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Original Research Article

Pooled nasopharyngeal swab collection in a single vial for the diagnosis of SARS CoV-2 infection: An effective cost saving method

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ABSTRACT

Background: Pool testing is one of the strategy to expedite testing capacities while simultaneously conserving various diagnostic kits, reagents and consumables and time. In the present study, we investigated potential role of combined specimen collection technique for the diagnosis of SARS-CoV-2 virus infection where five nasopharyngeal swabs were collected from different individuals and pooled together in a single viral transport medium (VTM).

Material and methods: This pilot study was conducted on different cohorts of Delhi state. Two nasopharyngeal swabs were collected from each enrolled individual. One swab was put into VTM vial to be further used for individual swab testing (ID). The other swab was put into a fresh VTM for pool swab collection. Each pool comprised five swabs collected from five different patients in one VTM vial. Both IDs and pools were tested in parallel for the detection of SARS-CoV-2 using real time PCR.

Results: A total of 46 pools were collected from 230 enrolled individuals. Among 230 ID tested, 60 were found to be positive for both E and RdRp gene. Among 46 pools, 25 pools included all negatives samples and remaining 21 pools included one or more positives. Comparing ID with pool results, overall concordance was seen in 42 pools (91.3%). Four pools showed false positive results as all included samples on ID testing were found to be negative. Considering ID results as reference, swab pool showed 100% sensitivity, 84% specificity, 84% positive predictive value and 100% negative predictive value.

Conclusion: The pooling of swab strategy could be beneficial only among asymptomatic in low prevalence areas.

Introduction

Beginning of the new decade saw the emergence of a new disease corona virus disease 2019 (COVID-19) caused by the SARS-CoV-2 virus [1]. COVID-19 has rapidly spread across the globe to cause the first corona virus pandemic in history [2] affecting over 213 countries and territories with 18,614,177 confirmed cases and 702,642 deaths [3]. Early diagnosis of COVID-19 is paramount in the containment of the pandemic and breaking the chain of transmission of the virus.

The laboratory diagnosis of SARS-CoV-2 is based primarily on nucleic acid amplification test (NAAT) like real time reverse transcriptase PCR (RT-qPCR) [4]. With a majority of countries across the globe having imposed lockdown there is an acute shortage of diagnostic kits [5,6] and

reagents which is a major bottle neck in the testing of COVID-19 infection. This unprecedented situation has led to testing initially asymptomatic individuals and leaving out asymptomatics and carriers, detection of whom is the key in containing the spread of infection into the community.

Furthermore densely and heavily populated countries like India have abysmally low test rates per capita in the world [7] and the number of cases are expected to shoot up post lockdown. So the present situation demands countries to scale up their testing and design innovative strategies to conserve diagnostic kits and reagents.

Pooling test strategy, already been applied in other infectious diseases [8,9], is one such feasible option by which a large number of population can be tested simultaneously and various consumables, diagnostic kits,

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reagents can also be conserved to an extent. This pooling method requires mixing of samples and testing it as a single pool, and subsequent individual tests are made only if the pool test found to be positive.

In the present study, we investigated the potential role of combined specimen collection technique in the diagnosis of SARS-CoV-2 virus infection, where five nasopharyngeal swabs were collected from different individuals and pooled together in a single viral transport medium (VTM) vial. Both the pooled and individual testing were performed simultaneously to compare the results.

Methodology

Studied population

This was a pilot study conducted to determine utility of pooling of swabs on three different cohorts in Delhi state. Cohort A included symptomatic individuals admitted in a tertiary care government hospital with history of close contact with group of known positives ($n = 100$). Cohort B included previously untested and asymptomatic contacts of known positives quarantined for a period of at least ten days ($n = 50$). The last cohort C included asymptomatic population with no history of contact with confirmed case ($n = 80$).

Specimen collection and transport

Two nasopharyngeal swabs from each individual were collected by a trained health care worker. One swab was put into a VTM vial for individual (ID) swab testing. The other swab was put into a fresh VTM for pool swab collection. Each pool comprised 5 swabs collected from 5 different patients in one VTM vial (Fig. 1). All necessary biosafety precautions were exercised while making pools so as to avoid any cross contamination during the sample collection. All the collected samples were transported to our tertiary care institute maintaining appropriate cold chain and biosafety precautions.

