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A COMPARATIVE STUDY OF DIAGNOSTIC ACCURACY OF A POINT-OF-CARE SCREENING TEST VIS-À-VIS A GOLD STANDARD TEST FOR DIAGNOSIS OF COVID-19 IN CASES OF SURGICAL EMERGENCIES

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ABSTRACT

In this COVID – 19 pandemic, there's an urgent need for a sensitive and specific test which provides results in a short span of time. Rapid Antigen Tests, have the potential to replace RT-PCR tests as a confirmatory test. A comparative study of Point-of-Care test vis-à-vis gold standard test was carried out by the Surgical Department of a secondary level care hospital. A total of 112 patients, with various surgical emergencies were tested, by RADT and by RT-PCR for confirmation. Patients were managed based on RADT results as per the institutional algorithm. 107 RADTs tested negative while five tested positive. 105 RT-PCR tested negative while seven tested positive. Two patients reported negative by RADT were reported as RT-PCR positive. Although RADTs have the potential, their sensitivity is highly variable. To establish them as diagnostic tests of choice, RADTs need to be developed further so as to ensure that their sensitivity is high and results are reproducible across multiple centers.

KEYWORDS

COVID – 19, RT – PCR, Rapid Antigen Detection Tests (RADTs), Surgical emergencies

1. INTRODUCTION

Tedros Adhanom Ghebreyesus, the Director General of World Health Organization, said, "You cannot fight a fire blindfolded. Our key message is test, test, test." [1] Test interpretation using Rapid Antigen Point-of-Care (POC) test as per ICMR advisory is as depicted in Fig 1. [2]

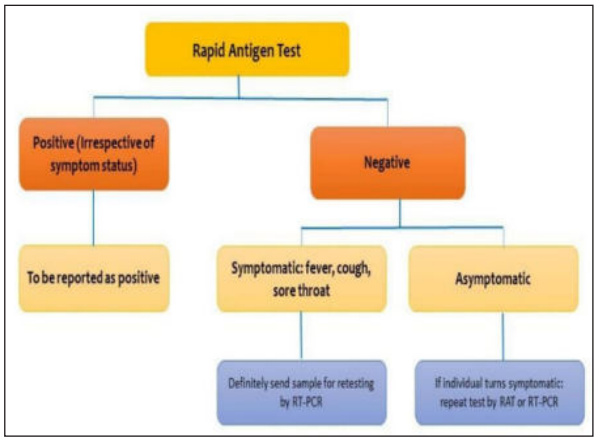

Fig 1. Algorithm for COVID – 19 test interpretation using Rapid Antigen Point-of-care test (Reproduced from ICMR – Advisory on Strategy for COVID – 19 Testing in India. 2020; (Ver VI:14-7). (Permission awaited)

A search for studies for efficacy of RADTs as pre-operative screening test through archives such as PubMed and Google Scholar produced very few results. On narrowing the search to Indian demographics, we were unable to find any study so far. Therefore, the present study was designed to analyze the efficacy of RADT [Covid – 19 Ag RespiStrip (Coris Bio-Concept, Gembloux, Belgium)] used at this center vis-à-vis the RT-PCR test [Truenat™ COVID-19 RT-PCR Testing Kit (Molbio Diagnostics Private Ltd, Goa, India)] which is considered as the gold standard for the purpose of this study.

2. OBJECTIVES OF THIS STUDY

(a) To assess diagnostic criteria/ accuracy parameters of Point-of-Care diagnostic test in detection of COVID – 19 in surgical emergency setting of a secondary level care hospital.
(b) To formulate strategy/ standard protocol for covid testing pre-operatively in cases of surgical emergencies.

