IJPSR (2022), Volume 13, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 13 April 2022; received in revised form, 19 May 2022; accepted, 03 June 2022; published 01 December 2022

COMPARISON OF SARS CoV-2 RT-PCR TEST WITH RAPID ANTIGEN DETECTION KIT AND OTHER NON SPECIFIC TEST

Ria Murugesan, Bhuvaneshwari Gunasekar * and Neelusree Prabhakaran

Saveetha Institute of Medical and Technical Sciences, Thandalam, Chennai - 602105, Tamil Nadu, India.

Keywords:

COVID-19, RT-PCR, Rapid Antigen SARS-CoV-2, CRP, IL-6, Procalcitonin

Correspondence to Author:
Bhuvaneshwari Gunasekar

Assistant Professor,
Saveetha Institute of Medical and Technical Sciences, Thandalam, Chennai- 602105, Tamil Nadu, India.

E-mail: riamurugesan@gmail.com

ABSTRACT: Background: Severe Acute Respiratory Syndrome Corona Virus Disease 2019 (COVID-19) is responsible for the COVID-19 pandemic causing respiratory illness worldwide. Hence there is an urgent need for simple, rapid, and accurate tests for diagnosis. Performance characteristic of Rapid Antigen detection test for identifying sensitivity and specificity with gold standard Real-Time Polymerase Chain Reaction and correlate the significance of non-specific parameters like CRP(C- Reactive Protein), IL-6(Interleukin-6), Procalcitonin for diagnosis of COVID-19. **Methods:** The Rapid Antigen Detection test was compared with Real-Time Polymerase Chain Reaction to detect SARS CoV-2 in respiratory specimens. 100 respiratory samples (mainly nasopharyngeal and throat swab) and for CRP, IL6, and Procalcitonin serum samples were obtained from COVID-19 suspected cases, mostly in-patients at Saveetha Medical College from April 2021- to September 2021. **Results:** Out of 100 samples, 67% were positive, 33% were negative for SARS-CoV2 RNA by RT-PCR assay. When compared with Rapid Antigen Test, the RT-PCR test showed 83.8% sensitivity and 59.3% specificity, while non-specific parameters correlation in diagnosis of COVID-19 showed CRP insignificant, IL-6 and Procalcitonin significant. **Conclusion:** RT-PCR is considered the standard gold method for diagnosing COVID-19. On comparing RT-PCR with other non-specific tests like CRP, Procalcitonin, IL-6 showed Procalcitonin and IL-6 can be considered non-specific tests for diagnosing COVID-19.

INTRODUCTION: Severe Acute Respiratory Syndrome Corona Virus 2(SARS-CoV2) is the strain of Coronavirus that cause Corona Virus Disease 2019 (Covid-19), and it is responsible for the Covid-19 pandemic causing the respiratory illness. On 30th January 2020, World Health Organization (WHO) officially declared the Covid-19 pandemic as a public health emergency of international concern [16].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(12).5050-56

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(12).5050-56

The emergence of SARS-CoV-2, since the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) 2002 and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) 2012, marked the third introduction of a highly pathogenic and large-scale epidemic coronavirus into the human population in the twenty-first century. Importantly, increasingly evidence showed sustained human-to-human transmission.

SARS is an airborne virus and can spread through small droplets of saliva, similar to colds and influenza. Hence there is an urgent need for simple, rapid, and accurate tests for diagnosis. Performance characteristic of Rapid Antigen detection test for identifying sensitivity and specificity with gold

standard Real Time Polymerase Chain Reaction and correlate the significance of non-specific parameters like CRP(C- Reactive Protein), IL-6(Interleukin-6), Procalcitonin for diagnosis of COVID-19.

Aim: To compare Rapid antigen detection method with Real-Time Polymerase Chain Reaction (RT-PCR) and to correlate the significance of non-specific parameters like C-Reactive Protein, Interleukin 6 and Procalcitonin for the diagnosis of Covid-19.

Objectives:

1. Nasopharyngeal and oropharyngeal swab received to Molecular Diagnostics and Research Laboratory from Covid-19 suspected patient will be subjected to RT- PCR (Real Time Polymerase Chain Reaction) and Rapid antigen detection test.

