

## Study of Antibody-based Rapid Card Test in COVID-19 Patients Admitted in a Tertiary Care COVID Hospital in Southern Rajasthan

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### Abstract

**Background:** COVID-19 also known as SARS CoV-2, is now a pandemic which started in December 2019 in China. RT-PCR based nucleic acid detection is currently the standard diagnostic method for COVID-19, but certain shortcomings make it unfeasible for use as a screening test.

**Aims and objectives:** To study the dynamics of IgM and IgG antibody, establish its role in diagnosis and prognosis of COVID-19 patients.

**Methods:** It was a cross-sectional study conducted over 100 RT-PCR confirmed COVID-19 patients admitted in various wards of a dedicated Corona hospital, RNT Medical College, Udaipur, Rajasthan, India over a period of 2 months – from April 2020 to May 2020.

**Results:** We performed an anti-SARS-CoV-2 IgG/IgM test on 100 confirmed COVID-19 patients and found that 61% patients had antibody positivity. Dynamics of antibody show that seroconversion, peaking, and disappearance of IgM antibody occur at end of 1st week, 2nd week, and 3rd week respectively, while for IgG seroconversion was seen at the end of 2nd week, and was persistently positive up to 32nd day of illness in our study. Patients with development of anti-SARS-CoV-2 antibodies had a mild degree of illness with positive outcome and vice versa.

**Conclusion:** Our study concludes that serological responses have been observed in COVID-19 patients, and the dynamic pattern of these responses is consistent with acute viral infection which is useful to see the immune status of these patients and diagnosis of COVID-19.

**Keywords:** COVID-19, RT-PCR, antibody-based card test.

### Introduction

Several cases of pneumonia of unknown aetiology have been reported from Wuhan, Hubei province, China, in December 2019<sup>1</sup>. It was later named COVID-19 (corona virus disease 2019) after genomic sequencing. COVID-19 also known as SARS CoV-2 is caused by an enveloped, positive sense, single-stranded RNA with a size varying between 26 kb and 32 kb<sup>2</sup>. The name corona was derived from the Latin word coronae or crown which means a coloured circle around a luminous body such as sun or moon. Current evidence suggests spread to humans occurred via transmission from wild animals illegally sold in the Huanan Seafood Wholesale Market<sup>3</sup>.

Up till now, 6 human corona virus species were known among which HCoV 229E, HCoV OC43, HCoV NL63, and HKU1 cause only mild upper respiratory disease<sup>4</sup>. Two recent

outbreaks of beta corona viruses had seen, causing epidemics were SARS-CoV and MERS-CoV. In 2003, the world had experienced the Severe Acute Respiratory Syndrome (SARS) caused by a new corona virus (SARS-CoV) whose outbreak started in Guangdong, South China, resulting in 8,700 cases and 744 deaths with case fatality rate of 9.5%<sup>5</sup>. In June 2012, another respiratory illness outbreak occurred named as MERS (middle-east respiratory syndrome) started in Saudi Arabia with the total of 2,040 cases and 712 deaths with case fatality rate of 34.90%<sup>6</sup>.

WHO declared COVID-19 as a pandemic on 11th March, 2020, and in India the first case was reported on 30 January, 2020 from Kerala. As on 01 June 2020, the total number of cases reported was 6,057,853 with 3,71,166 deaths worldwide, whereas in India the total number cases was 1,90,535 with 5,394 deaths<sup>7</sup>.

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Transmission of the corona virus is usually via airborne droplets to the nasal mucosa in closed environments and through close contact between people and touching contaminated surfaces, with an incubation period of 2 - 14 days and basic reproduction ratio of 2.2<sup>8</sup>. It causes a spectrum of clinical features which ranges from asymptomatic or mild symptom such as cough, cold, sore throat to acute respiratory distress syndrome, and multi-organ failure. COVID-19 affects people of all ages, but older people (more than 60 years) and those with underlying medical illness are at higher risk of getting severe infection with the death rate at 3.4%. Poor prognosis calculated by Mulbsta score<sup>9</sup>.

