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Prevalence and detection of SARS-CoV-2 by RT-PCR: an analytical study

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Abstract

Background: The COVID-19 pandemic has significantly affected the healthcare system. RT-PCR has played a major role in the confirmation of SARS-CoV-2 thus helping in an early diagnosis, clinical interventions and patient isolation. This study was conducted to ascertain the importance of RT-PCR testing among symptomatic and asymptomatic cases in different age groups with association to infection.

Methods: This retrospective cohort study was conducted from August 2020 to August 2021, in the Department of Microbiology, Autonomous State Medical College and Sarojini Naidu Memorial Hospital, Firozabad, Uttar Pradesh, India. Samples were collected from a total of 342, 281 cases which comprised of symptomatic patients (ILI, SARI), asymptomatic contacts, those seeking hospitalization, travelers and were subjected to testing by RT-PCR. The cases were divided into group A of patients presenting with symptoms ≤7 days, group B of patients with signs and symptoms >7 days and group C comprised of asymptomatic cases. The symptoms of patients were associated with the Ct values of the E/N screening gene and the RdRp/ORF1ab confirmatory genes. The Chi-square test was done to test the statistical significance of association of symptomatic patients with the outcome of the test.

Results: The number of positive samples were 4,342 showing a prevalence of 1.3%. The maximum prevalence of infection was found in the age group of 20-29 years followed by the age group 30-39 years (p-value <0.05). The maximum positivity and high viral load were seen in the patients who presented with symptoms ≤7 days with Ct values ≤25.

Conclusion: The maximum infection was found in the young age group. The screening and confirmatory genes could be detected in the samples of asymptomatic cases also which was helpful in isolating them and breaking the chain of further spread of the virus.

Keywords: ACE 2 receptors, Bio Safety Lab, COVID-19, Cycle Threshold, RTPCR, India

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Background

The Coronavirus disease 2019(COVID-19) pandemic has made a record-breaking devastation in the history of mankind, not only in the terms of mortality but also morbidity, psychological disorders, emotional stress and breakdown, financial loss by disrupting the business worldwide and sending the education system for a toss. Studies show a significant deterioration in the psychological behavior of people due to the ecosystem disturbance owing to sudden lifestyle changes [1]. There has been loss in learning in about 50% of the expected academic level due to absenteeism [2]. The global economy had been hit by the pandemic and contracted sharply by 3% in 2020 [3]. However, the pandemic has revamped the position of laboratory medicine much higher in hierarchy in the healthcare systems to deal with such critical situations [4]. One of the most commonly used methods of detecting the virus has been the RT-PCR [5]. In the beginning there were very few reference laboratories and Institutes in India which were detecting the COVID-19 virus. However, with the increase in the cases many government funded laboratories of the Biosafety Level 2 (BSL-2) were developed in the state of Uttar Pradesh, India [6]. With the advent of these, the samples which were being processed in distant reference laboratories were diverted to the new established labs which minimized the reporting time to a great extent and hence facilitated in the early isolation of patients, treatment and combating the further spread of the infection. This study was conducted to discuss the prevalence of SARS-CoV-2 in different

age groups, gender and confirmation of the virus in samples by RT-PCR technique.

Methods

Study design and participants

This retrospective cohort study was conducted from August 2020 to August 2021 in the Department of Microbiology at Autonomous State Medical College and Sarojini Naidu Memorial Hospital, Firozabad, Uttar Pradesh, India. Samples were collected from 342, 281 cases from District Firozabad and the neighboring areas of Kasganj, Tundla, Amroha and Mathura. The age, gender and signs and symptoms of the cases were recorded and they were divided into 3 groups viz. group A of patients who presented with symptoms ≤ 7 days, group B of patients who presented with signs and symptoms > 7 days and group C comprising of asymptomatic patients [7,8].