2. Data will be analyzed statistically to conclude the sensitivity and the specificity of rapid antigen detection with the gold standard RT-PCR gene detection.

Background: Real-Time Polymerase Chain Reaction is the gold standard molecular test that analyzes upper respiratory specimens for diagnosis of the genetic material of SARS-CoV. The average time taken is around 4-5 ho from receipt of sample to the generation of the result. Three steps are involved in detecting the result - extraction, amplification, and analysis. It targets the specific gene for SARS-CoV-2 that is RNA dependent RNA polymerase (RdRp), Open reading frames (ORF1ab), N2 nucleocapsid. Rapid and accurate tests for diagnosis of SARS-CoV-2 screening are essential to expedite disease prevention and control and screening during pre-operative management for invasive procedures. Lateral flow immunoassays is done by using monoclonal anti-SARS-CoV-2 antibodies, which target SARS-CoV-2 antigens. They can be the complementary screening tests if their accuracy was comparable to that of the real-time RT-PCR assays. Thus this study is taken up to determine the performance characteristic of rapid SARS-CoV2 antigen detection test evaluation and compared with gold standard Real Time Polymerase Chain Reaction(RT- PCR)test for diagnosis of Covid-19 disease.

METHODS:

Ethical Issues: A prospective study was carried out from April 2021 to September 2021 at Saveetha Medical College and Hospital in Chennai, Tamil Nadu, India. This study was approved by the Institutional Review Board of Saveetha Medical College and Hospital with Institutional Review Board (IRB) Number-SMC/IEC/2021/06/017.

Clinical Specimens:

1. The fever clinic will collect respiratory samples in the fever clinic, mainly nasopharyngeal and oropharyngeal swabs.

2. Samples will be transported at 4-8℃ to Molecular Diagnostics and Research Laboratory for processing with a particular detailed label sample.

3. For CRP, IL-6, and Procalcitonin - serum samples received to Clinical Microbiology Laboratory will be collected.

Inclusion Criteria:

1. For Covid-19 diagnosis, nasopharyngeal and oropharyngeal swab in Viral Transport medium transported in 4-8℃ to Molecular diagnostics and research laboratories were included.

2. For CRP, IL-6, and Procalcitonin- serum samples received from Clinical Microbiology Laboratory were included.

Exclusion Criteria:

1. Swab with no identity (Name, Age/Sex, hospital number) will be excluded.

2. Leakage in packing, and transportation-temperature (4-8℃) not maintained will be excluded and informed for further re-sampling.

3. Other patient request forms that were not requested for all 4 parameters were excluded; likewise, samples that do not abide by Universal precautions were excluded.

Real-Time Polymerase Chain Reaction (RT-PCR): Real Time-Polymerase Chain Reaction technique is based on two consecutive reactions:

A. Conversion of RNA into complementary DNA (cDNA) through reverse transcription enzyme and

B. Amplification of the cDNA sample by Polymerase Chain Reaction using gene-specific primers and fluorescent-labeled hydrolysis probes. The first step of DNA template production to be used in the second step, where the copy number of the DNA is increased throughout repeated thermal cycles. Gene-specific primers guide the second reaction for amplifying only the selected region on genome while the probes produce fluorescent signals upon each successful amplification of the gene regions, allowing a quantifiable reaction system. It targets the specific gene for SARS-CoV-2 that is RNA dependent RNA polymerase (RdRp), Open reading frames (ORF1ab), N2 nucleocapsid. An illustration for RT-PCR based on well-known TaqMan hydrolysis probes [6, 7].

