Original Article

Journal of Microbiology and Related Research
Volume 8 Number 2, July - December 2022
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.8222.1

Demographic Profile of RT-PCR in Diagnosing COVID-19 Patients for a Period of two years at Virology Laboratory

Megha S[1], Venkatesha D[2]

How to cite this article:
Megha S, Venkatesha D/Demographic Profile of RT-PCR in Diagnosing COVID-19 Patients for a Period of two years at Virology Laboratory/J Microbiol Relat Res. 2022;8(2): 37–42.

Abstract

Introduction: Corona virus belongs to the family of Coronaviridae, in the order of Nidovirales. It is formed by a positive sense single stranded RNA, usually appears spherical with size of 80-120nm and with crown like spikes on the surface. This large family of virus is commonly circulating among vertebrates, such as camels, cats and bats. Novel corona virus COVID-19 has been identified as new strain of corona virus. It can cause viral pneumonia and dyspnea in humans.[1]

Objective: Objective of this study is to analyse demographic aspect of the number of RT-PCR tests performed in the virology laboratory, Department of Microbiology, AIMS, B.G. Nagara for a period of two years i.e, July 2020 To June 2022.

Materials and Methods: Nasopharyngeal and oropharyngeal samples collected from patients were subjected for RT-PCR testing using meril kit results were tabulated based based on different demographic profiles.

Conclusion: In the present study with the overall data the strain causing the second wave was highly communicable and account for the highest infection rate and mortality. Least virulent strain was Omicron that caused third wave. Positivity rate of our district was highest in the month of May 2021 (11.1%). Positivity was high in the age group of 40 years and above.

Keywords: SARS Cov-2; COVID-19; RT- PCR.

Author Affiliation: [1]Assistant Professor, [2]Professor and Head, Department of Microbiology, Adichunchanagiri Institute of Medical Sciences, B.G. Nagar 571448, Mandya, Karnataka, India.

Corresponding Author: Megha S, Assistant Professor, Department of Microbiology, Adichunchanagiri Institute of Medical Sciences, B.G. Nagar 571448, Mandya, Karnataka, India.

E-mail: meghaptl88@gmail.com

Received on: 03.09.2022 **Accepted on:** 04.10.2022

INTRODUCTION

Coronavirus belongs to the family of Coronaviridae, in the order of Nidovirales. It is formed by a positive sense single stranded RNA, usually appears spherical with size of 80-120nm and with crown like spikes on the surface. This large family of virus is commonly circulating among vertebrates, such as camels, cats and bats. Novel corona virus (COVID-19) has been identified

as new strain of corona virus. It can cause viral pneumonia and dyspnea in humans.[1]

The first case of COVID-19 was reported on 31st December 2019, cluster of pneumonia cases of unknown etiology was linked to the seafood market of Wuhan, China.[2,3] China's Centre for disease Control (CCDC) identified the causative agent to be SARS-CoV-2 causing coronavirus disease.[4] World health organization (WHO) Declared it as Public Health Emergency of international concern (PHEIC) on 30th January 2020.[5] The infection spread rapidly affecting 113 countries; over a period of 3 months; thus the infection was declared pandemic on 11th March 2020.[6] Compared to 2002-2003 SARS–Cov and 2012-2014 MERS–CoV epidemics, COVID-19 coronavirus spread rapidly to other parts of world.[7]

In India, first case of COVID-19 was reported when one of the medical students returning from Wuhan University was tested positive in Kerala on January 30, 2020.[8] Indian Council of Medical Research (ICMR) has been leading India's Laboratory surveillance of COVID-19 testing.[8] In the initial phases testing for SARS CoV-2 was conducted through 78 selected national reference laboratories.[9]

The infrastructure for testing included ICMR institutes and partners through the Virus Research and Diagnostics Laboratories (VRDL) Network of the Department of Health Research, Ministry of Health and Family Welfare, New Delhi.[10] This network was established for enhancing India's capacity to diagnose and detect viruses of public health importance (ICMR).[6] Subsequently, on March 21, 2020, the ICMR guidelines allowed testing by private laboratories meeting the stipulated criteria. By April 12, 2020, the ICMR augmented the plan to fast-track COVID-19 testing laboratories and issued revised guidelines to use TruNAT-beta-CoV tests on April 14 and Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) using Cepheid® Xpert® Xpress SARSCoV-2 on April 19, 2020.