Diagnosis of COVID-19 is clinically done by history of contact and clinical features, while laboratory confirmation is done by RT-PCR (reverse transcription polymerase chain reaction) based oropharyngeal swab testing.

RT-PCR based nucleic acid detection is currently the standard diagnostic method for COVID-19, but certain shortcomings mentioned below make RT-PCR unfeasible to use as a screening test, which limit our COVID-19 containment efforts:-

1. Long turnaround time (2 - 3 hour).
2. The RT-PCR tests require certified laboratories, expensive equipment, and trained technicians to operate.
3. High false-negative results.
4. Expensive costing around INR 4,500 (Indian rupees) per test.

Therefore, there is an urgent need for a rapid, simple, sensitive, cheaper, and accurate test to quickly diagnose COVID-19 infected patients and to see the immune status, and to establish usefulness in prognosis and outcome of these patients.

## Aims and objectives

1. To study antibody status in RT-PCR confirmed COVID-19 patients.
2. To establish timeline of IgM and IgG in RT-PCR confirmed COVID-19 patients.
3. To establish relation between antibody appearance and clinical features, severity, and outcome in RT-PCR confirmed COVID-19 patients.

## Material and methods

### Definition of confirmed case

A person with laboratory confirmation of COVID-19 infection, irrespective of clinical signs and symptoms.

## RT-PCR testing

Oropharyngeal/nasopharyngeal swab samples were taken of suspected patients and transported via virus transport medium (VTM) for RNA extraction. In the microbiology lab, the sample is tested by RT-PCR based technique as directed by the National Institute of Virology, Pune.

## Antibody-based card testing

**Principle:** Immune chromatographic assay.

**Test kit:** Provided by SIDAK Life Care Ltd.

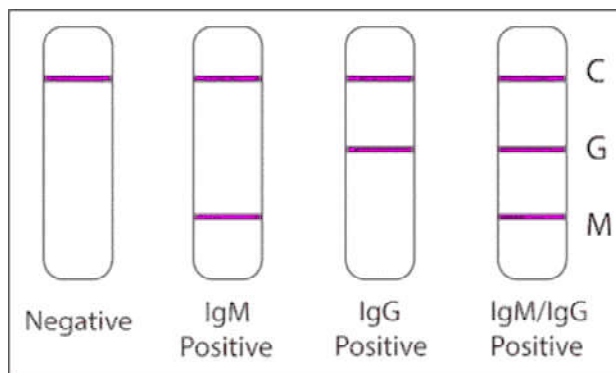
### Procedure:

1. Place test kit on flat surface.
2. Load 2 drops of blood into the sample well, then add 1-2 drops of buffer.
3. Interpret the result at 15 - 20 minutes.

**Interpretation of results:** as shown in Fig. 1.

1. Negative Result: if only C band is visible. The absence of any pink line in zones 1 and 2 indicate that no antibodies are present.
2. Positive result:
  - a. Along with C band if band at zone 1, indicates presence of IgM antibodies.
  - b. Along with C band if band at zone 2, indicates presence of IgG antibodies.
  - c. Along with C band if band at both zones 1 and 2, indicates presence of both IgM and IgG antibodies.
3. Invalid

If C band does not appear, the assay is invalid.



**Fig. 1:** Showing interpretation of antibody-based rapid card test.

## Data collection and study design

It was a cross-sectional study conducted over 100 RT PCR confirmed positive. COVID-19 patients admitted in various wards of the corona dedicated hospital, RNT Medical

College, Udaipur, Rajasthan, India, over a period of 2 months – from April 2020 to May 2020.

