Original Article

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Performance and Cost-effectiveness of Pooling COVID-19 Samples Using Real time Polymerase Chain Reaction Test

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ABSTRACT

Background: Rapid detection of Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) by real-time polymerase chain reaction (RT-PCR) is the most reliable method used worldwide. Although the incidence of the disease has increased globally, the limited availability of PCR kits has become the major bottleneck for the diagnosis of COVID positive patients.

Methods: Random samples were pooled for two months in group of two-five and tested for SARS-CoV-2. If the pool was negative, all individuals in the pool were reported negative. If the pool was positive, then the individual samples were retested to identify the positive individual.

Results: The mean cycle threshold (Ct) value of pooled samples was not significantly different with that of individual samples for N, ORF-1ab and E genes. Also, pooling saved more than 60% of reagents, time and effort, workforce and cost.

Conclusions: In this study, the positivity rate was around 5% and saving of reagent, cost, time and manpower was more than 60%.

Keywords: RT-PCR; Sample Pooling; SARS-CoV-2

INTRODUCTION

Rapid detection of Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2), previously known as the 2019- novel Coronavirus (2019 nCoV), that causes COVID-19 (Coronavirus disease 2019), by real-time polymerase chain reaction (RT-PCR) is the surveillance tool in containing the pandemic. Although the incidence of the disease has increased globally, the limited availability of PCR kits has become the major bottleneck for the diagnosis of the COVID positive patients .[1]This could result in delay in identification of the infection and reduce the effectiveness of the measures taken to restrain the disease.

Solution to this is pooling of specimens. One of the ways of pooling is sample pooling. Here the nasopharyngeal/ oropharyngeal samples are pooled before ribonucleic acid (RNA) extraction and then the pools are tested. If the pool resulted positive for the virus, only then retesting of the individual specimen is done from the pool to identify the positive sample.[2]

Sample pooling strategy can be applied when the positivity rate is less than 10% and save substantial PCR reagent, personnel time,[3,4] cost[5] and thus make maximum use of the limited resources. In our case, the positivity rate was around 5% and we pooled samples with pool size of 2-5.

This study aimed to study the effectiveness of sample pooling for detection of SARS-CoV-2 in terms of saving cost, reagents, workforce, time and effort.

METHODS

The data of July and August 2020, when the samples of suspected COVID-19 individuals were pooled and tested by using two test kits, Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) (Sansure Biotech Inc, Hunan Province, People Republic of China) and Light Mix Modular kits with Lyophilized 1-step RT-PCR Polymerase Mix (TibMolbiol Syntheselabor GmbH, Berlin Germany) at Molecular laboratory, Patan Hospital, Patan Academy of Health Sciences, were analyzed. The samples that were not pooled were excluded from the study.

Sansure kit detects Open Reading Frame-1ab (ORF-1ab)

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gene and Nucleoprotein (N) gene, both specific for SARS-CoV-2. TibMolbiol kit detects Envelope (E) gene and RNA Dependent RNA Polymerase (RdRp) gene. E gene is common to both severe acute respiratory syndrome (SARS) and 2019 nCoV whereas RdRp gene is specific for only 2019 nCoV. The cutoff cycle threshold (Ct) value for ORF-1ab gene and N gene is 40 and whereas the Ct value for E gene is 36 while that of RdRp gene is 40, according to the manufacturers' instructions.

The RT-PCR machine used was BIO RAD CFX96 Real-Time System and the cycle was extended to 45 in order to detect the low viral loads as well.[6]

First, random samples were pooled in the group of two to five and the pooled samples were then tested for SARS-CoV-2. For this, 200 ul of individual sample was taken and mixed together and from this homogeneous solution 200 ul was then extracted for PCR. For examples, for pooling 5 samples, we mixed 200 ul of individual sample together forming a total volume of 1000 ul. This 1000ul solution was vortexed properly and then from this homogeneous solution 200 ul was extracted for PCR. However, most of the samples were pooled in groups of three with respect to the convenience of our laboratory. If the pooled sample had Ct values below the cutoff, it was interpreted as positive and only then the individual samples in the pool were retested to identify the positive sample. The pooled sample that had Ct value greater than the cutoff was declared negative and no further testing was done. So, all the individuals in the negative pool were provided with negative test results. Statistical tests were applied only for the positive pooled samples. Chi square test for homogeneity of proportion was used to compare the homogeneity between the two genes of each test kit. The t-test was used to compare the means of Ct values between the positive pooled samples and their individual positive samples of both the PCR kits and between individual samples of Tim Molbiol Kit.

Net saving of reagents, time, effort, manpower and cost was calculated as difference between saving in negative pools and expending for individual positive samples in positive pool.

Ethical approval was taken from Internal Research Committee (IRC) of Patan Academy of Health Sciences (bss2105071521). Confidentiality of the data is maintained with high privacy by the researcher in password-protected electronic devices in the department and used solely for the research purpose only.

