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Pooling samples: a testing option for SARS-CoV-2 during a supply shortage

Hossein Salimnia [a,b], Robert Mitchell [a], Angela Gundel [a], Alicia Cambell [a], Fadi Gammou [a], Teena Chopra [a,c], Marilynn Fairfax [a,b,*]

[a] Detroit Medical Center University Laboratories, 4201 St Antoine, Detroit, MI 48201
[b] Department of Pathology, Wayne State University School of Medicine, East Canfield, Detroit, MI 48201
[c] Epidemiology and Infection Control, Detroit Medical Center, 4201 St. Antoine, Detroit, MI 48210

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ABSTRACT

Pooling of 1 positive sample with up to 5 negative samples prior to testing with the Cepheid GenXpert SARS-CoV-2 assay did not adversely impact detection of positive samples. At our current prevalence of 2%, it could save up to 70% of the test kits.

Shortages of reagents and kits for SARS-CoV-2 (CoV-2) tests may limit testing as hospitals reopen to patients at low risk of COVID-19. We wished to validate pooling nasopharyngeal samples collected in viral transport medium from such patients to allow large-scale CoV-2 testing while conserving reagents (Abdalhamid et al., 2020; Hogan et al., 2020). US blood collection agencies routinely pool 16 donor samples to perform molecular-based screening for hepatitis B and C and human immunodeficiency viruses (Dwyre et al., 2011). The US Food and Drug Administration has not issued emergency use authorization for pooling samples for CoV-2, although they say that "we realize that pooling and asymptomatic testing are critical to ending this pandemic and we want to ensure that recommended validation approaches are appropriately designed to provide sufficient data in a least burdensome manner" [email from Yvonne Shea, on 06/12/2020 (yvonne.shea@fda.hhs.gov)]. In addition, they have recommended a comment to be added if sample pooling is utilized (https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/covid-19-frequently-asked-questions 6/16/2020). We wanted to validate the use of pooling using the Cepheid GenXpert SARS-CoV-2 assay (CoV-assay), a reverse transcriptase polymerase chain reaction (PCR) assay, in an atmosphere of low positivity rates. This assay detects 2 targets genes: E (envelope) and N2 (nucleocapsid). A result is interpreted as positive if N2 is positive regardless of whether E is detected (Cepheid, 2020).

Our medical system (7 hospitals; 1 each specializing in pediatrics and cancer; 2000 beds) experienced a CoV-2 positivity rate > 50% at the peak of the Detroit epidemic when we were testing up to 150 samples per day from symptomatic patients. This rate has fallen to around 2% as our hospitals reopen to patients at lower risk of COVID-19, but we are testing approximately 400 samples per day. Testing 100 samples, in pools of 5, as opposed to individually would require 20 test kits followed by 10 more to test individual samples in the 2 anticipated positive pools, conserving 70 kits. This may prove essential as Cepheid is limiting the amount of testing supplies they will send us.

We determined the impact of sample pooling on detection by retesting 15 previously frozen CoV-2–positive samples initially submitted for patient testing. The samples were chosen based on the presumed concentrations of virus, reflected by their initial cycle or crossing threshold (Ct) value with the E target. Ct values decrease as the viral load decreases. Five had Ct <25 (high virus concentration), 5 had Ct 25–33 (intermediate), and 5e had Ct >33 (low). The manufacturer provides a Ct value for positive but not negative samples, and the cutoff value is proprietary (Cepheid, 2020).

Each sample was first retested to ensure that storage and 1 freeze/thaw cycle had not altered the positivity. The apparent viral load

Abbreviations: CoV-2, SARS CoV-2; NA, not available.

* Corresponding author. Tel.: +1-313-507-3531.
E-mail address: mfairfax@dmc.org (M. Fairfax).

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Table 1
Crossing thresholds and interpretations of 15 samples when pooled with various numbers of negative samples.