Specimen Collection

All samples were collected as per the Centre for Disease Control and Prevention (CDC) guidelines by trained healthcare workers who followed infection control guidelines and used recommended personal protective equipment (PPE) which included N95 masks, face shields, shoe covers, gloves and gowns while sample collection, handling, transport and processing [9]. Samples were collected from the OPD patients in COVID Walk in Sample Kiosk (WISK). Nasopharyngeal swabs (NPS) were collected from the nostril using Dacron swab from the posterior nasopharynx by tilting back the patients' head at 70 degrees and it was inserted gently and slowly until resistance was encountered or the distance equivalent to that from the ear to the nostril. While oropharyngeal swabs (OPS) were collected from both sides of the throat wherein, swab was inserted into the posterior oropharynx and the tonsillar area and rubbed over both tonsillar pillars. Both the swabs were put in a single Viral Transport Medium (VTM) vial to maximize test sensitivity. All the collected samples were transported to the laboratory in a cold chain maintained at 2-8°C. If there was an unavoidable delay the samples were stored at -70°C.

Inclusion and exclusion criteria

As per the Indian Council of Medical Research (ICMR) strategy for Covid-19 testing in India, all the suspected Covid-19 symptomatic patients, follow up patients, asymptomatic cases with history of contact with positive patients, history of international travel in the last 14 days, people from containment zones, people post quarantine period, pre operational cases, health care workers/front line workers, all patients of severe acute respiratory infections (SARI) and patients with influenza like illness (ILI) were included [10]. Asymptomatic people with no contact history with positive cases and no travel history were not included in the study.

RNA Extraction

The samples were processed for RNA extraction by manual method using QIAgen (spin column based), Genetix GeneMag Viral DNA/RNA Purification kit (Non- Prefilled), Genuine Biosystem (GB)'s aura pure and applied biosystems MagMaxTM Viral/Pathogen Nucleic Acid Isolation kit. Depending on the sample load, RNA extraction of some samples was also done by automated (ThermoFisher Scientific automated extractor machine) methods using commercially available kits Genuine Biosystem (GB)'s aura pure, applied biosystems MagMaxTM Viral/Pathogen Nucleic Acid Isolation kit (magnetic bead based) following the kit manufacturers' instructions.

Manual extraction:

Lysis buffer (100 µl) was prepared per reaction, to which 08 µl carrier RNA and 10 µl Proteinase K was added thus obtaining a total volume of 118 µl to which sample (200 µl) was added. These were mixed properly by vortexing for 30 seconds and kept on the shaker/rocker for 10 minutes at 56°C on thermomixer. Thereafter, ethanol (270 µl) and 40 µl GB's aura pure beads were added and mixed well by pipetting the lysate up and down 5-6 times and the lysate was then put on the thermomixer for 5 minutes at 37°C. The magnetic beads were separated by placing the tube in a magnetic stand and the entire supernatant was removed by gentle pipetting. The tube was removed from the stand and wash buffer 1 (500 µl) was added, vortexed and kept on thermomixer for 3 minutes at 37°C. The magnetic beads were separated by placing the tube in a magnetic stand and removing the entire supernatant by gentle pipetting. The step was repeated with wash buffer 2. Following this, the supernatant was completely removed and the beads were fully dried at 65°C for 5-6 minutes. Elution buffer (60µl) was added to tubes containing magnetic beads, vortexed and incubated for 5 minutes at 56°C. The beads were separated by keeping the tubes on the magnetic stand and the supernatant containing the viral RNA was transferred to new microcentrifuge tube and the extracted RNA was kept at 4°C.

Automated Extraction:

For automated RNA extraction kit manufacturers' instructions were followed wherein 04 deep well plates (one each for samples with lysis buffer, 2nd and 3rd wash buffer 1 & wash buffer 2 and 4th plate for elution buffer) were used on Thermo Fisher Scientific automated RNA extractor machine. The RNA extraction done by automated methods took approximately 30 minutes and manual method approximately 2-3 hrs. Samples were processed inside the BSL-2 laboratory following all the standard safety precautions [11]. According to the ICMR guidelines, the samples were discarded in a biohazard bag containing 2% lyzol or 5% freshly prepared sodium hypochlorite solution after sealing the bag [12].