RESULTS

For Sansure kit, there were 20 positive pools with 3 individual samples in each pool. However, out of those 60 individual samples, only 38 samples were positive for both the genes. In one sample, N gene was positive with Ct value 37.37 while the ORF-1abgene was negative. However, it was reported positive with respect to the N gene. For Tim Molbiol kit, there were 13 positive pools with 3 individual samples in each pool. Out of those 39 individual samples, 28 samples were positive for both the genes. The RdRp value for one sample was not available as the test was done with a different kit due to some technical reasons, the result however was positive for COVID-19. Chi-square test showed that the two genes in both the kits were not significantly different in detecting the positive samples as shown in Figure 1.

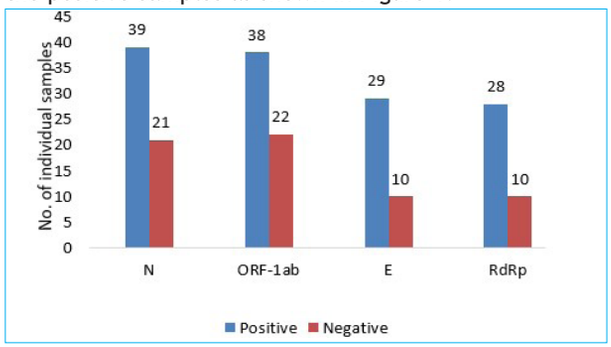

Figure 1. The number of positive and negative outcomes according to the gene type of the two kits.

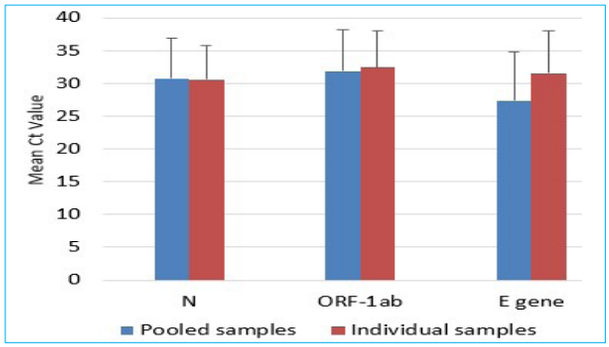

Figure 2. The mean Ct values of the mentioned genes for the pooled and individual samples.

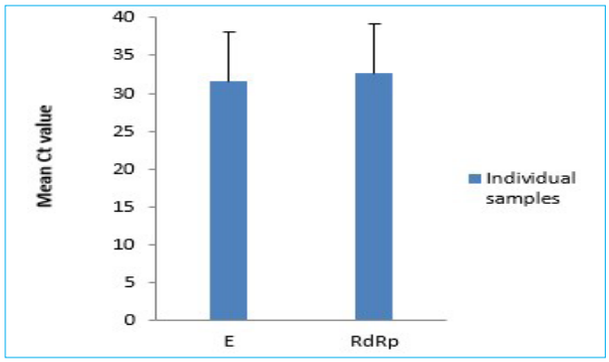

Figure 3. The mean Ct values of E and RdRp genes for individual samples.

The mean Ct values of pooled samples were not significantly different with the mean Ct value of their individual samples for N, ORF-1ab and E genes (Figure 2). No samples were pooled for RdRp gene as the gene is used to confirm COVID-19 positive individual samples. After a pool becomes positive for E gene, individual samples were retested for both E and RdRp genes. Although, the E gene is the screening gene but mean Ct value of E gene for individual samples was not significantly different from the mean Ct value of RdRp gene for individual samples (Figure 3).

In the month of July and August, 68% of reagents, both for extraction and RT-PCR, was saved in negative pools whereas 25% of the reagents was consumed more in positive pools and therefore the net saving was 64% (Table 1).

Similarly, time and effort, cost and manpower savings were 63% (Table 2,3 and 4).

Table 1. Saving of Reagents.

Saving in Negative Pool Only

Month	Total Individual sample	No.of pooled sample	Reagent demand for Individual sample	Reagent used	Reagent saving	Saving %
July	1946	617	1946	617	1329	68
August	556	184	556	184	372	67
Total	2502	801	2502	801	1701	68

Consumption in Positive Pool Only

Month	Total Individual sample	No.of pooled sample	Reagent demand for Individual sample	Reagent used	Reagent consumed (additional)	Additional Consumption %
July	30	10	30	40	10	25
August	69	23	69	92	23	25
Total	99	33	99	132	33	25

Net Saving

Month	Total Individual sample	Net saving	% Saving
July	1976	1319	67
August	625	349	56
Total	2601	1668	64

Table 2. Saving of Time.

Month	No.of Negative pool	No. of Individual negative sample	No. of Positive pool	No. of Individual positive sample	Total batch of Individual negative sample	Total hrs saved from Individual negative sample	Total sample processed	Total batch of sample processed	Time consumed (hrs)	Net time saved (hrs)	Time saved (%)
July	617	1946	10	30	20.70	62.11	657	6.99	20.97	41.14	66
August	184	556	23	69	5.91	17.74	276	2.94	8.81	8.94	50
Total						79.85				50.07	63

Note: 1 batch =94 samples, Minimum time required for 1 batch =3 hrs

Table 3. Saving of Cost.

