

## Comparative Evaluation of RT-PCR Results of Covid-19 With True-Nat And Rapid Antigen Test

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### ABSTRACT

The present study aim is to explore the performance of other Covid-19 testing method such as RAT and True- Nat as an alternative diagnostic test to identify the SARS- CoV-2 virus infection. For the prevention of future waves, an ideal diagnostic test with high sensitivity and specificity and rapid results may help in better management and containment of such pandemic. The study was conducted on 378 naso-oropharyngeal swabs samples and was tested by RAT, True-Nat and RT-PCR. The performance of these tests was analyzed by comparing the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio, diagnostic accuracy and kappa coefficient. The sensitivity and specificity of RAT was found to be 54.96% and 65.99% respectively. The sensitivity and specificity of True-Nat was 81.68% and 82.59% respectively. The positive predictive value and negative predictive value of RAT was 46.15% and 73.42% respectively. True-Nat test had higher value of positive predictive value and negative predictive value with 71.33% and 89.47% respectively. Considering RT-PCR as Gold standard, the comparative results showed that the True-Nat is a better diagnostic tool than the Rapid antigen test with higher diagnostic accuracy than the RAT, when compared with the RT-PCR results. But the significance of RAT cannot be ignored with respect to the convenience regarding mass testing and emergency situations.

**KEYWORDS:** RT-PCR, True-Nat, Rapid antigen Test, Sensitivity, Specificity.

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### INTRODUCTION

The corona virus, Covid-19, is caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2). It was first reported in Wuhan, China in December 2019, after which it rapidly spread around the world within few months and ultimately declared as Pandemic by World Health Organization in March 2020 [1]. The first case of Covid-19 in India was reported on January 30, 2020, in a student who had travelled from Wuhan, China. [2]. SARS-CoV-2 has positive-sense, single-stranded RNA (+ssRNA) which is packed within envelope. The virus has four main structural proteins i.e. envelope, spike, nucleocapsid proteins and membrane proteins. [3]

The COVID-19 infection is mainly transmitted by the respiratory droplets that are formed by coughing or sneezing. The viruses also transmitted by the fomites which are deposited on the various surfaces in which the viruses remain active for up to 4 days

[4]. The virus enters the lungs through the spike proteins which have higher affinity towards the lower respiratory tract of lungs due to the higher expression of angiotensin-converting enzyme 2 (ACE-2) on them [5]. Common symptoms of Covid-19 include dry cough, fever, chills, shortness of breath, muscle or body aches, Fatigue, diarrhea, loss of smell or taste etc. The incubation period of Covid-19 infection ranges from 2-14 days [6]. Unlike most of the viral diseases, Covid-19 infection also does not have specific treatment but can be controlled by applying preventive measures such as isolating the infected, symptomatic or individuals those who came in contact of infected persons. Common medicines such as paracetamol or ibuprofen are given for symptomatic relief. Other medication includes antiviral drugs like remdesivir, oseltamivir combined with antibiotics and oxygen support [7]. Community transmission can be managed by wearing face masks, avoiding social gathering, maintaining social distancing and personal hand hygiene. The Silent asymptomatic carriers which are capable of transmitting the Covid-19 infection are a major challenge to the controlling and mitigating efforts done by the government. As such with no specific medicine available for Covid-19 treatment, the management of Covid-19 pandemic depends mainly on social distancing, vigorous screening and separating the asymptomatic infected person is an important step to break the chain of transmission.

Initially, the sole accepted diagnosis test for Covid-19 was RT-PCR but in August 2020, ICMR gave acceptance to the use of RAT as one of the Screening tests. In September 2020, ICMR advised the RT-PCR as the best detecting method followed by, True-Nat and RAT for Covid-19 detection [8]. Therefore, in this paper, we evaluate the performance of all three tests for detection of Covid-19 infection.

## MATERIAL METHOD

The study was conducted in BSL-2 Laboratory, Microbiology Department, Rajarshi Dashrath Autonomous State Medical College, Ayodhya, Uttar Pradesh, India, between June 2022 and August 2022.