Real Time PCR

Principle of RT-PCR:

Real-time PCR combines single-step amplification and detection through fluorescence capture technique by using dyes to determine genomic product concentration, which is amplified to fluorescence intensity [13]. The point where the target amplification is first detected in the exponential phase is known as the "cycle threshold" (Ct). This indicates the time at which detectable fluorescence intensity is greater than background fluorescence. In another words, the more the genetic material present in the clinical sample, the earlier significant increase in fluorescence signal will occur, resulting in a lower Ct value [14,15]. Real time PCR was performed using various ICMR approved RT-PCR kits. The GCC Biotech DIAGsureTM nCoV-19 Detection kit, Genetix COVISure COVID 19 RT-PCR kit, GB

SARS-CoV-2 RT-PCR kit and Meril Covid-19 one step RT-PCR kit. This test was performed with primers and probes provided by the ICMR targeting the Envelope gene (E gene), Nucleocapsid protein (N gene), RNA dependent RNA polymerase (RdRP gene) and Open Reading Frame (ORF1ab gene) genomic region of SARS-COV-2 and Internal Control (IC) Human Ribonuclease P (RNAseP).

Preparation of Master Mix (per reaction):

Master mix was prepared as per the kit manufacturers' instructions. To the master mix (6µl), Primer Probe mix A (2µl) for ORF1ab, Primer Probe mix B (2µl) for RNaseP and Primer Probe mix C (2µl) for N-Gene were added and a total volume of 12µl master mix was obtained. Master mix was dispensed in PCR tubes/wells and 8µl of extracted RNA was added separately to each tube and a Positive control (PC) and Negative control (NC/NTC) each were added. The plate was sealed with optically clear sealers. After centrifugation the tube/plate were placed in the BioRadCFX96 real time system. The amplification steps included reverse transcription at 50°C for 15 minutes, initial denaturation at 95°C for 3 minutes, denaturation at 95°C for 10 seconds and annealing/extension at 60°C for 30 seconds.

Result interpretation:

It was done as per the kit manufacturers' instructions. A Ct value ≤35 of the internal control indicated a valid test. When both the E/N gene and RdRp/ORF1ab gene showed Ct< 35, the specimen was considered positive for SARS-CoV-2. When the test result showed graph only for the internal control the sample was considered as negative. When only the screening genes were detected, the sample was considered to be positive for any other virus from the Coronaviridae family. If all 3 plots for E/N, RdRp/ORF1ab and IC were not seen, RNA extraction and amplification of the same sample was repeated. If retest results were the same as initial test, the samples were rejected and resampling of the patient was advised.

The samples showing screening and confirmatory genes' Ct values ≤25 was grouped as those with High viral load. Ct values between 26–30 exhibited a significant reduction of viral load and samples were grouped as Medium viral load. The samples with Ct values more than 30 were grouped as low viral load [16].

The positive samples were preserved at -80°C for further molecular studies.

Statistical analysis

All the categorical variables were presented in the form of frequencies and percentages besides tabulation was done for the summary. Thereafter, the Chi-square test was done to test the statistical significance of association of symptomatic patients with the outcome of the RTPCR test. The association of categories of Ct values and symptomatic patients was tabulated and its significance checked using Chi-square test. All the analyses were performed using R-4.3.1 and MS Excel 2007.

Results

Of the total samples collected from 342, 281 cases 216, 193 (63.2%) were from males and 125, 999 (36.8%) from females. The maximum samples were tested in males of the age group of 20-29 years followed by 10-19 years (Table 1).

Table 1: Age group and gender wise (percentage) testing of Coronavirus by RT-PCR

Age Group	Female	Male	Transgender	Grand Total	p-value and significance
0-9	3690(1.1%)	4982(1.5%)	1(0%)	8673(2.5%)	Independence of RT-PCR result and age-group p-value=0.02×10^{-14} (<0.05) Significant
10-19	21705(6.3%)	42719(12.5%)	8(0%)	64432(18.8%)	
20-29	46855(13.7%)	66755(19.5%)	46(0%)	113656(33.2%)	
30-39	22103(6.5%)	40295(11.8%)	15(0%)	62413(18.2%)	
40-49	14498(4.2%)	27208(7.9%)	10(0%)	41716(12.2%)	
50-59	9205(2.7%)	18168(5.3%)	1(0%)	27374(8%)	
60-69	6049(1.8%)	11379(3.3%)	3(0%)	17431(5.1%)	
70-79	1538(0.4%)	3820(1.1%)	2(0%)	5360(1.6%)	
80-89	310(0.1%)	770(0.2%)	2(0%)	1082(0.3%)	
90-99	46(0%)	97(0%)	1(0%)	144(0%)	
Grand Total	**125999(36.8%)**	**216193(63.2%)**	**89(0%)**	**342281(100%)**	

