Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/17221
DOI URL: http://dx.doi.org/10.21474/IJAR01/17221

RESEARCH ARTICLE

A COMPARATIVE STUDY OF RAPID SARS COV 2 ANTIGEN DETECTION TEST & REAL TIME PCR TEST FOR DIAGNOSIS OF COVID-19 IN TERTIARY CARE INSTITUTE AT KOTA, SOUTH–EAST RAJASTHAN, INDIA

Dr. Harshad Singh Naruka[1], Dr. Dinesh verma[2], Dr. Preetam Singh Mandawat[3] and Dr. Bhupendra Kumar Mandawat[4*]

1. Senior Demonstrator, Department of Microbiology and Immunology, Government Medical College-Kota, Kota, Rajasthan, INDIA.
2. Professor, Department of Microbiology and Immunology, Government Medical College-Kota, Kota, Rajasthan, INDIA.
3. Senior Demonstrator,Department of Pathology,Government Medical College-Kota, Kota Rajasthan, INDIA.
4. Professor, Department of Microbiology and Immunology, Government Medical College- Kota, Kota, Rajasthan, INDIA.

Manuscript Info

Manuscript History
Received: 10 May 2023
Final Accepted: 14 June 2023
Published: July 2023

Key words:-
RT-PCR (Reverse Transcriptase Polymerase Chain Reaction),RAT(Rapid Antigen Test),COVID-19 (Corona Virus Infection Disease-2019), SARS Cov 2 Virus (Severe Acute Respiratory Syndrome Coronavirus 2 Virus), PPV(Positive Predictive Value), NPV(Negative Predictive Value)

Abstract

Background: The 2019 (Covid19) pandemic of coronavirus disease was caused by SARS Cov 2 Virus. The emergence of the disease was started from the Wuhan city of China in December 2019[1]. This infection causes asymptomatic and mild diseases more than severe pneumonia. A Rapid Lateral flow immunoassays using monoclonal anti-SARS CoV- 2 antibodies, targeting SARS-CoV-2 antigens, can be used as complementary screening tests if their accuracy comes comparable to that of gold standard real-time RT-PCR assays.

Objective: The present study has been conducted retrospectively on the data of patients who underwent for Covid 19 diagnosis by RAT at the Microbiology Department Central Laboratory, Maharao Bhim Singh Hospital Nayapura Kota.

Material and Method: Respiratory samples from suspected 5219 COVID-19 patients, mainly nasopharyngeal and throat swabs were collected. COVID-19 Ag Confirmit™ kit (Alpine biomedicals) is a rapidchromatographic immunoassay for the detection ofSARS-CoV-2 nucleoprotein (N) antigen in respiratoryspecimens. Multiplex Real-time RT-PCR kit (TRIVITRON healthcare) was used, whichtargets envelope gene (E), and Orf1ab gene of SARS-CoV-2 genome and RPP30 Human gene as internal control, was used for SARS-CoV-2RNA detection. Descriptive statistics were used to describe the usefulness of rapid diagnostic tests. For that online MedCalc's Diagnostic test evaluation statistical tool[10].

Result: Among all 5219 samples (n=5219) tested by RT-PCR 785 (15.4%) samples were positive and 4434 (84.95%) were negative. Samples tested by RAT 813 (15.57%) samples were positive & 4406 (84.42%) were negative, in our study we found discordant results in 36 samples. The sensitivity and specificity of the COVID-19 Ag

Corresponding Author:- Dr. Bhupendra Kumar Mandawat
Address**:-** Professor, Department of Microbiology and Immunology, Government Medical College-Kota, Kota, Rajasthan, INDIA.

Confirmit™ kit (Alpine biomedicals) were 99.49% and 99.28% respectively.
Discussion: Out of 785 RT-PCR-positive samples in our study, the 4 false negative results were tested for SARS-CoV-2 antigen seven days after disease onset. The RT-PCR result of these samples had relatively high Ct-values. Without the present population prevalence of COVID-19, the positive and negative predictive values (PPV and NPV) of the assay could not be accurately calculated.
Conclusions: There is comparable Sensitivity and Specificity between Rapid assay for SARS-CoV-2 antigen detection (COVID-19 Ag Confirmit™ kit) and real-time RT-PCR assay.

