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Original Research Article

Evaluation of Dry Swab Direct RTPCR Method for Detection of SARS-Cov2 in a Tertiary Care Hospital

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Abstract:

Introduction: The ongoing SARS-CoV-2 pandemic has led to shortages of laboratory-based testing kits and reagents worldwide and manufacturers have developed simple testing strategies for easy use and convenience. The Dry Swab-based Direct RT-PCR is a simple and quick method for SARS-COV2 detection which has been developed by Meril Diagnostics Pvt. Ltd. This method is a simple variation of the existing RT-PCR testing, making the extraction step easy and can expedite the testing capacity and reduces the turnaround time.

Objectives: The aim of this study was to assess the diagnostic accuracy (sensitivity, specificity, positive predictive value and negative predictive value) of Meril extraction-free dry swab kit and compare its diagnostic performance with the usual RTPCR testing aided with extraction process using swabs collected in viral transport medium.

Materials and Methods: This is a cross sectional (facility-based study) conducted at a tertiary care hospital in South India.

The nasopharyngeal or oropharyngeal dry swab was taken under aseptic precautions, kept in a sterile collection tube and sent to the laboratory for further testing. The dry swab was processed as per the manufacturer's instructions and proceeded with RTPCR testing as per the PCR kit protocol.

The same individual's VTM swab samples were also processed using the extraction kit and then proceeded with RTPCR protocol and the comparisons between the two test methods were done.

Results: Among the 133 patients who were included in the study, RT-PCR testing with conventional extraction was positive for 19 individuals, with a prevalence of 14.29% and negative for 114 patients (85.71%).

The Meril extraction free dry swab kit test was positive for 21 patients (15.79%) and negative for individuals112 (84.21%). The comparison analysis shows a Sensitivity of 94.7%, Specificity 97.4%Positive Predictive Value 85.7% And Negative Predictive Value 99.1%. Area under the Curve (AUC) indicates that the dry swab kit was able to distinguish between true positive and true negative very efficiently.

Conclusion: Nasopharyngeal and oropharyngeal swabs stored in dry collection tubes area robust and inexpensive method for SARS-CoV-2 testing. The efficiency is almost equal to RTPCR testing. It can be deployed for large scale testing considering the advantages.

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Introduction

The novel Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2), causing Corona Virus Disease 2019 (COVID-19) was first identified in Wuhan Jinyintan Hospital, China, in December 2019. Since then, it has spread widely, reaching the status of global pandemic as declared by the World Health

Organization on 11 March 2020 [1]. As of January 2023, 44,682,719 cases have been reported and 530,740 deaths due to COVID 19, have been reported in India.[2]

The emergence of the COVID-19 pandemic resulted in an unparalleled need for RT-PCR-based molecular diagnostic testing, placing a burden on the logistics of commercial PCR testing kits and reagents. There was a high demand for COVID-19 diagnostic kits and reagents around healthcare centers due to which many kits were approved through emergency use approval (EUA) mode because of limited availability of other COVID 19 testing kits. Due to this measured supply, there is an increase in global demand for alternative approaches which is economical and also needed lesser time to process the samples. While long-term prevention goals include quick diagnosis and implementation of vaccines, the urgency of this rapidly developing crisis all over the world has prioritized the shorter turnaround time of diagnostic testing. The heavy social and economic toll of the coronavirus disease 2019 (COVID-19) pandemic has led to burgeoning of testing strategies for the causative pathogen, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[3]

The initiative to accelerate new testing regimens has eased the usual stringency that new assays would normally undergo before release. So, there is a need to carefully assess the overall performance of the various rapidly emerging testing platforms prior to implementation. Currently, most diagnostic tests depend on the reverse transcriptase polymerase chain reaction (RT-PCR) to detect viral ribonucleic acid (RNA). This method needs extraction process which is cumbersome and needs trained personnel for testing.[4]

There are also difficulties with logistics of key virological testing components such as swabs and viral transport medium which have led to variations in practice with respect to collection of samples. These disadvantages have led to the exploration of alternative techniques for virus detection. This includes modifications of the PCR technique, by eliminating the need for collection of samples ina viral transport medium and skipping the use of regular RNA extraction step.[5]

