EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH
www.ejpmr.com

DIAGNOSIS OF COVID-19: DRY VERSUS WET SWAB TECHNIQUE

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ABSTRACT

Currently, people all over the world have been suffering due to the coronavirus disease 2019 (COVID-19), which is the fifth pandemic after the Spanish Flu pandemic occurred in 1918. The symptomatology of those patients, including fever, malaise, dry cough, and dyspnea, was diagnosed as viral infection. There have been recent advances in methods for detection of the COVID-19 out of which; the most sensitive method is real-time polymerase chain reaction (RT-PCR). For any RT-PCR test, the first step is extraction of nucleic acid (Sample Extraction) which is achieved by spin column and magnetic beads based extraction. These methods have prominent drawbacks such as prolonged turn-around time, consumption of resources and tedious mass handling of samples in laboratory. We have presented a novel method of extraction known as The Dry swab test. In this article, we have compared both, the conventional spin column of extraction with dry swab extraction method. The Dry swab method was equivalent with spin column methods for isolation of total RNA from patient samples.

KEYWORDS: RT-PCR, COVID-19, SARS CoV-2, Dry Swab, RNA Extraction.

INTRODUCTION OF COVID-19 AND SARS-COV-2

Currently, people all over the world have been suffering due to the coronavirus disease 2019 (COVID-19), which is the fifth pandemic after the Spanish Flu pandemic occurred in 1918. As of now, the first report and subsequent outbreak from a cluster of novel human pneumonia cases can be traced to Wuhan City, China, from late December 2019. The symptom onset can be traced from 1st December 2019. The symptomatology of those patients, included fever, malaise, dry cough, and dyspnea, which was diagnosed as viral infection. Previously, the disease was called Wuhan pneumonia by the press because of the location of emergence and pneumonic symptoms.[1,2]

Whole-genome sequencing results indicated that a novel corona virus may be the causative agent. Therefore, SARS-CoV-2 is one of the seventh members of the coronavirus family to infect humans. The World Health Organization (WHO) temporarily termed the new virus 2019 novel coronavirus (2019-nCoV) on 12 January 2020 then officially named this communicable disease coronavirus disease 2019 (COVID-19) on 12 February 2020. Later, the International Committee on Taxonomy of Viruses (ICTV) officially designated the virus as SARS-CoV-2 supported phylogeny, taxonomy and established practice.[3]

- **Structure and Genetic make-up**

SARS-CoV-2 is an enclosed and globular particle approximately 120 nm in diameter containing a positive-sense single-stranded RNA genome which is supposed to be in consortium with a nucleoprotein within anucleoprotein within a capsid comprised of matrix protein. The envelope bears club-shaped glycoprotein projections. Some coronaviruses have exhibited a presence of hem agglutinin-esterase protein (HE). It belongs to the subfamily Coronavirinae, family coronavirdiae, and order nidovirales.[4,5]

Coronaviruses possess the most important genomes (26.4 - 31.7 kb) among all known RNA viruses, with GþC contents varying from 32% to 43%. Variable numbers of small ORFs are present between the varied, yet conserved genes (ORF1ab, spike, envelope, membrane and nucleocapsid) and, downstream to the nucleocapsid gene in different coronavirus lineages. The viral genome contains distinctive features, including a singular N-terminal fragment within the spike protein. Genes for the main structural proteins altogether coronaviruses occur within the 5' to 3' order as S, E, M, and N.[6]

A typical CoV contains a minimum of six ORFs in its genome. Apart from Gammacoronavirus that lakes nsp1, the firstORFs (ORF1a/b), about two-thirds of the entire genome length, encode 16 nsps (Non-Structural Proteins, nsp1-16). ORF1a and ORF1b contain a frameshift in

between them which produces two polypeptides: pp1a and pp1ab. Either virally encoded chymotrypsin-like protease (3CLpro) or Main protease (MPro) and one/two papin-like proteases Process these Polypeptides into 16 nsps. All the structural and accessory proteins are translated from the sgRNAs of CoVs while ORFs 10, 11 encode the four main structural proteins contain spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins on the one-third of the genome near the 30-terminus.[7,8]