Rapid SARS-CoV-2 Antigen Detection Assay: Rapid antigen detection test is a rapid Chromatographic immunoassay for the detection of SARS-CoV-2 nucleocapsid (N) antigen in respiratory specimens. This test has two pre-coated lines: the "C" Control line and the "T" Test line. The control region (C) is coated with mouse monoclonal anti-chicken Igγ antibody; the test (T) region is coated with mouse monoclonal anti-SARS-CoV-2 antibody against SARS-CoV-2 N antigen. The detectors for the SARS-CoV-2 N antigen present in the specimen are mouse monoclonal anti-SARS-CoV-2 antibodies conjugated with color particles. Antigen-antibody colored complex migrates through capillary force and is captured by the mouse monoclonal anti-SARS-CoV-2 antibody coated on the test (T) region. The colored test line (T) intensity depends on the amount of SARS-CoV-2 N antigen presented in the sample. C indicates the control; hence, the colored band of both the test line and control line is considered positive. Antigen test typically takes only 15-30mins [2].

Human Il-6 Elisa Test: Human IL-6 test was performed by using ELISA (Enzyme-linked immunosorbent assay). A Capture antibody is considered highly specific for IL-6 coated in microtiter strip plate wells. Binding of IL-6 samples and known standards capture antibodies and subsequent bindings of the Biotinylated anti-IL-6 secondary antibody to the analyte are completed during the same incubation period. The HRP (high reactive protein) conjugate solution is added to every well, including the zero well following incubation; excess conjugate is removed by careful washing. The addition of chromogen substrate to all wells results in progressive blue color complex development with the conjugate. The colour development is then stopped by adding acid and turning the final product yellow. The intensity of producing colour complex is directly proportional to the concentration of IL-6 present in samples. The color complex's absorbance is measured, and each standard's generated optical density value is plotted against excepted concentration forming a standard curve. This standard curve is then used to accurately determine the concentration of Interleukin-6 in the sample tested [17].

CRP (C-Reactive Protein): CRP test was performed by using CLIA (Chemiluminescence immune assay) method. C-Reactive Protein kit based on the immunological reaction between CRP Antisera bound to biologically inert latex particles and CRP in the test specimen. When serum CRP is equal or greater than the Reagent sensitivity (indicated on the latex vial label), the visible agglutination occurs [4].

Procalcitonin: Procalcitonin test was performed by using the CLIA (Chemiluminescence immune assay) method, this test measures the level of procalcitonin in your blood. A high level may indicate serious bacterial infection, such as sepsis. Sepsis occurs when an infection in one area of your body (mainly skin or urinary tract) spreads into your bloodstream. This triggers an extreme immune reaction.

It causes a rapid heartbeat, shortness of breathing, decreased blood pressure, and other symptoms. Without immediate treatment, sepsis can lead to organ failure or even death. A procalcitonin test help to determine if you have sepsis or another serious bacterial infection in the early stages. This may help you get treated promptly and avoid life-threatening complications [5].

RESULT: This cross-sectional study was carried out from April 2021 to September 2021 at Saveetha Medical College and Hospital in Chennai, Tamil Nadu, India. A total number of 100 Covid-19 suspected samples with both sexes (male and female) of all age groups were tested. In this study, 52 were female, and 48 were male. Out which 67 (67%) had tested evidence of Covid-19 positive infection and 33(33%) negative cases in Real-Time Polymerase Chain Reaction.

TABLE 1: SEX AND AGE-WISE DISTRIBUTION

		Male (48)	Female (52)	Total
Age Wise	0-14	6	12	18
	15-59	17	21	38
	60+	25	19	44
	Total	48	52	100

The chi-square statistic is 3.0842. The p-value is .213935. The result is not significant at p<0.05.

TABLE 2: COMPARISON OF KIT- 1 AND KIT- 2 SENSITIVITY AND SPECIFICITY

	Kit - 2			
Kit- 1		**Positive**	**Negative**	**Total**
	Positive	61 a	5 b	66
	Negative	7 c	27 d	34
	Total	68	32	100

The Comparison of KIT-1 and KIT-2 sensitivity, specificity, positive predictive value, and negative predictive values are- 89.7%, 84.3%, 92.4%, 79.4%; from the above calculation, we conclude that KIT-2 is more sensitive and specific when compared to KIT- 1.