A total of 378 samples were taken from people from hospital and some random samplings were done as per the screening protocol given by the government from various regions of Ayodhya. Samples obtained via oropharyngeal and nasopharyngeal swabs were tested by Rapid antigen test and True-Nat and their performance was compared with the gold standard RT-PCR.

Samples were taken from persons between age group varying from 4 years and 90 years. The collected samples were processed in BSL-2 lab RDASMC, Ayodhya, as per the protocol approved by ICMR, New Delhi.

### Sample collection:

Combined Nasopharyngeal and oropharyngeal swabs were collected by healthcare workers across various collection centers and transported the samples in a 3ml viral transport media (VTM) maintaining proper cold chain and collected at the BSL-2 lab RDASMC Ganja campus, Ayodhya, Uttar Pradesh.

### Rapid Antigen test

For RAT, few drops of samples were directly put on the SDAK Lifecare diagnostic Simplified RAT card. RAT is based on Lateral Flow Chromatographic Immunoassay. It directly detects SARS-n CoV viral nucleoprotein produced by replicating virus in respiratory secretions and gives the results within few minutes to maximum 30 minutes [9].

### True-Nat

True-Nat test is a Chip based test used for the semi quantitative detection of SARS-CoV-2 RNA in the respiratory secretions. It is portable, light weight, battery powered equipment which can be used in remote area even with poor infrastructure facilities [10].

### Principle of True-NAT

True-Nat works on Principle of Real Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) based on Taq Man chemistry. The RNA from the samples were first extracted using Trueprep AUTO/AUTO v2 Universal Cartridge based Sample Prep Device and Trueprep AUTO/AUTO v2 Universal Cartridge based Sample Prep kit. 6ul of isolated purified RNA was dispensed in the reaction well of True-Nat COVID-19 chip which was then inserted in True lab Real Time quantitative microPCR Analyzer for RT-PCR. At the end of the reaction, E/ORF1 gene of SARS Cov-2 virus was amplified in positive case.

### RNA Isolation for Real time RT-PCR

RNA is automatically isolated using the Kingfisher Flex Magnetic automated extractor (Thermo Fisher Scientific). For the isolation of RNA, three 96 deep well plates, one 200ul microplate and one tip comb was used. First plate contained 200ul sample (individual or pooled) and 600ul lysis buffer. Second plate contained 600ul wash buffer and third plate had 50ul elution buffer in which final RNA is eluted.

Covid-19 viral nucleic acid extraction was done by using Q-line Molecular Viral RNA extraction kit based on magnetic bead method of RNA extraction. All the 3 plates with the Tip comb are placed in the KingFisher Flex machine according to the Q-line\_Extraction\_Kit program set in the automated RNA extraction machine. After a 14 min run of the given program, the elution plate with RNA is ready for further processing.

### Real time RT-PCR

Till date, the gold standard test for the Corona virus-19 disease (Covid-19) diagnosis is Real time PCR (RT- PCR) which detects the viral genome in naso-oropharyngeal swab fluids, but it requires well equipped biosafety laboratory along with skilled trained manpower [11].

For the detection of SARS-CoV-2 from the isolated RNA, The DiAGSureTMnCoV-19 Detection Assay kit was used. The principle involved the Taq Man chemistry for the detection of nucleic acid from the SARS-CoV- 2 virus in human samples. This three plex RT-PCR included the detection of dual targets E-gene and ORF1 ab gene, and an endogenous RNase-P internal control in a single tube reaction. The RT-PCR reaction was run on BIO-RAD CFX96Real-Time System.

#### Statistical tools:

The number and percentage (%) were used to represent the categorical variables and mean and SD were used to present the continuous variables. To compare the qualitative variables Chi- Square test was used. GraphPad calculator was used to calculate sensitivity, specificity, LR+, LR-, NPV and PPV etc. in 2 x 2 contingency table. A p value of <0.05 was considered statistically significant. The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 23.