- **Transmission of the Disease**

Airborne transmission via aerosols formation is suspected to be the primary mode of transmission. Aerosols are particles under 100 μm in diameter.[9] Thus, their minute size and suspension within the air may ease direct contraction of the virus. Aerosols could also be formed during various surgical and dental procedures or could also be formed as droplet nuclei while talking, coughing, and sneezing by an infected patient. During a study by Li et al., eight healthcare staff and five post-operational patients tested positive for COVID-19 after being in close contact with an infected patient. This means that droplet formation is a potent mode for human-to-human transmission.[10]

- **Window period of Infection**

The incubation Period for covid-19 is 0-14 days assumed by WHO whereas 2-12 days assumed by the ECDC.[11]

- **Infection rate**

COVID-19 features a very steep age gradient for risk of death.[12] Moreover, many, and in some cases most, deaths in European countries that have had large numbers of cases and deaths[13] and within the USA[14] occurred in nursing homes. Locations with many home deaths may have high estimates of the infection death-rate, but the infection death-rate would still be low among nonelderly, non-debilitated people. Asian countries have reported very low infection fatality rates which is accredited towards younger population in these countries (excluding Japan) exhibiting previous immunity from exposure to other coronaviruses, genetic diversity, hygiene practices, lower infectious load and other unknown factors.[15,16,17,18]

However, comorbidities, poverty, frailty (e.g. malnutrition) and congested urban living circumstances may have an adverse effect on risk and thus increase infection death-rate.[19]

- **Diagnosis and Treatment**

Rapid and accurate detection of COVID-19 is crucial to regulate outbreaks within the community and in hospitals. Current diagnostic tests for coronavirus include reverse-transcription polymerase chain reaction (RT-PCR), real-time RT-PCR (rRT-PCR), Fluorescent Immmuno-assay (FIA), Rapid Antigen Tests, reverse transcription loop-mediated isothermal amplification (RT-LAMP).[20]

Treatment of Covid-19 includes Antiviral Agents, Chloroquine and Hydroxychloroquine, corticosteroids, antibodies, convalescent plasma transfusion and Vaccines.[20]

The COVID-19 pandemic has scaled up strategies for determining the quantitative levels of the causative pathogen; Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The use of direct molecular diagnostic testing based on sequencing of SARS-CoV-2 proved considerable support in identifying infected individuals, and it was deemed to be acceptable by the FDA, leading to their inclusion in recommendations by both the CDC and the FDA (https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/faqs-testing-sars-cov-2#testingsupply).[21-22] To detect coronavirus, quantitative real-time polymerase chain reaction (q-RT-PCR) is essential. Prior to q-RT-PCR, the viral nucleic acids must be isolated. During the beginning of the pandemics, nasal swab collection method in VTM was well-established and accepted for detection of COVID-19.[24-26] There are numerous kits available for extraction of viral samples which includes seven to eight steps. However, this method has few demerits. Firstly, it is expensive, and subsequently time consuming to produce results and also have a significant false-negative rate and numerous sample extractions becomes difficult. These disadvantages lead to exploration of alternative techniques available for extraction of Nucleic acid samples. This includes modifications of the PCR technique; by eliminating the transport medium or leaving out the RNA extraction step. To meet the exponential demand in testing, companies have developed the dry swab techniques, that is, those incubated in dry conditions. These strategies have raised the possibility of reducing the testing time and the expense of testing. The current study deals with the advantages of using dry swab samples to extract, store and detect the virus, as compared to wet swabs, to reduce costs while improving safety.

METHODOLOGY

Swabs were inoculated in viral stocks of known titer just like samples collected from patients. The swabs were clustered in two groups to be processed separately. The first was the group stored as dry swabs; the second comprised swabs stored in virus transport medium.

Dry Swab Elution: Elution of genetic material from the dry swabs was achieved by adding 400μL of DS Buffer (Meril Diagnostic Pvt Ltd) to each screw top tube containing dry swab performing a 30 second vortex with intermittent pulsing. After vortexing samples were incubated for 30 mins. Thereafter 50μL aliquot of DS buffer in 0.2 ml PCR tubes was heated at $98°C$ for 6 mins using PCR thermocycler. After cooling and a brief spin the samples were processed for RT-PCR with ICMR approved Meril amplification kit, using DS buffer as RNA template.