Virology laboratory for SARS-CoV-2 testing at Adichunchanagiri Institue of Medical Sciences was established and inaugurated on 3 June 2020 by Poojya Nirmalananda Mahaswamiji. Our laboratory is Accredited and ICMR approved. With a capacity of performing 1000 RT-PCR tests per day. Our laboratory has performed 367541 RT-PCR tests over a period of two years. Laboratory is equipped with One 96 well plate RT-PCR machine, one 32 well pate automated RNA extractor, 3 biosafety class 2A cabinets.

OBJECTIVE

Objective of this study is to analyse demographic aspect of the number of RT-PCR tests performed in the virology laboratory, Department of Microbiology, AIMS, B.G. Nagara for a period of two years i.e, July 2020 To June 2022.

MATERIAL AND METHODS

Samples were received from Various districts of Karnataka i.e, Mandya, Bangalore, Hassan, Tumkur, Davangere and Kalburgi. Patients who were suspected of COVID-19 having initial respiratory signs (including sore throat, shortness of breath), fever, cough, Muscle ache and head ache were included in the study. Suspected ILI(Influenza like illness) and SARI (severe acute respiratory illness) were tested for SARS CoV-2 virus by RT-PCR method.

Data (clinical information and results of RT-PCR for SARS–CoV-2 viral nucleic acid detection) were collected from Laboratory information system. The following information were collected for analysis: (1) Demographic characteristics such as age and gender; (2) Clinical characteristics such as ILI/SARI infection; (3) SARS-CoV-2 RT-PCR characteristics. (4) Month wise positivity rate; Throat and/ or nasal swabs were collected for the SARS Co-2 viral nucleic acid detection in sequential time points.

Real time RT-PCR kit used in our laboratory is Meril COVID-19 one step RT-PCR kit which is ICMR approved. RNA extraction done by EX-RNATM–MAG manufactured by Coral Clinical Solutions.

Sample Requirement:[1]

A separate area has been designated for the collection of COVID-19 samples in the hospital premises.

1. ***SampleType:*** Nasopharyngeal, Oropharyngeal swab specimen, Throat swabs, serum and virus preservation buffer,
2. ***Sample collection:*** collected in accordance with conventional sample collection method.
3. ***Sample storage and transportation:*** Sample to be tested will be processed immediately, or stored at -20±5 C. Avoid repeated thawing and freezing. Sample should be transported with refrigerant packs in sealed Styrofoam

box or ice chest.

RNA Extraction: [11]

RNA extraction is done by EX-RNATM-MAG RNA extraction kit for RT-PCR testing (Magnetic beads method) manufactured by Coral Clinical Systems.

Specimen is mixed with the Lysis/binding Buffer and Magnetic beads during which the specimen is lysed and releases substantial amountof RNA from the cells which binds to magnetic beds, in the next step the RNA bound to the beads are washed with wash Buffer A and B sequentially to remove the salts and proteins. Finally, the Elution Buffer is added to elute the RNA from beads, Eluted RNA can be used for RT-PCR and other molecular testing.

RT-PCR Molecular testing

The kit used is Meril COVID-19 One-step RT-PCR Kit

1. Reagent preparation

1.1 Master mix preparation:

Take out the components from the box and let it thaw at room temperature until equilibrated. Resuspend the lyophilized Enzyme Mix in 400µL Enzyme buffer. Add 500µL RNase-free water and gently pipette up and down. Avoid generating air bubbles. Wash the wall of tube by pipetting to prevent lyophilized powder from remaining. Place the tube aside for 30 min.

