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SARS-CoV-2 Pooled Testing Methodology for PCR Testing Applied in Private Laboratory in Armenia

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Abstract

Since the beginning of COVID-19 pandemics many countries were facing challenges with testing capacity recourse limitations. Throughout the waves of the pandemic countries were trying to address the existing constrains exploring solutions to increase the testing capacity with more cost-effective approaches. Pooled methodology was one of the methods which many have validated and used. It is evident that in case of pooled sample testing the sensitivity becomes lower, however the variation highly depends on the pool size as well as the incidence rate at the certain point. Armenia as well as many other countries has adopted regulations for mandatory COVID-19 PCR testing for all the travelers. Current study aimed to explore the efficiency of COVID-19 pooled PCR testing for nasopharyngeal swabs of individuals with no symptoms in a time period with good epidemiological state of the infection. Nasopharingeal swab samples from individuals were collected. The manual extraction of RNAs of samples was performed after pooling up to 5 samples. The pools with Cycle Threshold (CT) of < 37 were considered positive and were retested individually. In total 28,015 samples were grouped in 667 pools of which 57 were positive. The total number of positive samples was 65. The median difference (CT-pool – CT samples) was 2.4 (ranging from –3.0 to 8.9). The correlation of CT of pools and positive samples was positive. The correlation coefficient r = 0.84, P < 0.000, 95% CI range 0.7423 to 0.9243). The total economic saving when using pools compared to the individual testing was 72%. The minor difference between CT values of pools and samples can be explained by the dilution effect in the pool. However, the positive correlation between the values as well as the amount of cost saving demonstrate that pooling on nasopharyngeal samples for COVID-19 PCR testing can be a good method for efficient screening with significant resource saving. One of the most important advantages of the proposed method is the fact that samples

are pooled prior extraction, which avoids the possibilities with misinterpretation of IC due to low yield of RNA in the extraction process.

Keywords

COVID-19, Screening, Grouped Testing

1. Background

In December 2019 severe acute respiratory syndrome (SARS-CoV-2) virus was detected in China [1]. Virus named as COVID-19 rapidly spread throughout the globe resulting to pandemics [1]. According to World Health Organization by September 2023 around 770 million cases of infection were identified which resulted to around 6.9 million cumulative deaths worldwide (https://covid19.who.int/) [2]. Since the beginning of COVID-19 pandemics many countries were facing challenges with testing capacity recourse limitations [3]. There was a severe need of finding solutions for performing testing of contacts, travelers as it was evident that even asymptomatic individuals can spread the infection [4] [5].

Throughout the waves of the pandemic countries were trying to address the existing constraints by exploring solutions to increase the testing capacity with more cost-effective approaches [3] [6]. Testing, contact tracing and isolation was a known strategy in many countries, however the testing capacity was limited as the reagents, PPE and other necessary supply chains suffered as a result of lock down as well as human and equipment recourses were limited [7] [8]. RT-PCR was developed for primary diagnosis of the infection [9], however even developed countries faced challenges in fulfilling the necessary reagent supplies [10].

Pooled testing is conducted by comprising multiple samples and testing them as a single sample followed by individual testing in case of positive result of the pool. This method allows to test much larger volume of samples in shorter time period with less resources [11] [12]. Pooled methodology was first described by Robert Dorfman in 1943, when screening patients with syphilis in US military forces [12]. The method began to be utilized wider in different infectious disease screenings such as HIV [13].

Since then, many scientists offered various methodologies for performing pooled testing [13] [14]. Robert Dorfman's pooling methodology is the simplest, where number of individual samples are grouped into the pool and if the pool tests positive it is being followed with separated testing [13]. Dorfman's pooling methodology is the most preferred type of pooling in clinical setting [13].

Pooled methodology was one of the methods which many have validated and used during pandemics [14] [15] [16] [17] [18]. It is evident that in case of pooled sample testing the sensitivity becomes lower, however the variation highly depends on the pool size as well as the incidence rate at the certain point [19] [20] [21]. Armenia as well as many other countries has adopted regulations

for mandatory COVID-19 PCR testing for all the travelers [22]. Current study aimed to explore the efficiency of COVID-19 pooled PCR testing for nasopharyngeal swabs of individuals with no symptoms in period with good epidemiological state of the country. To our knowledge it is the first study conducted in Armenia to explore the plausibility of utilization of pooled testing methodology for COVID-19 diagnostics tying to address limitations in number of qualified specialists, test kits.