Of the total samples tested, positive samples were 4,342 (1.3%), negative samples were 3,31091 (96.7%), equivocal samples were 9 (0%) and samples requiring repeat sampling were 6,839 (2%). The maximum prevalence of infection was found in the age group of 20-29 years followed by 30-39 years. The positivity percentage was 1.3% (Table 2-Appendx-1). Of these, 0.1% positivity was found in the group of patients who were tested fortravel purpose and 1.2% positivity was found in people other than travel purpose. In the case of other than travel purpose the contacts of positive cases showed maximum positivity 0.8% (Table 3-Appendex-2). The maximum positivity was seen in the patients who presented with symptoms ≤7 days with Ct values ≤25 of E and ORF1ab gene (18.6%), E and RdRp genes (2.2%) and N and ORF1ab gene (1.2%) of these patients were found with high viral load (Table 4).

Discussion

The present study was done to evaluate the performance of RT-PCR assay for screening and confirmation of SARS-CoV-2. In the present study the maximum RT-PCR testing was done in males of the age groups of 20-29 years followed by 10-19 years($p<0.05$). This was due to more locomotion of these age groups for professional and educational purposes. The least number of people were tested in the age groups above 60 years. This could be due to their adherence to the advisory issued by the National Human Rights Commission which restricted the unnecessary locomotion of the elderly [17].

Table 4. Association of symptoms in different groups with the E and ORF1ab genes, E and RdRp genes and N and ORF1ab genes positivity detected by RTPCR

Gene Value	Group A (Symptomatic <=7 days)	Group B (Symptomatic >7 days)	Asymptomatic	Total	p-value
	E gene & ORF1ab gene				
<=25	808(18.6%)	442(10.2%)	103(2.4%)	1353(31.2%)	Chi-square=243.87 p-value =0.0000002 (<0.05) Significant
26-30	622(14.3%)	531(12.2%)	128(2.9%)	1281(29.5%)	
>30	254(5.8%)	234(5.4%)	202(4.7%)	690(15.9%)	
Total	1684(38.8%)	1207(27.8%)	433(10%)	3324(76.6%)	
	E gene & RdRp gene				
<=25	97(2.2%)	28(0.6%)	12(0.3%)	137(3.2%)	Chi-square=32.39 p-value =0.0000015 (<0.05) Significant
26-30	134(3.1%)	88(2%)	35(0.8%)	257(5.9%)	
>30	169(3.9%)	121(2.8%)	83(1.9%)	373(8.6%)	
Total	400(9.2%)	237(5.5%)	130(3%)	767(17.7%)	
	N gene & ORF1ab gene				
<=25	51(1.2%)	29(0.7%)	6(0.1%)	86(2%)	Chi-square=11.84 p-value =0.01 (<0.05) Significant
26-30	43(1%)	28(0.6%)	9(0.2%)	80(1.8%)	
>30	42(1%)	23(0.5%)	20(0.5%)	85(2%)	
Total	136(3.1%)	80(1.8%)	35(0.8%)	251(5.8%)	
Grand Total	2220(51.1%)	1524(35.1%)	598(13.8%)	4342(100%)	

