



## Original Research Article

# Molecular diagnosis of covid-19 through application of highly sensitive and specific reverse transcriptase Allplex™ 2019-nCoV RT- PCR assay validated with clinical specimens

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## ABSTRACT

**Introduction:** COVID-19 is a pandemic caused by the SARS-CoV-2, and it is now playing havoc to the life of several people and has been big impact and blow to the world economy. Early detection of the symptomatic and asymptomatic patients having COVID-19 through application of Real-time PCR helps in preventing the spread of the virus.

**Aim:** In the present pilot study for the month of June 2020, we evaluated the application of RT-PCR in detection of SARS-CoV-2.

**Materials and Methods:** A total of 49,033 nasopharyngeal swabs were collected comprising 29, 685 at the individual level and 19,348 pooled samples and evaluated using Allplex™ 2019-nCoV Assay for detection of COVID-19.

**Results:** A total of 1364 cases (2.7%) were detected positive for the SARS-CoV-2 RNA and the remaining samples of total 47,441 were negative for the above mentioned virus.

**Discussion:** The pilot study analysis of detection of COVID-19 using Allplex™ 2019-nCoV Assay is very useful in detecting the suspected COVID-19 cases at the earliest and helps in preventing the spread of the infection.

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## 1. Introduction

Corona virus is a positive sense single standard RNA viruses and these viruses are known to cause infections mainly in animals and a part to humans also.<sup>1</sup> In December 2019, World Health Organization was informed from China the occurrence of a cluster of cases of pneumonia of unknown etiology. It was named as the novel Coronavirus Disease 2019 (COVID-19) caused by SARS-CoV-2, and it was found to be closely related to the severe acute respiratory syndrome coronavirus (SARS-CoV). The patients infected with the COVID-19 have been known to suffering with sudden onset of fever, myalgia, cough, dyspnea along with atypical pneumonia.<sup>2</sup> The clinical picture was supported by

the radiological evidence of the ground glass opacities of the lungs. WHO has declared the COVID-19 as a pandemic and the case fatality rate ranges from 1% to 12%.<sup>3</sup> The major concern of the COVID-19 is the spread through person to person contact and several countries in more than 200 are implementing the containments strategies as advised by the WHO to prevent the spread of the viruses among individuals.<sup>5</sup> Scientists in various countries are working out and clinical trials are underway for the development of effective vaccines for COVID-19.

Early detection of the SARS-CoV-2 in symptomatic and asymptomatic patients is one of the best way to prevent the spread of this virus. Several countries are evaluating the detection of antigen and antibody, but the major problem in establishing the effective assay is the cross reactivity with the SARS-CoV.<sup>4</sup> There is a need of a novel technique which

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has an ability to detect the etiology of the COVID-19 with high sensitivity and specificity for early testing, diagnosing, and ability to detect both symptomatic and asymptomatic carriers of the virus. Worldwide recent studies pointed out that the most reliable diagnostic test for the detection of the COVID-19 is the reverse transcription-PCR (RT-PCR). The access of the complete genome has led to the development of RT-PCR assay targeting the RNA-dependent RNA polymerase (RdRp), envelope (E), and nucleocapsid (N) genes of SARS-CoV-2.<sup>5</sup>

In this present pilot study we evaluated the Allplex™ 2019-CoV assay RT-PCR for the detection of the SARS-CoV-2 in symptomatic patients visited in our hospital.

## 2. Materials and Methods

### 2.1. Clinical specimen

The pilot study has been conducted from the department of Microbiology, Kurnool Medical College, Andhra Pradesh. The pilot study was conducted for the month of June 2020, the nasopharyngeal swabs were collected from the patients showing clinical symptoms. A total of 49,033 were collected comprising 29,685 at the individual level and 19,348 pooled samples.

### 2.2. Nucleic acid extraction for SARS-CoV-2

RNA extraction was performed from the clinical specimens using HiPurA® Viral RNA Purification Kit (Hi media, Netherlands). After the elution, the RNA was stored at -20°C till further analysis using RT-PCR.

### 2.3. RT-PCR

Real-time RT-PCR assay for SARS-CoV-2 RNA detection was performed using Allplex™ 2019-nCoV Assay (Seegene, Seoul, Republic of Korea), according to the instructions given and RT-PCR was performed in the thermocycler RT-PCR Biorad cfx96 (—). The thermal profile included 1<sup>st</sup> Hold temperature of 50°C for 20 min, 2<sup>nd</sup> Hold temperature of 95°C for 15 min and PCR 45 cycle which includes 94°C for 15 sec and 58°C for 30 sec. Fluorescence is detected at 58°C. The flurophore used includes FAM for E gene, HEX for the internal control, Cal Red 610 for RdRp gene and Quasar 670 for N gene respectively. The data was analysed through application of the Seegene Viewer software, Applied Biosystems™ 7500.

