Correspondence

Hospital laboratory experience with SARS-CoV-2 (COVID-19) molecular assay sample pooling method in New York City

The ongoing SARS-CoV-2 (COVID-19) pandemic has been a huge challenge for healthcare systems worldwide. Laboratories have been confronted with rapidly increasing testing demands that exceed testing capacity. Regions with a low SARS-CoV-2 molecular assay positivity rate are looking for ways to increase testing capacity and prevent a second wave while returning to 'normal' life. One aspect of this process is to reopen non-COVID-19 related medical services that were shut down due to SARS-CoV-2 transmission risk.

Sample pooling method has been used for mass testing of several infectious diseases for decades and is currently proposed as a new strategy to increase COVID-19 testing capacity in low-risk regions.[1–5] New York State Department of Health (NYSDOH) requires patients to be tested for COVID-19 using a molecular assay for detection of SARS-CoV-2 RNA before medical procedures. While the number of medical procedures were rapidly increasing at our hospital, the supply of SARS-CoV-2 molecular assay reagents has been insufficient and inconsistent. To meet the need of testing before medical procedures in a timely manner and prevent possible delays in patient care, Lenox Hill Hospital laboratory implemented the sample pooling method for patient testing. We, herein, report our 6-week experience with pool testing for COVID-19.

In the beginning of July 2020, we validated the pooling testing method for COVID-19 using the Cepheid Xpert Xpress SARS-2 CoV-2 (EUA) cartridges on GeneXpert System (Cepheid, Sunnyvale, California, USA) by using the recent guidelines published by the Food and Drug Administration and NYSDOH.[6 7] The SARS-CoV-2 positivity rate at Lenox Hill Hospital of Northwell Health was <3% over the last month with the majority of the days being 0%–1%. Briefly, a sample pool was created by combining 200 µL of Universal Transport Medium from each patient sample into a labelled tube using a pipettor or sterile transfer pipette. The pooled sample was then vortexed for 5–10 s to mix. From this pooled mixture, 300 µL was tested on the GeneXpert System. Linkage between pooled sample and original patient samples was maintained by log sheet. The validation set consisted of 16 pools (15 positive, 1 negative) with each having five samples. Including five samples in each pool as the optimal method was determined as previously shown.[3 7] The positive pools had one known positive and four known negative samples. The negative pool included only viral transport medium. Among 15 known positive samples, nine were previously tested by GeneXpert System with a cycle threshold (Ct) values from 13.3 to 29.8 for the envelope target (E) and from 15.7 to 33.2 for the nucleic acid target (N2). The Ct value is inversely proportional to the viral load. The reminder of the six positive samples were previously tested by the ePlex SARS-CoV-2 assay (GenMark Diagnostics) (Ct value is not generated). Additionally, two of the positive pools were tested in triplicate runs to evaluate the reproducibility of the pooling method.

After implementing the pooling method at our laboratory, asymptomatic patients who require a non-elective medical procedure (eg, cardiac catheterisation) were included in the pool testing during the 6-week period (2 July 2020–12 August 2020). The majority of the pools included five samples; however, some pools included <5 samples by considering multiple factors such as the number of samples waiting for testing, the type and urgency of procedures, as well as the risk of COVID-19 stated by the clinical team. For quality assurance (QA), all positive samples and a negative pool including their individual samples randomly chosen by the laboratory staff each day were sent to an outside laboratory for testing by alternative testing method (Aptima SARS-CoV-2 Assay (Panther System)). This work was determined to meet the criteria of quality improvement project; therefore, an approval from the institutional review board was not requested.

During the validation, all 15 positive pools were confirmed as positive on GeneXpert System. The Ct value for both E and N2 targets was higher in all pool samples compared with the individual samples, as expected. The increase in Ct value ranged from 1.3 to 3.5 for target E and from 1.8 to 4.9 for target N2 (figure 1). The two positive pools that were tested thrice showed positive result in all three runs, and lastly, the negative control pool showed a negative result.