Sample number, concentration, initial Ct with E and N2 Targets	Repeat result			Pool of 3 samples			Pool of 5 samples			Pool of 6 samples				
	E	N2	Result	E	N2	Result	E	N2	Result	E	N2	Result	E	N2
1 High	22.5	24.9	POS	23.3	25.6	POS	25.1	27.4	POS	25.1	27.5	POS	25.4	27.6
2 High	14.6	17.9	POS	13.4	16.2	POS	15.7	18.1	POS	16.1	18.4	POS	16.5	18.7
3 High	15.6	18.4	POS	14.9	17.4	POS	16.4	18.6	POS	17.1	19.4	POS	17.7	19.9
4 High	20.8	23.7	POS	17.7	20.2	POS	19.6	21.9	POS	20.2	22.7	POS	20.5	22.9
5 High	19.3	21.7	POS	18.1	20.5	POS	20.1	22.5	POS	20.7	23.3	POS	21.1	23.4
6 Int	26.2	28.8	POS	26.1	28.8	POS	28.0	30.4	POS	28.4	30.9	POS	28.5	31.2
7 Int	28.3	31.3	POS	31.6	34.4	POS	33.4	36.6	POS	34.6	37.1	POS	30.3	33.3
8 Int	29.6	32.5	POS	30.2	32.8	POS	32.0	35.0	POS	34.2	35.8	POS	30.2	32.9
9 Int	23.0	25.2	POS	21.6	23.7	POS	23.6	25.7	POS	24.3	26.4	POS	24.4	26.4
10 Int	31.2	34.0	POS	30.2	32.8	POS	32.2	35.4	POS	32.7	36.0	POS	32.7	35.8
11 Low	33.4	36.7	POS	31.1	33.8	POS	32.9	36.3	POS	32.4	35.1	POS	34.8	36.8
12 Low	33.2	36.4	POS	27.5	30.2	POS	33.4	36.6	POS	36.2	37.9	POS	35.6	39.3
13 Low	38.5	41.9	POS	33.7	36.9	POS	0.0	42.6	POS	0.0	41.2	POS	NA	41.0
14 Low	35.8	38.0	POS	38.5	38.3	POS	41.6	42.3	POS	37.8	43.2	POS	NA	42.2
15 Low	35.7	39.3	POS	35.8	39.4	POS	39.9	42.1	POS	40.5	41.4	POS	NA	41.9

The initial sample was pooled with negative samples to give the indicated pool sizes. The Ct values of the samples (based on the initial value obtained with the E target) were as follows: high, >25; int, 25–32; low, <33. NA = not available; the instrument does not provide Ct values for samples deemed negative, and the manufacturer's cutoff values are proprietary.

occasionally increased (the Ct decreased), possibly due to random fluctuations or to disaggregation of sample and tissue clumps in the freeze/thaw process. Samples were deidentified and given a testing number. Each sample was then retested repeatedly after pooling with 2–5 negative samples (Table 1). To prepare a pool of 3 samples, 0.5 mL from a positive sample was pooled with an equal volume taken from each of 2 negative samples. Pools containing 1 positive and up to 5 added negative samples were prepared similarly. Negative samples were used only once. Each pool was vortexed for 5 s, and 300 μL was used for CoV-2 testing according to the manufacturer's instructions (Cepheid, 2020).

Each pool remained positive regardless of initial Ct or of whether the pool contained 2, 4, or 5 negative samples. However, the E target became undetectable in the 3 samples with the highest initial Ct values (lowest virus titers) when they were pooled with 5 negative samples (pool size 6 samples; Table 1). This suggested that further dilution could cause samples with low titers of CoV-2 to become undetectable.

There are 2 general groups of CoV-2 molecular diagnostic assays from multiple manufacturers. The first requires extraction and purification of the nucleic acid prior to the assay, which could theoretically facilitate concentration and pooling of larger numbers of specimens but may exhibit prolonged turnaround time (TAT). The second group, which includes our assay, is designed for direct from the sample testing and should have a shorter TAT. Sample concentration is not an option, so pooling requires sample dilution and may be more consequential. Before pooling is utilized, initial studies should be performed with each assay to determine how many samples can be pooled without impacting the detection of positive samples. A theoretical calculation by Abdalhamad et al. (2020) concluded that with a sensitivity of 95% or 100%, a specificity of 100%, a lower limit of detection of 1–3 copies/μL, and a prevalence of 5%, the optimal pool size was 5 samples, and their experimental validation supported this. Hogan et al. pooled 10 samples, but our data suggest that such a pool size may be too great for samples with low viral loads tested in our assay. A further consideration is that approximately half of our current testing is ordered stat, as the person being tested is going for a procedure or to the operating room or is in the process of delivering a baby. If such a sample is in a pool that exhibits a positive result, the required retesting would double the in-lab testing time. Thus, pooling of samples may not be appropriate for stat tests.

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Author statement

None of the authors of this paper has a conflict of interest or competing interest to declare.

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