 1747.4 | 5.97 |
| 21 | 6508.6 | 19.79 |
| 22 | 1930.1 | 7.59 |
| 23 | 2122.3 | 5.28 |
| 24 | 2431.6 | 2.80 |
| 25 | 2584.6 | 11.20 |
| 26 | 1174.3 | 4.05 |
| 27 | 2926.3 | 4.56 |
| 28 | 529.7 | 1.05 |
| 29 | 1415.6 | 3.06 |
| 30 | 2872.2 | 9.00 |
| 31 | 4246.8 | 7.01 |
| 32 | 2351.4 | 11.28 |
| 33 | 781.6 | 1.58 |
| 34 | 367.3 | 1.21 |
| 35 | 813.1 | 1.84 |
| 36 | 1792.5 | 17.20 |
| 37 | 813.1 | 4.88 |
| 38 | 1356.9 | 4.02 |
| 39 | 167.5 | 0.83 |
| 40 | 254.4 | 0.82 |
| 41 | 222.6 | 0.37 |
| 42 | 290 | 0.69 |
| 43 | 122.0 | 0.23 |
| 44 | 451.4 | 0.64 |
| 45 | 6542.6 | 0.34 |
| 46 | 117.8 | 0.19 |
| 47 | 204.3 | 0.51 |

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| | | |
|---|---|---|
| 48 | **1415.6** | **0.37** |
| 49 | **110.3** | **0.30** |
| 50 | **352.2** | **0.60** |

# Sl no 1 -18: Infected before vaccination, Sl no 19 -48 vaccinated and not infected, Sl no 49-50: Infected after vaccination and Sl no 39 -40: Equivocal by qualitative assay.

**Table2: Comparison of levels of Ig G antibodies against the S1 RBD of SARS-CoV-2 among those infected before vaccination by quantitative and qualitative assay.**

| SL NO | SARS-CoV-2 IgG II Quant assay by CMIA method (qualitative and quantitative) CUT OFF < 50.0AU/ml - Negative ≥ 50.0 AU/ml - Positive. | SARS-CoV-2 IgG assay by CLIA (qualitative) CUT OFF REACTIVE : >1 EQUIVOCAL : >0.80- <1.00 NON REACTIVE : <0.80 |
|---|---|---|
| 1 | **18794.6** | **21.67** |
| 2 | **19000** | **35.80** |
| 3 | **20543.3** | **22.33** |
| 4 | **11996.5** | **45.60** |
| 5 | **9719.3** | **14.74** |
| 6 | **7166.7** | **31.24** |
| 7 | **6542.6** | **16.23** |
| 8 | **6542.6** | **27.02** |
| 9 | **9540.6** | **15.27** |
| 10 | **12584.5** | **24.29** |
| 11 | **10549.3** | **19.96** |
| 12 | **5473.7** | **10.58** |
| 13 | **5473.7** | **26.72** |
| 14 | **18262.8** | **27.73** |
| 15 | **9770.7** | **27.69** |
| 16 | **13197.4** | **47.42** |
| 17 | **51890** | **60.57** |
| 18 | **>80000.0** | **69.08** |

Out of 18 samples, antibodies were detected in all samples by both the methods.

**Table 3: Comparison of levels of Ig G antibodies against the S1 RBD of SARS-CoV-2 among those vaccinated and not infected by quantitative and qualitative assay.**

| SL NO | SARS-CoV-2 IgG II Quant assay by CMIA method (qualitative and quantitative) CUT OFF < 50.0AU/ml - Negative ≥ 50.0 AU/ml- Positive. | SARS-CoV-2 IgG assay by CLIA (qualitative) CUT OFF REACTIVE : >1 EQUIVOCAL : >0.80- <1.00 NON REACTIVE : <0.80 |
|---|---|---|
| 1 | **547.2** | **1.79** |
| 2 | **1747.4** | **5.97** |
| 3 | **6508.6** | **19.79** |
| 4 | **1930.1** | **7.59** |
| 5 | **2122.3** | **5.28** |
| 6 | **2431.6** | **2.80** |
| 7 | **2584.6** | **11.20** |
| 8 | **1174.3** | **4.05** |
| 9 | **2926.3** | **4.56** |
| 10 | **529.7** | **1.05** |
| 11 | **1415.6** | **3.06** |