The positivity rate of the present study was 1.3% and in the maximum cases infection was found in the age group of20-29 years followed by 30-39 years. Similar findings were found in other studies too [18,19]. As the younger generation pursued work and other activities there was an increase in the positivity in this age group [20]. However, in another study the commonest age group affected was 40-49 years [21]. Their results were in concordance with other studies in which the middle age group people were affected more by the pandemic [22-25]. A study conducted in Italy, also found maximum SARS-CoV-2 positive cases in the age group of 40-59 years [26]. This could be because Italy is a home to the elderly with underlying pathologies [27]. The pandemic has affected men and that too adults at higher rates [28]. It could be due to more exposure levels of males due to more locomotion. Further, it could be attributed to difference in lifestyle viz. higher levels of smoking and drinking among men compared to women or a more responsible attitude of women while following the preventive measures [29]. The Angiotensin-converting enzyme-2 (ACE 2) encoded by the ACE 2 gene is the receptor for the SARS-CoV [30]. Therefore, the higher the expression of ACE 2, greater the pathogenesis of Coronavirus. Studies have shown higher expression of ACE 2 in Asian males than females [31]. Also, the expression of ACE 2 in human lungs was expressed more in Asian males [32]. Another reason could be sex-based immunological differences driven by sex hormone and X chromosome [33,34]. In this study 0.1% positivity was found in the group of patients who were tested for travel purpose and 1.2% positivity was found in people who were tested for other than travel purpose. In the group of other than travel purpose 0.8% positivity was found among the contacts of positive cases. In a study conducted by Zhang et al. [35] in China, 2.7% of the close contacts were positive for SARS-CoV-2. In this study, maximum people who got tested for travelling purpose were found positive in the age group of 30-39 years followed by 20-29 years which is an age of more locomotion due to educational and professional reasons. Since the cellular proteases and human airway trypsin (HAT) like protease provide a good platform for the Coronavirus entry [36,37,38], nasopharyngeal and oropharyngeal swabs were the ideal specimens of choice [39]. The E gene which is the integral membrane protein facilitating the assembly of the viral particle [40,41] and the helical N gene which is found abundantly making them ideal diagnostic markers [42] were used for screening in the present study. The samples of patients who presented with symptoms for <7 days showed low Ct values of screening and confirmatory genes. In patients presenting with symptoms >7 days, the samples showed medium and high Ct values. The association between Ct values and the duration of symptoms was found to be inversely proportional ($p<0.05$). The maximum positivity was seen in the patients who presented with symptoms ≤7 days with Ct values ≤25 ($p<0.05$). A low Ct value indicated an elevated level of genetic material, which has been associated with a high risk of infection [43]. A high Ct value, indicated less probability of infectivity due to low concentration of viral genetic material [44]. In another study by Soria et al. [45] also a correlation between low Ct values and higher disease severity, higher mortality, a higher chance of developing severe illness and lower Ct values being associated with worse outcomes in COVID-19 patients was observed. However, some studies did not find any association between Ct values and disease severity [46,47]. Of the total positive samples, 13.8% cases were asymptomatic and approximately 2.4% showed Ct values ≤ 25. This could be due to the immune response to the infection wherein the T cells may have efficiently worked against the SARS-CoV-2 in the acute phase, indicating that high viral load may not always be a distinct factor for severity of infection [48]. Sajjad et al emphasized the role of microbiota composition as a significant factor for varied immune response due to its ability to modulate it [48]. The limitation of the study was that some samples had to be repeated due to invalid results which could be due to improper sampling etc. in non-cooperative patients.

Conclusion

The RT-PCR technique has been considered a gold standard assay for the detection of SARS-CoV-2 and has good sensitivity and specificity. In this retrospective study the screening and confirmatory genes could be detected in the samples of asymptomatic cases as well. This indicates a very great advantage of the technique because it helped in isolating such cases and breaking the chain of further spread of the virus. Although it is expertise dependent and requires well equipped labs, the government left no stone unturned in the pandemic time to provide the facility of this otherwise expensive test at every district level. The labs shared the sample load thus making reporting faster and easier for the general public. Besides, the use of indigenously prepared kits was a boost to the countries' scientific community.

