Effectiveness of the pool testing of COVID-19 suspects in a resource limited country

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ABSTRACT

BACKGROUND
The novel coronavirus (CoV) has resulted in a global pandemic despite drastic measures to avoid contagious spread. A reliable and accessible diagnosis of active SARS-CoV-2 infection is the foremost priority to facilitate efficient public health interventions. India being a resource-limited country, various group testing strategies are being applied to cover a larger population with the aim to compare the laboratory results from pooled testing (5 samples) with individually tested samples using standard qPCR to ensure that detection rate is not compromised.

Material and Methods: This was a retrospective cross-sectional study done from 14th May to 15th June 2020 at COVID-19 testing laboratory at UPUMS, Saifai, Etawah. During the study duration, we screened 319 positive pools which correspond to 1595 of Nasopharyngeal (NS) and Oropharyngeal swabs (OS). Ct value of PCR of pools and individual samples were compared for effectiveness of pooling strategy.

RESULT
61 pools didn’t have any positive sample after deconvolution, 188 pools had only one positive sample and 70 pools had more than one positive sample. Mean Ct value for E gene of pools was 28.30 whereas mean Ct value for positive samples was 26.53.

CONCLUSION
India being a resource limited country with the second largest population; group testing i.e., pooling the samples for higher productivity seems to be the crucial step to curb the COVID-19 pandemic.

Keywords: Coronavirus disease 2019, pool testing, real-time polymerase chain reaction, severe acute respiratory syndrome coronavirus 2

INTRODUCTION
In December 2019, a new coronavirus emerged in Wuhan, China which caused a pandemic named as Coronavirus disease 19 (COVID-19) while the virus was called Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2). COVID-19 is characterised by flu like symptoms mainly fever, cough, fatigue, shortness of breath, pneumonia, and other respiratory tract symptoms, and in many cases progresses to death.¹ As of July 16, 2020, more than 14 million cases have been confirmed in the world with India being the third most affected country in the world after United States of America and Brazil.²
A reliable and accessible diagnosis of an active SARS-CoV-2 infection is the foremost priority to facilitate efficient public health interventions. The gold standard test for the diagnosis of COVID-19 involves three main steps, viral inactivation and lysis, extraction of viral RNA, and reverse transcription (RT)-PCR. Due to the rapid spread of the virus and the increasing demand for tests, the limited availability of test reagents has become a major bottleneck as the pandemic expands. \(^3\)

To combat this challenge, group testing i.e., pooling the samples together was advised by Indian Council of Medical Research (ICMR) based on a study conducted at Virus Research & Diagnostic Laboratory (VRDL) at King George's Medical University (KGMU), Lucknow. \(^4\) They demonstrated that performing real-time PCR (qPCR) for COVID-19 by pooling 5 samples is feasible. Deconvoluted testing was recommended if any of the pool tested positive and all the negative pools were to be considered as negative.

This study compares the laboratory results from pooled testing (5 samples) with individually tested samples using standard qPCR to ensure that detection rate is not compromised.

**MATERIALS AND METHODS**

This is a retrospective study done from 14\(^{th}\) May to 15\(^{th}\) June 2020 at COVID-19 testing laboratory of our institute. During the study duration, we screened 319 positive pools which corresponds to 1595 of Nasopharyngeal (NS) and Oropharyngeal swabs (OS).

Two samples both NS and OS were collected from each patient suspected of SARS-CoV-2 infection wearing Personal Protective Equipment (PPE) and both the swabs were put into the same Viral Transport Medium (VTM). This VTM was transported in a triple layer packing to the COVID-19 testing laboratory of our institution under cold chain.

After receiving samples in the laboratory, these were kept at -20°C until processed.

Pool testing: Pools were created using 200 \(\mu\)L of VTM from each sample to make a final volume of 1 mL. Nucleic acid (NA) extraction was performed on each pool using QIAamp\textsuperscript{TM} Viral RNA extraction kit (Qiagen, Germany). Real-time reverse transcription PCR for the detection of E gene was performed on the extracted NA using SuperScript\textsuperscript{TM} III Platinum\textsuperscript{TM} One-Step qRT-PCR kit following the manufacturer’s instructions.

