COMMENTARY

Interpretation of Diagnostic Tests for COVID-19 (SARS-COV-2)

Sadaf Shafique[1], Farheen Aslam[2], Raheel Khan[3], Asma Shaukat[4]

ABSTRACT:
The knowledge regarding diagnostic testing for SARS COV-2 is still at hit-and-trial phases, all over the world. Evolving day by day through ongoing research and extensive trials, use for SARS-COV-2 infections- Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and IgM/ IgG serology by Enzyme Linked Immunosorbent Assay (ELISA) or Electro-Chemiluminescent Immunoassay remain the main stay of diagnosis. However, the time course for the PCR positivity and seroconversion seem to vary in children and adults both, which also includes a huge population of asymptomatic individuals who are potentially labelled negative hence posing a great threat to the surrounding community.

KEYWORDS: SARS COV-2, Immunoglobulins, Enzyme Linked Immunosorbent Assay (ELISA).

How to Cite This:
Shafique S, Aslam F, Khan R, Shaukat A. Interpretation of diagnostic tests for COVID-19 (SARS-VOV-2). Biomedica. 2020; 36 (COVID19-S2): 93-6.

BACKGROUND

COVID-19, i.e. abbreviation for Coronavirus disease 2019 is now a days a health threat emerging on a global level. On March 11th,2020 its spread was declared as a pandemic disease by the Director of World Health Organization.[1] The viral pathogen which causes this disease belongs to a Coronaviridae family, finally being defined as Severe Acute Respiratory Syndrome Corona virus 2 (SARS-COV-2). It has close identical sequence match with the homologous virus (SARS-COV-1) that had previously caused the SARS outbreak in 2003.[2] The knowledge regarding diagnostic testing for SARS COV-2 is still on initial level and evolving day by day through researches and extensive trials on patients and exposed community. It is very important to have a clear and crisp understanding of the nature of tests and interpretation of their findings which may help a great deal in the management of patients. This commentary therefore explains how to interpret the two types of diagnostic tests which are in common use for SARS-COV-2 infections; Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and IgM/ IgG serology by techniques of Enzyme Linked Immunosorbent Assay (ELISA) or Electro-Chemiluminescent Immunoassay as their results can vary over time.

1. Sadaf Shafique
Assistant Professor, Department of Pathology
Quaid-e-Azam Medical College, Bahawalpur – Pakistan.
2. Farheen Aslam
Assistant Professor, Department of Pathology
Quaid-e-Azam Medical College, Bahawalpur– Pakistan.
3. Raheel Khan
Senior Registrar, Department of Medicine
Quaid-e-Azam Medical College/Victoria Hospital, Bahawalpur– Pakistan.
4. Asma Shaukat
Professor, Department of Pathology
Quaid-e-Azam Medical College, Bahawalpur– Pakistan.

Correspondence to:
Dr. Sadaf Shafique
Assistant Professor, Department of Pathology
Quaid-e-Azam Medical College, Bahawalpur– Pakistan.
Email: drsadafshafiq@gmail.com

DETECTION of VIRAL RNA by RT-PCR:

This is the most commonly used and considered as a reliable test performed for the diagnosis of COVID-19. It is performed by using nasopharyngeal swabs or other upper respiratory