……

Introduction:-

The coronavirus disease 2019 (Covid19) pandemic was caused by SARS Cov 2 Virus. The emergence of the disease was from the Wuhan city of China in December 2019[1]. On January 27 2020 first confirmed case of Covid19 was reported from Kerala in India from a 20-year-old female travelling from Wuhan City, China[2]. Since then as on October 28 2022 in India 4464880 Corona Virus infection cases have been reported to WHO with a death toll of 528987 (India: WHO Corona Virus Disease Dashboard)[3,4]. Usually SARS-CoV-2 virus causes asymptomatic and mild diseases rather than severe pneumonia. Severe cases may develop acute respiratory distress syndrome (ARDS) with an average mortality rate of 6% (range 1–14.4%). Lateral flow immunoassays using monoclonal anti-SARSCoV- 2 antibodies, which target SARS-CoV-2 antigens, can be used as complementary screening tests if their accuracy were comparable to that of the real-time RT-PCR assays. Initially, only RT-PCR was done for diagnosis of Covid19 but after May 2021 Rapid antigen test (RAT) for diagnosis of SARS-Cov2 was started, after that any symptomatic person who comes positive by RAT was considered a positive but symptomatic person who come negative by RAT were confirmed by RTPCR if suggested by the physician. For screening or diagnosis of early infection a nasopharyngeal (NP) swab and/or an oropharyngeal (OP) swab are often recommended. The swab must be inserted deeply into the nasopharyngeal cavity, to properly obtain an NP swab specimen. Apart from RTPCR test, rapid diagnostic tests (RDTs) should be prioritized, since they are timely, easy to perform, and can serve as point-of-care testing [(5)].

The present study has been conducted retrospectively on data from patients who have undergone for Covid 19 diagnosis by RAT at Microbiology Department Central Laboratory, Maharao Bhim Singh Hospital Nayapura Kota. The data from 20 May 2021 to 27 Oct 2022 has been taken of all OPD and IPD patients who have undergone RAT Cov2 followed by RTPCR Cov2. 5219respiratory specimens have been processed during this period.

Material and Method:-

The present study has been approved by our institutional ethical committee after that retrospective data of 5219 samples from 20 May 2021 to 27 Oct 2022 has been taken of all OPD and IPD patients who undergone RAT Cov2 followed by RTPCR Cov2 in the Microbiology department Central Laboratory Maharao Bhim Singh hospital Government Medical College Kota. We evaluated COVID-19 Ag Confirmit™ kit (Alpine biomedicals), a rapid SARS-CoV-2 antigen detection test, using 5219respiratory specimens. The performance of which was compared with the SARS-CoV-2 RT-PCR assay.

Sample Collection: As per guidelines respiratory samples, mainly nasopharyngeal and throat swabs, were collected from 5219 suspected COVID-19 cases, including pre-operative patients. Samples were mixed in 2 mL of viral transport media (VTM), consisting of Hanks' balanced salt, 0.4% fetal bovine serum, HEPES, antibiotic, and antifungal agents. then Samples were transported at 2–8 °C to the PCR laboratory, department of Microbiology Government Medical College Kota, for processing. All specimens were processed in biosafety level-3 (BSL-3) and biosafety level-2 enhanced (BSL-2 +) facilities with full personal protective equipment.[6]

Rapid SARS- CoV- 2 antigen detection assay:COVID-19 Ag Confirmit™ kit (Alpine biomedicals) is a rapidchromatographic immunoassay for the detection ofSARS-CoV-2 nucleoprotein (N) antigen in respiratoryspecimens. The rapid antigen test device has two pre-coatedlines on the result window: control (C) and

test(T) lines. On conjugate pad, the monoclonal antibody against the Covid-19 antigen was conjugated with colloidal gold and deposited and immobilized on the test zone of the nitrocellulose membrane. Sample containing COVID-19 antigen bind with antibody and forms antigen-antibody- gold complex which flows toward the T line where this complex is captured by immobilized antibodies forming a pink line in positive samples. To serve internal control, a control band was designed to indicate that the test is performed properly.7

Viral RNA Extraction:
HiGenoMB Insta NX® Mag96 automated extraction platform (Hi-media) using HiPura® Super 11 Pre-Filled Plates for Insta NX Mag96was used to extractSARS-CoV-2 RNAs from 200 µl of nasopharyngeal andthroat swabs. Extraction was performed according to themanufacturer's instructions. Viral RNA was eluted with50 µl buffer and used for RT-PCR assay[8].

