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Original Research Article

Clinical evaluation of a rapid antibody test kit "iFinDx COVID-19 IgM/IgG" for the diagnosis of SARS-CoV2 infection

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ABSTRACT

The disease COVID-19 caused by SARS-CoV-2 that led to serious health problems since late 2019, and WHO announced as pandemic. The only diagnostic strategy available during pandemic was RT PCR used as the gold standard method, due to its high throughput procedure and high cost, the screening of patients would be difficult. Hence the antigen and antibody detecting rapid diagnostic kits were developed, that can be used as the point of care testing. The assessment of iFInDx COVID-19 IgM/IgG test kit was evaluated the sensitivity and specificity of the diagnostic kit with a total samples of 427 compared with ELISA and RT PCR method.

1. Introduction

The novel COVID-19 infectious disease became a pandemic in early 2020s which was caused by the virus SARS-CoV-2 virus. Since its emergence and its wide spread nature across the countries very rapidly, the WHO declares the infection as pandemic infection of severe respiratory disease and it was named by international taxonomy on viruses.[1] The most common symptoms of COVID-19 patients comprise of fever, cough, breathlessness, and dyspnea. Among these cases, SARS-COV-2 infection can lead to pneumonia, kidney failure, and even more death.[2]

The spread of disease can be highly controlled by rapid diagnosis in accuracy. SARS-COV-2 is an RNA virus that can be diagnosed by molecular methods reverse transcriptase-polymerase chain reaction (RT-PCR) that can be referred as gold standard method as reference method.[3]

Apart from RT-PCR test, antibody testing can also have complementary role. The role of antibodies IgM and IgG as latent markers for COVID-19 has been evaluated and they may be act as a reliable target for COVID-19 diagnosis. The immune system produces specific antibodies (IgM and IgG) during viral. The antibody IgM can become detectable during the first week and second week of illness after the onset of symptoms. Similarly, IgG antibodies toward different SARS-CoV-2 antigens first become detectable during the third week; more than 90% of patients with mild or severe COVID-19 have detectable IgG antibodies.[1,2]

The antibody tests that have been produced based on recombinant nucleocapsid (N), spike (S), S1 subunit, or receptor binding domain (RBD) SARS-CoV-2 should need to have high sensitivity and specificity in diagnosis. This is also important that may suggest the persons with previous asymptomatic or mild SARS-CoV-2 infections may have a weaker antibody response to SARS-CoV-2 than moderately to severely ill patients.[2]

The sensitivity of gold-standard RT PCR method is usually depending on the pre-analytical, analytical, sampling and transportation of samples. The recent studies also revealed that the serological test were used as only

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an additional aid for molecular testing which showed negative repeatedly but suspecting the infection for positive. Similarly in immune-competent patients, the rise of IgA and IgM antibodies can be found in the acute phase of infection and IgG appears at the later phase of infections, which helps the time of sample collection for molecular testing and also help for the treatment.[4,5]

In addition to this, the viral antibody studies showed that the specific antibody IgM of SARS CoV-2 level would drop by 50% in five months and IgG can also last upto 8 to 24 months after infection. These finding would make interest in developing new diagnostic techniques to control the epidemics.[6,7]

Since the emergence of detecting the antibodies of SARS-CoV-2, the point of care (POC) testing has been the rising concern for the laboratory technicians and clinicians. The development of antibodies IgG and IgM can detect the asymptomatic and acute phase infections within 10 minutes by rapid diagnostic tests (RDT), also they can be used to identify the patients has undergone infections and developed antibodies. Hence the present study would describe the clinical evaluation of iFInDx COVID-19 IgM/IgG test with standard reference test as ELISA test and RT PCR.

2. Materials and Methods

The assessment of diagnostic accuracy of iFInDx COVID-19 IgM/IgG test kit was compared to standard comparator RT-PCR test of COVID-19 with COVID-19 IgM/IgG ELISA method from the multiple sites of Rao's pathlab, Salem. The samples were collected from the patients with signs and symptoms of COVID-19. The study was reviewed and approved by institutional review board.