Nucleic acid extraction using spin column technique
Nucleic acid extraction was performed by adding 400 μL lysis buffer (Meril) to 200 μL of sample and incubating at room temperature for 10 minutes. 450 μL of binding buffer was added to it, 600 μL of the same was spin at 14 – 16000 rpm for 1 min. The flow through was discarded and the column placed back into a fresh collection tube. 400 μL Wash buffer 1 and 600 μL Wash buffer 2 were added simultaneously one by one following spin at 14 – 16000 rpm for 1 min. Spin column was dried for 3 min by spinning at 14 – 16000 rpm. 50 μL of elution buffer was added to the centre of the column and was allowed to stand for 3 min and centrifuging 14 – 16000 rpm for 1 minute to elute the nucleic acid.
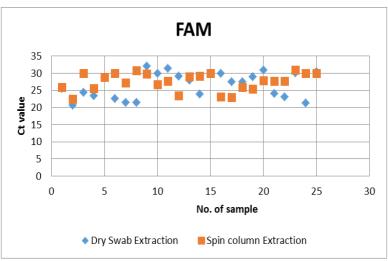
RT-qPCR
Final volume of RTPCR was 20 μL, each containing 9 μL Master mix and 1 μL primer probe along with 10 μL sample.RT-qPCR was then performed on Real Time Thermocycler (50°C for 15 minutes, 95°C for 3 minutes, , followed by 40 cycles of 95°C for 15 seconds, 55°C for 40 seconds). Reported Ct values were obtained from the onboard analysis using the auto-determined cycle thresholds. Data was analyzed using Microsoft Excel.
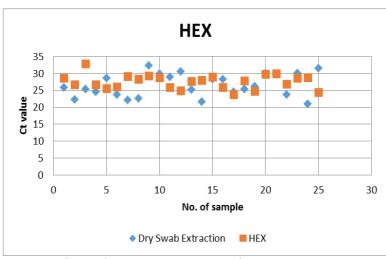
PCR amplification and analysis
Amplification was done using PCR amplification kit (Meril Diagnostics). Raw cycle thresholds were obtained. Samples with indeterminate or spurious amplification signals were designated a cycle threshold (Ct) value of 40 cycles. Ct values were recorded for the SARS-CoV-2 targets (N and ORF1ab) by interpretation of graphs obtained for FAM (ORF 1 ab) HEX (N gene) and ROX (Internal Control) channel.

RESULT
Here, we compared ct values of dry swab extraction method and Spin column extraction method. Graph 1 shows ct value of orf 1ab gene, which is detected at FAM channel there is no significant difference in ct value. Graph 2 shows N gene ct value where detection is achieved by HEX channel the results of dry swab test and spin column are almost similar. Graph 3 shows ct value of IC where detection is achieved by ROX channel here also there is no significant difference in the Ct values of both method.
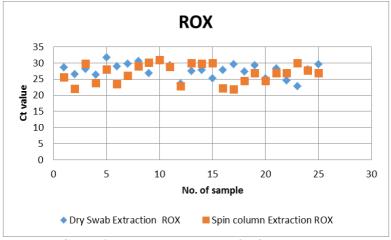

Graph 1: Represents ct value of FAM channels.

Graph 2: Represents ct value of HEX channels.

Graph 3: Represents ct value of ROX channels.

DISCUSSION
In the present study of dry swab test and spin column method were compared for elution of RNA of SARS COv-2from patient's sample. Dry swab test is the most simple, less time consuming and easy to perform. Extraction was performed using positive titre, in dry swab method just adding buffer and heating elutes viral RNA and spin column includes certain step such as lysis, binding washing and drying. These eulted RNA was used for qualitative determination in RT-PCR and from ct value comparison was made.

Results showed dry swab and spin column extraction method there is no significant difference. Hence Dry swab test can be used as a extraction procedure where it reduced the time, cost effective and ease for user.

CONCLUSION
The present study has conclusively shown that the results of Rt-PCR by both wet and dry swab are similar and comparable. There is however an added advantage of the latter that the need for.

VTM is obviated and the entire process has become easier, faster and cheaper due to the bypassing of the step of extraction of genetic material. Hence it is recommended that the dry swab technique should be adopted because of a faster TAT (Turn around Time) and lesser cost with no variation in the results. It is also beneficial looking to the vast numbers being tested as well as in economically deprived countries where cost effectiveness is also crucial. It shall prove highly beneficial if more centres adopt this technique especially now when the spectre of a third wave is looming large on the horizon.

ACKNOWLEDGEMENT

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