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RAPID DIAGNOSIS OF SARS-COV-2 VIRUS WITH REAL TIME MICRO PCR SYSTEMIN AMBEDKAR NAGAR DISTRICT OF UTTAR PRADESH: A NEAR CARE APPROACH

Health Science

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ABSTRACT

Background & Objectives – There is an urgent need to use a rapid and easy to perform molecular test to detect coronavirus to stop spreading a pandemic disease. Thus in this study we see the prevalence of covid 19 cases in eastern part of Uttar Pradeshby a rapid diagnostic method. **Methods-** We evaluated the performance of a chip- based nucleic acid amplification test in the detection of Coronavirus in oropharyngeal swabs from 1110 patients. The test involved processing using nanoparticle-based protocol run on a battery-operated device and real-time PCR performed on the Quattro Real Time micro PCR Analyzer (handheld, battery-operated thermal cycle). **Results –** Of 1110 subjects tested for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in their oropharyngealswab during July-December 2020, 74 (6.7%) were positive. **Conclusion-** This preliminary study shows that this miniature RT PCR test allow detection in approximately one hour and can be utilized as a quick and accurate diagnostic method.

KEYWORDS

Coronavirus, SARS-CoV-2, miniature RT PCR, Covid 19, nucleic acid amplification.

INTRODUCTION:

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a RNA virus belonging to the β Coronavirus family, began in Wuhan, Hubei province, China, in December 2019 that caused the current pandemic of the disease[1]. On May 17, 2020, the day when this analysis was undertaken, 4,717,079 subjects in 214 countries have been diagnosed to have COVID-19, of whom 312,388 (6.6%) died. The first patient with coronavirus disease (COVID-19) was confirmed in India on January 30, 2020[2]. There are three types of covid-19 tests that is molecular test to detect the genetic material of SARS-Cov-2; antigen tests that can detect fragments of the virus from nasal swabs quickly; third is antibody test that tells about the past infection. Real –time RT-PCR, gold standard test, continues to be the most accurate method to detectSARS-CoV-2 virus. Real Time micro PCR system, also a molecular test, can return results faster time than the standard RT-PCR tests. Indigenous portable workstations, previously used and recommended by WHO for tuberculosis[3] and also deployed for detection of Nipah virus disease (unpublished) and leptospirosis (unpublished), are now being used for detection of SARS-CoV-2. Beta CoV E-gene screening assay and SARS-CoV-2 RdRp gene-confirmatory assaywere earlier validated as a two-step test[4]. A multiplex assay combining E-gene screening and Orf1a-gene confirmatory assay has also been validated recently[5]. All three of these assays exhibited 100% sensitivity and specificity, and positive and negative predictive value when compared with the gold-standard RT-PCR test[6]. In battery operated micro PCR test, the RNA from patient sample was first extracted using Universal cartridge based Sample prep Device and AUTO Univeral cartridge based Sample prep kit. Six (6) µL of this clear solution is then pipetted outusing the same pipette and tip and dispensed into the reaction well of the Beta CoVchip and the test is inserted in the Real Time Quantitative microPCR Analyzer where the RNA is first converted into complementary DNA (cDNA) bythe RT enzyme and further thermal cycling takes place. The machine is portable and can be carried in a briefcase, is battery operated with one charge lasting ten hours. To control this spread of this pandemic disease, rapid detection is a great need.Thus in this study, portable RT PCR machine is used for rapid detection of asymptomatic carriers to prevent spread of infection.

MATERIALS

Settings-

Sample collection, micro PCR test was performed at Mahamaya Rajkiya Allopathic Medical College, Ambedkarnagar.

Study population and specimens -

This was a single site, blinded study to determine the prevalence of covid 19 cases by a rapid method. Specimens were taken from patients presenting routinely to our hospital. Standard diagnostic follow was performed on all patients.

Sample processing protocol on Trueprep AUTO Sample Prep Kit - Nucleic Acid extraction using Trueprep Auto Universal Cartridge based Sample Prep Device -

Oropharyngeal or nasopharyngeal specimens were collected using a standard nylon flocked swab as per standard procedures. Collected swab with specimen was the inserted into provided transport medium in swab specimen tube and mixed well by repeatedly twirling the swab in the buffer solution. After mixing, squeezing out the excess liquid from the swab, the swab was disposed off as per guidelines.0.5ml of swab sample was transferred into the lysis buffer tube using 1 ml transfer pipette. Further the entire content of lysis buffer tube was transferred to the sample chamber of cartridge using 3 ml transfer pipette. After inserting the cartridge in Trueprep Auto device, it takes 20 minutes to extract the RNA. This extracted RNA was the collected in Elute chamber which was pierced with the provided transfer pipette and whole volume of extracted RNA was transferred into Elute collection tube (ECT).