2. Methodology

Before pooling the samples, we took into consideration the epidemiological situation in the country, based on the percentage of the positive samples in our laboratory and comparing them with the percentages that were published by the MOH of Armenia. Study was conducted from the period of January 4th to January 12th 2022, when the country had a good epidemiological state, however the demand for testing was high due to travel requirements. Only individuals without acute respiratory disease symptoms were included in the pool. Pooling of samples was carried out based on the reasons of getting tested: travel, work, health check.

Nasopharingeal swab samples from individuals were collected. The manual extraction of RNAs of samples was performed after pooling up to 5 samples. The pooled samples were then amplified using Real-Time PCR method. The kit was able to detect two Covid-19 specific genes: E-gene, N-gene. The study was carried out using the Ct values of the N-gene. The pools with Cycle Threshold (CT) of <37 were considered positive and were retested individually.

3. Results

In total 28 015 samples were grouped in 667 pools of which 57 were positive **Figure 1**. The total number of positive samples was 65.

The median difference (CT-pool – CT samples) was 2.4 (ranging from −3.0 to 8.9). The correlation of CT of pools and positive samples was positive (**Figure 2**). The correlation coefficient r = 0.84, P < 0.000, 95% CI range 0.7423 to 0.9243).

Considering the approximate time needed for extraction of 28,015 sample is

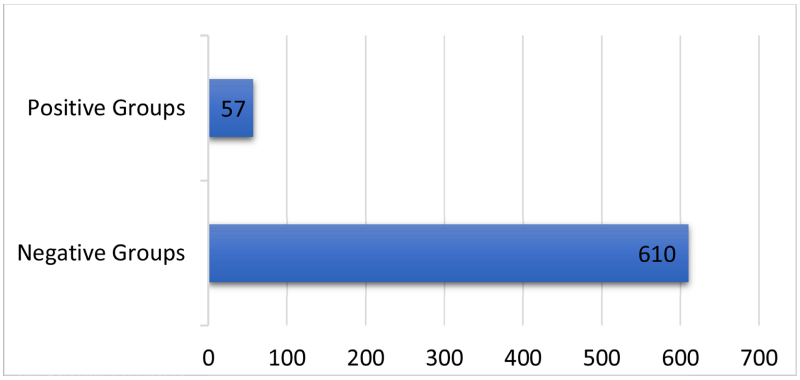
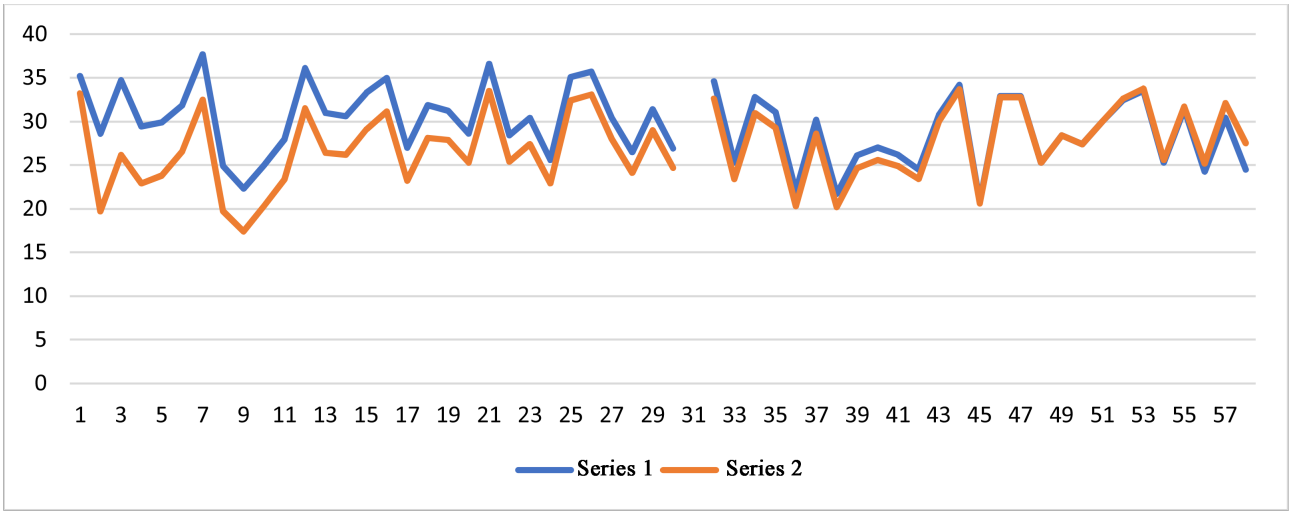