## RESULTS

A total of 378 patients were taken randomly for this study with or without the symptoms. Patients were mostly from Ayodhya district of the Uttar Pradesh. The individuals under study showed mild to moderate grade of symptoms. Samples were tested for performance evaluation of Rapid antigen Test, True-Nat and Real-Time RT-PCR among subjects. After the samples were received at BSL-2 Lab, Rapid antigen test was performed from nasopharyngeal swab followed by True-Nat and Real-Time PCR respectively.

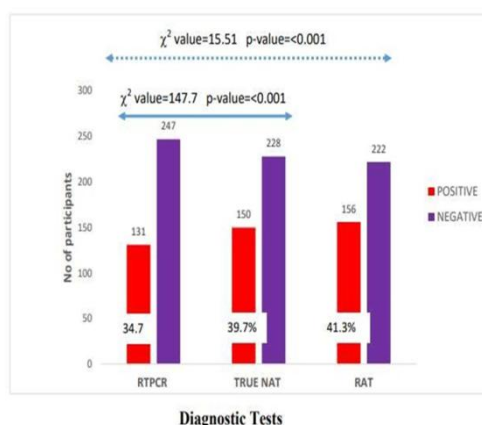


Fig 1. Diagnostic performance of True-Nat and Rapid Antigen test by performing The Chi-Square test.

#### Demographic details of individuals understudy

Characteristics		Number(n =378)	Percentage (%)
Age Groups(Years)	<18	44	11.6
	18 to 35	160	42.3
	36 to 50	93	24.6
	51 to 65	60	15.9
	>65years	21	5.6
Mean(±SD)Age& Range(Years)	36.73±16.95&04-90		
Gender	Male	232	61.4
	Female	146	38.6
RAT Result	Positive	156	41.3
	Negative	222	58.7
TRUENAT Result	Positive	150	39.7
	Negative	228	60.3
qRT-PCR Result	Positive	131	34.7
	Negative	247	65.3
Clinical Condition	Asymptomatic	164	43.4
	Symptomatic	214	56.6

Table 2 show the results of asymptomatic and symptomatic individuals

		Clinical Conditions				P value
		Asymptomatic		Symptomatic		
		n=164	Percentage (%)	n=214	Percentage (%)	
Age Groups( Years)	<18	20	12.2%	24	11.2%	0.947
	18 to 35	69	42.1%	91	42.5%	
	36 to 50	42	25.6%	51	23.8%	
	51 to 65	26	15.9%	34	15.9%	
	>65years	7	4.3%	14	6.5%	
Mean(±SD)Age& Range(Years)		36.73±16.95&04-90				
Gender	Male	103	62.8%	129	60.3%	0.756
	Female	61	37.2%	85	39.7%	
RT-PCR Result	Positive	28	17.1%	103	48.1%	<0.001
	Negative	136	82.9%	111	51.9%	
True-Nat Result	Positive	21	12.8%	129	60.3%	<0.001
	Negative	143	87.2%	85	39.7%	
RAT Result	Positive	59	36.0%	97	45.3%	<0.001
	Negative	105	64.0%	117	54.7%	

Table 3 show the comparative diagnostic performance of True-Nat and RAT

		RT PCR Result					
		Positive		Negative		Total	
		N	%	N	%	N	%
True-Nat	Positive	107	81.7%	43	17.4%	150	39.7%
	Negative	24	18.3%	204	82.6%	228	60.3%
	Total	131	100.0%	247	100.0%	378	100.0%
RAT	Positive	72	55.0%	84	34.0%	156	41.3%
	Negative	59	45.0%	163	66.0%	222	58.7%
	Total	131	100.0%	247	100.0%	378	100.0%

Table 4 show diagnostic performance of True-Nat and RAT

	True-Nat	RAT
Sensitivity	81.68%(74.0to87.89)	54.96%(46.03to63.66)
Specificity	82.59%(77.28to87.11)	65.99%(59.72to71.88)
LR+	4.69(3.53to6.23)	1.62 (1.28 to 2.04)
LR-	0.22 (0.15 to 0.32)	0.68 (0.55 to 0.84)
PPV	71.33%(63.43 to 78.45)	66.39%(61.01to71.37)
NPV	89.47%(84.71to93.14)	54.52 (49.30 to 59.64)
Accuracy	82.30%(78 to 85.95)	59.93%(54.79to64.90)
Cohen's Kappa (k)	0.622(0.54to0.70)	0.201(0.10 to 0.30)
Standard Error (p-value)	0.041(<0.001)	0.051(<0.001)

Table 5 show the Percentage of COVID-19 positive and negative in asymptomatic and symptomatic individual.