A total of 427 subjects were included in this study, of which 206 COVID-19 positive and 221 COVID-19 negative patients were included in this study, samples collected were whole blood, serum or plasma to detect the COVID antibody for the evaluation by RDT and ELISA method. Nasopharyngeal / Oropharyngeal swabs were collected and stored in Viral Transport Media (VTM) and tested with reference assay as RT-PCR.

2.1. RT – PCR

The VTM samples were collected and subjected to extract viral RNA by manual method, the extracted RNA then tested for the presence of SARS-CoV-2 confirmatory genes such as N-gene and Rdrp gene by Qiagen QiAquant 96 5 Plex RT-PCR machine. The amplification curves and Ct-values were noted.

2.2. iFInDx COVID-19 IgM/IgG test method

Allow the test strip to equilibrate to room temperature, 10μl of serum, or plasma or whole blood sample were dropped in the sample area on the strip. Then 2 drops of diluents were dropped on the same sampling area. The results were read after 15-20 minutes (Figure 1).

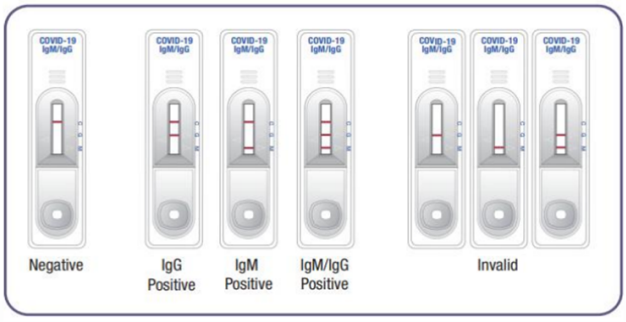

Fig. 1: Interpretation of test results of iFinDx COVID-19 IgM/IgG

2.3. ELISA

Positive, negative and cut-off controls were also tested in duplicate along with serum or plasma samples. Dilute 1:100 of all the samples with Sample Dilution Buffer for SCoV-2. The 50 μl of diluted samples and controls were dispersed in the SCoV-2 Antigen Coated Microtiter Strip plate (ELISA plate). The plates were incubated in dark at 37°C for 1 hour. After incubation the plates were washed with 1X wash buffer for 6 times with 300μl for each well.

The 50μl of 1X conjugate buffer was added to the each well and again the plates were incubated in dark at 37°C for 30 minutes. After incubation, the plates were again washed with 1X wash buffer for 6 times. Then 75μl of TMB substrate was added to the wells and kept at room temperature in dark for 20 minutes. Then add 50 μL per well of Stop Solution into all appropriate wells. Then the plates were read for the absorbance at 450nm.

The results of all the three parameters were interpreted according to the standard procedures and compared; it is also statistically calculated for sensitivity and specificity of iFInDx COVID-19 IgM/IgG test kit. The data were also analyzed statistically for diagnostic sensitivity, positive predictive value (PPV) and diagnostic specificity, negative predictive value (NPV).

3. Results and Discussion

A total of 428 samples were included in this study, the samples were collected from the patients who visit for the various health centres for the treatment. The patient's consent was obtained by informing about the study. The institutional ethical clearance was obtained and the IRB number for the study is IORG0010284.

Out of these 428 samples, 283 samples were taken from the patients with symptoms such as fever, breathlessness, body pain, diarrhoea, and vomiting. The remaining 145 samples were obtained from the asymptomatic patients who

Table 1: Distribution and types of samples

Symptomatic /Asymptomatic	Days of onset of symptoms	VTM	Serum	Plasma	Whole blood
Symptomatic	0-3 days	132	69	-	63
	4-6 days	14	35	-	15
	7-14 days	39	21	18	-
	More than 14 days	60	16	08	36
Asymptomatic	-	145	10	121	14

were either vaccinated or recovered from Covid infections. The distribution and types of samples were shown in the Table 1.

