PROJECT COMPLETION REPORT

Project Title: Proposal for pilot study for efficacy of dry swab method of RT-PCR for COVID-19 tests that is reliable, reproducible, fast and cost-effective as well.

REPORT:

The coronavirus disease 2019 (COVID-19) pandemic has led to upscaling of testing strategies for the causative pathogen the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The use of direct molecular diagnostic testing based on sequencing of SARS-CoV-2 proved significant in identifying infected individuals and were deemed 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) [1].

During the beginning of the pandemics, nasal swab collection method in VTM was well establish and accepted for detection of COVID-19 [1, 2].Therefore most diagnostic tests use the nasopharyngeal or oropharyngeal samples from patients and perform the reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) to detect viral ribonucleic acid (RNA). However this method encompasses few demerits. First it is expensive, subsequently it is slow to produce results and also have a significant false-negative rate. These disadvantages have led to the exploration of alternative techniques for virus detection. 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. This has also, with the above modifications, raised the possibility of reducing the testing time and the expense of testing. The current study deals with the feasibility of using dry swab

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samples to 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 Elusion: elution of material from the dry swabs was achieved by adding 400µL of DS Buffer (Meril) 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 standard technique: Nucleic acid extraction was performed from 200 µL of sample added to 400 µL lysis buffer (Meril) 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 supernatant was collected in another tube and centrifuged again for 1 min at 14 – 16000 rpm.

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 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. Results showed ct value of orf 1ab gene, which is detected at FAM channel there was no significant difference in ct value. N gene ct value where detection is achieved by HEX channel the results of dry swab test and spin column are almost similar.

Again 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.

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 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.

The data was published and shared for reference (Publication attached)

References:

1. Tu YP, Jennings R, Hart B, Cangelosi GA, Wood RC, Wehber K, et al. Swabs collected by patients or health care workers for SARS-CoV-2 testing. N Engl J Med. 2020; 383(5):494–6. https://doi.org/10.1056/NEJMc2016321 PMID: 32492294

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2. Altamirano J, Govindarajan P, Blomkalns AL, Kushner LE, Stevens BA, Pinsky BA, et al. Assessment of sensitivity and specificity of patient-collected lower nasal specimens for sudden acute respiratory syndrome coronavirus 2 testing. JAMA Netw Open. 2020; 3(6):e2012005. https://doi.org/10.1001/jamanetworkopen.2020.12005 PMID: 32530469

Dr Neha Jaiswal
Principal Scientist (Dry Swab Project)
Scientific Officer
Central Research Lab
IMCHRC, Indore

FORWADED AND APPROVED

Dr Sanjeev Narang
Principal Investigator (Dry Swab Project)
Prof. and Head
Dept. of Pathology
IMCHRC, Indore

H. O. D.
Department of Pathology
Index Medical College, Hospital
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Meril
Diagnostics

LETTER OF COLLABORATION

Date: 16/03/2022

TO WHOMSOEVER IT MAY CONCERN

This is to certify that **Dr. Neha Jaiswal** – Scientific Officer, Index Medical College Hospital and Research Centre, Indore; has performed validation of Meril Extraction Free Dry Swab kit under the guidance of **Dr. Sanjeev Narang** – Head of Department, Pathology, Index Medical College Hospital and Research Centre. They have received financial support of Rs. 48000 along with the Extraction free Dry Swab Kit, Meril one step RTPCR kit, VTM costing Rs 100000. They have completed the Validation with utmost precision and on time, and we hope to collaborate with them in future.

Thank you,

With Best Regards,

Dr. Jalaram Thakkar,
Head, Research and Development
Meril Diagnostics pvt ltd, Vapi

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Project Title: Pilot study to evaluate the efficacy of dry swab method of RT-PCR for COVID-19.

Principal Investigator: Dr. Sanjeev Narang

Designation: Prof. and Head, Dept. of Pathology, IMCHRC, Indore

Co-Principal Investigator: Dr. K Ramanath

Designation: Prof. and Head, Dept. of Microbiology, IMCHRC, Indore

Principal Scientist: Dr. Neha Jaiswal

Designation: Scientific Officer (Central Research Lab, IMCHRC, Indore)

Introduction: COVID-19 is one of the most devastating pandemics which have affected almost the entire world. With a very high infection rate it spread rapidly through silent carriers who transmitted the disease manifold. Thus early and accurate diagnosis of suspected patients is a must, using technique to be less time and cost effective. The nose swab PCR test for COVID-19 is the most accurate and reliable test at present for diagnosing COVID-19. The standard method of RT-PCR for COVID patients includes sample collection, extraction preceded by Polymerization in a Thermal Cycler. In April 2021 ICMR-CCMB Hyderabad introduced a new PCR Protocol for COVID patients namely Dry Swab technique which excluded the costly and time consuming process of extraction of genetic material and simplified it by using EDTA with proteinase K enzyme. Being speedy and cost effective this technique was very shortly followed by many clinical diagnosis based bio industries, Meril being one among them. This study aims to evaluate the two protocols namely the standard method and the dry swab method by comparing their CT values using Meril diagnostic kit for the same.

Specific Aim: To evaluate the efficacy of dry swab technique over standard method (using VTM tubes) in nasopharyngeal/ oropharyngeal samples of COVID-19 patients.