Processing of specimens

Both IDs and pool were tested in parallel in the laboratory. Briefly, a total of 200 μ l of VTM was taken for nucleic acid extraction using Qiasymphony DSP Virus/Pathogen mini kit (Qiagen GmbH, Germany) as per the manufacturers' instructions. RT-qPCR for screening of SARS-CoV-2 targeting E gene was performed with primers and probe (E_Sarbeco-F: ACAGGTACGTTAATAGTTAATAGCGT, E_Sarbeco-R: ATATTCGACGCA GTACGCACACA and E_Sarbeco-P: FAM-ACACTAGCCATCCTTAC TGGCGTTCG-BBQ) using LightMix® SarbecoV E-gene (TIB MOLBIOL) and SIII qRT PCR kit Invitrogen enzyme (Thermo Fisher Scientific) on Applied Biosystem (ABI) 7500 Real Time PCR system. The cycling conditions were as follows: reverse transcription at 55 °C for 5 min, denaturation at 95 °C for 5 min and then 45 cycles of amplification at 95 °C for 5 s and at 60 °C for 15 s. The screening test was considered positive if Ct value was found to be ≤ 40 . If screening test was found to be positive, confirmatory test targeting RdRp gene was performed with primers and probe (RdRp_SARSr-F: GTGARATGGTCATGTGTGGCGG, RdRp_SARSr-R: CARATGTTAAASACACTATTAGCATA and RdRp_SARSr-P2: FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ) using LightMix® Modular SARS-CoV-2 RdRp (TIB MOLBIOL) and SIII qRT PCR kit Invitrogen enzyme (Thermo Fisher Scientific) on Applied Biosystem (ABI) 7500 Real Time PCR system. The cycling conditions were similar as for screening test. The confirmatory test was also considered positive if Ct value was found to be ≤ 40 . The study was approved by the Ethics Committee of the Institute.

Statistical analysis

The results so obtained was statistically analysed using SPSS version 22 (IBM Corp Ltd. Armonk NY). Data on Ct value is shown in

mean \pm standard deviations. Paired t-test was applied to compare Ct value of ID with pool. Intraclass correlation coefficient (ICC) between ID and pool Ct values was also calculated. A Bland-Altman analysis was performed to assess bias and agreement in Ct value. A p value of ≤ 0.05 was considered statistically significant.

Results

Total number of pools collected

From enrolled population, 46 pools (each containing 5 swabs) were collected among which 20, 10 and 16 were obtained from cohort A, B and C respectively (Fig. 2).

Overall ID vs pool test results

Among 230 ID tested, 60 were found to be positive for both E and RdRp gene and remaining 170 were negative giving an overall positivity rate of 26.08% for SARS CoV 2 infection. Among 46 pools, 25 pools had all negatives samples and in remaining 21 pools there was one or more positive sample (Table 1).

Comparing ID with pool results, overall concordance was seen in 42 pools (91.3%). Four pools showed false positive results as all included samples on ID testing were found to be negative. Considering ID results as reference, swab pool showed 100% sensitivity, 84% specificity, 84% positive predictive value (PPV) and 100% negative predictive value (NPV). The overall mean Ct of ID and pool were 31.39 and 29.63 respectively and no statistically significant difference was observed between them on t-paired test (p value 0.095). Overall ICC of 84.9% (95% CI; 45.8–94.7) was documented between ID and pool which was statistically significant (p < 0.01). A strong level of agreement was also seen between them on Bland Altman analysis (Fig. 3).

ID vs pool results in cohort A

Among 100 individuals included in cohort A, 25 were found to be positive for both E and RdRp gene making an overall positivity rate of 25%. Among 20 pools so obtained, 11 pools included all negatives and remaining 9 pools included one or more positive (Table 1). The overall concordance of 90% was observed between pool and ID results. Two pools showed false positive result as included samples in them were negative on ID testing. Considering ID results as reference, swab pool showed 100% sensitivity, 81.8% specificity, 81.8% PPV and 100% NPV. The overall mean ID and pool Ct values were 34.89 and 34.03 respectively and no statistically significant difference was observed between the two on paired t-test (p value 0.137). ICC of 91.4% (95% CI, 61.8–98.1) was documented between ID and pool which was statistically significant (p value < 0.05).

ID vs pool results in cohort B

None of 50 individuals included in cohort B was found to be positive for E gene. A total of 10 pools so obtained included all negatives on

Table 1
Details of pool composition from different cohorts.