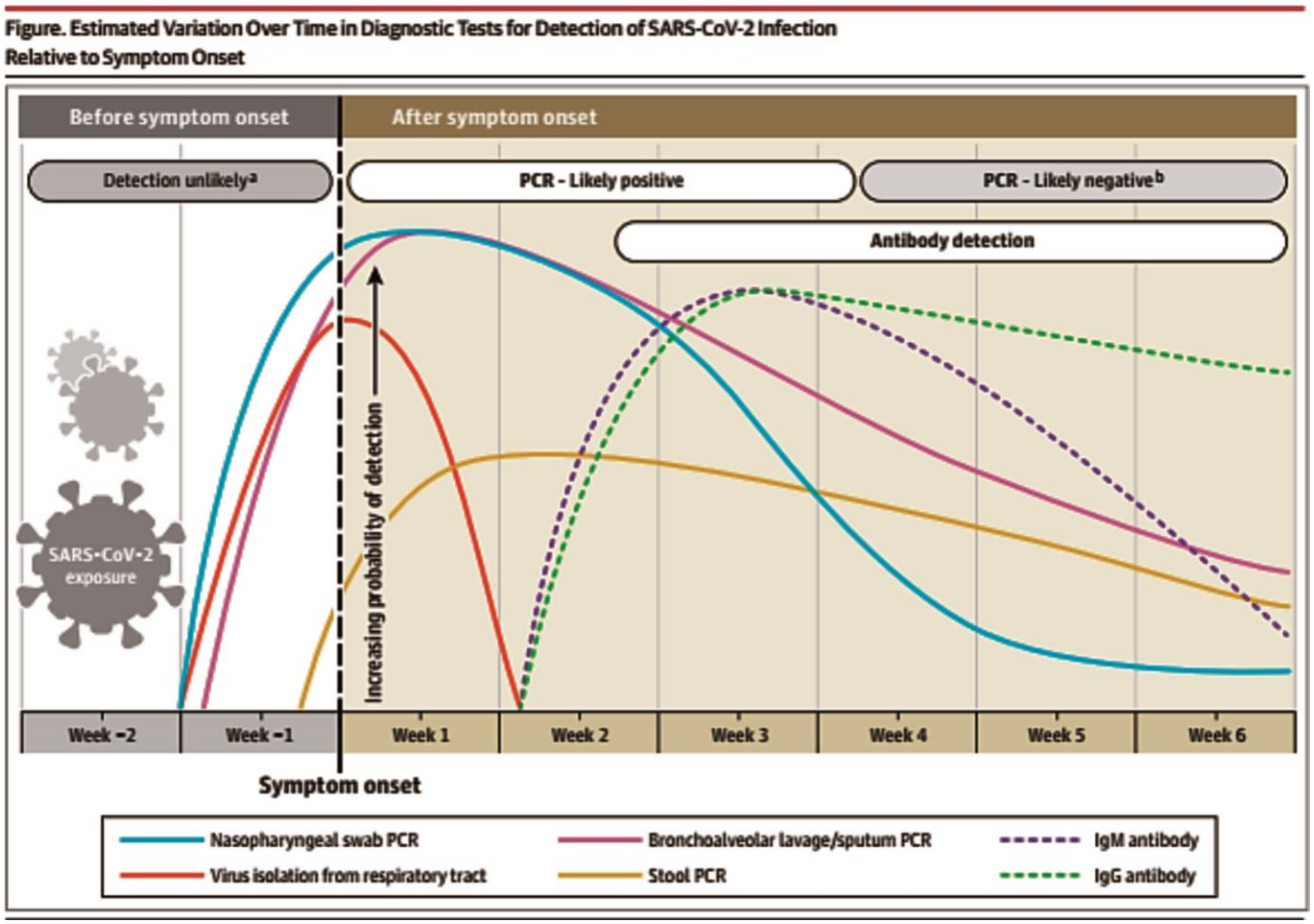

Fig.1: Adapted from: COVID-19: Screening a. COVID-19: Screening, testing, PUI, and returning to work – REBEL EM – Emergency Medicine Blog [Internet]. REBEL EM – Emergency Medicine Blog. 2020 [cited 17 May 2020]. Available on line at: https://rebelem.com/COVID-19-screening-testing-pui-and-returning-to-work/

tract specimens including throat swab or saliva recently. Different manufacturers use a variety of RNA gene targets. Most of the tests target 1 or more of the Envelope (Env), Nucleocapsid (N), Spike (S), RNA dependent RNA polymerase (RdRp) and ORF1 genes.[3] In most symptomatic patients with COVID-19, viral RNA may become positive as early as day 1 of symptoms and peaks within a week. It becomes undetectable by week 3 and then undetectable. In few cases, viral RNA has been detected by RT-PCR even 6 weeks after the first positive test whereas in some other cases they have been reported to be positive after 2 consecutive negative performed 24 hours apart.[4] This may entirely be due to testing error, re infection or re activation. The timeline of PCR positivity is different for specimens other than nasopharyngeal swabs. it has been noticed that in sputum the PCR positivity has declined more slowly and have been still positive after the nasopharyngeal swabs are negative.[5] According to a study of 205 patients with confirmed COVID-19 infection, RT-PCR positivity was highest in bronchoalveolar lavages (93%) followed by sputum (72%), nasal swab (63%) and pharyngeal swab (32%).[6] False negative results have also been seen due to inappropriate timing of sampling in relation to onset of illness, its faulty technique especially of nasopharyngeal swabs. The specificity of most of the RT-PCR tests is 100% as the design of primer is specific to the genomic sequence of SARS-COV-2. False positive results may also occur due to technical errors or contamination of reagents.