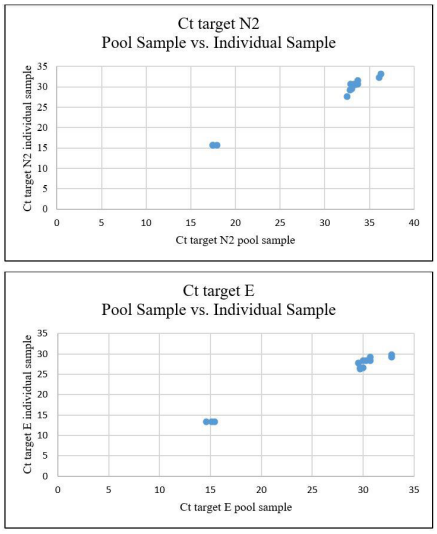

Figure 1 Cycle threshold (Ct) values of nucleic acid (N2) and envelope (E) targets for the validation samples.

A total of 1597 patient samples were tested in 343 pools during the 6-week period. The number consisted of 5 pools of 2 samples, 23 pools of 3 samples, 54 pools of 4 samples and 261 pools of 5 samples (figure 2). Thirty-five out of 343 pools detected SARS-CoV-2. Individual testing of these 35 positive pools showed 23 concordant (22 pools: 1 individual sample positive, 1 pool: 2 individual samples positive) and 12 discordant results (all individual samples negative on two different testing systems). The majority of discordant pools showed low viral load with a median Ct value of 40.3 (range: 25.9–44.3). Among 22 concordant tests, the individual samples in 17 pools were tested by GeneXpert System (Ct value is generated), and five of them were tested by the GenMark ePlex assay (Ct value is not generated). Both the individual and pool samples in 13 of 17 tests showed low viral load (Ct value >35). The Ct value in the remaining four concordant tests ranged from 25.8 to 34.7 (figure 3, table 1) Consistent with the validation results, the Ct values were higher in pool samples compared with the individual samples in all concordant positive tests (range in increase: 0.7–3.4).

A total of 187 samples were sent to an outside laboratory for QA. The concordance rate for negative pools (Cohen's kappa=1) and negative individual samples (Cohen's kappa=0.97) were perfect. None of the QA samples reported negative by the pooling method were detected as positive by alternative testing method. Lastly, the overall SARS-CoV-2 positivity rate (23/1597; 1.4%) at our hospital

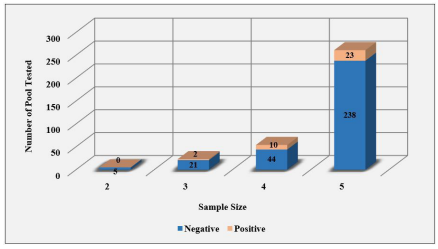

Figure 2 Distribution of the number of COVID-19 PCR pool test according to sample size and test result.

remained constant after the implementation of pool testing.

In order to overcome the challenge of reagents supply shortage and improve the testing turnaround time in favour of patients and clinical team, we successfully validated, implemented, and maintained the pooling method for SARS-CoV-2 molecular assay at our laboratory. In 6 weeks, a total of 1597 patient samples were tested with 494 test cartridges for both pool testing (n=343) and confirmatory individual tests (n=151) for positive pools. The pooling method with a maximum number of five samples/pool allowed our laboratory to preserve 69% ((1597–494) / 1597×100) of the test cartridges that would have otherwise required for individual testing. All 13 positive individual samples with low viral load from study samples were also tested positive with pooling test in our study, which eliminates the main concern of missing positive individual samples with low viral load in pooling method due to dilution.