CCMB developed Dry Swab-based Direct RT-PCR which has been approved by ICMR after suitable validation. The dry swab-based method can help in augmentation of the testing process due to the elimination of regular extraction process. [6]

The use of dry swabs makes collection of samples easier by avoiding the use of liquid transport media and thereby reduces costs. The method of RNA extraction is also simple and quick, rendering the process of testing faster, cheaper and safer. The benefit of this method lies in its simplicity as it needs no sophisticated instruments for RNA extraction. Also, labs with limited technical expertise can efficiently perform the test.[7]

Materials & Methods

Study design and setting

A prospective cross-sectional study was conducted in a COVID 19 testing center situated in a tertiary care hospital in South India.

Participants

Consecutive individuals presenting with symptoms or individuals who wanted to get tested for travel purposes or for rejoining work and academic institutions including pre-operative patients for a period of two months from were included in the study. A total of 133 patients were tested after obtaining proper informed consent.

Eligibility Criteria

Patients of any age group and gender who are getting tested for the reasons listed below were included this study.

a) Diagnostic purposes-suspected SARS-CoV-2 infection
b) Patients presenting with influenza like illness (ILI)
c) Screening purposes (preoperative and travel)

Ethical Committee Approval

The study protocol was approved by the Institutional Human Ethics Committee (IHEC NO: PMCHRI-IHEC-046) and all participants who were included in the study have been briefed about the research protocol and proper informed consent was obtained.

Sample Collection

Nasopharyngeal swab

The swab was inserted into the nostril of the patient parallel to the palate till resistance was felt and swabbed over the surface of the posterior nasopharynx, leaving the swab for 10 seconds to absorb the secretions. [8]

Oropharyngeal swab

The swab tip is swabbed over the tonsil area quickly and firmly to obtain a good sample.[8]

Dry swab extraction free testing method

The Dry swab is then cut with a sterile razor blade and added in sample collection tubes so that it could be closed and sent to the laboratory for further testing.DS buffer is added to each tube containing dry swab and was vortexed for 30 seconds. Then it was incubated at room temperature for 30 minutes with occasional tapping of tube. Aliquots of DS buffer are transferred to PCR tubes. The aliquots are heated at 98 ºC for 6 minutes using heating block. After cooling, a brief spin for 10 seconds was given. Then it was proceeded with COVID-19 RTPCR testing following the manufacturer recommended protocol using the heated DS buffer aliquots as RNA template [9]

Regular RTPCR testing

Real time PCR assay was done to confirm the presence of COVID- 19. Specimens were processed in level 2 biosafety cabinets. The RTPCR assay was done according to the manufacturers' instructions by trained laboratory technicians. The conventional method of sample extraction was done using QIAGEN sample extraction kit.

Pathodetect COVID-19 qualitative PCR kit is RTPCR assay which is used for the detection of COVID-19 in respiratory specimens, using E as screening gene detected in the red fluorescence channel and RdRp and N as confirmatory genes detected in a green fluorescence channel. The endogenous control is also used in the testing to check the efficiency of sample collection procedure.[10]

Bias

The bias was prevented by blinding the results of the Meril dry swab kit test from the technician who was performing the regular RTPCR testing.

Statistical Analysis

Patient characteristics were presented as numbers (percentages). For qualitative data, Pearson chi square test and diagnostic test with ROC curve were used. Concordance, specificity and sensitivity with 95% confidence intervals, and positive predictive value and negative predictive value (PPV/NPV) of the dry swab kit test were calculated using the RT-PCR results as the reference test. A "p" valueless than 0.05 was considered as statistically significant. The statistical analysis was done by SPSS for Windows 17. There was no patient dropout rate and all the selected participants accepted to participate in the study.