SARS‑ CoV‑ 2 RNA detection:
COVIDsureMultiplex Real-time RT-PCR kit (TRIVITRON healthcare) was used, whichtargets envelope gene (E), and Orf1ab gene of the SARS-CoV-2 genome and RPP30 Human gene as internal control, was used for SARS-CoV-2RNA detection according to the manufacturer's instructions.In the master mix5 µl of extracted RNA was added to 10 µlof 2X RT-PCR Mix, 2 µl of Primer-Probe Mix (Orf1ab-FAM, E-HEX; RPP30-ROX), and 3 µl of RNase free water. A total of 20 µl of reaction mix per sample was amplified usingCFX-96 real-time thermal cycler (Bio-Rad Laboratories,Inc., Hercules, CA, USA). Theconditions consisted of 1 cycle of 15 min at 46 °C, 2 minat 95 °C and followed by 40 cycles of 10sec at 95 °C, 30secat 58 °C. The result was interpreted as a cycle threshold value (Ct value)≤ 36 for Viral genes along with the control gene was defined as a positiveresult[9]. The mean (average) cycle threshold (Ct) values in COVID-19 positive cases were 23.79±6.69 (min 10.49, max 35.02) for the E gene, 26.73±6.55 (min 13.41, max 39.20) for RPP30 gene, and 24.09±6.47 (min 12.07, max 37.17) for Orf1ab gene.

Statistical analysis:-
Descriptive statistics were used to describe the usefulness of rapid diagnostic tests. Data were presented categorically in numbers at a 95% confidence interval (95% CI). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using an online MedCalc Diagnostic test evaluation statistical tool[10].

Result:-

All suspected persons of covid-19 attending OPD & IPD were sampled as per guidelines & tested by Rapid test for antigen detection followed by confirmatory gold standard RTPCR test as a national/ State guidelines for laboratory diagnosis of Covid-19[11,12].

A total of 5219 nasopharyngeal / throat swab from 20 May 2021 to 27 Oct 2022 were collected in VTM for RTPCR and in buffer solution for rapid antigen detection test for Covid -19. Among all 5219 samples (n=5219) tested by RT-PCR 785 (15.4%) samples were positive and 4434 (84.95%) were negative. Samples tested by RAT 813 (15.57%) samples were positive & 4406 (84.42%) were negative, in our study we found discordant results in 36 samples among them 32 samples were false positive and 4 samples were false negative by RAT test. Among 32 false positives, 28 were weakly positive & 4 were positive by RAT. The Ct value of RTPCR for 4 false negative samples was higher (upper limit) than the mean ct value.

We evaluated the performance characteristics of SARS Cov-2 Antigen detection with gold-standard real-time RT-PCR for the detection of viral RNA. The sensitivity and specificity of the COVID-19 Ag Confirmit™ kit (Alpine biomedicals) were 99.49% and 99.28% respectively (Table-1)

Table1:- The sensitivity and specificity of the 37 COVID-19 Ag Confirmit™ kit (Alpine biomedicals).

COVIDsureMultiplex Real-time RT-PCR kit (TRIVITRON healthcare)			
Positive	**#Negative**	**Total**	
COVID-19 Ag Confirmit™ kit (Alpine Biomedicals)			
Positive 781	32	813	
Negative	4	4402	4406
Total	785	4434	**5219**
Sensitivity	99.49% (781/785; 95%CI, 98.71% to 99.86%)		

Specificity	99.28% (4402/4434; 95%CI, 98.99% to 99.51%)
[#]Negative RT-PCR is defined as having Ct-values of E, RPP, and Orf greater than 40	

Table 2:- A total evaluation report of the COVID-19 Ag Confirmit™ kit (Alpine biomedicals).