Table5			RT-PCR Result			
			Positive		Negative	
			N	%	N	%
Asymptomatic	True-Nat result	Positive	6	21.4%	15	11.0%
		Negative	22	78.6%	121	89.0%
	Rat	Positive	2	7.1%	57	41.9%
		Negative	26	92.9%	79	58.1%
Symptomatic	True-Nat result	Positive	101	98.1%	28	25.2%
		Negative	2	1.9%	83	74.8%
	RAT	Positive	70	68.0%	27	24.3%
		Negative	33	32.0%	84	75.7%

**Table6. Diagnostic performance analysis of True-Nat as compared to RT-PCR in asymptomatic and symptomatic patients.**

RTPCR vs TRUE NAT	ASYMPTOMATIC	SYMPTOMATIC
Sensitivity	21.43% (8.30% to 40.95%)	98.06% (93.16% to 99.76%)
Specificity	88.97% (82.46% to 93.69%)	74.77% (65.65% to 82.54%)
LR+	1.94 (0.83 to 4.57)	3.89 (2.82 to 5.36)
LR-	0.88 (0.72 to 1.08)	0.03 (0.01 to 0.10)
PPV	34.60% (18.37% to 55.43%)	99.50% (99.32% to 99.64%)
NPV	80.62% (77.26% to 83.58%)	42.72% (15.84% to 74.71%)
Accuracy	74.52% (67.13% to 80.99%)	97.62% (94.57% to 99.21%)
Cohen's Kappa (k)	0.115 (-0.061 to 0.292)	0.722 (0.632 to 0.811)
Standard Error (p-value)	0.09 (0.134)	0.046 (<0.001)

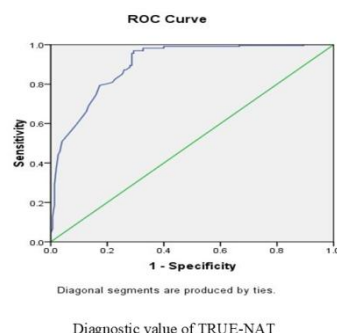
**Table7. Diagnostic performance analysis of RAT as compared to RT-PCR in asymptomatic and symptomatic patients.**

	Asymptomatic	Symptomatic
Sensitivity	7.14% (0.88% to 23.50%)	67.96% (58.04% to 76.82%)
Specificity	58.09% (49.33% to 66.49%)	75.68% (66.62% to 83.32%)
LR+	0.17 (0.04 to 0.66)	2.79 (1.96 to 3.98)
LR-	1.60 (1.34 to 1.91)	0.42 (0.31 to 0.57)
PPV	1.29% (0.34% to 4.78%)	85.58% (80.65% to 89.43%)
NPV	89.11% (87.29% to 90.71%)	52.64% (45.15% to 60.02%)
Accuracy	54.47% (46.52% to 62.25%)	70.43% (63.83% to 76.46%)
Cohen's Kappa(k)	-0.242 (-0.342 to -0.141)	0.437 (0.317 to 0.558)
Standard Error (p-value)	0.051 (<0.001)	0.061 (<0.001)

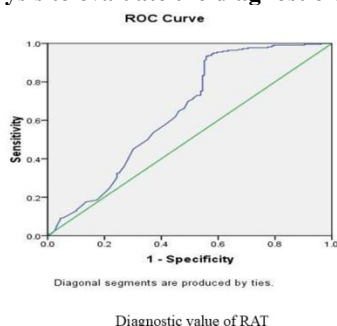
**Table 8 show comparisons between RT-PCR, True-Nat and RAT**

