Original Research Article

Detection of SARs CoV-2 during initial phase of pandemic from population of banas kantha district of Gujarat-India with special emphasis on pooled samples

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ABSTRACT

Corona virus (SARs CoV-2) has caused immense effect on morbidity and mortality of the population globally. We undertook this study as we are a part of one of the network laboratories of ICMR to test the patient's sample by RT PCR for the ORF 1 ab gene of corona virus.

Materials and Methods: For a period of one and half months (14th April to 31st May 2020) we tested the nasopharynx and oro-pharynx swab samples sent to us in VTM from the assigned districts of Gujarat. All the samples were subjected to RT PCR method by following standard methods.

Results: Total of 9.04%(256/2833) population was positive and 4.73%(139/2833) belonged to age groups 21-40 and 2.33% (66/2833) to 41-60yrs. Above the age of 60yrs there were only 0.95% (22/2833) cases which were positive. It was advantageous to pool the samples. Out of the number of pools prepared, we reported around 80% negative and rest were positive in pools. The study also included association of viral load and infectivity. We found that 12% of the asymptomatic people and 5.1% of symptomatic individuals had high viral load.

Conclusion: It is seen that the incidence of Novel corona virus -19 detection by RT PCR is a reliable method and the establishment of the Ct value and infectivity of the patient to the health care workers and relatives needs to be taken care of. Also, the study presents asymptomatic patients having high viral loads being highly infective.

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1. Introduction

The current pandemic of upper respiratory infection and pneumonia due to Corona virus (SARS CoV-19) has caused morbidity and mortality worldwide.[1] There is well-aware fact that this respiratory disease began in china and has spread globally.[2]

The coronaviruses were recognized as independent family in 1968 and infection resembled that of the chicken respiratory virus and mouse hepatitis virus. Electron microscopic structure showed a spherical particle surrounded by a halo (Latin: Corona) of surface projections. They have diameter of 80-200 nm & surface is covered with bulbous projection 20nm long and 10nm wide at their distal end[3] Corona virus invade the respiratory tract via the nose with an incubation period in 3 to 5 days & symptoms of common cold, nasal obstruction, sneezing, and running nose and occasionally cough. There are different methods for the diagnosis of corona virus infection like viral culture, electron microscopy detection, serology includes laboratory diagnosis by collecting paired sera, (acute & convalescent phase of disease) and to test by ELISA for the rise in antibodies.[4]

The Indian Council of Medical Research (ICMR) has been leading India's laboratory surveillance testing for COVID-19. As a part of the network laboratories of ICMR,

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The Microbiology Laboratory of the Gujarat Cancer and Research Institute, Ahmedabad was appointed as a nodal laboratory for testing the samples of the symptomatic patients and non-symptomatic population. Thus, we take this opportunity to generate the results of COVID-19 infection of the different categories of population under study and assess the infectivity of the virus based on the cycle threshold value (Ct value).

2. Materials and Methods

The Microbiology Laboratory (NABL accredited) of The Gujarat Cancer and Research Institute (GCRI), Ahmedabad applied formally for the grant of permission to start the testing of COVID-19 from the Indian Council of Medical Research, New Delhi. After getting permission, the protocol sheets were prepared for extraction and amplification $^{(protocol sheet)}$. The Research and Technical staff of the GCRI were given formal training on RT PCR testing method. The SOP for laboratory safety guidelines as per ICMR were prepared and the staff was trained in using PPEs and other safety precautions. The study and data accumulation were carried out with the approval from the institutional review board/Committee (IRB/C).

Population under study

As per Government of Gujarat directives the different districts were assigned to us for receiving nasopharyngeal / oropharyngeal samples. The districts allotted to us were Anand, Kheda, Mehsana, Sabarkantha, Patan and Banaskantha. The population were symptomatic suspected cases, asymptomatic groups having high-risk contacts or high-risk health care workers and also asymptomatic population from green zone, as per the categories defined by ICMR (7 categories)

Method: After having received the samples in viral transport media (VTM), the integrity of the samples was checked, they were double checked with the patient's details with the sample referral form (SRF). The observation of any mismatch or leak of the media lead to sample rejection. The laboratory testing processes started with giving proper Lab ID numbers and sampling from the VTM was performed as described by ICMR protocol such that adequate RNA is fetched from the media. For the extraction of the RNA we used kits from Hi-Media and for amplification we used BGI kits respectively from ICMR depot situated at NIOH, Ahmedabad. The BGI kit detects the ORF1 ab gene of COVID-19 by RT PCR. The protocols and test procedures for both was prepared and was readily made available to the staff involved. The method given in the kit literature was followed to perform the test.