Once the pool came positive for the E gene it was deconvoluted to perform further testing. We followed the guidelines given by ICMR to consider all the samples in the pool negative if the pool is negative for E gene.

Sample testing: All the pools which were flagged in the PCR were deconvoluted and NA extraction was performed using QIAamp\textsuperscript{TM} Viral RNA extraction kit (Qiagen, Germany). Real-time reverse transcription PCR for the detection of E gene was performed followed by detection of RdRp gene on the extracted NA using SuperScript\textsuperscript{TM} III Platinum\textsuperscript{TM} One-Step qRT-PCR kit (Invitrogen life technologies) following the manufacturer’s instructions. Sample was labelled as positive only when both the genes were detected.

The Ct (Cycle threshold) value of PCR of the pools and individual samples were compared to know the effectiveness of the pooling strategy.

**RESULT**

During the study period, screening of 319 positive pools was done, which corresponded to 1595 samples. Out of the total positive pools, 61 pools didn’t have any positive sample after deconvolution, 188 pools had only one positive sample and 70 pools had more than one positive sample. (Fig 1)

Among 319 positive pools which corresponds to 1595 samples only 419 samples were positive making it 26.6%. Fig 2 shows the pattern of Ct values of E gene between all the positive pools which reproduced only one positive sample (188) after deconvolution and the Ct value of the positive sample. The mean Ct value for E gene of the pool was 28.30 whereas the mean Ct value for the positive sample was 26.53.

Among the pools with >1 positive sample (70), 52 pools had two positive samples and 19 had three. The mean CT value for E-gene was 26.62 and 25.76 respectively. (Fig 3)

**DISCUSSION**

With the current increasing trend of COVID-19 in the world, widespread testing is needed to get this outbreak under control. But in many regions, there’s a shortage of the chemicals needed to run tests. In
several countries, health officials have started using a strategy that was first proposed in the Second World War: group testing. By testing samples from many people at once, this method can save time, chemical reagents and money.\(^5\) India is among a few countries that are using the group testing strategy for the early identification of COVID-19 cases and this study was aimed to know the effectiveness of pooling for screening the samples of suspected COVID-19 cases.

We followed the guidelines given by ICMR that is based on a study conducted at KGMU, Lucknow which stated that the PCR screening of a specimen pool comprising 5 individual patient specimens must be followed by individual testing (pool deconvolution) only if a pool screens positive and all individual samples in a negative pool are to be regarded as negative. This results in substantial cost savings when a large proportion of pools tests negative.\(^4\)

In this study we screened 319 positive pools and after deconvolution we found that 61 (19.12%) pools didn’t have any positive sample, 188 (58.93) pools had only one positive sample and 70 (20.05%) pools had more than one positive sample.

Fig 3 shows that as the number of positive samples in a pool increases the Ct value of the pool decreases. The mean Ct value for E gene was maximum (28.30) when the pool only had one positive sample. According to Wacharapluesadee S, et al. more positive specimens in a pool could decrease the sensitivity of qPCR as it would result in too many viral copies, which will cause an insufficiency of PCR enzymes and other reagents in the mix to amplify all the viral copies.\(^7\) However, this didn’t affect the overall testing results, since positive pools were deconvoluted and the samples were tested individually.

Among the 188 positive pools which reproduced only one positive sample the mean of the Ct value of the pool and the sample was 28.30 and 26.53 respectively. The difference in the Ct value being 1.77. This could be explained by the result of a study done in Japan which concluded that pooling of 5 samples decreases the viral loads from original value to 0.7 log10 thus increasing the Ct value by 2.3\(^5\) as the process of pooling multiple patient samples into one tube inevitably causes dilution of the RNA of each individual sample leading to higher Ct value.\(^6\)

In this study, we concluded that since the COVID-19 infectivity is growing exponentially in India and qRT-PCR is the gold standard for its diagnosis, testing and identifying the infected cases is the only way to control the infection. India being a resource limited country with the second largest population; group testing i.e., pooling the samples for higher productivity seems to be the crucial step to curb the COVID-19 pandemic.

REFERENCES
Fig 1: Distribution of positive pools

Fig 2: Difference between the Ct value of pool and sample
Fig 3: Difference in the mean Ct values of the pool