Month	No.of Negative pool	No. of Individual negative sample	No. of Positive pool	No. of Individual positive sample	Total cost (NRs) saved from Individual negative sample	Total sample processed	Cost for processed samples(NRs)	Net cost saved (NRs)	Cost saved (%)
July	617	1946	10	30	8562400.0	657	2890800.00	5671600.0	66
August	184	556	23	69	2446400.0	276	1214400.00	1232000.0	50
Total					11008800.			6903600.0	63

Note: 1RT-PCR test report =NRs 4,400/-

Table 4. Saving of Manpower.

Month	No. of Individual negative sample	No. of batch	No. of manpower demand	No.of Negative pool	No. of batch	No. of manpower used	No. of Individual positive sample	No. of Positive Pool	No. of batch	No. of manpower used for pool	Actual Manpower saving	Manpower saved(%)
July	1946	20.70	62.11	617	6.56	19.69	30	10	0.43	1.28	41.14	66
August	556	5.91	17.74	184	1.96	5.87	69	23	0.98	2.94	8.94	50
Total			79.85			25.56				4.21	50.07	63

Note: 1 batch =94 samples, Minimum manpower required for 1 batch =3

DISCUSSION

This study was done when the positivity rate was around 5% and the resources saved were more than 60%. Studies have shown that at a very low positivity rate (1%), the pool size could be up to 50 with saving of 58% of reagents. Even at moderately high positivity rate of 10%, reagents saving could reach around 40% with the pool size of 3.[7]

In this study, most of the positive pools contained both lower (≤ 35) and higher Ct value samples or samples with higher and lower viral loads respectively. The means of Ct values in pooled samples and individual samples were not significantly different in this study which was similar to a study done in Thailand.[8] However, in a study done in India, the mean of pooled Ct values was higher than the mean of individual samples.[4]

In a study done in Germany, it was suggested that 30 samples in a pool can be detected with sufficient accuracy which would also increase the test capacity with the available equipment and reagents. However, their concern was with the borderline positive which could escape detection in such a large pool due to the dilution effect. Nevertheless, it was also found that such borderline positives were in their convalescent stage. [9] In our study, we pooled two-five samples only and therefore even when all the individual samples in the pool had Ct values greater than 36, the pool was still positive. Even in the case of only one positive sample with a Ct value higher than 37 in the pool, the pool of three samples was still positive and the Ct values between the pooled and individual samples were comparable (37.89 vs 37.44 respectively). This was similar to a study[3] where in a pool of five samples with only one positive sample with Ct value greater than 37, the pool was positive. Thus, the effect of sample dilution was less when the pool size was small. One exception however was where the only positive sample in the pool with Ct value 34.63 for ORF-1ab had a very high pool Ct value of 40.71. However, for the same sample, Ct value of N gene between the pooled and individual samples was similar. However, in other studies, Ct value discrepancy was absent only when the individual sample in the pool had Ct value lesser than 35. When the Ct values of individual samples were greater than 35, discrepancies were observed between Ct values of pooled and individual samples. [8,10] This could be due to the fact that in our case, fresh samples were pooled first and only if the pool tested positive, individual samples were retested but in other studies known positive samples which were stored for future were used. Thus, the effect of dilution could be less when samples were processed immediately. Therefore, specimen pooling did not affect the sensitivity of detecting SARS-CoV-2 even when only one sample with lower viral load was present (Ct value around 37), accuracy was also maintained as the means of Ct values were also similar

There was no discrepancy between N gene and ORF-1ab (Sansure Kit) in detecting positive samples when the Ct value of individual sample was less than 37.44 but inconsistencies were observed when the Ct values of the individual samples were greater than38.5±1.1. The N gene was detected in slightly more positive samples than the ORF-1ab gene (39/60 vs 38/60 respectively), although the difference was not statistically significant. However, studies done in China using the commercially available RT-PCR kits, it was shown that the most conserved region ORF-1ab gene had lower sensitivity compared to other genes like N which was less conserved but more sensitive.[11,12]

Detection of number of positive samples by both E and RdRp gene (Tib Molbiol Kit) was equal, and the Ct value of E gene was lesser than the Ct value of RdRp gene, when compared for individual samples. However, the difference in mean Ct value of individual samples between the two genes was not statistically significant. In a study done at Sheffield Teaching Hospitals National Health Service (NHS) Foundation Trust (UK), it was found that E gene was significantly better than RdRp gene. [13]

By pooling 2601 samples in the month of July and August 2020, we could save and thus extend our testing to 1668 more samples. In a sample pooling study in Nebraska Medical Center, the reagents were saved by 63% which is

very close to that found in this study.[3] In another study, saving of reagents was 47.3%, tests cost saving was 43.4%, and labor cost saving was 23.7%.[5] In a study done in India[4] RT-PCR reagents and personnel time saving with sample pooling was similar to that found in our study.

The limitation of the study was that sample pooling was done only for two months and so the sample size was small.

CONCLUSIONS

In this study, the positivity rate was around 5% and therefore, we could save more than 60% of cost, time and manpower with the pool size of 2-5.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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