Abbreviation

RTPCR: Real-Time Reverse Transcription- Polymerase Chain Reaction; COVID 19: Coronavirus Disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2;SARI: Severe Acute Respiratory Infections; ILI: Influenza Like Illness; BSL-2:BioSafety Level 2;CDC: Centre for Disease Control and Prevention; PPE: Personal Protective Equipment; N95: Non-oil; WISK: Walk in Sample Kiosk; NPS: Nasopharyngeal swabs; OPS: Oropharyngeal swabs; VTM: Viral Transport Medium; ICMR: Indian Council of Medical Research; GB: Genuine Biosystem; Ct: Cycle Threshold; E gene: Envelope gene; N gene: Nucleocapsid protein; RdRP gene: RNA dependent RNA polymerase; ORF1ab gene: Open Reading Frame; IC: Internal Control; RNAseP: Human Ribonuclease P; PC: Positive control; NC: Negative control; ACE 2: Angiotensin-converting enzyme-2;HAT: Human Airway Trypsin

Declaration

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Availability of data and materials

Data will be available by emailing tuli_lekha@rediffmail.com

Authors' contributions

Lekha Tuli (LT) designed the study, supervised the study, conceived the idea, did data analysis, curated and drafted the manuscript. Mohd Shariq (MS) did the practical work, data analysis and drafted the manuscript. Rohit Patawa (RP) did the statistical analysis and interpreted the data in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

We conducted the research following the declaration of Helsinki. The ethical approval **(No Objection Certificate)** was obtained from the office of the Chief Medical Superintendent, A.S.M.C., and S.N.M. Hospital, Firozabad, India.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

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Appendix 1

Table 2. Percentage positivity of Coronavirus in different age groups detected by RT-PCR with travel details

Age Group (in years)		0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-99	Grand Total
Equivocal	For travel purpose n (%)	0(0%)	0(0%)	0(0%)	1(0%)	0(0%)	0(0%)	0(0%)	1(0%)	0(0%)	0(0%)	2(0%)
	Other than travel n (%)	0(0%)	0(0%)	3(0%)	1(0%)	1(0%)	1(0%)	0(0%)	0(0%)	1(0%)	0(0%)	7(0%)
	Total n (%)	0(0%)	0(0%)	3(0%)	2(0%)	1(0%)	1(0%)	0(0%)	1(0%)	1(0%)	0(0%)	9(0%)
Negative RT-PCR	For travel purpose n (%)	753(0.2%)	5861(1.7%)	12341(3.6%)	7988(2.3%)	6262(1.8%)	3878(1.1%)	1540(0.4%)	373(0.1%)	54(0%)	3(0%)	39053(11.4%)
	Other than travel n (%)	7709(2.3%)	57165(16.7%)	97845(28.6%)	52071(15.2%)	33876(9.9%)	22325(6.5%)	15203(4.4%)	4741(1.4%)	972(0.3%)	131(0%)	292038(85.3%)
	Total n (%)	8462(2.5%)	63026(18.4%)	110186(32.2%)	60059(17.5%)	40138(11.7%)	26203(7.7%)	16743(4.9%)	5114(1.5%)	1026(0.3%)	134(0%)	331091(96.7%)
Positive RT-PCR	For travel purpose n (%)	3(0%)	20(0%)	78(0%)	89(0%)	72(0%)	59(0%)	33(0%)	9(0%)	1(0%)	0(0%)	364(0.1%)
	Other than travel n (%)	66(0%)	357(0.1%)	1076(0.3%)	875(0.3%)	627(0.2%)	473(0.1%)	331(0.1%)	139(0%)	29(0%)	5(0%)	3978(1.2%)
	Total n (%)	69(0%)	377(0.1%)	1154(0.3%)	964(0.3%)	699(0.2%)	532(0.2%)	364(0.1%)	148(0%)	30(0%)	5(0%)	4342(1.3%)
Repeat sampling required	For travel purpose n (%)	8(0%)	66(0%)	206(0.1%)	130(0%)	98(0%)	70(0%)	27(0%)	5(0%)	1(0%)	0(0%)	611(0.1%)
	Other than travel n (%)	134(0%)	963(0.3%)	2107(0.6%)	1258(0.4%)	780(0.2%)	568(0.2%)	297(0.1%)	92(0%)	24(0%)	5(0%)	6228(1.8%)
	Total n (%)	142(0%)	1029(0.3%)	2313(0.7%)	1388(0.4%)	878(0.3%)	638(0.2%)	324(0.1%)	97(0%)	25(0%)	5(0%)	6839(2%)
Grand Total n (%)		8673(2.5%)	64432(18.8%)	113656(33.2%)	62413(18.2%)	41716(12.2%)	27374(8%)	17431(5.1%)	5360(1.6%)	1082(0.3%)	144(0%)	342281(100%)