3. MATERIALS AND METHODS

(a) Study Type/Design. Comparative study of diagnostic accuracy parameters of point-of-care test vis-à-vis gold standard test.
(b) Study Setting. Surgical Department of a secondary level care hospital.
(c) The present study was approved by the Institutional Ethical Committee letter number 15/2021 conducted on 05 April 2021.
(d) Operational definitions. For the purpose of this study,
(i) Emergency surgical patient is defined as "Any patient requiring surgical evaluation (operative/ non-operative) for diseases within the realm of general surgery as defined by American Board of Surgery". [3] For our study, emergency surgical case is that patient who is admitted with a diagnosis that warrants emergency surgical procedure.
(ii)'RADT' is defined as "Immunoassays that detect the presence of specific SARS-CoV2 Ag in upper and lower respiratory specimens such as Nasopharyngeal / Oropharyngeal swabs, Sputum, Bronchoalveolar lavage, etc. which indicates current infection. [4] The brand used in this study was Covid – 19 Ag Respi Strip manufactured by Coris Bio-Concept, Gembloux, Belgium and all specimens were collected from Nasopharynx as recommended by the Company.
(iii)rRT-PCR is defined as real time – Reverse Transcription – Polymerase Chain Reaction (rRT-PCR) test for both qualitative and quantitative detection of nucleic acid from SARS-Cov2 in upper and lower respiratory specimens such as Nasopharyngeal / Oropharyngeal swabs, Sputum, Bronchoalveolar lavage, etc. In our study, Truenat™ COVID-19 RT-PCR Testing Kit manufactured by Molbio Diagnostics Private Ltd, Goa, India was used. Samples were collected from oropharyngeal swabs as per company's literature recommendation.
(e) Sample Size and Sample Selection Procedure. All patients who were admitted to the hospital for undergoing emergency surgical procedure and consented were included in the study. Assuming the proportion of patients requiring emergency surgery as a function of/ upon all surgical patients (p) as 0.6 (60%), with an acceptable deviation (d) + of 10% (0.1), power of study (β) as 0.8 (80%) and level of significance (α) as 5% (0.05), sample size was computed as 92.16, approximated to 92.

Formula used was

$$n = \frac{p*q*(Z_{1-\alpha/2})^2}{d^2}$$

where $Z_{1-\alpha/2}$ is the score in table corresponding to value of 1-α/2

Considering the constraints imposed by the pandemic on time, resources and allied institutional factors, the required sample was met by accrual/ enrolment method. Patients meeting the inclusion criteria were enlisted in the study until the optimal sample size (that is 92) was fulfilled.

Inclusion Criteria:
Patients posted for emergency surgery and willing to participate in the study.

Exclusion Criteria:
Patients undergoing elective surgical procedures and/or those unwilling to be part of the study.

(f) Data Collection Techniques.
RADT test was conducted on all patients included in the study, by taking their nasopharyngeal swab. Simultaneously, RT-PCR test was also conducted on all these patients using throat swab (oropharyngeal sample as recommended by the company). While the results of RADT were available within 20 minutes, in a time frame befitting wheeling the patient to Emergency OT, the results of RT-PCR takes an average of four to six hours and for patients admitted in the late evening, even up to 18 hours. Hence as a mid-way measure to balance covid protection protocols with those of emergency life-saving surgery, those positive with RADT were assigned to conservative management as much as feasible and only life or limb threatening emergencies were taken up for surgery with all COVID precautions. Such patients who were to undergo conservative management were admitted to isolation ward, for covid treatment. However, those found RADT negative were taken up for emergency surgery, even while the RT-PCR result was awaited. Again, complete COVID precautions were followed during the emergency surgery (assuming the patient to be covid positive until RT-PCR results were reported). If the RT-PCR result came as negative, the patient was admitted to non-covid post-operative ward as a normal case and the subsequent post-surgery protocols were followed. If the RT-PCR result came as positive, the patient was shifted to covid ward and treated as per covid treatment protocols. The algorithm that was followed is depicted in Fig 2.