S.no.	Statistic	Value	95% CI
1	Positive Likelihood Ratio	138.85	98.31 to 196.12
2	Negative Likelihood Ratio	0.01	0.00 to 0.01
4	Positive Predictive Value (*)	96.08%	94.52% to 97.31%
5	Negative Predictive Value (*)	99.91%	99.77% to 99.98%
6	Accuracy (*)	99.31%	99.05% to 99.52%

(*) These values are dependent on disease prevalence and vary with the prevalence rate.

Discussion:-

To confirm SARS-CoV-2 infection, molecular tests are the gold standard laboratory diagnosis; In COVID-19 diagnostic laboratories RT-PCR assays for SARS-CoV-2 RNA detection in clinical specimens are widely used. Rapid antigen immunoassays with equivalent sensitivity and specificity to real-time RT-PCR assays will help to speed up disease screening. In this study, the commercially available rapid SARS-CoV-2 antigen detection kit (COVID-19 Ag Confirmit™ kit (Alpine biomedicals)) was compared with the RT-PCR assay (COVIDsureMultiplex Real-time RT-PCR kit (TRIVITRON healthcare)) for detection of SARS-CoV-2 infection.

Out of 785 RT-PCR-positive samples in our study, the 4 false negative results were tested for SARS-CoV-2 antigen seven days after disease onset. The RT-PCR result of this sample had relatively high Ct-values in Table- 3 which may explain the negative result of the COVID-19 Ag Confirmit™ kit.

Table 3:- Results of 4 false negative case of during our study.

Sample	Orf1ab gene Ct value	E gene Ct value	RPP30 gene Ct value
1	35.54	31.18	39.24
2	36.21	32.05	36.22
3	35.85	31.97	34.57
4	36.84	34.21	38.22

Soon after the symptom onset SARS-CoV-2 viral load in upper respiratory specimens was detected at a higher level[13]; thus, having higher chance of positive antigen detection at the early phase. So this SARS-CoV-2 antigen detection kit might be recommended for patients at the early onset of symptom where higher viral loads are anticipated. Some other factors such as clinical manifestation, duration from disease onset to laboratory test, type of specimens, and process of specimens collection and processed (sample handling and processing techniques) potentially affect the result interpretation.

Our results showed higher sensitivity (99.49%) than other rapid antigen tests previously reported (98.33% by Standard Q COVID-19 Ag test), 50.0% by COVID-19 Ag Respi-Strip CORIS®,93.9% (95% CI, 86.5–97.4%) by Fluorescence Immunochromatographic Assay for 2019-nCoV Ag Test (Bioeasy Biotechnology Co., Shenzhen, China), and 11.1–45.7% by BIOCREDIT COVID-19 Ag (BioVendor Research and Diagnostic Products)[1,14,15,16]

Without the present population prevalence of COVID-19, the positive and negative predictive values (PPV and NPV) of the assay could not be accurately calculated. However, we found 32 false-positive samples tested by the COVID-19 Ag Confirmit™ kit. The PPV for this test may be low in a low COVID-19 prevalence area, due to low infectious burden or sampling variability,there is concern that test may give the variability of viral loads in COVID-19 patients and antigen detection may miss cases[16]. Thus, the COVID-19 Ag Confirmit™ kit might be useful in the high prevalence area. The advantage of the COVID-19 Ag Confirmit™ kit as a screening for COVID-19 is its simple procedure and quick results with high NPV, but its disadvantage is low PPV in a low prevalence area. Thus, RTPCR test for SARS-CoV-2 gene detection, which is more sensitive and specific than rapid lateral flow immunoassay, is still a standard test for COVID-19 diagnosis. Even with its limitations, the rapid SARS-CoV-2 antigen test can benefit all healthcare workers in managing infected individuals in time effectively, especially in rural and outbreak areas. Therefore, before the implementation a prospective study of the rapid SARS-CoV-2 antigen test in these fields should be performed.