Initially the samples tested were individual. Later they were pooled into 8, 10 & 16 which were received from the green zones in successive batches. The design of the spread sheet for sampling, extraction and amplification is illustrated in Protocol sheet.

2.1. Pooled Sampling

ICMR: Recommendations for sample pooling for real-time RT-PCR screening for COVID-19 are as follows: Advised to use only in areas with low prevalence of COVID-19.[5]

DMER-Gandhinagar: Laboratory test and pooling.[6]

Throat/nasal swabs to be collected for RT-PCR tests Samples should be tested in a onetime pool of 25. Results of this sample pooling is only for surveillance purposes. It should not be used for diagnosis of individual patients.

2.2. Method followed by laboratory for pooling

1. Label Each VTM Tube with Sample ID
2. Arrange them as per label in tube stand in a set of 8 or 10 or 16 separately at different occasions labelled in serial identification numbers
3. Arrange such rows in tube racks/stand. It depends on how many samples you are taking at a time. Accordingly, arrange 2 ml MCTs
4. Take 200μl of sample from each VTM tube in falcon tube. The total volume will be as per the numbers of pools. From this total aspirate 200 ul in 2 ml MCT containing HRL buffer and carrier RNA.
5. Follow rest of the steps of RNA extraction as per kit instructions.
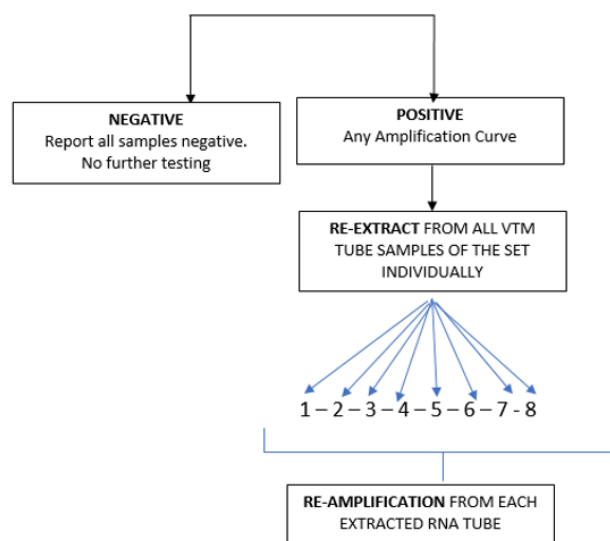6. For each pooled extracted RNA, perform amplification by RT-PCR.

Fig. 1: Flow Chart for Pool of 8 samples

3. Results

The testing was performed from 14th April onwards. The result analysis is taken for a period of one and half months i.e. from 14th April to 31st May 2020. In all 9.04%

(256/2833) were positive and 89.27%(2529/2833) were negative. 1.69% (48/2833) were rejected due to sample and form irregularities. In the age group 21-40 yrs. there was 4.73%(134/2833) positive cases followed by 2.33% (66/2833) in 41-60 yrs. COVID -19 positive was 0.95% (27/2833) in patients who were more than 60yrs.(Table **??**)

Of the total samples tested (the ICMR data was available), 33.39% (n=1692) patients were asymptomatic direct and high-risk contact of confirmed case - family member (cat.5a), and 3.37% (57/1692) were positive for corona virus. Symptomatic contact of lab confirmed case (cat.2) were 6.03% and 2.25% were positive, 2.78% belonged to symptomatic travellers in last 14 days (cat.1) and 0.06% were positive. Of the hospitalized SARI patients (cat.1) 0.77% were positive and rest were negative. Amongst the others category (48.52%) there were 4.31% (73/1692) patients were positive for corona virus infection. (Table 2)

The purpose of pooled samples is to increase capacity of the laboratories to screen increased numbers of samples using molecular testing for COVID-19 for the purpose of surveillance. Hence, it may help to use the pooled samples for screening. A pooled testing algorithm involves the PCR screening of a specimen pool comprising multiple individual patient specimens, followed by individual testing (pool deconvolution) only if a pool screens positive. As all individual samples in a negative pool are regarded as negative, it results in substantial cost savings when a large proportion of pools tests negative.