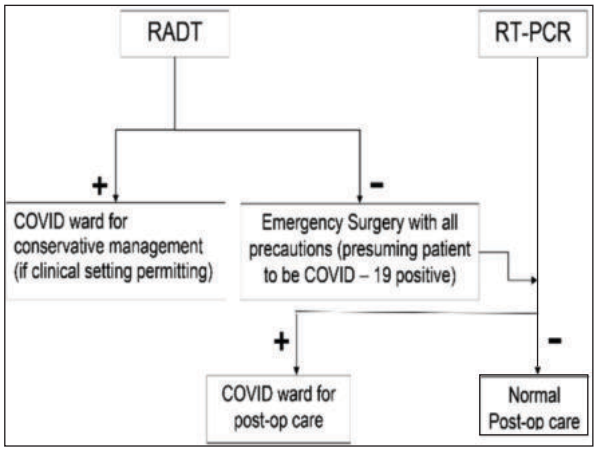

Fig 2. Algorithm of Patient Testing

(g) Data Entry and analysis.
Data was entered into MS Excel worksheets and analyzed using Epi-Info software. Results were computed in terms of percentage, proportion, rates and ratios. They were displayed as pie charts, bar diagram, tables and figures.

4. RESULTS
A total of 224 samples were collected and tested, 112 each by Rapid Antigen Testing and by RT-PCR. RADT samples were obtained from Nasopharynx while RT-PCR samples were obtained from Oropharyngeal swabs as recommended by the literature specifications provided with the kit. Most patients (75.5%) were males. Median age of patients was 30.5 years, with Inter-Quartile Range (IQR) being 16.75 years (Table 1).

	Male		Female		Total (n=112)	
Age (in years)	Nos	Percentage	Nos	Percentage	Nos	Percentage
0 - 10	5	5.95	3	10.71	8	7.14
11 – 20	7	8.33	1	3.57	8	7.14
21 – 30	32	38.10	8	28.57	40	35.71
31 – 40	18	21.43	11	39.29	29	25.89
41 – 50	3	3.57	1	3.57	4	3.57
51 – 60	3	3.57	2	7.14	5	4.46
61 – 70	12	14.29	2	7.14	14	12.50
71 - 80	4	4.76	0	0.00	4	3.57
Total	84		28		112	

Table 1. Age – Sex distribution of study group.

Overall Test Positivity rate (TPR) was 95.5%. Test Positivity Rate was highest in females tested by RADT (96.3%), and least for females tested by RT-PCR (92.6%). Distribution of patients as per age, gender and TPR is depicted in Figures 3 and 4.

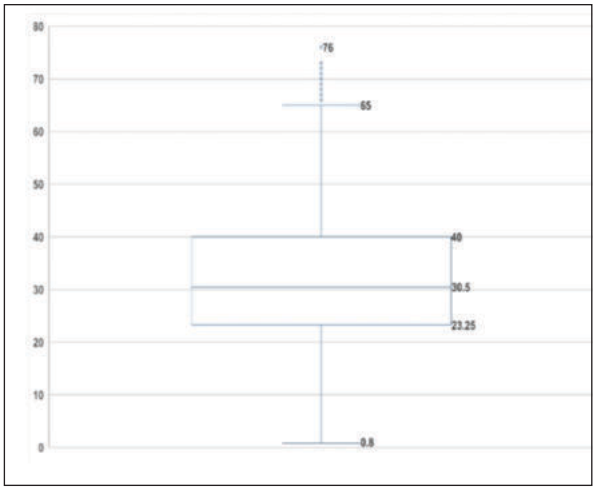

Fig 3. Box and Whisker Plot – Age Distribution of Emergency Surgery Patients.

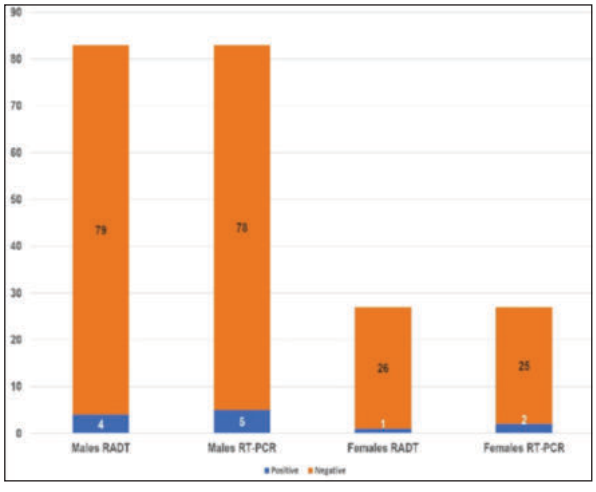

Fig 4. Test Positivity Rate – Distribution as per Test Type

Out of the 112 RADTs conducted, 107 tested negative while five tested positive. Similarly, out of the 112 RT-PCR tests conducted, 105 tested negative while seven tested positive. Out of the seven that tested positive by RT-PCR, five had tested positive by RADT as well. However, two patients tested negative in RADT were found to be RT-PCR test positive (Table 2).