## 3. Results

In a 1 month June 2020, a total of 49,033 were collected comprising 29,685 at the individual level and 19,348 pooled nasopharyngeal swabs received from all over the Kurnool District of Andhra Pradesh were evaluated for the presence of COVID-19 agent through application of RT-PCR. A total

of 1364 cases (2.7%) were detected positive for the SARS-CoV-2 RNA and the remaining samples of total 47,441 were negative for the above mentioned virus. All the 3 genes were detected in the assay. The persons positive were informed and these patients were subjected to immediate quarantine to prevent the spread of the virus.

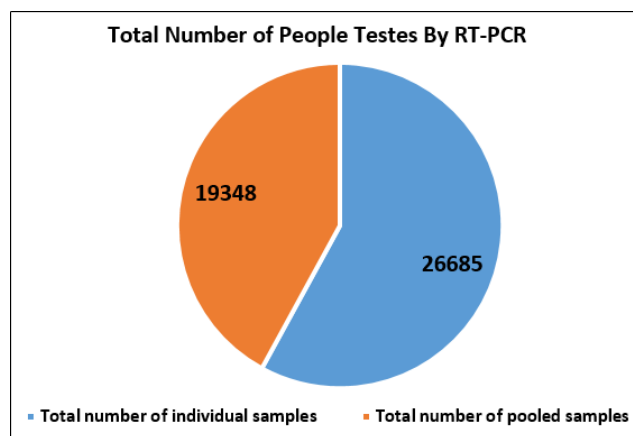


Fig. 1: Total number of people testes by RT-PCR

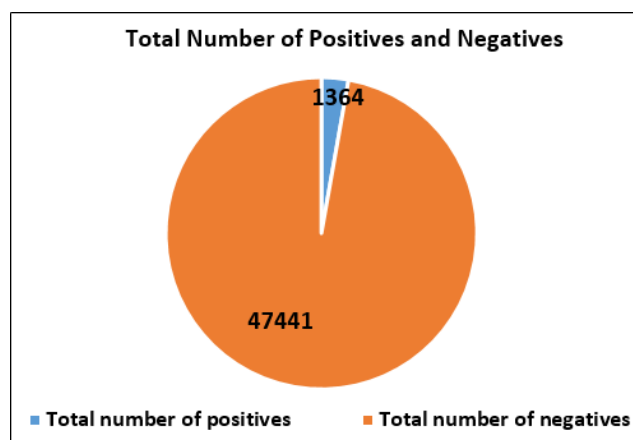


Fig. 2: Total number of positives and negatives

## 4. Discussion

The COVID-19 has affected the several countries and WHO has declared it as a pandemic affecting the persons irrespective of caste, creed, rich or poor. Several countries are in following the instructions and guidance provided by the WHO to contain the spread of the COVID-19 virus. Early detection of virus through application of RT-PCR helps to prevent the spread of the COVID-19 at the earliest. In the present pilot study we evaluated the presence of this virus in larger population in our district, and was evaluated using Allplex™ 2019-nCoV Assay (Seegene,

**Table 1:** Showing total number of people tested, both individual samples and pooled samples with total number of positives and negatives using target genes (E, RdRP and N genes) for detection of SARS-CoV-2 by RT-PCR

| Total number of people tested | Total number of individual samples | Total number of pooled samples | Total number of positives | Total number of negatives |
|-------------------------------|------------------------------------|--------------------------------|---------------------------|---------------------------|
| 49,033                        | 26,685                             | 19,348                         | 1,364                     | 47,441                    |

Seoul, Republic of Korea). The advantage of this kit, that it has an ability to identifies 3 different target genes (E, RdRP and N genes) simultaneously in a single reaction tube which allows for highly accurate results. In the present study we found 2.7% of cases were positive for COVID-19. Similarly a study conducted by Drew and co-workers using the using Allplex™ 2019-nCoV Assay found the 17% were positive for 3 genes, 4 (1.4%) samples were positive for 2 genes (all N gene and RdRP gene), 8 (3%) samples were positive for 1 gene (all N gene only).<sup>6</sup> In another study through application Allplex™ 2019-nCoV Assay found no cross reactivity and found reliable for the detection of COVID-19.<sup>7</sup>

We conclude the present pilot study analysis of detection of COVID-19 using Allplex™ 2019-nCoV Assay is very useful in detecting the suspected COVID-19 cases at the earliest and helps in preventing the spread of the infection. Further study with larger sample size is warranted.

## 5. Source of Funding

None.

## 6. Conflict of Interest

The authors declare that there is no conflict of interest.

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