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Decision to Close a Factory in the COVID-19 Pandemic: Only One Hour of Work

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Abstract

Aim: Accurate and rapid screening with polymerase chain reaction (PCR) is important for epidemic management. In our study, samples taken at a local factory were evaluated to report cases of Coronavirus disease-2019 (COVID-19). In this study, we show the importance of the pooling method.

Materials and Methods: In this study, samples were taken from 840 people with suspected COVID-19 in a textile factory in Turkey. COVID-19 RT-qPCR Detection Kit (Bio-Speedy®) performing one-step reverse transcription (RT) and real-time PCR (qPCR) (RT-qPCR) targeting the RNA-dependent RNA polymerase (RdRp) gene Severe acute respiratory syndrome (SARS)-CoV-2 The specific region was used. Non-sigmoid curves and curves below the threshold level were considered negative. The result was interpreted as SARS-CoV-2 (2019-nCoV) positive if RdRp was positive and as SARS-CoV-2 (2019-nCoV) negative if RdRp was negative.

Results: Among the pooled samples, 20 (23%) were found to be positive, and when the samples were studied individually, 102 (12%) were positive. Since all the samples fit on a single test plate with the aid of pooling method, it was possible to work at once and the results were obtained 1 h after the samples arrived at the laboratory. Thus, the separation of positive and negative persons was carried out in 1 h by pooling and it was 12 times shorter.

Conclusion: In cases where large screening groups need a rapid diagnosis, pooling is thought to be beneficial in terms of preventive medicine and social and economic aspects.

Keywords: COVID-19, pandemic, polymerase chain reaction, pooling

Introduction

Coronavirus disease-2019 (COVID-19) is an infectious disease that appeared in December 2019 in Wuhan, China, which emerged as pneumonia of unknown etiology and caused a pandemic in a short time. The disease primarily affects the respiratory system, and non-specific symptoms such as fever, cough, myalgia, headache, hemoptysis, diarrhea, and dyspnea can also be seen. Coronaviruses (CoV) as a member of the Coronaviridae family can progress to different clinical situations, from flu to lower respiratory tract and lung infections, depending on the patient's immune system (1,2). Although lung computed tomography, high C-reactive protein level, and low leukocyte count are helpful in the diagnosis of the disease, they are not sufficient to distinguish it from other pneumonia forms (3). Also, the symptoms observed in the patients are nonspecific and other respiratory system infections may present with a similar clinical picture.

Molecular tests are the most widely used method to prove the presence of the virus, and many nucleic acid and antibody detection kits have been approved. Today, reverse transcriptase-polymerase chain reaction (RT-PCR) is the most frequently used method for detecting the agent in respiratory tract samples (4). RT-PCR tests have some limitations, test results can be obtained for a long time, and they are expensive and technically difficult.

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In addition, the risk of contamination is high and the process requires specialized equipment and experienced personnel. In addition to methodological handicaps, it is possible to obtain false-negative results depending on the characteristics of the agent and infection. The results are affected by the timing of the sample (being taken in the early or late period of the disease), sample quality, transportation conditions, and test technique (eg virus mutation, inhibition in the test) (5). When the first case is detected in crowded communities in social life and workplaces, studies should be carried out for RT-PCR in the form of rapid and collective screenings so that work and social life are not affected. However, when this sudden workload is far beyond the capacity of the laboratory, the diagnostic time becomes longer.

In cases of limitation, closure, and mass quarantine, determination of how much of the scanned community is infected is important in terms of limiting transmission. In the diagnostic process, the isolation rules and the increasing restrictions on both business and social life made it necessary to detect patients immediately. Samples can be examined one by one or by the pooling method, which is simple, practical, and valuable in that it uses minimal research resources (6).

In our study, we used samples taken for the decision to close a local factory with suspicion of disseminated disease. The results of the RT-PCR performed with the pooling method and the studies performed for each patient were compared later on, and we aimed to reveal the importance of the pooling method.

Materials and Methods

In this study, samples taken upon the detection of COVID-19 cases in a textile factory in Mardin city were evaluated. Samples were taken from 840 factory workers to close the factory due to the epidemic or to take the quarantine decision of the contacts. Before starting the study, the necessary permission was obtained from the Ethics Committee of the Ankara City Hospital no. 2 with the decision dated 02.03.2022 and numbered E2-22-2493. After evaluating the RT-PCR results performed using the pooling method in the first hour, the results were verified by working the samples one by one.