TABLE 3: COMPARISON OF RT-PCR (KIT-2) AND RAPID ANTIGEN DETECTION TEST REPORTS

		Rt-Pcr (Real-Time Polymerase Chain Reaction)		
Rapid		Positive	Negative	Total
Antigen	Positive	57	13	70
Detection	Negative	11	19	30
Test	Total	68	32	100

The sensitivity and specificity of RT-PCR, when compared to Rapid Antigen Detection test, are 83.8% and 59.3%. The positive and negative predictive values of RT-PCR compared to the Rapid Antigen Detection test are 81.4 % and 63.3%.

TABLE 4: CORRELATION OF NON-SPECIFIC PARAMETER WITH RT-PCR COMPARATIVE STUDY OF RT-PCR (KIT-2) WITH CRP (CHI-SQUARE TEST)

		RT- PCR		
CRP (C-reactive protein)		**Positive**	**Negative**	**Total**
	Positive	50 a	21 b	71 A
	Negative	16 c	13 d	29 B
	Total	66 C	34 D	100

The chi-square statistic is 2.1339. The p-value is 0.14407. Not significant at p <0.05. Thus null hypothesis is accepted.

TABLE 5: COMPARATIVE STUDY OF RT-PCR WITH PROCALCITONIN (CHI-SQUARE TEST)

		RT-PCR		
Procalcitonin		**Positive**	**Negative**	**Total**
	Positive	47	10	57 A
	Negative	18	25	43 B
	Total	65 C	35 D	100

The chi-square statistic is 17.755. The p-value is 0.000025. Significant at p <0.05. Thus null hypothesis is rejected.

TABLE 6: COMPARATIVE STUDY OF RT-PCR WITH IL6 (CHI-SQUARE TEST)

		RT-PCR		
IL6(Interleukin-6)		**Positive**	**Negative**	**Total**
	Positive	42	17	59 A
	Negative	15	26	41 B
	Total	57 C	43 D	100

The chi-square statistic is 11.816. The p-value is 0.000587. Significant at p <0.05. Thus null hypothesis is rejected.

DISCUSSION AND CONCLUSION: Molecular tests confirm the standard laboratory diagnosis for SARS-CoV-2 infection; RT-PCR assays for SARS-CoV-2 RNA detection in clinical specimens are widely used in Covid-19 diagnostic laboratories. In this study, out of 100 randomly selected Covid-19 suspected patients sample, 52 were female, and 48 were male. Out which 67 (67%) had tested evidence of Covid-19 positive infection and 33(33%) negative cases in Real-Time Polymerase Chain Reaction. If Rapid antigen immunoassays with equivalent sensitivity and specificity to real-time RT-PCR assay will help speed disease screening. In this study, the commercially available rapid SARS-CoV-2 antigen detection method was compared with the RT-PCR (Real-Time Polymerase Chain Reaction) assay to detect SARS-CoV-2 infection. For the master mix step in RT-PCR, two kits were processed separately-KIT-1 and KIT-2. KIT -1 showed the N gene and ORF1ab, while KIT-2 showed RdRp and N-gene during analysis for final reports in the RT-PCR machine.

When tested with KIT-1, Samples reported 66 positives and 34 negative cases, While KIT-2 reported 68 positives and 32 negative cases. In comparing the sensitivity and specificity between KIT-1 and KIT-2, we observed sensitivity of 89.7% and specificity of 84.3%; also, the positive and negative predictive values were 92.4% and 79.4%. Thus we prefer KIT-2 for the comparative study as it is more sensitive and specific when compared to KIT-1. Similarly, in the article Evaluation of the RealStar® SARS-CoV-2 RT-PCR kit RUO performances and limit of detection Benoit Visseaux [11] concluded the comparison of the RealStar® SARS-CoV-2 assay with the reference WHO assay; he observed a slightly better sense of the RealStar® assay.

On comparing RT- PCR and Rapid antigen detection test reports, 57 positive and 19 negative cases were common in both tests. Calculating the sensitivity and specificity, we observe 83.8% of sensitivity and 59.3% of specificity; that is RT-PCR is 83.8% more sensitive and 59.3% specific when compared to the Rapid Antigen detection method. Hence from the study, we can consider RT-PCR a gold standard method because of its sensitivity and specificity when compared to the Rapid Antigen Detection kit. The article Rapid SARS-CoV-2 antigen detection assay in comparison with real-time RT-PCR for laboratory diagnosis of Covid-19 in Thailand-By Chutikarn Chaimayo(8), concluded that The rapid assay for SARS-CoV-2 antigen detection showed comparable sensitivity (98.33%; 95% CI, 91.06–99.96%) and specificity (98.73%; 95% CI, 97.06–99.59%) with Real-Time PCR assay.