All the VTM samples (total no. of samples 283) were tested for Covid RT PCR, out of 283 VTM samples, 224 (79%) samples were found to be positive by RT PCR. 192 (67%) of samples were tested positive for both IgG and IgM antibody, irrespective of their RT PCR Covid results. Out of 283 samples tested, 132 samples were got positive only in RT PCR, but none of the samples were positive for antibodies either by ELISA or by rapid method. The 50 samples collected after 4-6 days of onset of symptoms, the PCR amplification for the genes were found to be positive and none of the samples were positive for antibodies by both methods.

During any viral infections, the specific antibodies can be produced in most of the patients but not in immune compromised patients. According to CDC 2019, Antibodies including IgM, IgG, and IgA against S protein and its subunits of SARS–CoV-2 can be detected in serum within 1-3 weeks of post infection. Following SARS-CoV2 infection, the IgM can be detectable by first or second week of illness after the onset of symptoms.[2] The present study observed that 132 samples collected during 0-3 days of onset of symptoms and 50 samples collected between 4-6 days of onset of symptoms were negative for any antibodies.

The samples (39 samples) collected from 7-14 days post infection showed 20 (51%) samples were positive for the antibody IgM detected only in serum and plasma samples by ELISA and RDT method. The antibody IgM rouse from the day 7 of symptoms onset, and it will begin to diminish from day 21. The plasma samples (11 samples) collected after 13^{th} day of onset of symptoms showed positive for IgM and IgG by both the methods as well as positive for RT-PCR also.[8] Hence, the samples collected post 13^{th} day of onset of symptoms attained 100% sensitivity with RT-PCR, ELISA and RDT. A total of 58 Nasopharyngeal and oropharyngeal samples were collected after 14 days of onset of symptoms and simultaneously from the same patients 16 serum, 8 whole blood and 34 plasma samples were collected and tested for the presence of antibody by ELISA and RDT method. The results found that, all the 58 samples were negative in the RT-PCR test and it's found to be positive for the antibody IgG in both ELISA and RDT. These results further confirm that RDT kit shows the 100% sensitivity compared with ELISA.

The antibodies not detected may be linked to various characteristics like antibody production kinetics, antibody response, antibody affinity, or assay characteristics such as the antigen nature and preparation, or antigen-antibody incubation time. The discrepancies in antibody results were due to mild or asymptomatic patients or weak antibody.[9,10]

The iFinDx COVID-19 IgM/IgG test kit was qualitatively analyzed based on the specific SARS CoV-2 antibodies present in serum of symptomatic patients. The analysis was done in comparison with the US FDA EUA authorized Real-Time PCR Kit (EURO Real Time SARS-CoV-2) as the reference assay and was characterized the specimens with US FDA EUA authorized ELSIA kit (InBios SCoV-2 Detect IgG & IgM ELISA). A total of 428 patients were tested at the POC site with consent for specimen collection and provision of information. The iFinDx COVID-19 IgM/IgG kit showed 94.64% of sensitivity (95% CI: 90.83% to 97.20%) and 99.02% of specificity (95% CI: 96.50% to 99.88%) with serum specimens, among the positive and negative nasopharyngeal swab specimens confirmed by Real-Time PCR. Also, as a result of testing by ELISA assay to validate the detection of anti-SARS-CoV-2 antibodies, a result of 100% sensitivity and 100% specificity was obtained.

4. Conclusion

"iFinDx COVID-19 IgM/IgG rapid kit will be a useful diagnostic product, that quickly and accurately tests the antibody formation (IgM and IgG)following SARS-CoV-2 virus infection.

5. Conflict of Interest

The authors declare no relevant conflicts of interest.

6. Source of Funding

None.

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