### Inclusion criteria

1. All RT PCR confirmed symptomatic and asymptomatic COVID-19 patients above the age of 18 years.

### Exclusion criteria

1. COVID-19 patients already on chronic steroid, immunosuppressants and chemotherapy.
2. Known case of PLHA and other immune-deficient diseases.
3. COVID-19 patients not giving consent for study.

All these patients included in the study were evaluated by a set protocol in the form of detailed history, physical examination, systemic examination if needed.

All patients underwent routine investigations which included complete blood count, renal function test, liver function test, chest X-ray, electrocardiography.

These patients were divided into 4 groups according to duration of illness, i.e., 0 - 7 days, 8 - 14 days, 15 - 21 days, > 21 days.

### Data analysis

The collected data were entered in a Microsoft Excel Sheet. Graphs and tables were generated using Microsoft Word and Microsoft Excel. Quantitative data were studied using Mean, Median, Mode and Standard Deviation (SD).

### Results and observation

In our study, out of 100 patients 45 were male and 55 were female having mean age of 37 years with 22 ( 22 %) patients having co-morbidities which included type 2 diabetes mellitus, thyroid disorder, hypertension, and malignancy. In view of residence, 88 patients (88 %) were from urban locality while the rest of 12 (12%) were from rural areas. Clinical features as depicted in Fig. 2, show that 76 patient were asymptomatic and diagnosed because of contact tracing. In 24 symptomatic patient, 6 (25%) had cough, 5 (20%) had fever, 5 (20%) had both cough and fever, 7 (30%) patients had shortness of breath, 1 (5%) had other features.

Table I shows that out of 100 patients, 61 tested positive and 39 negative for antibodies. Among 61 antibody positive patients, IgM positivity is seen in 21 patients (34%), IgG positivity in 30 patients (49%), both IgM and IgG positivity in 10 patients (17%).

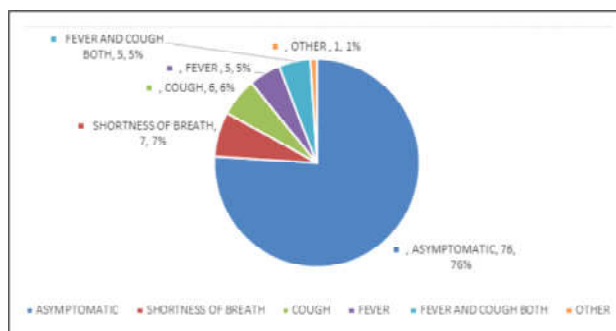


Fig. 2: Clinical presentation of COVID-19 patient.

Table I: Categorisation of total patients tested according to positivity.

S.No	Category of patients	Number	%
1.	Total number of patients tested	100	
2.	Total patients having positivity	61	
1.	IgM positive patients	21	34
2.	IgG positive patients	30	49
3.	Both IgM and IgG positive patients	10	17

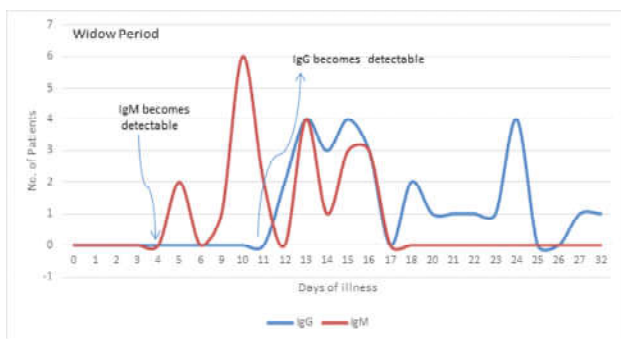
Table 3 and Fig. 3 show that between 0 to 7 days of illness, out of 23 patient tested only 2 (8.69%) patients developed IgM antibody and none of them had IgG antibody test positive. In the next 7 days (between 8 - 14 days) of illness, among 27 patients tested, 11 (40.2%) patients developed IgM and 9 patients (33.3%) developed IgG. In 15 - 21 days group of illness, out of 36 patients tested 8 (22.2%) developed IgM where rest 12 patients (33.3%) developed IgG and 10 patients (27.7%) developed both IgM and IgG antibody. In the later days of illness, i.e., beyond 21 days, on testing 14 patients, only 9 patients (64.2%) developed IgG and no one had IgM antibody.