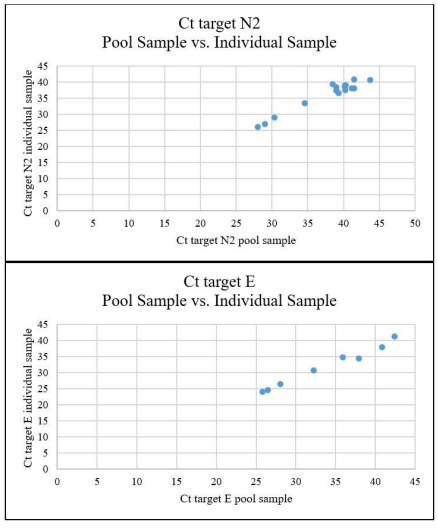

Figure 3 Cycle threshold (Ct) values of nucleic acid (N2) and envelope (E) targets for the samples from the patients scheduled for a non-elective medical procedure in 6-week period.

Table 1 Cycle threshold (Ct) values of the individual and pool samples for the validation and study groups

Pool size	Pool sample Ct target E	Individual sample Ct target E	Pool sample Ct target N2	Individual sample Ct target N2
Validation samples*				
5	29.5	27.7	33.4	30.6
5	30.0	26.6	33.0	29.5
5	29.7	26.3	32.5	27.6
5	29.7	26.6	32.8	29.2
5†	14.6	13.3	17.5	15.7
5†	15.1	13.3	17.5	15.7
5†	15.4	13.3	17.9	15.7
5	30.7	29.2	33.7	31.5
5	32.8	29.8	36.3	33.2
5†	30.0	28.4	32.9	30.7
5†	30.3	28.4	33.3	30.7
5†	30.7	28.4	33.7	30.7
5	32.8	29.3	36.1	32.3
Study samples with positive pool results				
4	36.0	34.7	39.1	37.4
4	0	42.3	40.2	38.5
3	32.3	30.6	34.7	33.3
5‡	0	0	38.7	0
4	0	0	38.5	39.2
5	0	40.9	40.3	38.8
3‡	0	0	42.9	0
5	0	35.5	40.3	37.4
5	0	0	42.2	+
5	35.3	+§	38.1	+
5‡	0	0	41.4	0
5	0	0	41.6	40.6
5	36.7	+	37.2	+
5‡	0	0	43.4	0
5	35.9	+	40.3	+
5	36.2	+	39.5	+
5	0	0	43.7	+
4‡	38	0	39.9	0
2‡	0	0	42.7	0
5	0	35.8	41.6	38.0
5	38.0	35.3	39.4	36.5
4	40.9	37.7	39.1	38.4
4	0	0	43.8	40.4
5	25.9	23.8	28.1	25.8
5	0	0	41.2	38.0
5	26.6	24.5	29.1	26.8
5‡	39.5	0	38.0	0
5	27.6	+	29.9	+
5‡	0	0	42.7	0
5‡	0	0	43.1	0
5	0	0	40.4	38.7
4‡	0	0	44.3	0
5	42.5	41.1	0	0
4‡	43.7	0	40.7	0
5	28.1	26.2	30.4	28.9

*Only 9 of 15 positive validation samples with available Ct values were included in the table.
†Validation samples tested thrice to evaluate the reproducibility of the pooling method.
‡Discordant study samples showing positive pool tests and negative individual tests. The negative results of all the individual samples were confirmed at an outside laboratory.
§Ct values are not generated for positive samples with '+' sign.
E, envelope; N2, nucleic acid.

Also, reviewing the clinical notes, serology results and QA results of the patients with discordant results revealed that there was no clinically significant adverse effect on test performance and patient care as QA results showed a perfect correlation, particularly for negative tests.

This study is limited to a relatively low number of validation and study samples collected from a single laboratory in a short duration.

In conclusion, while health systems are pursuing to implement the SARS-CoV-2 molecular assay pooling method for large-scale population-based screenings, its use in clinical settings should also be considered in a proper context. Regardless, the pooling strategy would make a huge impact on testing capacity, shortage of test supplies and the overall battle against COVID-19.

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