		RT-PCR	
		Positive	Negative
RADT	Positive	5	0
	Negative	2	105

Table 2. Summary of results of RADT and RT-PCR.

The patients with surgical emergencies that required emergency surgical intervention and their diagnoses are depicted in Table 3 and Figure 5.

Sr	Diagnosis	Total	Percentage	RADT		RT-PCR	
				Positive	Negative	Positive	Negative
a.	Abscess AAW	1	0.89	0	1	1	0
b.	Abscess Breast	8	7.14	0	8	0	8
c.	Abscess Gluteal Region	13	11.61	0	13	0	13
d.	Abscess Lower Limb	3	2.68	0	3	0	3
e.	Abscess Neck	5	4.46	0	5	0	5
f.	Abscess Perineal Region	8	7.14	1	7	1	7
g.	Acute Appendicitis	23	20.54	2	21	2	21
h.	CLW	12	10.71	0	12	0	12
i.	Crush Injury	6	5.36	1	5	1	5
j.	Degloving Injury	2	1.79	0	2	0	2
k.	Diabetic foot	11	9.82	0	11	0	11
l.	Infected Pilonidal Sinus	3	2.68	0	3	0	3
m.	Miscellaneous	10	8.93	0	10	1	9
n.	Tendon Injury	5	4.46	1	4	1	4
o.	Traumatic Small Bowel Perforation	2	1.79	0	2	0	2
	Total	**112**		**5**	**107**	**7**	**105**

Table 3. Types of surgical emergencies.

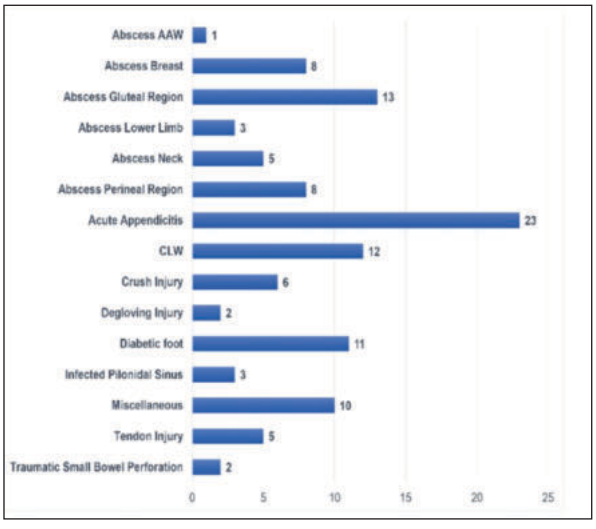

Fig 5. Distribution of diagnosis at presentation.

34% were abscesses of various locations warranting incision and drainage, 21% of them were Acute Appendicitis, and nearly 11% were Contused Lacerated Wounds (CLW). Miscellaneous group, which included Obstructed Hernias, Foreign body removal, acute rectal bleed and Necrotizing Soft Tissue Infection (NSTI), contributed to approximately 9% of surgical emergencies.

Statistical parameters of RADT, the POC diagnostic test vis-a-vis RT-PCR, are as brought out in Tables 1 and 2. RADT had 71.4% sensitivity, 100% specificity, 100% Positive Predictive Value and 98.1% Negative Predictive Value.

5. DISCUSSION

In a secondary level care hospital with a regular inflow of surgical emergencies warranting hospital admission and urgent surgical intervention, a POC testing for COVID – 19 is essential, the result of which would determine the ward to which the patient gets admitted, the need for isolation and associated hospital logistics requirements, handling of patient and his / her specimens by health care workers, etc. In addition, whether the patient would be taken up for surgery or placed on conservative management (clinical situation permitting) depends to a large extent on the COVID status of the patient. Therefore, the POC test should be able to produce reliable results in shortest span of time, preferably within 20 to 30 minutes.