Samples were taken along with the same test strip, first as an oropharyngeal swab and then as a nasopharyngeal swab. After visualizing the posterior wall of the oropharynx, the tip of the test stick was rubbed against the posterior pharyngeal wall with a rolling motion without touching the tongue, base of the tongue, tonsils, or soft palate. After it was observed that the tip of the swab was wetted with secretion, the swab was then taken out without touching the surroundings, and the nasopharyngeal sampling phase was started. The test stick was passed through the nostril and advanced parallel to the palate from the inferior meatus under the inferior concha, and the tip of the test stick was wetted by secretion. The test rod was advanced to the distance from the nostrils to the level of the external auditory canal (8-10 cm) and it was ensured to reach the nasopharynx. The test strip was held in this area for a few seconds to absorb the secretions and then slowly removed. After the samples were taken, the swab was placed in the transport container and the excess part was broken off and discarded. The samples were placed in a VNAT (viral nucleic acid buffer) (Bioeksen, Turkey) solution and delivered to the microbiology laboratory without breaking the cold chain.

After the samples reached the microbiology laboratory, necessary sorting and recording procedures were performed. In the isolation room, 100 µL was withdrawn from each of the 10 samples and transferred to the same tube and this was accepted as a new sample. All 84 samples were studied in single cycle.

The samples were taken into class 2 biosafety cabinet. The COVID-19 RT-qPCR Detection Kit targeting the SARS-CoV-2-specific RdRp (RNA-dependent RNA polymerase) gene region was used for nucleic acid isolates obtained in the medium. The kit was run with the Biorad CFX96 system, and the detection limit for the RdRp gene was based on 3.8 copies-RNA/reaction. The number of thermal cycles was determined as 40. Non-sigmoid curves and subthreshold curves were considered negative. The RNase P gene in the kit was used as the internal control of the test. If the result was RdRp positive, it was interpreted as SARS-CoV-2 (2019-nCoV) positive, and if the result was RdRp negative, it was interpreted as SARS-CoV-2 (2019-nCoV) negative. If the target gene and internal control amplification were invalid, the test was repeated. After the results were obtained, the relevant authorities were notified and a preliminary report was prepared in terms of the factory's decision to continue to work. The final results were reported to the relevant administrative units.

Statistical Analysis

This study was designed retrospectively. Data were analyzed using the Statistical Package for Social Sciences (SPSS) 20.0 for Windows (SPSS Inc., Chicago, IL). The results of the samples studied one by one and the data obtained because of pooling method were evaluated with descriptive statistical methods.

Results

In this study, samples were taken from 840 people working in a textile factory for COVID-19 screening. The samples taken from 840 people were examined by pooling method and 20 (23%) of the pools were found to be positive. Later on samples were studied individually and 102 (12%) were positive. When the

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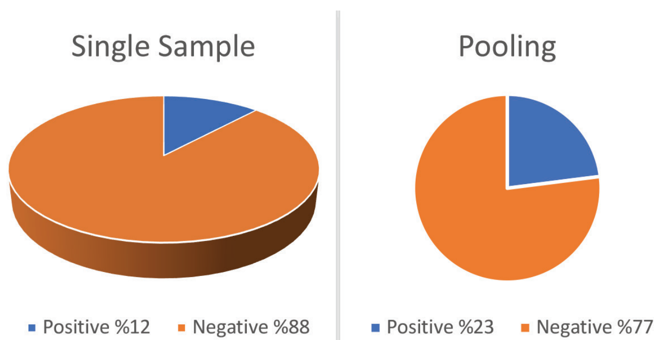
negative pools were opened and studied one by one, no positive samples were detected (Graphic 1).

When working with pooling method, the results were obtained 1 h after the samples reached the laboratory. All samples were studied in one run because they fit on one test plate. A preliminary report was prepared in 1 h for 840 samples. When the samples were run individually, 840 samples were run in 9 runs and the results were reported after approximately 12 h (Table 1).

Discussion

With the provisional guide published on March 2, 2020, the World Health Organization has determined an algorithm on how diagnostic tests can be applied in different transmission scenarios in the COVID-19 outbreak. Accordingly, in countries where the virus is rare, two steps are required to confirm the diagnosis of the first case. In the first stage, a positive result should be obtained with the NAAT test targeting at least two different regions in the virus genome, and then partial or whole genome sequencing of the virus should be performed. In countries where SARS-CoV-2 is common, screening with RT-PCR using a single distinctive target region has been recommended. However, one or more negative results will not exclude the possibility of infection (7).

Loeffelholz and Tang (8) (2020) evaluated the literature data and presented general information about the usage of COVID-19 tests. In the study, it was stated that although the nasopharyngeal swab usually the collection method, it may miss some cases. To prevent this, they suggest taking deeper samples, for example, by bronchoscopy. Alternatively, repeated testing may be used to increase the possibility of SARS-CoV-2 demonstration. It was emphasized in the study that various integrated, random access, point-of-care molecular devices have been developed for the rapid and accurate diagnosis of SARS-CoV-2 infection. It was suggested that these assays are simple, fast, and safe and can be used in local hospitals and clinics that carry the burden of identifying and treating patients.



Graphic 1. Positive rate of samples in the case of pooling and a single sample study

Indeed, RT-PCR tests have many limitations such as the delay in results, technical time-consuming, need for special equipment-experienced personnel, expensiveness, and risk of contamination. Negative results are obtained depending on the pathogenesis of the agent infection and the methodological handicaps. The nature of the material in the sample, sample collection time, improper transportation, and technical problems (e.g., virus mutation, inhibition in the test) affect the test result (9).