1.2 Reaction Mix Preparation:

The recommended sample volume used in the reaction is 5µL or 10µL. Refer to of the columns below to prepare the reaction mix.

1x Volume Required		
Description	**For 5µL Sample**	**For 10µL**
Resuspended master mix	9 µL	9 µL
ORF 1ab/N/ICON Primer and probe(FAM/HEX/ROX)	1 µL	1 µL
RNase- free water	5 µL	–
Total volume	15 µL	10 µL

1.3. Aliquot 15µL (or 10µL, depending on sample volume) of the above reaction mix into the PCR plate of the chosen PCR platform. Aliquot into wells according to the number of samples to be tested, include one well for the positive control and one well for the negative control. Transfer the reaction mix to Sample Processing area.

2. Sample Adding (Performed in Sample Processing Area)

2.1 For 5µL Sample: Add 5µL of the following into appropriate wells according to plate setup: Samples, Positive control, Negative Control.

2.2. For 10µL sample: Dilute positive control with 5µL DEPC treated water to total volume of 10µL. Add 10µL of the following into the appropriate wells according to plate setup: Sample, Diluted Positive control and negative control.

2.3 After adding the samples, cover the lid immediately. Spin down briefly using a centrifuge to remove air bubbles. Transfer the mixture to amplification area.

3. PCR Amplification (Performed in Amplification and Analysis area)

3.1. Place the tubes on the sample holder in the instrument. Setup the test panel according to the position of the positive control and RNA samples.

3.2. Select the detection channels as following:

a) Select FAM (ORF-1ab gene) and HEX (N gene) channels to detect COVID-19 RNA.

b) Select ROX channel to detect internal control.

Step	Temp.	Time	Cycle
Reverse Transcription	50˚C	15 Mins	1
cDNA Initial Denaturation	95˚C	3 Mins	1
Denaturation	95˚C	15 sec	40
Annealing, Extension and Fluorescence measurement	55˚C	40 sec	–
Cooling	25˚C	10 sec	1

3.3 Enter the amplification program Recommended as below:

Save the file and run the reaction.

4. Result Interpretation:

4.1. After the reaction is completed, the results are automatically saved and the amplification curves of the detected target DNA and the internal control are analyzed separately.

5. Quality Control:

5.1. COVID-19 PCR Negative control:

No amplification should be observed in FAM, HEX and internal control (ROX) channel.

5.2. COVID-19 PCR Positive Control:

FAM, HEX and internal control channels Ct≤35

5.3. Internal control (R NaseP):

Internal control (ROX) channels Ct ≤35

5.4. The above requirements must be met same time

same experiment. Otherwise, this experiment is invalid and needs to be repeated.

This assay runs for 40 Cycles.

RESULT ANALYSIS

Target	Ct Value	Interpretation
ORF 1 ab gene (FAM)	Ct≤40	2019-nCov ORF 1ab gene positive
Nucleoprotein N gene	Ct≤35	2019 –nCov Nucleoprotein gene positive
Internal control	Ct≤35	Internal control positive

ORF 1ab gene (FAM)	Nucleoprotein gene (HEX)	Internal control (ROX)	Result interpretation	Action to be taken
Positive	Positive	Positive	SARS-CoV-2 Positive	Reports results to sender and appropriate health authority
Positive	Negative	Positive	SARS-CoV-2 Positive	Repeat the extraction and retest. If again getting Ngene negative, the interpretation is positive
Negative	Positive	Positive	SARS–CoV-2 Negative	Repeat the extraction and retest, If again getting ORF 1ab gene negative, the interpretation is negative
Negative	Negative	Positive	SARS–CoV-2 Negative	Report results to sender
Negative	Negative	Negative	Invalid	Sample should be repeated once again with fresh extraction. If a second failure occurs, it should be reported to sender as invalid and sample recollection is recommended if patient is still clinically indicated.