Coronavirus Disease 2019 (COVID – 19) is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2, previously 2019-nCoV). This virus was first registered in Wuhan City, Hubei province, China[5]. Transmission to humans is not just through droplets from coughing and sneezing but during speech also [6,7].

SARS-CoV-2 is an enveloped, positive-sense single-stranded genomic RNA virus (+ssRNA) which is taxonomically classified into the Coronaviruses family and Sarbecovirus subgenus. To date, six other viruses of this family have been responsible for infection in humans. SARS-CoV-2 virus diameter lumen is approximately 80 nm and the genome is ~30kb which contains genes encoding orf1ab polyproteins and structural proteins which are of four types, namely surface glycoprotein (S), an envelope protein (E), membrane protein (M), and nucleocapsid (N) proteins[8].

Majority of the commercially available SARS-CoV-2 virus detection kits employ this knowledge to detect presence of infection in an individual by this virus. Real-Time Reverse Transcription Polymerase Chain Reaction Test and Isothermal Nucleic Acid Amplification Test identify specific viral gene regions using Nucleic Acid Amplification Techniques (NAAT). Similarly Rapid Antigen Detection Test detect presence of viral components such as S Glycoprotein, M Protein or N Protein, which act as antigens in the body [9].

In RT-PCR, SARS-CoV-2 RNA undergoes reverse transcription into a complementary DNA (cDNA). The next step is designing primers and a fluorophore-quencher probe for amplifying specific parts of the cDNA and obtaining quantified results about the presence of SARS-CoV-2. A mixture containing the extracted RNA, all necessary buffers enzymes, primers, probes, and precursors, including nuclease-free water, reverse transcriptase, polymerase, additives, forward and reverse primers, a fluorophore-quencher probe, and nucleotides is put in a thermocycler and the series of temperatures and time periods are set to run the cycles. In each cycle, the cleavage of the fluorophore-quencher probe results in a fluorescent signal which is detected by the thermocycler to give information on the process in a real-time manner. In the test kit that we used, Truenat™ COVID-19 chip based RT-PCR Testing Kit, a positive amplification causes the dual labeled fluorescent probe to release the fluorophores in an exponential manner which is then captured by the built-in opto-electronic sensor and displayed as amplification curve on the analyzer screen, on a real time basis during the test run[10,11].

The Rapid Antigen Testing Test that we used, "Covid – 19 Ag Respi Strip" (Coris Bio-Concept, Gembloux, Belgium), is an immunochromatographic test (ICT) for detection of the SARS-CoV-2 antigen in secretions from the nasopharynx. The ICT strip consists of nitrocellulose laminated on a plastic backing, with colloidal-gold conjugates being dried on a conjugate pad overlapping the bottom of the nitrocellulose. Based on previous research on SARS-CoV, it was established that the nucleoprotein was the best target for a sensitive diagnostic sandwich assay using monoclonal antibodies. Thus, for this test, monoclonal antibodies directed against the SARS-CoV and SARS-CoV-2 highly conserved nucleoprotein antigen are coated on the nitrocellulose. The sample, along with the buffer, is let to stand for ~15 minutes after which the test is read. Presence of a Test line along with the Control line indicated positive result. The intensity of the test line may vary depending on the quantity of antigens found in the sample[12,13]

As per the manufacturer, this testing kit was compared with RT-PCR test on 189 nasopharyngeal specimens in GP Clinic settings in USA and on 231 nasopharyngeal specimens in two Reference Hospitals:

University Hospital Laboratory of Brussels and the University Hospital Laboratory of Liège. Overall sensitivity, in the case of GP Clinic setting, was 91.2% and in the case of specimens from the two reference hospitals, it was 60.1%. Specificity for both the setups was 99.4% and 99% respectively[14]. A Cochrane review of literature determined the average sensitivity and specificity of this strip as 39.7% and 98.3% in overall patients (n = 7; 1781 samples, 707 cases), 34.1% and 100% in symptomatic people (n = 3; 780 samples, 414 cases) and 28.6% and 100% in asymptomatic people (n = 1; 45 samples, 14 cases)[15].