Results

A total of 133 individuals were included in the study. Out of which 54.88% were males and 45.12% were females. The patients were divided into different groups based on their age. The mean age was 32.96 ± 12.59. The RTPCR test was positive for 19 patients (14.28%) and negative for 114 patients (85.72%). The mean CT value of RdRp gene was 22.34 ± 5.6 and E gene was 24.04± 5.73 among the RTPCR positive samples. Dry swab testing was positive for 21 patients (15.79%) and negative for 112 patients (84.21%). The mean CT value of RdRP gene was 22.15 ± 5.91 and E gene was 24.03 ± 5.57 among the dry swab positive samples (Table 1)

Table 1: Characteristics of the study population

Characteristics	Frequency	Percentage
Gender		
Males	73	54.88
Females	60	45.12
Age Group		
<= 20 Years	7	5.26
21 - 40 Years	97	72.93
41 - 60 Years	23	17.29
> 60 Years	6	4.51
RTPCR positive Mean CT	19 RdRp-22.34 ± 5.6 E-24.04± 5.73	
Dry swab positive Mean CT	21 RdRp-22.15±5.91 E-24.03±5.57	

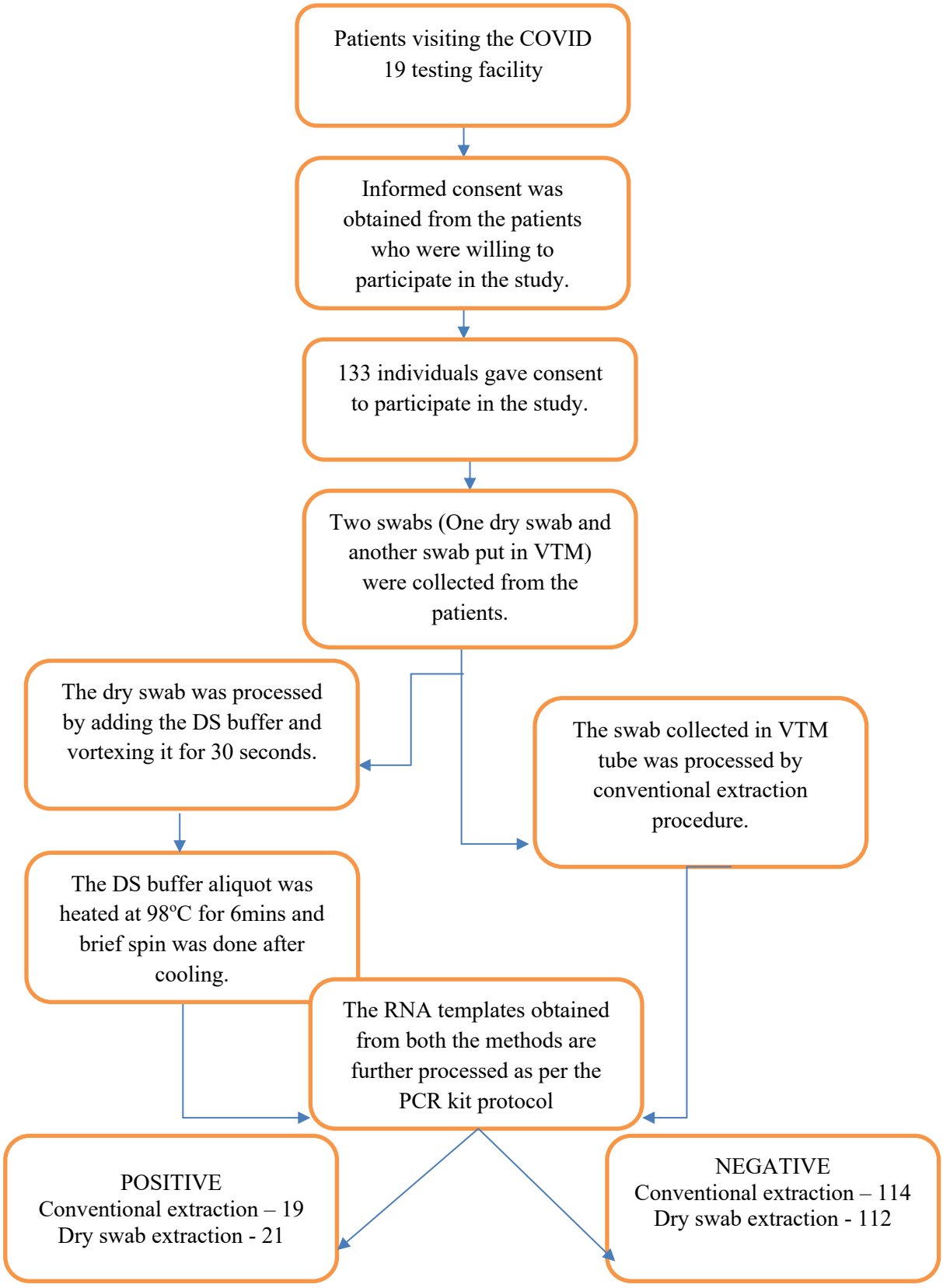

Figure 1: Schematic Diagram of Sample Flow

Table 2: Crosstabs between QIAGEN Extraction and Dry Swab RT- PCR method

			Regular Extraction RTPCR		Total
			Positive	Negative	
Dry Swab - RTPCR Result	**Positive**	Count	18	3	21