Yan et al. (9), point out the importance of nucleic acid tests (NAT) in the diagnosis of COVID-19 and stated that the most important and first key to responding to epidemics is early diagnosis and that laboratory tests play a major role in the early detection of infected people, enabling the identification of the source of infection and cutting the transmission route. It has been emphasized that RT-PCR is the preferred and most widely used method for NAT due to its easy methodology and extensively validated standard operating procedure (10). The pooling of PCR test samples is simple, practical, and valuable because it uses minimal research resources (6). In our study, the COVID-19 RT-q PCR detection kit was used; only 840 people working in the specified factory were included and no additional samples were taken from other applications.

Because clinical studies used different RNA extraction methods and different RT-PCR kits, it would be difficult to compare their results. Virtually, it will be necessary to conduct experiments to validate the current kit and extraction method before pooling the samples. In addition, the possibility of catching positivity by pooling may vary according to the country, region, and even the specific group tested. Therefore, the size of the pooled tests and the positivity rates of the specific groups tested should be regional at the laboratory level. Pooling should not be done in groups with high probability of positive results and in such circumstances samples should be studied one by one.

Table 1. Comparison of pooling method and single sample studies

	Pooling	Single sample run
Result time	1 hour	12 hour
Positivity rate	23%	12%
Consumable usage	1 time	9 times
Positivity	20 wells x 10 pools: 200	102 original case

In mathematical modeling, it shows that the sample pooling strategy will work better in environments with low prevalence and in asymptomatic individuals (11-13).

When large numbers of individuals should be evaluated, pooling of 5 samples is better than pooling of 10 samples to reduce false negativity and false positivity. Similar to our study, samples of 10 patients were pooled in the USA, Spain, and Chile studies (14-16). Another factor affecting our pooling plan is that factory workers are considered to have a low prevalence.

In particular, samples with a cq value greater than 33 cycles are more unsuitable for pooling (14-16). In the USA, Spain, and Chile studies, it was observed that positive samples above 30 cq were found to be false negatives, especially when clinicians pooled 10 samples (14-16) (Table 2). In our study, 10 samples were collected in 1 pool and sigmoid curves below 33 cq, which passed internal controls, were considered positive. When the negative pools were studied one by one, no positive cases were found. After we studied 840 samples in our study, a preliminary report was prepared and 102 positive cases were detected.

By using the pooling method, positive and negative people were separated in as little as 1 h, and the contact time was 12 times shorter than the individual study.

Patients infected with SARS-CoV-2 are the main source of infection. These patients should either be isolated at home or kept in the hospital according to the severity of the disease, in line with the recommendations of the health personnel. When isolation is preferred at home, patients should be kept in a single room and contact with those living at home should be minimized. The items used should be disinfected, and the room should be kept clean and ventilated appropriately (17).

Conclusion

An accurate and rapid diagnosis of COVID-19 is important for correct epidemic management. Scanning with PCR at factories, soccer matches, or concerts are key to the continuation of these activities. In such cases it takes days to run thousands of samples. Hence, individuals should be evaluated by pooling first, and samples should be studied one by one after the decision to continue the activity is made. When the factory population in our study was evaluated, no new cases were detected in the following days, and it was seen that the spread of the virus was prevented.

We conclude that pooling will be beneficial in terms of preventive medicine and social and economic aspects in cases where large screening groups need a rapid diagnosis.

Table 2. Examples of different pooling strategies and pools from different countries

Different pooling strategies from different countries			
Country	**Pooling strategy**	**The number of samples pooled**	**Reference**
Israel	Pooling of extracted RNA	32 samples/1 pool	(14)
Israel	Combined pooling strategies	348 samples/48 pools	(16)
Germany	Pooling of extracted RNA	4-30 samples/1 pool	(13)
Germany	Pooling of swabs directly in a pooling container	5 samples/1 pool	(15)
United States of America	1 pool of 5 samples of 50 µL each	5 samples/1 pool	(10)
Chile	Pooling of nasopharyngeal specimens from the transport medium	5 samples/1 pool	(9)
Spain	Pooling of nasopharyngeal specimens in transport medium	5-10 samples/1 pool	(11)

Ethics

Ethics Committee Approval: Permission was obtained from the Ankara City Hospital Ethics Committee with the date 02.03.2022 and number E2-22-2493. All procedures in this study comply with the 1975 Declaration of Helsinki, updated in 2013.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.S., Concept: A.T., E.S., Design: A.T., E.S., H.C., A.B., Data Collection or Processing: A.T., Analysis or Interpretation: A.T., H.C., A.B., Literature Search: E.S., H.C., A.B., Writing: H.C., A.B.

Conflict of Interest: No conflict of interest was declared by the authors.

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