DETECTION of ANTIBODIES to SARS-COV-2

Indirectly COVID-19 infection can be diagnosed by

measuring the immune response to SARS-COV2 infection. For patients with mild to moderate degree of illness, presenting late beyond 2 weeks of onset of illness, serological diagnosis plays a very important part. With growing needs for rapid diagnosis, it has become a vital tool to understand the extent of COVID-19 in the community and to identify immune individuals or who are potentially protected from becoming infected. IgM and IgG antibodies have been found to be positive as early as fourth day after the onset of symptoms; they are even at higher levels at second and third weeks of illness.[7] According to a study done by Xiang et al with 85 patients, IgM and IgG seroconversion occurred in all of these patients between the third and fourth weeks of clinical illness onset. By week 5 IgM begins to decline and reaches a lower level, disappearing almost by week 7 but levels of IgG persist beyond 7 weeks.[8] ELISA based IgM and IgG antibody tests have greater than 95% specificity for diagnosis of COVID-19. The diagnostic accuracy can be further increased by testing of paired serum samples with initial PCR and then second one 2 weeks later.[9] Various manufacturers have developed the rapid point of care tests for detection of antibodies with variable quality, sensitivity and specificity. The nature of antigens used by them is not revealed. These are purely qualitative in nature and only telling the presence or absence of SARS-COV-2 antibodies.[9]

Few popular companies like Roche have introduced the detection of antibodies through sandwich method using the popular and very sensitive technique of electrochemiluminescence immunoassay. The anti SARS-COV-2 assay uses the recombinant protein representing the Nucleocapsid (N) antigen for the determination of antibodies against SARS-COV-2. The overall specificity as mentioned in their literature is 99.81% with 95% lower confidence interval of 99.65% whereas the sensitivity ranges upto 65.5%-100% after 6 – 14 days of PCR confirmation.[10]

CONCLUSION

A very useful timeline of diagnostic markers for detection of COVID-19 has been devised for clinical correlation. The time course for the PCR positivity and seroconversion seem to vary in children and in other age groups, which also includes a huge population of asymptomatic individuals who are never diagnosed. A big question is still there which needs to be answered i.e. how long the potential immunity lasts in both asymptomatic and symptomatic individuals infected with the novel SARS-COV-2.

CONFLICT OF INTEREST

None to declare.

FINANCIAL DISCLOSURE

None to disclose.

REFERENCES

1. World Health Organization. WHO Virtual press conference on COVID-19. Available online at: https://www.who.int/docs/default-source/Coronaviruse/transcripts/who-audio-emergencies-Coronavirus-pressconference-full-and-fnal-11Mar2020.pdf?sfvrsn=cb432bb3_2. [Last accessed on 16thMay, 2020].
2. Lippi G, Plebani M. The critical role of laboratory medicine during Coronavirus disease 2019 (COVID-19) and other viral outbreaks. Clin Chem Lab Med. 2020; [Epub ahead of print].
3. Nalla AK, Casto AM, Huang MW. Comparative performance of SARS-CoV-2 detection assays using seven different primer/probe sets and one assay kit. J Clin Microbiol. 2020; [Epub ahead of print].
4. Zheng S, Fan J, Yu F, Feng B, Lou B, Zou Q, et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. BMJ. 2020; 369: m1443.
5. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al. Virologic assessment of hospitalized patients with COVID-2019.Nature.2020; April 1. [Epub ahead of print].
6. Wang W, Xu Y, Gao R. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA. 2020; 323 (18): 1843–4.
7. Lou B, Li T, Zheng S, Yingying S, Zhiyong L, Wei L, et al Serology characteristics of SARS-CoV-2 infection since theexposure and post symptoms onset. Med Rxiv. 2020; 03.23.20041707.
8. Xiang F, Wang X, He X, et al. Antibody detection and dynamic characteristics in patients with COVID-19. Clin Infect Dis. 2020; pii: ciaa461.

9. To KKW, Tsang OT, Leung WS, Owen TYT, Wai SL, Anthony RT, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis. 2020; 20 (5): 565-4.
10. Roche's cobas SARS-CoV-2 Test to detect novel Coronavirus receives FDA Emergency use authorization and is available in markets accepting the CE market. Available online at: https://www.roche.com/media/releases/med-cor-2020-03-13.htm. [Last accessed on 17thMay, 2020].

Author's Contribution

SS: Conception and design of study, Acquisition and analysis of data.

FA: Critical review and intellectual input.

RK: Acquisition of data, Intellectual input.

AS: Intellectual input and approval of the final version to be published.

Prof. Asma Shaukat (MBBS, DCP, MCPS, FCPS, CHPE, FRCPath) is a consultant pathologist and is Head of Department of Pathology at Quaid-e-Azam Medical College Bahawalpur. She has over 47 publications in peer reviewed indexed journals. She has keen interest in Chemical Pathology and has established biosafety lab level 3 in her institution with the help of her team to carry out diagnostic testing for COVID-19. She has also published many Editorials, short communications and commentaries regarding the topic since the outbreak has emerged and playing an important role as Editor in chief of Journal, Annals of QAMC.