		% within DRY SWAB - RTPCR RESULT	85.7%	14.3%	100.0%
		% within REGULAR EXTRACTION - RTPCR RESULT	94.7%	2.6%	15.8%
	Negative	Count	1	111	112
		% within DRY SWAB - RTPCR RESULT	0.9%	99.1%	100.0%
		% within REGULAR EXTRACTION - RTPCR RESULT	5.3%	97.4%	84.2%
Total		Count	19	114	133
		% within DRY SWAB - RTPCR RESULT	14.3%	85.7%	100.0%
		% within REGULAR EXTRACTION - RTPCR RESULT	100.0%	100.0%	100.0%

Table 3: Diagnostic accuracy of the dry swab extraction free kit

Parameter	Estimate	95% CIs
Sensitivity	94.74%	(75.36, 99.06)
Specificity	97.37%	(92.55, 99.10)
Positive Predictive Value	85.71%	(65.36, 95.02)
Negative Predictive Value	99.11%	(95.12, 99.84)
Diagnostic Accuracy	96.99%	(92.52, 98.82)
Likelihood ratio of a Positive Test	36.0	(18.62 - 69.61)
Likelihood ratio of a Negative Test	0.054	(0.008 - 0.384)

The sensitivity was found to be 94.7%, specificity was found to be 97.4%, positive predictive value was found to be 85.7% and negative predictive value was found to be 99.1%. (Table 2 & 3). Area Under the Curve (AUC) for this logistic regression model is 0.961 which is extremely high (Figure 2). It indicates that the dry swab kit can distinguish between the positive and negative very efficiently. (Table 4)