Overall pool composition	Frequency	Cohorts and pool composition frequency		
		Cohort A	Cohort B	Cohort C
5 negatives	25	11	10	4
1 negative+ 4 positives	2	1	0	1
2 negatives+ 3 positives	9	3	0	6
3 negatives+ 2 positives	3	3	0	0
4 negatives+ 1 positive	4	1	0	3
5 positives	3	1	0	2
Total	46	20	10	16

ID testing (Table 1). The overall concordance of 100% was observed between pool and ID results. Considering ID results as reference, swab pool showed specificity and NPV of 100% respectively.

ID vs pool results in cohort C

A total of 35 were tested positive for both E and RdRp gene among 80 individuals making an overall positivity rate of 43.75% in cohort C. Among 16 pools so obtained, 4 pools included all negative and remaining included one or more positive samples (Table 1). Overall 87.5% concordance was observed between pool and ID results. Two pools showed false positive result as on ID testing, all included samples were found to be negative. Considering ID results as reference, swab pool showed 100% sensitivity, 50% specificity, 85.7% PPV and 100% NPV. The overall mean Ct of ID and pool were 29.16 and 26.34 respectively and on paired t-test statistically significant difference was observed between them (p value 0.008). ICC of 43.5% was documented between ID and pool which was not statistically significant (p value 0.179).

Discussion

The present study evaluated the potential role of combined specimen collection technique where nasopharyngeal swabs collected from different patients were pooled together in a single VTM vial and compared with standard ID testing for the diagnosis of COVID-19.

The study was conducted on different cohorts depicting varying positivity rates of infection. Overall good concordance was observed between pool and ID results with 100% sensitivity and 81.8% specificity.

COVID-19 was declared as pandemic on March 11, 2020 by World Health Organization [10]. To curtail further spread, timely diagnosis and isolation of active cases and their contacts is an essential component. Real time PCR remains the gold standard for diagnosis of SARS-CoV-2. Due to unprecedented demands, there is woeful shortage of various diagnostic kits and consumables. Pool testing is one of the potential strategy in present ongoing lockdown scenario to enhance the diagnostic capacity while reserving resources and obtaining timely results. Pooling of specimens can be performed at different levels viz. pooling of VTM or extracted RNA [11]. Recently published studies by Gupta et al. and Khodare et al. had demonstrated the efficacy of RNA pooling strategy for COVID-19 [12,13].

In the present study, pooling was performed at the time of specimen collection from different cohorts. By this, apart from conserving cost of extraction and PCR reagents, a judicious cap into cost of VTM can also be achieved. Various studies had explored the feasibility, practicality and optimal size for pool testing recently [14–19]. Yelin et al. showed the detection of SARS-CoV-2 in pool of 32 samples and potentially in 64 samples [20]. Another study found 5 samples pool better than 10 samples pool with high concordance rate and less false negatives [21]. Maximum Ct value difference of five was reported between pool and individual testing by Lohse et al. [22]. In the present study, no statistical difference in Ct value between two was demonstrated.

An overall good concordance (93.1%) between pool and ID results was observed, however false positivity was seen in four pools, 2 each from Cohort A and C. This scenario could be attributed due to high positivity rate of 25% and 43.75% respectively despite exercising extreme precautions at the time of specimen collection. Furthermore, unlike in Cohort A, statistically significant difference between mean Ct value of pool and ID were observed in Cohort C which might be due to heterogeneity of the pool composition.

Indian Council of Medical Research (ICMR) had released advisory on VTM pool testing strategy and recommended the same only in areas with low prevalence rate of infection [23]. Our study is in agreement with ICMR advisory that pooling swab strategy could be a success only in asymptomatic individuals with areas of low prevalence of infection. The strategy does not prove to be beneficial in areas with high positivity rate as ID test need to be performed for each positive pool, which is again time

as well as resource consuming. Additionally pool testing had demonstrated 100% NPV in all the three cohorts in this study. This finding could be of immense help in releasing quick negative results, thereby reducing mental stress and agony of the tested population to an extent in crisis situation.

The present study has certain limitation. This was a pilot study conducted to evaluate the performance of pool swab testing in different cohorts. However, a mathematical computational model needs to be evaluated to determine the appropriate pool size. Secondly, this was a pilot study and follow up of the enrolled population could not be performed.

Conclusion

India and many other low and middle income countries with a dense population need to scale up their testing capacities. Pool testing is one such novel testing strategy where large number of samples can be tested and diagnosis of COVID-19 can be done in a timely manner, ultimately breaking chain of transmission.

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Declaration of competing interest

The authors declare no conflict of interest.

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