Table II: Timeline of IgM and IgG antibody with duration of illness.

Days of illness	Total patients tested	Patients having IgM positive	Patients having IgG positive	Patients having both IgM and IgG positive	Total patients positive
0 - 7	23	2 (8.69%)	0 (0%)	0 (0%)	2 (8.69%)
8 - 14	27	11 (40.2%)	9 (33.3%)	0 (0%)	20 (74%)
15 - 21	36	8 (22.2%)	12 (33.3%)	10 (27.7%)	30 (83.3%)
> 21	14	0 (0%)	9 (64.2%)	0 (0%)	9 (64.2%)

Table III given below showing correlation between IgM and IgG antibody with severity of disease.

Out of 100 patients studied, 76 patients were in the asymptomatic group where 17 patients were in the mild-



**Fig. 3:** Timeline of antibody response in COVID-19 patient.

to-moderate symptom group and 7 patients were in the severe symptom group; when studied further, it was found that antibody positivity in these group was 65%, 59% and 29% respectively. In respect to antibody type specific, IgM antibody positivity in asymptomatic, mild-to-moderate and severe group was 26%, 5.5% and 0%, while for IgG it was 26%, 48% and 29% and for both IgM and IgG it was 13%, 5.5%, 0% respectively.

**Table III: Antibody positivity and disease severity.**

S. No	Disease severity	Total patients	IgM positive	IgG positive	Both IgM and IgG positive	Total positive	Total negative
1.	Asymptomatic	76	20 (26%)	20 (26%)	9 (13%)	49 (65%)	27 (35%)
2.	Mild-to-moderate	17	1 (5.5%)	8 (48%)	1 (5.5%)	10 (59%)	7 (41%)
3.	Severe	7	0 (0%)	2 (29%)	0 (0%)	2 (29%)	5 (71%)

Table IV shown below depicts the co-relation between disease severity, antibody response and outcome of these patients in the form of death or discharge. In asymptomatic, mild and moderate symptom group, total antibody response was found 65% and 59% respectively and outcome was noted in form of 100% discharged, whereas in severe symptom group, antibody response was only 29% and major outcome was death (5 out of 7 patient studied) which was 71%.

**Table IV: Correlation between disease severity, antibody response, and outcome.**

S. No	Disease severity	Total number of patients	Patients with antibody response positive	Outcome (death/discharge)
1.	Asymptomatic	76	49 (65%)	100% discharge
2.	Mild-to-moderate	17	10 (59%)	100% discharge
3.	Severe	7	2 (29%)	5 deaths (71%)

## Discussion

Started as an epidemic in China, and then spreading to

other countries quickly, COVID-19 pandemic is an unexpected global health issue. Day by day COVID-19 information and understanding about its diagnosis, treatment and containment is changing.

Our study was aimed mainly to observe the antibody response, its dynamics and its clinical application in COVID-19 patients.

In the present study, a total of 100 RT-PCR confirmed cases were taken, out of which 45 (45%) were males whereas 55 (55%) were females with a median age of 37 years. Huang *et al*<sup>10</sup> did a similar type of study and found mean age of patients to be 49 years which was not resembling with our study due to the sample size which was smaller in our case. Among 100 patients studied, 76% patients were asymptomatic and the rest 24% patients were in the symptomatic group. The study done by Tian *et al*<sup>11</sup> and Wang *et al*<sup>12</sup> demonstrated 90 - 95% patients were symptomatic. These studies contradict the present study because of the fact that the clinical presentation, severity, and mortality varies from country to country and region to region because of the different viral strain, immunity status, age, co-morbidities, and various other epidemiological factors among peoples, which are not known to us.