Conclusions:-

The rapid assay for SARS-CoV-2 antigen detection (COVID-19 Ag Confirmit™ kit) showed comparable Sensitivity of 99.49% (781/785; 95%CI, 98.71% to 99.86%) and Specificity of 99.28% (4402/4434; 95%CI, 98.99% to 99.51%) with real-time RT-PCR assay. We concluded that there is a potential use of this rapid and simple SARS-CoV-2 antigen detection test as a screening assay, especially in a high-prevalence area.

Declarations

Conflict of interest:

The authors have no conflict of interest.

Ethics statement:

Ethics approval was taken and ethics approval number is IEC: 34/2023.

Human and Animal Rights and Informed Consent:

This article does not contain any studies with human or animal subjects performed by authors.

Bio-Safety:

All standard precautions, bio-safety measures & Biomedical Waste Management in our study according to Biological Waste Management's Rules 2016 and its new amendment were observed.

Data availability:

All datasets generated of analyzed during this study are included in the manuscript.

Financial support and Sponsorship

Nil.

Acknowledgments:-

I would like tothank the Institutional Ethics Committee for their guidance and approval of this study. I am also thankful to all my colleagues in Microbiology as well as other supporting staffs for extending all help for the fulfilment of this endeavor.

References:-

1. Chaimayo et al. Rapid SARS‐CoV‐2 antigen detection assay in comparison with real‐time RT‐PCR assay for laboratory diagnosis of COVID‐19 in Thailand. Virology Journal. (2020) 17:177
2. M.A. ANDREWS ET AL. FIRST CONFIRMED CASE OF COVID-19 INFECTION IN INDIA: A CASE REPORT INDIAN J MED RES. 2020 MAY; 151(5): 490–492.
3. World Health Organization. Coronavirus disease (COVID-19) Weekly epidemiological update and weekly operational update. https://www.who.int/emerg encie s/disea ses/novel -coron aviru s-2019/situa tion-repor ts.Accessed 27 Oct 2022.
4. Worldometer. Coronavirus Cases. https ://www.world omete rs.info/corona virus/. Accessed 27 Oct 2022.
5. Kishan P. Patel Transmission of SARS-CoV-2: an update of current literature. Eur J Clin Microbiol Infect Dis (2020) 39:2005–2011
6. Guidelinesand SOP COVID-19virus testing in NHS laboratories,https://www.rcpath.org/uploads/assets/90111431-8aca-4614-b06633d07e2a3dd9 /guidance-and SOP-COVID-19 testing-NHS-laboratories.pdf
7. Confirmit one step novel coronavirus (COVID-19) antigen detection kit, Alpine Biomedicals. instructions for use.
8. HiPurA Super 11 Pre-filled Plates for Insta NX Mag96. HiGenoMB, Himedia, product code MB615MPF9611 pg1-5. mb@himedialabs.com
9. COVIDsure Multiplex Real-time RT- PCR Kit, instructions for use. Trivitron healthcare.
10. MedCalc's Diagnostic test evaluation calculator. https://www.medcalc.org/calc/diagnostic test. php
11. ICMR Advisory on Use of Rapid Antigen Detection Test for COVID-19 Dated: 14 th June 2020:P 1-3
12. Advisory on Strategy for COVID-19 Testing in India (Version VI, dated 4th September 2020) Recommended by the National Task Force on COVID-19: P1-4

13. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med. 2020;382(12):1177–9.
14. Lambert-Niclot S, Cufel A, Le Pape S, et al. Evaluation of rapid diagnostic assay for detection of SARS-CoV-2 antigen in a nasopharyngeal swab. J Clin Microbiol 2020; JCM.00977-20. doi https://doi.org/10.1128/JCM.00977-20.
15. Porte L, Legarraga P, Vollrath V, et al. Evaluation of novel antigen-based rapid detection test for the diagnosis of SARS-CoV-2 in respiratory samples. Int J Infect Dis. 2020;S1201–9712(20):30405–7. https ://doi. org/10.1016/j.ijid.2020.05.098.
16. Mak GC, Cheng PK, Lau SS, Wong KK, Lau C, Lam ET, et al. Evaluation of rapid antigen test for detection of SARS-CoV-2 virus. J Clin Virol.2020;129:104500.