Appendix 2

Table 3. Percentage positivity of Coronavirus in different patient categories detected by RT-PCR

Age Group		0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-99	Total
Equivocal	SARI	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	ILI	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Seeking hospitalization	0(0%)	0(0%)	1(0%)	0(0%)	0(0%)	1(0%)	0(0%)	0(0%)	0(0%)	0(0%)	2(0%)
	Contacts	0(0%)	0(0%)	2(0%)	1(0%)	1(0%)	0(0%)	0(0%)	0(0%)	1(0%)	0(0%)	5(0%)
	Total	0(0%)	0(0%)	3(0%)	1(0%)	1(0%)	1(0%)	0(0%)	0(0%)	1(0%)	0(0%)	7(0%)
Negative RT-PCR	SARI	193(0.1%)	2307(0.7%)	1089(0.3%)	1252(0.4%)	936(0.3%)	620(0.2%)	397(0.1%)	233(0.1%)	94(0%)	8(0%)	7129(2.1%)
	ILI	289(0.1%)	2051(0.6%)	961(0.3%)	1055(0.3%)	1021(0.3%)	733(0.2%)	485(0.1%)	272(0.1%)	95(0%)	10(0%)	6972(2%)
	Seeking hospitalization	1253(0.4%)	8203(2.4%)	18518(5.4%)	9030(2.6%)	6469(1.9%)	5694(1.7%)	4274(1.2%)	1360(0.4%)	219(0.1%)	26(0%)	55046(16.1%)
	Contacts	5974(1.7%)	44604(13%)	77277(22.6%)	40734(11.9%)	25450(7.4%)	15278(4.5%)	10047(2.9%)	2876(0.8%)	564(0.2%)	87(0%)	222891(65.1%)
	Total	7709(2.3%)	57165(16.7%)	97845(28.6%)	52071(15.2%)	33876(9.9%)	22325(6.5%)	15203(4.4%)	4741(1.4%)	972(0.3%)	131(0%)	292038(85.3%)
Positive RT-PCR	SARI	1(0%)	21(0%)	29(0%)	26(0%)	20(0%)	17(0%)	12(0%)	7(0%)	2(0%)	1(0%)	136(0%)
	ILI	2(0%)	28(0%)	39(0%)	24(0%)	22(0%)	19(0%)	10(0%)	9(0%)	3(0%)	0(0%)	156(0%)
	Seeking hospitalization	29(0%)	104(0%)	276(0.1%)	211(0.1%)	139(0%)	71(0%)	78(0%)	46(0%)	7(0%)	1(0%)	962(0.3%)
	Contacts	34(0%)	204(0.1%)	732(0.2%)	614(0.2%)	446(0.1%)	366(0.1%)	231(0.1%)	77(0%)	17(0%)	3(0%)	2724(0.8%)
	Total	66(0%)	357(0.1%)	1076(0.3%)	875(0.3%)	627(0.2%)	473(0.1%)	331(0.1%)	139(0%)	29(0%)	5(0%)	3978(1.2%)
Repeat sampling required	SARI	2(0%)	39(0%)	48(0%)	29(0%)	21(0%)	20(0%)	11(0%)	4(0%)	2(0%)	1(0%)	177(0.1%)
	ILI	4(0%)	58(0%)	71(0%)	35(0%)	21(0%)	19(0%)	9(0%)	6(0%)	2(0%)	0(0%)	225(0.1%)
	Seeking hospitalization	59(0%)	281(0.1%)	587(0.2%)	362(0.1%)	194(0.1%)	99(0%)	70(0%)	30(0%)	6(0%)	1(0%)	1689(0.5%)
	Contacts	69(0%)	585(0.2%)	1401(0.4%)	832(0.2%)	544(0.2%)	430(0.1%)	207(0.1%)	52(0%)	14(0%)	3(0%)	4137(1.2%)
	Total	134(0%)	963(0.3%)	2107(0.6%)	1258(0.4%)	780(0.2%)	568(0.2%)	297(0.1%)	92(0%)	24(0%)	5(0%)	6228(1.8%)