He believes that this rapid and simple SARS-CoV-2 antigen detection test is a potential use as a screening assay, especially in a high prevalence area. Another Clinical article assessment of SARS-CoV-2 antigen rapid detection compared with RT-PCR assay for emerging variants at a high-throughput community testing site in Taiwan by Ming-Jr Jian, Cherng-Lih Perng [12] discussed that Considering the short turnaround times and lower costs, this simple SARS-CoV-2 antigen detection test for rapid screening combined with RT-PCR as a double confirmatory screening tool to facilitate the prevention of community transmission during Covid-19 emergencies.

Other non-specific tests like CRP(C reactive protein), IL-6(Interleukin-6), and Procalcitonin were also analyzed. IL-6 is the primary trigger for cytokine storms. In peripheral blood, IL-6 levels could be used as an independent factor to predict the progression of Covid-19and may increase during infection. C - reactive protein is a non-specific acute-phase protein induced by IL-6 in the liver and a sensitive biomarker of inflammation, infection, and tissue damage. The expression level is usually low but increases rapidly and significantly during acute inflammatory responses. PCT is made of glycoprotein without hormonal activity and the precursor of calcitonin. Serum levels of PCT are usually low or undetectable.

PCT levels increase with bacterial infections and are relatively low with viral infections and, therefore, can be used to distinguish between bacterial and viral infections. In comparing RT-PCR with CRP, we observed 50 common positives and 13 common negative cases in both methods. The chi-square statistic is 2.1339, and the p-value is 0.14407, which is Not significant at $p < 0.05$. Thus C-Reactive Protein test is insignificant when compared with RT-PCR.

We observed 47 common positives and 25 common negative cases in comparing the RT-PCR test with Procalcitonin. The chi-square statistic is 17.755. The p-value is 0.000025, which is Significant at $p < 0.05$. Hence the Procalcitonin test is significant when compared with the RT-PCR test.

In the comparative study of RT-PCR with IL-6 (Interleukin-6), we observed 47 common positives and 25 common negative cases in both tests. The chi-square statistic is 11.816, and the p-value is 0.000587, significant at $p < 0.05$. Thus by the calculation and observation, the IL-6 test is significant compared to the RT-PCR test.

In the article Interleukin-6 in Covid-19: A systematic review and meta-analysis by Hourmazd Haghbayan [14], he demonstrates that serum levels of IL-6 are significantly elevated in the setting of complicated Covid-19 disease and increased IL-6 levels to be in turn significantly associated with adverse clinical outcomes. This suggests that the progression of initial SARS-CoV-2 infection to complicated disease may result from host immune response and autoimmune injury.

While in the article- Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in Covid-19 by Tobias Herold[13] , concluded that the maximal level of IL-6, followed by CRP level, was highly predictive of the need for mechanical ventilation, the possibility of using IL-6 or CRP level to guide escalation of treatment in patients with Covid-19-related hyper-inflammatory syndrome. The study showed Procalcitonin test is significant to Covid-19. Similarly, the article Procalcitonin levels in Covid-19 patients by RuiHu, ChaofeiHan, Shiyao Pei, MingzhuYin, Xiang Chen [15] also concluded that PCT may be an indicator of disease severity and may contribute to determining the severity of patients with Covid-19. In addition, serial PCT measurements may be useful in predicting the prognosis. The study shows that Procalcitonin and IL6 test is significant with the SARS-CoV-2 RT-PCR test. Thus Procalcitonin and IL-6 tests will be helpful in further treatment of Covid-19 suspected patients. RT-PCR is considered to be the standard gold method for the diagnosis of COVID 19. In comparing RT-PCR with other non-specific tests like CRP, Procalcitonin, and IL-6(Interleukin-6), we conclude that the CRP test is insignificant with COVID 19 diagnosis. At the same time, it is significant to compare Procalcitonin and IL-6 (Interleukin-6).