4. Result of pooled samples

On an average we performed pooling of samples in numbers of 8, 10 & 16. There were 48 pools of 8, in which 14 pools were positive and 34 were negative. In the deconvolution of these pools, 14 pooled samples (112/384 individual samples) were further subjected to re-extraction and re-amplification. There was 11% positive and 89% were negative. Next, there were 31 pools of 10, in which only 2 pools were positive and 29 were negative. Out of these 2 pools (20/310 samples) 0.64% (2 samples) were positive while deconvoluting 99.35% were negative. Similarly, 32 pools of 16 samples showed 7.42% positive and 92.58% were negative.

4.1. Importance of C_t Value

The C_t (cycle threshold) is the number of cycles required for the fluorescent signal to cross the threshold (i.e. exceeds background level). C_t levels are inversely proportional to the amount of target nucleic acid in the sample (i.e. the lower the C_t level the greater the amount of target nucleic acid in the reaction (Figure 3).

The viral load is classified based on the C_t (cycle threshold) value of the amplification of the Coronavirus

RNA in the reaction of RT PCR. As per Figure 3 the categories are 17-24 Ct as high viral load, 24-31 as moderate viral load and 31-38 as low viral load. Of the 175 selected positive person's samples, 12% of the asymptomatic people had higher viral load, 18.9% had moderate viral load and 37.7% had low viral load. Whereas in symptomatic patients 5.1% had high viral load, 10.9 % had moderate and 14.9% had low viral load. In total 17.1% had high viral load and 29.7 had moderate viral load and 52.6% were having low viral load.

GCRI-Microbiology created protocol sheet for sampling, extraction and amplification

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* Created by D.Parijat N.Goswami

5. Discussion

Coronavirus disease 2019 (covid-19) is cause by several acute respiratory syndrome corona virus -2 (SARS-Cov-2) was first reported in Wuhan, Hubei Province, China in late December2019.

During the current corona virus pandemic, the testing done by RT-PCR for the Orf gene detection for a period of one and half month showed 9.04 % case were positive. The study conducted by Wen-Hua Kong.[7] The states of India Showing highest positivity were 10.6% in Maharashtra, 7.8% in Delhi. Madhya Pradesh 6.1% & west Bangor 5.8% as per the report of given by ICMR Covid study group.

Table 1: Age group and detectionof Covid-19 RNA by RT PCR

Age Group	Positive		Negative		Reject sample		Total	
	Total	%	Total	%	Total	%	Total	%
0 - 1	2	0.07	16	0.56	1	0.035	19	0.67
01-14	10	0.35	133	4.69	4	0.141	147	5.19
15-20	17	0.60	223	7.87	10	0.353	250	8.82
21-40	134	4.73	1419	50.09	26	0.918	1579	55.74
41-60	66	2.33	604	21.32	5	0.176	675	23.83
>60	27	0.95	134	4.73	2	0.071	163	5.75
TOTAL	256	9.04	2529	89.27	48	1.694	2833	100.00

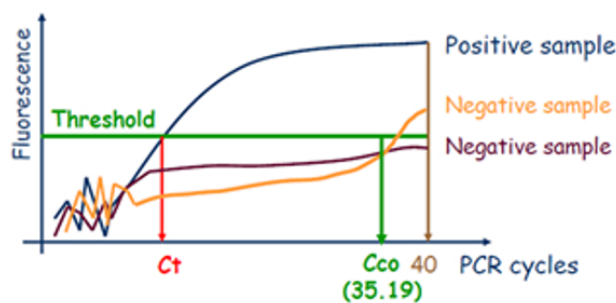**Table 2:** Profile of individuals tested for COVID-19 from 26th April to 31st May 2020.