Table8. Comparison between Rapid antigen test, True-Nat and RT-PCR				
S.No	Properties	RAT	True-NAT	RT-PCR
1	Swab	Nasopharyngeal and oropharyngeal	Same as RAT	Same as RAT
2	Specimen for test	Sample fluid	RNA processed by machine	RNA processed either manually or automated
3	Lab required	Not compulsory	Not compulsory	Compulsory BSL-2/BSL-3 Lab
4	Protocol	Easy	Moderate	Tough
5	Trained person	Not required	Not compulsory	Required
6	Time duration	10-30min	1-2hrs	3-4hrs
7	Results form	Stripes on the card	Ct-values	Ct-values
8	Sensitivity	Low	Medium	High
9	Specificity	High	High	High

10	Cost	Lowest	Moderate	Highest
11	Better used for	For surveillance	Diagnosis	Diagnosis
12	Implementation at community level	Possible	Not possible	Not Possible



**Fig 2. ROC analysis to evaluate the diagnostic value of True-Nat**



**Fig 3. ROC analysis to evaluate the diagnostic value of RAT**

Upon studying the demographic details of the 378 individuals involved in this study, we observed that most of them fall under the age group of 18-35 years (42.3%) and the least were in the age group above 65 years (5.6%). About 61.4% were male and 38.6% were female. Out of 378 individuals, 214 (56.6%) were symptomatic having one or more COVID-19 disease-related symptoms like high-grade fever, loss of taste, loss of smell, cough, sneezing, myalgia etc., while 164 (43.4%) were asymptomatic. Out of 214 symptomatic cases, 129 were positive and 85 were negative when tested by True-Nat. Among 164 asymptomatic cases, 21 were positive and 143 were found to be negative by True-Nat (Table 2). While 97 were tested positive and 117 negatives by RAT in symptomatic. Along with 59 positive and 105 negative in asymptomatic persons by RAT (Table 2). Most of the symptomatic cases were present in the 18–35 years of age range presenting 42.5% (n=91) of the total symptomatic cases (Table 2). The Mean ( $\pm$ SD) Age & Range (Years) was  $36.73 \pm 16.95$  & 04-90 years (Table 2).

In a study of 378 samples, True Nat identified 107 true positives (81.7%) and 204 true negatives (82.6%), with 43 false positives (17.4%) and 24 false negatives (18.3%). This resulted in a total of 150 positive and 228 negative cases. RAT results showed 72 true positives (55.0%) and 163 true negatives (66.0%), with 84 false positives (34.0%) and 59 false negatives (45.0%), leading to 156 positive and 222 negative cases (Table 3).

Upon comparison of diagnostic efficiency, the RAT sensitivity was found to be 54.96% and specificity was 65.99%. The positive predictive value (PPV) was 46.15% and the negative predictive value (NPV) was 73.42%. The diagnostic accuracy between RAT and RT-PCR techniques was found to be 62.17% with a kappa coefficient of 0.201 (SE: 0.051 and CI at 95%: 0.10 to 0.30). The likelihood ratio for positive result (LR+) of RAT was 1.62 and the likelihood ratio for negative result (LR-) of RAT was 0.68 (Table 4).

The True-Nat sensitivity was found to be 81.68% and specificity was 82.59%. The positive predictive value (PPV) was 71.33% and the negative predictive value (NPV) was 89.47%. The accuracy between True-Nat and RT-PCR techniques was found to be 82.30% with a kappa coefficient of 0.622 (SE: 0.042 and CI at 95%: 0.54 to 0.70) (Table 4). The likelihood ratio for positive result (LR+) of True-Nat was 4.69 and the likelihood ratio for negative result (LR-) of True-Nat was 0.22 (Table 4).

In asymptomatic patients, the positivity rate was higher in True-NAT (21.4%) as compared to RAT which showed only 7.1%, while the symptomatic patients also had a much higher positivity rate by True-Nat (98.1%) as compared to only (68%) by RAT. These results showed that the True-Nat showed much better performance than RAT when compared to RT-PCR (Table 5).

The True-Nat shows higher sensitivity (98.06%), LR+ (3.89), PPV (99.5%) and diagnostic accuracy (97.62%) in symptomatic patients with significant p value as compared to asymptomatic patients, while the specificity (88.97%), NPV (80.62%) was higher in asymptomatic patients upon testing with True-Nat. Hence, True-Nat can better detect the COVID-19 infection in symptomatic patients as compared to asymptomatic patients (Table 6).