Figure 1. Positive and negative pool counts.



*The CT values of pools (Series 1) and positive samples (Series 2) and their differences can be seen in the figure below. The correlation between the CT of samples and CT of pools can be seen in the correlation figure.

Figure 2. The cycle threshold (CT) of pools and samples.

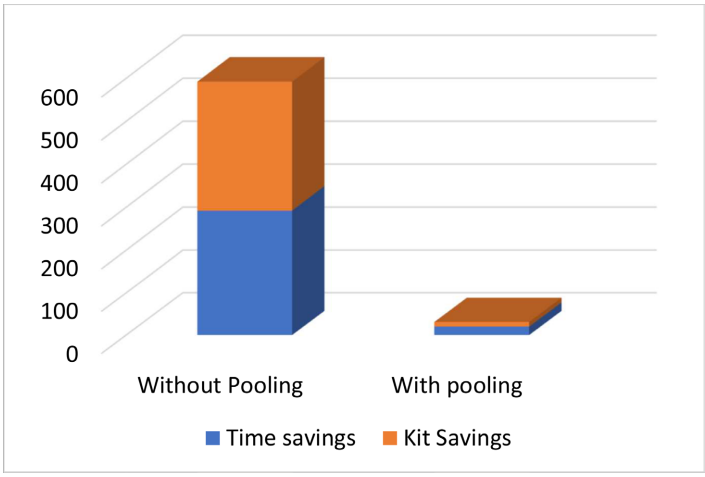


Figure 3. Time and Kit savings with pooling methodology.

300 hours and the extraction of 667 negative groups and 57 groups as individual is 20 hours. 300 kit is needed for the amplification of 28015 samples, whereas only 11 kit was needed for the amplification of 952 negative and positive pool samples. The total economic saving when using pools compared to the individual testing was 72%. The illustration of economic saving is presented in **Figure 3**.

4. Conclusion

The main value of the current study was the fact that it was performed first time in Armenia, and was able to replicate the same type of consistent and trustful results both in case of testing accuracy as well as recourse savings. The robust pooling methodology that we have provided in this paper demonstrated its value for accuracy and recourse saving. The national policy makers could consider implementing the experience from this study in a routine surveillance for

screening large groups of population. This study may allow policy makers to be more trustful towards pooled methodology for COVID-19 testing as pooled methods are shown to be successfully utilized in public health settings [17]. This methodology involves asymptomatic individuals only, however the results can be used for further development of risk based pooling approach. The optimal and accurate pooling method can be developed in national levels considering the resource limitations which the setting is facing. Before validation of any pooling methodology and implementing for wide use it is important to perform sensitivity calculation based on the existing models in literature [17] [18]. The minor difference between CT values of pools and samples can be explained by the dilution effect in the pool [3] [17] [18]. However, the positive correlation between the values as well as the amount of cost saving demonstrate that pooling on nasopharyngeal samples for COVID-19 PCR testing can be a good method for efficient screening with significant resource saving [10] [15]. One of the most important advantages of the proposed method is the fact that samples are pooled prior extraction, which avoids the possibilities with misinterpretation of IC due to low yield of RNA in the extraction process. Yet several studies are published using pooling methodologies for COVID-19 diagnostics, it is not used as a universal method. The main limitation of the current study is that the testing was conducted during epidemiologically convenient time period. Further studies may help to combine experience developed by different universities or research centers around the globe and apply pooled methodology as a widely used strategy for surveillance to test large volumes of individuals and save resources.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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