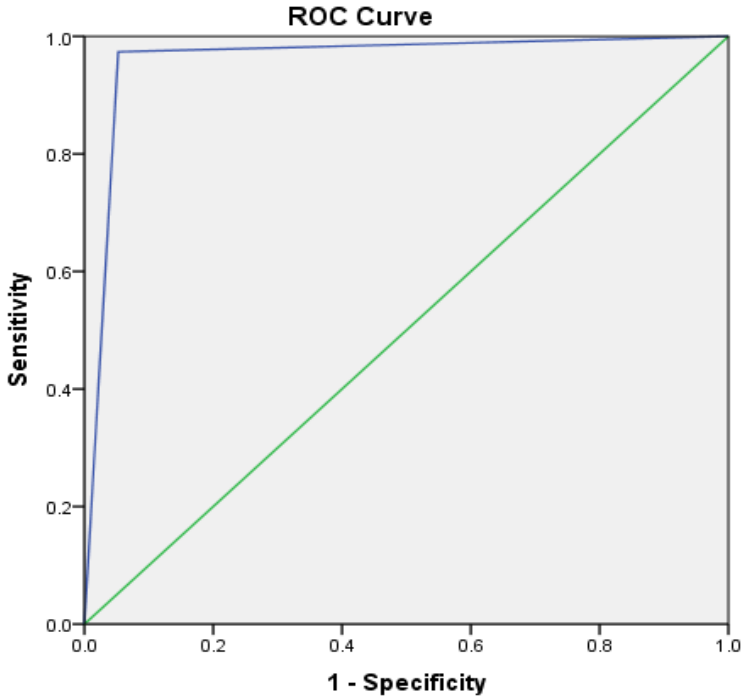

Figure 2: ROC curve

Table 4: Area Under the Curve

Test Result Variable(S): Dry Swab Extraction - Rtpcr Result				
Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.961	0.031	0.000	0.900	1.000

Discussion

Meril Extraction Free Dry Swab Kit eliminates the process of extraction which in turn reduces the man-power, cuts off the cost of expensive automated extractor and also saves the time. Increased rate of testing and giving the result promptly contributes to the decrease in the transmission rate of COVID-19. The cost of testing reagents and problems in transportation hinders the efforts of achieving the required level of testing. Hence this Dry Swab Kit can be used in small scale molecular testing lab and resource limited settings where it is difficult to procure materials for regular RTPCR testing.[11] Out of 133 samples tested from patients, male patients were in high numbers (54.88%) than female patients (45.12%) which is not in accordance with the study conducted by Dhakad MS et al in which female patients were higher in number[12]. The mean age of the patients involved in our study is 32.96 years whereas the mean age of male and female is 38 years and 35 years respectively [12].

We evaluated the diagnostic performances of a novel diagnostic kit used for COVID-19 detection, the Meril Extraction Free Dry Swab Kit (Meril Diagnostics), to verify if this method is suitable for implementation. The sensitivity, specificity, positive predictive value & negative predictive value of Meril Extraction Free Dry Swab Kit (Meril Diagnostics) were evaluated; moreover, the test results were compared to the RTPCR COVID-19 test, a confirmatory gold-standard assay for detecting COVID-19 infection.

The sensitivity of our study kit was found to be 94.7%, specificity was found to be 97.4%, which is more efficient than the other dry swab-based kit evaluated by Dhakad MS et al which was found to have sensitivity and specificity of 51.43% and 81.08% respectively. Another study done by Alcoba-Florez et. al reported the sensitivity for seven kits in which two other company kits (TaqMan Fast Virus 1-Step Master Mix kit sensitivity was reported to be 95.9% &LightMix1 Modular SARS-CoV kit sensitivity was reported to be 97.9%) were reported to have a sensitivity closer to our study.[13]

The mean CT value for regular RTPCR testing of RdRp gene was 22.34 ± 5.6 and E gene was 24.04 ± 5.03. The mean CT value for extraction free dry swab RTPCR testing of RdRp gene was 22.15 ± 5.91 and E gene was 24.03 ± 5.57. Area Under the Curve (AUC) for this logistic regression model is 0.961 which is extremely high. It indicates that the dry swab kit can distinguish between the positive and negative very efficiently.

This kit was found to be a suitable alternative for the RTPCR testing, considering the high sensitivity and specificity and can be deployed in settings where the infrastructure and consumables are difficult to be procured.

Limitations

Our study has several limitations, we did not evaluate in extremes of temperature or atmosphere or other conditions that may affect stability, nor did we evaluate swabs other than the commonly used flocked versions.

We evaluated the kit using nasopharyngeal and oropharyngeal swabs only. Other samples like broncho alveolar lavage or sputum or saliva was not used for evaluating the kit.

Any of these variables could affect the performance of testing from “dry swabs” and requires additional study prior to implementing this method.

Conclusion

The Meril dry swab extraction free kit sensitivity was found to be 94.7%, specificity was found to be 97.4%, positive predictive value was found to be 85.7% and negative predictive value was found to be 99.1%. Area Under the Curve (AUC) indicates that the dry swab kit was able to distinguish between true positive and true negative very efficiently. Hence the kit is validated for diagnostic use as an alternative for regular extraction aided RTPCR kits.

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