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| | | |
|---|---|---|
| 12 | **2872.2** | **9.00** |
| 13 | **4246.8** | **7.01** |
| 14 | **2351.4** | **11.28** |
| 15 | **781.6** | **1.58** |
| 16 | **367.3** | **1.21** |
| 17 | **813.1** | **1.84** |
| 18 | **1792.5** | **17.20** |
| 19 | **813.1** | **4.88** |
| 20 | **1356.9** | **4.02** |
| 21 | **167.5** | **0.83** |
| 22 | **254.4** | **0.82** |
| 23 | **222.6** | **0.37** |
| 24 | **290** | **0.69** |
| 25 | **122.0** | **0.23** |
| 26 | **451.4** | **0.64** |
| 27 | **6542.6** | **0.34** |
| 28 | **117.8** | **0.19** |
| 29 | **204.3** | **0.51** |
| 30 | **1415.6** | **0.37** |

Out of 30 samples, quantitative assay were positive in all 30. In qualitative assay, 20 were reactive, 2 were equivocal and 8 were non reactive.

**Table 4: Comparison of levels of Ig G antibodies against the S1 RBD of SARS-CoV-2 among those infected after vaccination by quantitative and qualitative assay.**

| **SL NO** | **SARS-CoV-2 IgG II Quant assay by CMIA method** (qualitative and quantitative) **CUT OFF < 50.0AU/ml - Negative ≥ 50.0 AU/m l-Positive.** | **SARS-CoV-2 IgG assay by CLIA** (qualitative) **CUT OFF REACTIVE : >1 EQUIVOCAL : >0.80-<1.00 NON REACTIVE : <0.80** | **HISTORY** |
|---|---|---|---|
| 1 | **110.3** | **0.30** | VACCINATED AND INFECTED AT THE TIME OF TESTING(SARS Ig M WAS POSITIVE) |
| 2 | **352.2** | **0.60** | VACCINATED AND INFECTED AT THE TIME OF TESTING(RT PCR WAS POSITIVE), HAD FEVER |

Out of 2 samples, all were detected positive by quantitative assay. 2 samples were non reactive, who were infected after vaccination and were in acute infection at the time of testing by qualitative assay.

## DISCUSSION

Limitation of this study is small sample size. Plaque reduction neutralization tests (PRNT) are used to quantify the titer of neutralizing antibodies for a virus, which is gold standard test, but it is time consuming, expensive and needs BSL3. So there is need for fast, automatable and affordable serological tests that can quantify the titer of neutralizing antibodies, which gives an idea of the immune status of an individual.

As the antibody titer was high in those 18 samples, from subjects who had acquired the infection prior to vaccination, both the assays were able to identify them. Out of 50, 10 samples were negative by SARS-CoV-2 Ig G qualitative assay by CLIA.

A negative result can occur if the quantity of antibodies for the SARS-CoV-2 virus present in the specimen is below the detection limit of the assay. Out of 10 samples, 2 were in early infection at the time of testing. The sensitivity of the SARS-CoV-2 Ig G qualitative assay by CLIA, early after infection is unknown. For the two samples with equivocal results, the subjects were advised to repeat the test after two weeks.

The SARS-CoV-2 IgG II Quant assay by CMIA method detected the specific antibodies in all people who were infected before vaccination, those who were vaccinated, and those who got infected after vaccination.

## CONCLUSION

To understand the vaccine efficacy, serological testing to detect anti-SARS-CoV-2–specifc antibodies is important. We conclude that, there is a need for quantitative assay for quantitative measurement the antibody levels in the blood post vaccination rather than the qualitative test which distinguish between positive and negative results [4]. This study plays a valuable role for choosing the better serological testing method.

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