Category	Grand Total		Positive		Negative		Sample Rejected	
	Total	%	Total	%	Total	%	Total	%
Cat 1: Symptomatic travellers in last 14 days	47	2.78	1	0.06	46	2.72	0	0
Cat 2: Symptomatic contact of lab confirmed case	102	6.03	38	2.25	64	3.78	0	0
Cat 3: Symptomatic Healthcare worker / Frontline workers	31	1.83	3	0.18	28	1.65	0	0
Cat 4: Hospitalized SARI patient	59	3.49	13	0.77	46	2.72	0	0
Cat 5a: Asymptomatic direct and high-risk contact of confirmed case - family member	565	33.39	57	3.37	503	29.73	5	0.3
Cat 5b: Asymptomatic healthcare worker in contact with confirmed case without adequate protection	28	1.65	1	0.06	27	1.6	0	0
Cat 6: Symptomatic Influenza Like Illness (ILI) patient in Hospital	27	1.6	6	0.35	21	1.24	0	0
Cat 7: Pregnant woman in/near labour	12	0.71	4	0.24	8	0.47	0	0
Others	821	48.52	73	4.31	748	44.21	0	0
Total	1692	100	196	11.58	1491	88.12	5	0.3
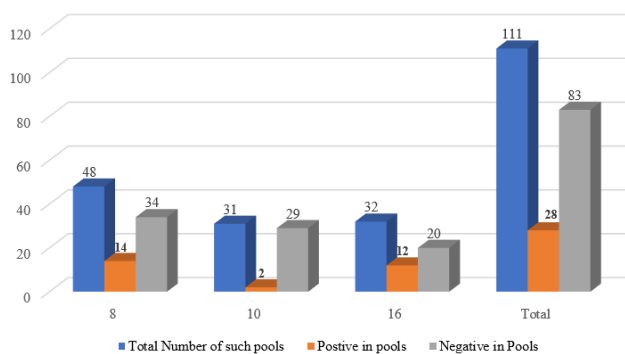
Fig. 2: Result of pooled sample

Fig. 3: Significance of Cycle threshold (C$_t$) value.

Contrary to the reports published ae found highest detection of corona virus RNA in age groups 21-40 and41-60 years. (4.73% & 2.33%) respectively and it was less (0.95%) in >60 years of age.

Many cases whose samples were collected(33.3%) belonged to asymptomatic high risk contact of confirmed family member and the detection of corona RNA was 3.73%

Which is similar to the report published by ICMR study group for reasons unknown , The detection of the virus was less (2.25%) in symptomatic contact of laboratory confirm case & symptomatic travellers (2.78%).

To avoid dealing with the large number of sample load receiving from districts for Covid-19 testing we pooled the samples. Pooling the samples lessened the burden of work with limited staff and economising on the reagents. This has been beneficial in samples received from green zone or areas

Ct value and Grade of Viral Load-of 196 positive samples
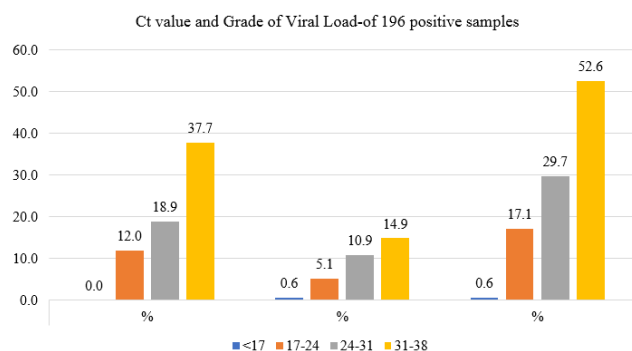
Fig. 4: RT - PCR C_t Value and its probable association with patients having Corona Virus infection.

where the incidence of infection is <5%. We found 11%, 0.64% and 7% detection of Covid-19 from 8, 10 and 16 pooled samples. During the literature search we could not find any such results with other studies if at all done for comparison.

In a press release in The Time of India 3rd June 2020, the Ahmedabad based ICMR- National Institute of Occupational health (NIOH), studies 2000 individuals of which 140 were COVID- 19 positive (7%). They concluded infected patients do not transmit the virus uniformly. They observed that infected cases bearing high viral load spread the infection at a rate almost eight time higher than cases with low viral load. In their study of the 140 positive cases, those with high viral load were limited to only 7% of the infected population, and 9% had moderate viral load and 84% had low viral load. Unlike their report, we found 17.1% patients with high viral load, 29.7% with moderate viral load and 52.6% having low viral load. Further in addition to the above, we found that the high viral load is more (12%) in asymptomatic than when compared with symptomatic patients (5.1%). In another study in China showed that probably only 10% of the population with high viral load spread 80% of the infection.

6. Conclusion

The current Corona virus disease pandemic caused by SARS-CoV2 viruses reported first from China in December 2019 and has already engulfed the world. A high number of COVID-19 test confirmed cases account for 84% in 10 states of India. Gujarat reported more than 20,000 confirmed cases. Our study showed around 9% confirmed cases. Based on the Ct value, the high and moderate viral load patients should be isolated and hospitalized on priority. Those with low viral load should take all precautions for containing the disease. Moreover, health care professionals at COVID-19 hospitals are exposed to patients with high viral load and are prone to get infected. Therefore, they should take necessary precautions to protect themselves.

8. Conflict of Interest

The authors declare that there are no conflicts of interest in this paper.

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