The present study was carried-out over 100 patients. 61 (61%) patients had positive antibody response whereas 39 (39%) patients had no antibody response. Among these 61 antibody positive patients, IgM positivity was seen in 21 patient (34%), IgG positivity was in 30 patients (49%) and both IgM and IgG antibody positivity was in 10 patients (17%). Long *et al*<sup>13</sup> observed antibody response in 96.8% patients and the proportion of patients with positive virus-specific IgM and IgG seen in 94%, and 100% patients respectively. Higher percentage of positivity in the Long *et al* study is because of multiple times antibody testing in a single patient during the course of illness while we conducted it only a single time.

The present study was carried-out in 100 COVID-19 patients and correlated with duration of illness and antibody response. These patients were grouped according to duration of illness, i.e., 0 to 7 days, 8 - 14 days, 15 - 21 days and more than 21 days respectively. Between 0 to 7 days of illness out of 23 patients tested only 2 (8.69%) patients developed IgM antibody. In next 7 days (between 8 - 14 days) of illness among 27 patients tested, 11 (40.2%) patients developed IgM and 9 patients (33.3%) developed IgG. In 15 - 21 days group of illness, out of 36 patients tested 8 (22.2%) developed IgM where the rest 12 patients (33.3%) developed IgG, and 10 patients (27.7%) developed both IgM and IgG antibody. In the later days of illness, i.e., beyond 21 days, on testing 14 patients, only 9 patients (64%) developed IgG whereas no one had IgM antibody.

Regarding antibody response and peaking, it was observed that IgM appearance was noted after 7 days with peak in between 10 to 14 days, and IgM disappeared by the end of 3rd week, while IgG seroconversion was seen at the end of 2nd week and it persistently remained positive up to 32nd day of illness. Similar type of results were observed by a study done by Hou *et al*<sup>14</sup>.

Comparing antibody positivity with severity of illness and outcome, we observed that, as severity of illness increases antibody detection decreases and outcome worsens, i.e., % of antibody positivity in asymptomatic, mild and severe disease is 65%, 59% and 29% respectively and outcome was observed in form of 100% discharged in asymptomatic, and mild/moderate symptomatic group whereas 71% mortality observed in severe symptomatic group in which antibody response was only 29%.

In respect to antibody type specific, IgM antibody positivity in asymptomatic, mild-to-moderate and severe group is 27%, 5.5% and 0% while for IgG it is 26%, 47% and 29% and for both IgM and IgG it is 12%, 5.5%, 0% respectively which is contrary to the study conducted by Hou *et al* in which mild, severe and critical groups IgM was detected in 81.3%, 82.9% and 82.7% of cases, IgG was detected in 90.6%, 92.7% and 88% of cases, and both IgM and IgG were detected in 79.7%, 77.9% and 80% of cases.

Our results may explain that patients who develop protective antibody IgM and IgG had a mild severity, early RT-PCR negativity, short course of illness, rapid recovery and better outcome while vice versa for those who do not develop antibody. The significance of antibody response in COVID-19 is important, not only in the diagnosis but also prognosis. Specific antibodies, including IgG antibodies and neutralising antibodies, are important for protecting the host from infection by blocking viral entry into host cells after viral infection<sup>15</sup>.

As greatly said "Sky has no limit" this study is our little contribution from our clinical experience to add to the literature of COVID-19, a novel disease with an evolving course.

## Conclusion

From the present study, we conclude that like other viral diseases, significant antibody response was observed in COVID-19 patients and its positive response can be directly correlated with severity and outcome of disease. Further antibody detection by rapid card may be a useful diagnostic

tool which provide valuable information regarding diagnosis, severity, and outcome of COVID-19 patients. Moreover, it is a less expensive, less time consuming, easy to perform, and least cumbersome tool in this pandemic scenario.

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