Mertens et al, in their study, found this testing kit to have an overall sensitivity and specificity of 57.6% and 99.5% respectively [12].

In our study, we established the RADT to be 71.43% sensitive and 100% specific. For confirmation, RT-PCR testing was done for these patients on the same or subsequent day during the post-op period. This was done keeping the RT-PCR tests results, carried out at our Centre, as the gold standard. For the same, we used Truenat™ COVID-19 RT-PCR Testing Kit (Molbio Diagnostics Private Ltd, Goa, India). This is a chip-based Real Time duplex Reverse Transcription Polymerase Chain Reaction (RT PCR) test for the semi quantitative detection of SARS CoV-2 RNA in human oropharyngeal and nasopharyngeal swab specimen and aids in detection and confirmation of SARS CoV-2 infection and diagnosis of COVID-19. The test detects the E and Orf1a genes of the virus.

As per the manufacturer's data, the test kit was evaluated clinically at the State VRDL lab at Bangalore Medical College, Bengaluru, India. Truenat™ COVID-19 assay kit was able to correctly detect all the 45 confirmed SARS CoV-2 positive cases and the 74 confirmed RT-PCR negative samples thus indicating 100% sensitivity, specificity and 100% overall concordance to reference gold standard assay[11,16].

WHO brought forth the general recommendations regarding the use of RADT for detection of COVID – 19. The minimum performance requirements that any RADT must achieve is ≥80% sensitivity and ≥97% specificity as compared to a NAAT reference assay[17]. Based on different studies, the RADT kit that we used for our study, shows sensitivity from as low as 57% to as high as 91%. Sensitivity of the same RADT kit was 71.43% as per our study.

Such a large variation in sensitivity can be attributed to various factors, these being, time from onset of infection, the concentration of virus in the specimen, the quality and processing of the specimen collected from a person, and the precise formulation of the reagents in the test kits. Furthermore, RADTs for COVID – 19 are most sensitive during periods of high viral loads, i.e., when the patients are most infectious, which is generally 1 – 3 days before the onset of symptoms and the first 5 – 7 days after the onset of symptoms. RADTs may be false negative when the viral load falls below the test's limit of detection, which corresponds to RT-PCR cycle threshold values of <30 – 35. [18,19]

This study's uniqueness lies in the fact that we were unable to find any other study that was exploring the potential of RADTs as a reliable screening test for emergency surgical patients. The strength of the study is that it is a single center study where sample collection was done for all patients by the same individual.

However, the study is not without its weaknesses. Since the sample size was small and restricted to a community confined to a localized region, it may not be adequately representative of the general population. Also, RADT samples were collected from the nasopharynx while RT-PCR samples were taken from the oropharynx either on the same day or the next day. The viral load of both the sites may be different and variable timings of sample collection as well may have affected the sensitivity of the tests.

6. CONCLUSION

RADTs have the potential to reduce testing time to a significant level. However, in our study, it fails to stand the test of our scrutiny to be a standalone investigation for pre-operative screening in cases of surgical emergencies because

(i) The same kit (Covid – 19 Ag Respi Strip) shows variable sensitivity in various centers as mentioned above.
(ii) WHO approves a screening test for COVID as valid if Sensitivity is >80% and Specificity >90%. In our study, though the specificity is 100%, sensitivity is 71.4%.

Also, our secondary objective to evolve a strategy / protocol for pre-operative screening for COVID-19 using RADT could not materialize for reasons mentioned above.

7. Recommendations

We recommend that the same study be repeated with bigger sample size, across multiple centers, using same or similar RADT kits to assess reproducibility of results. Also, the patients must undergo specimen collection from same site for both the tests viz RADT and RT-PCR and sample collection to be performed simultaneously to improve consistency. Until such time, interim recommendations may be formulated based on the ICMR's Advisory on Strategy for COVID-19 Testing in India as brought out schematically in Figure 2. The process of testing and diagnosing COVID – 19 is a dynamic challenge which can only be accomplished by our persistent efforts to fine tune our protocols and processes with the ever-growing knowledge about this disease.

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