Hence, Procalcitonin and IL-6 tests can be considered non-specific tests for diagnosing COVID 19.

Availability of Data and Materials: All data generated or analyzed during this study are included in this published article and its additional files.

ACKNOWLEDGEMENT: Nil

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Chutikarn Chaimayo and Bualan Kaewnaphan: Rapid SARS-CoV-2 antigen detection assay in comparison with real-time RT-PCR assay for laboratory diagnosis of Covid-19 in Thailand 2020; 17(1): 177.
2. CoviRAT-Rapid Test for Detection of SARS-CoV-2 Antigen in Human Nasopharyngeal Swab Specimen Device Instruction Manual- Data file Zephyr Biomedicals.
3. Interim Guidance for Rapid Antigen Testing for SARS-CoV-2, https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antigen-tests-guidelines.html
4. Atlas Medical- Atlas C-Reactive Protein (CRP) Latex Kit instruction manual-info@atlas-medical.com
5. Cloud-Clone Corp- Chemiluminescent Immunoassay Kit for Procalcitonin (PCT) Instruction manual- 7th Edition-www.cloud-clone.us
6. Meril Diagnostics - Meril COVID-19 One step RT-PCR Kit instruction manual.
7. BioMerieux- Argene SARS-CoV-2 R-gene instruction manual for Real time Polymerase Chain reaction (RT-PCR) Kit.
8. Apurba S Sastry, Sandhya BhatEssentials of MEDICAL MICROBIOLOGY -Third edition Published on 2019.
9. Gary W. Procop, Deirdre L. Church, Geraldine S. Hall, William M. Janda, Elmer W. Koneman and Paul C: Schreckenberger Koneman's Color Atlas and Textbook of Diagnostic Microbiology-Seventh Edition Published on june 2016.
10. Philip V'kovski, Annika Kratzel, Silvio Steiner and Hanspeter Stalder: Volker Thiel Coronavirus biology and replication: implications for SARS-CoV-2-Published 2020; 155–170.
11. Benoit Visseaux, Quentin Le Hingrat and Gilles Collin: Evaluation of the RealStar® SARS-CoV-2 RT-PCR kit RUO performances and limit of detection- Volume 129, August 2020; 104520.
12. Ming-Jr Jian, Cherng-Lih Perng, Hsing-Yi Chung and Chih-Kai Chang: Jung-Chung Clinical assessment of SARS-CoV-2 antigen rapid detection compared with RT-PCR assay for emerging variants at a high-throughput community testing site in Taiwan 2022; 115: 30-34.
13. Tobias Herold, Vindi Jurinovic, Chiara Arnreich, Brian J Lipworth, Johannes C Hellmuth, Michael von Bergwelt-Baildon and Matthias Klein: Tobias Weinberger-Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in Covid-19-Epub 2020; 146(1): 128-136.

14. Eric A. Coomes: Hourmazd Haghbayan -Interleukin-6 in Covid-19: A systematic review and meta-analysis-First published: 26 August 2020.https://doi.org/10.1002/rmv.2141
15. Rui Huab1 Chaofei Hanc1 Shiyao Peiab Mingzhu Yinab Xiang Chenab-Procalcitonin levels in Covid-19 patients Author links open overlay panel-Received 2 April 2020 https://doi.org/10.1016/j.ijantimicag.2020.106051
16. World Health Organization. Coronavirus disease (Covid-19) Weekly epidemiological update and weekly operational update. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situatio. Accessed 1 Sept 2020.
17. Diaclone Human IL-6 ELISA KIT instruction manual biotech group- www.diaclone.com

How to cite this article:
Murugesan R, Gunasekar B and Prabhakaran N: Comparison of Sars Cov-2 RT-PCR test with rapid antigen detection kit and other non specific test. Int J Pharm Sci & Res 2022; 13(12): 5050-56. doi: 10.13040/IJPSR.0975-8232.13(12).5050-56.