The RAT shows higher sensitivity (67.96%), specificity (75.68%), LR+ (3.89), PPV (85.58%) and diagnostic accuracy (70.43%) in symptomatic patients with significant p value as compared to asymptomatic patients, while only NPV (80.62%) was higher in asymptomatic patients upon testing with RAT. Hence, RAT can better detect the COVID-19 infection in symptomatic patients as compared to asymptomatic patients (Table 7).

Among both True-Nat and RAT, True-Nat has higher percentage of diagnostic performance as compared to RAT in symptomatic patients.

## DISCUSSION

The SARS-Cov2 virus is a novel virus which is consistently evolving to escape the immune system of human, thus making the vaccination less effective against curbing the Covid-19 infection. Therefore, rapid diagnosis and segregation of infected individuals are an important strategy for controlling the spread of Covid-19 infection. This study was conducted in BSL-2 lab, Rajarshi Dashrath Autonomous state Medical College, Ayodhya from duration June 2022 to August 2022 to analyze the comparative study of all the tests including Covid-19 Rapid antigen test, True-Nat and RT-PCR.

In this study the assay performance of Rapid Antigen test and True-Nat was compared with Real-Time RT-PCR. RT-PCR test is very sophisticated, time consuming test and also it requires well- equipped laboratory, and highly trained technicians. Therefore, there is a need for rapid test kit that can be used for screening of both asymptomatic as well as symptomatic individuals with less equipment and facilities. Since, Rapid antigen test is simple, with minimal technical expertise, basic infrastructure facilities and can be easily performed with some universal precautions, therefore RAT is popular in at places with minimal facilities and infrastructure and in remote areas. RAT in this paper has shown specificity of 65.99% and sensitivity of 54.96% respectively. The WHO recommended a minimum of 80% of sensitivity and 97% of sensitivity for RAT as diagnostic test [12]. Since, the sensitivity and specificity of RAT is very low as compared to RT-PCR, it cannot replace Real- Time RT-PCR for diagnosis and surveillance for SARS-CoV-2 [13]. True-Nat test for detection of SARS CoV- 2 was first introduced by Molbio Diagnostics, which suggest sensitivity of 100% and specificity of 98%. Later it was approved by Indian Council of Medical Research (ICMR) New Delhi for the widespread Covid-19 detection [14,15]. Upon comparison with RAT, the True-Nat results with the same 378 samples give better performance efficacy. We observed better sensitivity (81.68%) and specificity (82.59%) and also the diagnostic accuracy of True-Nat was found to be higher i.e. 82.30% as compared to 62.17% of RAT, which indicate True -Nat to be a better diagnostic method than RAT. The PPV and NPV of True-Nat are quite close to RT-PCR values making it good choice for screening of symptomatic and asymptomatic individuals. The chi-square test results also predict better performance of True-NAT as compared to Rapid antigen test with significant p values  $\leq 0.001$  (Fig 1). Studies have established that positive SARS-CoV-2 RT-PCR do not allow definitive conclusions whether the patient is still contagious or not [16].

Upon comparing the results with respect to RT-PCR, we get the lowest sensitivity in Rapid antigen test which detects the Covid-19 virus spike proteins synthesized by the replicating viruses in the respiratory secretions. Since there is no amplification of the target like that in the True-Nat and RT-PCR, therefore it is least sensitive as compared to the other two tests. RAT test was reported with 47% sensitivity with a specificity of 86%, even though the positive given by the RAT were considered as positive in the hospital for preventive measures. While the True-Nat shows better sensitivity of 73% and specificity of 90% with respect to RT-PCR as gold Standard [17].