Table 1: Distribution according to Month wise COVID data

Month	Number	Positivity%
Jul-20	5233	1.4
Aug-20	13648	3.7
Sep-20	10705	2.9
Oct-20	20532	5.6
Nov-20	25702	7.0
Dec-20	23625	6.4
Jan-21	19808	5.4
Feb-21	7729	2.1
Mar-21	1348	.4
Apr-21	13288	3.6
May-21	40856	11.1
Jun-21	30050	8.2
Jul-21	36308	9.9
Aug-21	39925	10.9
Sep-21	21931	6.0
Oct-21	13901	3.8
Nov-21	7370	2.0
Dec-21	1287	.4
Jan-22	26770	7.3
Feb-22	7429	2.0
Mar-22	54	.0
Apr-22	32	.0
May-22	0	0
June-22	0	0

Table 2: Distribution according Age, Sex, Nationality, State, waves, and year wise frequency of COVID 19

Age Group	Number	%
Less than 18 yrs	68765	18.7
18 - 44 yrs	175299	47.7
45 - 60 yrs	80479	21.9
Above 60 yrs	42988	11.7
Sex		
Female	182504	49.7
Male	185011	50.3
Transgender	16	.0
Nationality		
Anguilla	3	.0
Antarctica	2	.0
India	367090	99.9
Kazakhstan	8	.0
Sweden	1	.0
United States	1	.0
No Data	426	.1
State		
Karnataka	367375	100.0
Others	156	.0

Wave time		
June 2020 to Feb 2021 (First Wave)	126982	34.6
March 2021 to July 2021 (Second Wave)	121850	33.2
Aug 2021 to June 2022 (Third Wave)	118699	32.3
Total Samples Year Wise		
2020	99445	27.1
2021	233801	63.6
2022	34285	9.3

Table 3: Distribution according to district wise data in Karnataka State.

District Name	Number	%
Bagalkote	3	.0
Ballari	20	.0
Belagavi	23	.0
Bengaluru Rural	573	.2
Bengaluru Urban	9169	2.5
Bidar	7	.0
Chamarajanagara	18	.0
Chikkaballapura	19	.0
Chikkamagaluru	37	.0
Chitradurga	52	.0
Dakshina Kannada	12	.0
Davangere	856	.2
Dharwad	10	.0
Gadag	3	.0
Hassan	1690	.5
Haveri	3	.0
Kalaburagi	836	.2
Kodagu	21	.0
Kolar	7	.0
Koppal	2	.0
Mandya	346353	94.2
Mysuru	238	.1
Raichur	3	.0
Ramanagara	232	.1
Shivamogga	10	.0
Tumakuru	7167	2.0
Udupi	2	.0
Uttara Kannada	1	.0
Vijayapura	1	.0
Yadgir	5	.0
Mandya	2	.0
Other State	156	.0

Table 4: Distribution according to test results

Test Results	Number	%
Negative	341074	92.8
Positive	26227	7.1
Rejected	230	.06
Total	367531	100

Table 5: Distribution according to Vaccination status, type and doses of the study subjects

Vaccination Status	Number	%
No	343272	93.4
Yes	24259	6.6
Vaccination Type		
No	343272	93.4
Covaxin	7049	1.9
Covishield	17195	4.7
Other Vaccine	15	.0
1st Dose		
No	343272	93.4
Yes	24259	6.6
2nd Dose		
No	354790	96.5
Yes	12741	3.5

The validity and the interpretation of each specimen result according to the results in each channel are given below in tabular form:

If the internal control ROX channel failed to detect Ct or Ct> 35, it indicates that the concentration of tested sample is too low or there is an inhibitory reaction from the interfering substance. Users have to repeat the experiment.