Method:

The study will be performed in biosafety Lab level -2 designated for RT-PCR test of COVID-19 patients of Index Medical College Hospital and Research Centre, Indore.

VTM and dry tube nasopharyngeal/ oropharyngeal samples will be collected separately from same 100 patients suspected for SARS CoV-2 infection. The samples will be further processed as per the respective protocol.

Standard Method Protocol:

a. sample collection (as per indicated on kit. Product Code: MBTVTM-01)

b. extraction (as per indicated on kit. Product Code: MBTREK-08)

c. amplification (as per indicated on kit. Product Code: NCVPCR-01)

Dry swab technique Protocol:

a. Sample collection and nucleic acid elusion (as per indicated on kit. Product Code: MBTDSK-01)

b. Amplification (as per indicated on kit. Product Code: NCVPCR-01)

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Analysis:efficacy of dry swab technique will be done on the basis of CT (Cycle Threshold) value obtained after PCR amplification of the samples.

Project Type: Minor (Pilot Study)

Sample Size: 100 samples

Time Duration: 2 months

Funds: project is sponsored by Meril Diagnostics Ltd

Dr. Sanjeev Narang (PI)

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Dr. K Ramanath (Co-PI)

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Dr. Neha Jaiswal (RA)

Scientific Officer

Central Research Lab, IMCHRC, Indore

Application for submission of Research Project to I.E.C.

To,

The Chairman

Institutional Ethics Committee

Index Medical College Hospital & Research Centre, Indore

(Through Scientific Evaluation Committee)

Sub: request for approval from Institutional Ethics Committee (IEC) for conducting Pilot study to evaluate the efficacy of dry swab method of RT-PCR for COVID-19.

Respected Sir/Madam,

This is Dr. Neha Jaiswal, currently working as scientific officer in IMCHRC is hereby writing to bring to your kind notice that I under guidance of Prof Dr. S Narang is proposing a diagnostic study on samples from patients suspected for COVID-19. The trial is entitled as "Pilot study to evaluate the efficacy of Dry Swab method of RT-PCR for COVID-19" and sponsored by Meril Diagnostic Ltd.

Salient features of the study include:

1. evaluate the efficacy of Dry Swab method over standard method

I am herewith enclosing the project proposal of the project work.

I submit the following undertaking for this study:

1. I will start the study after obtaining the ethical clearance from the Institutional Ethics Committee.
2. The patients will be informed about this study prior to sample collection and we will get an informed consent signed by them.
3. I will carry out the work without detrimental to regular activity as well as without extra expenditure to the institution.
4. I will inform the committee in the occurrence of any change in the study procedure, site, investigation or guide.
5. I will not deviate from the area of work for which I have applied for ethical clearance.
6. I will abide by the rules and regulations of the institution.

Registrar

Recieved 3/8/24

7. I will complete the study within the specified period of I have applied for. If any extension of work is found then I will apply for the permission and again and continue for the work.
8. I will not ask for any type of funds from the institution while doing the work or on completion.

Thanking you

Yours Obediently,

Dr Neha Jaiswal
Scientific Officer

Date: 03/08/21

Forwarded by:

Dr Sanjeev Narang (Principal Investigator)
Prof. and head
Dept. of Pathology, IMCHRC, Indore.

Index Medical College, Hospital & Research Centre, Indore (M.P.)

Dr K Ramanath (Co-Principal Investigator)
Prof. and head
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Institutional Ethics Committee

Index Medical College Hospital and Research Centre, Indore, M.P.

(Registered under Rule 122 DD of the Drugs and Cosmetics Rules 1945)

Registration No. **ECR/708/Inst/MP/2015**

Ref. No.:- IMCHRC/IEC/2021/ 108 **Date:** - 07.09.2021

Principal investigator: Dr. Sanjeev Narang, Professor and HOD, Department of Pathology, Index Medical College Hospital and Research Centre, Indore (M.P.)

Institutional Ethics Committee of Index Medical College Hospital and Research Centre, Indore (M.P.) has reviewed and discussed your proposal to conduct the research work related to your dissertation entitled **"Pilot study to evaluate the efficacy of dry swab method of RT-PCR for COVID-19"**

The brief description of the proposal was reviewed and discussed.

The committee has approved the above mentioned study in the present form to be conducted by :

Principal investigator: Dr. Sanjeev Narang, Professor and HOD, Department of Pathology, Index Medical College Hospital and Research Centre, Indore (M.P.)
Co-Principal investigator: Dr. K. Ramnath, Professor and HOD, Department of Microbiology, Index Medical College Hospital and Research Centre, Indore (M.P.)

IEC reference number for future correspondence is IMCHRC/IEC/2021/ dated07.09.2021.

IEC expects to be informed about the progress of the study and any changes in the protocol.

Dr. D. K. Taneja
Chairperson, IEC
IMCHRC, Indore (M.P.)

Chairperson
Institutional Ethics Committee
Index Medical College,
Hospital & Research Centre, Indore

Dr. V. K. Arora
Chairperson, SEC
IMCHRC, Indore (M.P.)

Dr. Aarti Sahasrabuddhe
Member Secretary, IEC
IMCHRC, Indore (M.P.)

Member Secretary
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Index Medical College,
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