A 2025 cross-sectional study from the West region of Cameroon investigated antimicrobial susceptibility among urinary pathogens isolated from patients with urinary tract infections. The authors found a high prevalence of multidrug resistance in *Escherichia coli* and *Klebsiella pneumoniae*, with a large proportion of isolates producing extended-spectrum  $\beta$ -lactamases (ESBLs), contributing to extensive resistance against third-generation cephalosporins and several commonly used antibiotic classes. Molecular analysis revealed the dominance of genes conferring  $\beta$ -lactam resistance, underscoring the challenge these organisms pose to empirical UTI therapy in the region and highlighting the urgent need for continuous surveillance and tailored antimicrobial stewardship interventions [18].

In a multicenter study across nine hospitals in China, antimicrobial susceptibility patterns of *Streptococcus pneumoniae* isolates were evaluated against both traditional and newly developed antibiotics. Results showed uniform high resistance of all 208 isolates to macrolides and tetracycline, and notable penicillin non-susceptibility in a subset. Importantly, the novel agents — including eravacycline, omadacycline, and contezolid — exhibited low resistance rates ( $\leq 7.2\%$ ), suggesting maintained efficacy against resistant pneumococcal strains. Genetic analysis revealed specific resistance mechanisms, such as 23S rRNA mutations and tet genes, informing future clinical use and surveillance strategies for emerging antimicrobials [19].

A 2025 study from a pediatric hospital in Romania assessed antimicrobial resistance trends and ESBL prevalence among *E. coli* isolates from children with urinary tract infections. The analysis revealed a substantial proportion of ESBL-producing strains, with elevated resistance to multiple first-line antibiotics, complicating empirical treatment decisions. Resistance trends indicated persistent difficulty in treating pediatric UTIs with conventional agents like amoxicillin-clavulanate and fluoroquinolones, reinforcing the need for local antibiogram-guided therapy and enhanced antibiotic stewardship to curb the spread of resistant *E. coli* in this vulnerable population [20].

The Area under curve analysis (AUC) for the True-Nat test result is 0.899, with a standard error of 0.017. This high AUC indicates excellent discriminatory power between positive and negative cases. The 95% confidence interval ranges from 0.866

to 0.932, suggesting the result is precise and reliable. The p-value is 0.000, shows highly significant. (Fig2). The AUC for the RAT test result is 0.647, with a standard error of 0.031. This suggests moderate discriminatory power between positive and negative cases. The 95% confidence interval ranges from 0.587 to 0.707, indicating the result is fairly precise. The p-value is less than 0.001 (Fig 3). The table 8 showed the summary of comparative features of Rapid antigen test, True-Nat and RT-PCR. WHO had recommended the importance of molecular testing over antigen test. Though, all three tests have its own importance depending upon the infrastructure, availability of instruments, skilled persons and the place.

## CONCLUSIONS

Upon comparing all three testing method in our lab, it can be concluded that real time PCR has higher sensitivity and specificity over True-Nat or Rapid antigen test. In RT-PCR 96 samples can be processed simultaneously as compared to four and one samples in True- Nat and RAT respectively. WHO recommends >80% sensitivity for RAT but our lab RAT results has showed much lesser sensitivity [17]. Both True-Nat and RAT test kits studied here has shown less sensitivity and specificity than RT-PCR. But for saving time and in medical emergency RAT can be preferred upon other tests.

We can conclude that though RT-PCR is gold standard for detection of Covid-19 infection but the importance of RAT and True-Nat cannot be ignored altogether. RAT being the simplest and least time consuming with lowest sensitivity and specificity among all three tests but it is good for primary screening and surveillance. True-Nat is also a simple and easy to use, rapid molecular diagnostic test for detection of Covid-19 infection and can be performed with limited lab set up. Combination of these tests will help us in better and efficient detection of Covid-19 infection in hospitals and remote areas.

## Author Contributions

Conceptualization, S.S. and G.K.; methodology, L.G., and L.K.U.; statistical analysis, G.K., L.G. and L.K.U.; investigation, M.K. and R.S.; data curation, L.G. and L.K.U.; writing—original draft preparation, L.G. and L.K.U.; writing—review and editing, L.G., L.K.U., and G.K.; supervision, G.K. and S.S; project administration, S.S and G.K. All authors have read and agreed to the published version of the manuscript.

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## Conflict of Interest

The authors declare that there is no conflict of interest.

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