For positive samples and virus cultures, there is no requirement of the internal control results. For negative samples, the internal control should be positive. If the internal control is negative, the test result of the sample is invalid. The cause should be found and eliminated. Users should redo sampling and repeat the experiment.

DISCUSSION

For a period of 2 Years, our laboratory received 367531 samples for SARS CoV-2 RT-PCR testing, of which 367375 was from Karnataka state. Which were 99445 in the year 2020, 233801 in the year 2021 and 34285 in the year 2022. Wave time is divided into first second and third, starting from June 2020 to February 2021, March 2021 to July 2021 and Aug 2021 to June 2022 respectively. Total number

of samples received in the first wave was 126982, second wave was 121850 and third wave was 118699. Maximum number of sample was received in the month of may 2021 with positivity rate of 11% which falls in the second wave.

Among the population highest number of samples were received from 18-44 years age group, accounting for about 47%. Among the Karnataka state samples highest samples receive were from mandya district, which accounted for 94% followed by Bengaluru urban (2.5%) and Tumkur (2%). Of the total samples received for a period of 2 years, a total of 26227 were SARS-CoV-2 positive (7.1%). 230 samples were rejected.

Of per the vaccination received data, only 24259 (6.6%) patients of population were vaccinated with atleast one dose. 12741 (3.5%) were fully vaccinated with 2 doses and the rest of 343272 (93.4 %) population were not vaccinated as on the date of sample collection.

The present study has several limitations that should be taken into consideration. Firstly it's a retrospective study, the accuracy of SARS CoV 2 RT-PCR may vary (improvement of the detection protocol and gain of experience in sampling). Secondly the study investigated only the few dynamic profiles as age, gender and place of sampling while other factors were not taken into consideration.

CONCLUSION

In the present study with the overall data the strain causing the second wave was highly communicable and account for the highest infection rate and mortality. Least virulent strain was Omicron that caused third wave. Positivity rate of our district was highest in the month of May 2021 (11.1%). Positivity was high in the age group of 40years and above.

REFERENCES

1. Meril COVID-19 One step RT-PCR Kit Insert, Meril Diagnostics.
2. World Health Orgaization (2020) Rolling updates on coronavirus disease (COVID-19).
3. Wu YC, Chen CS, Chan YJ(2020) The outbreak of COVID-19: An overview. J Chinese Med Assoc 83:217-220.
4. Aylward, Bruce, Liang W (2020) Report of the WHO-China Joint Mission on coronavirus Disease 2019 (COVID-19).
5. World Health Organization (2020) International Health Regulations Emergency Committee on novel coronavirus in China.
6. World health organization (2020) Coronavirus disease 2019, situation report 101.14.
7. Rajab Mardani et al, Laboratory parameters in detection of COVID-19 Patients with positive RT-PCR; a diagnostic Accuracy Study. Ach Acad Emerg Med. 2020;8(1):e43.
8. Priya Abraham et al, Laboratory surveillance for SARS-CoV-2 in India: Performance of testing & descriptive epidemiology of detected COVID-19, January 22 - April 30, 2020. Indian J Med Res 151, May 2020, pp 424-437.
9. Indian Council of Medical Research. Press Release: Note on COVID-19 Laboratory Preparedness in India. New Delhi: ICMR; 6 March, 2020. Available from: https://www.icmr. gov.in/pdf/covid/labs/ICMR_PressRelease_COVID_19.pdf, accessed on May 8, 2020.
10. Department of Health Research. Establishment of a network of laboratories for managing epidemics and Natural Calamities (VRDL). New Delhi: Ministry of Health and Fmily Welfare, Government of India; 2020. Available from: https://dhr.gov.in/schemes/establishment-network-laboratories-managingepidemics-and-natural-calamities, accessed on May 2, 2020.
11. EX-RNA-MAG RNA extraction kit for RT-PCR Testing(Magnetic beads method) kit insert. Coral clinical systems.