Real-time micro PCR Protocol

6 µl of the elute collected in the elute collection tube was aspirated and dispensed to microtube containing freeze dried RTPCR reagents (mastermix). It was also incubated for 30 seconds at room temperature. 6 µl ofmastermix was then dispensed into the Truenat™ Beta CoV micro PCR chipand the real-time PCR was done using a pre-programmed profile on the Truelab™ Real Time Quantitative micro PCR Analyzer device. Results were observed on the screen. At the end of the test run, Beta CoV "DETECTED" or "NOT DETECTED" result was displayed and in samples that were detected with E- gene, extracted RNA was further put on Truenat™ SARS CoV-2 micro PCR chip for detection of RdRp gene and confirmation of the infected individual was done.

RESULTS

During a study period of six months, out of 1110 subjects tested for Covid-19, 74 (6.7%) gave positive result.

DISCUSSION

Early and correct identification of the beta coronavirus is important for

effective isolation, treatment and case management. In line with WHO recommendations, molecular diagnostics are currently the method of choice for such virus detection and differentiation. However, molecular tests for beta coronavirus have so far beenrestricted to centralized reference laboratories as they require skilled manpower and elaborate infrastructure. Also the turnaround time for results could take a few days. NAAT assays are developed and validated, confirmation of cases of novel virus infection will be based on specific detection of unique sequences. Among the laboratory testing methods developed for identifying patients with acute infection due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—the aetiological agent of coronavirus disease 2019 (COVID-19)—viral RNA amplification using real-time PCR (RT-PCR) is to date the standard method in many clinical virology laboratories[7]. However, RT-PCR-based assays are labour intensive and, when not completely automated, take hours to yield results. Conversely, rapid antigen detection assays—intrinsically less laborious and requiring a few minutes to results—have the potential to satisfy the pressing demand for an early SARS-CoV-2 infection diagnosis. COVID-19 Ag might be reliably used in the early phases of acute SARS-CoV-2 infection, e.g. within the first days after infection when Ct values are likely to still be below 25 (corresponding to higher viral loads)[8]as seen in few studies. D**iagnostic test** can show if you have an active coronavirus infection and should take steps to quarantine or isolate yourself from others. Since antigen testing doesn't involve any processes of amplifying the virus or its genetic material, a swab sample may have too little antigen to be detected. This could produce a *false negative* result. As a precaution, a negative test should be followed up by the more accurate RT-PCR test, to confirm a *true negative* for COVID-19. Accuracy is the single largest problem with antigen tests, which are much less sensitive than RT-PCR as a diagnostic tool. Antibody tests may not be able to show whether the virus is currently infecting the body. It takes at least 12 days after exposure for body to make enough antibodies to show up on a test thus also not an effective method in a time of pandemic in which limit of spread of infection is an utmost priority.As Conventional RT-PCR requires RNA extraction and analysis to be done in two different rooms backed with cold storage and trained experts handling laboratory designed equipment. TrueNat on the other hand is designed primarily to work at remote locations, and considered as 'last mile diagnostics'.This includes sample preparation, an RNA extraction system, an RT-PCR machine, and disposable kit components.

The workstation is a chip-based, real-time quantitative PCR system that is portable, battery-operated, and fully automated. This laboratory-in-a-suitcase can be used in remote areas and has network data transfer ability and an automated reporting system. Samples are collected in a viral lysis buffer with minimum biosafety and biosecurity requirements. Results from a single test are available in 45 minswith an added advantage with Quattro system to test four samples per run. The machine is portable and can be carried in a briefcase, is battery operated with one charge lasting ten hours. A few drops of the solution are then placed on a cartridge. On inserting this cartridge into a machine, a pre-programmed reaction is initiated, which extracts the nucleic acids or the genetic material from the samples. This has to be followed by an RT-PCR. The purified nucleic acid is added into a micro-tube containing freeze-dried RT-PCR reagents, and the solution is allowed to stand for about a minute. This solution is then applied to a microchip and the test is inserted into another machine, where the reverse transcription and PCR take place. These platforms have a quick turnaround time (30 -60 minutes). In this study, we found 74 samples were positive for RdRp gene and out of these random 10 samples were rechecked with nested RT-PCR test and concordant results were found.

The Real Time micro PCR System enables decentralization and near patient diagnosis of and monitoring of Beta Corona viral load. This is enabled by making the real time PCR technology rapid, simple, robust and user friendly, thereby offering “sample to result” capability even at resource limited settings.

CONCLUSION:

Miniaturization of the PCR platform would confer advantages such as reduction in cost of instruments and tests, faster turnaround times as slow turnaround time would mean that infected individuals will come in contact with others and spread the virus even while waiting for the test result. There are some new diagnostic tests available with alternative methods and benefits.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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