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Sample pooling on the Cepheid Xpert® Xpress SARS-CoV-2 assay

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ABSTRACT

The COVID-19 pandemic has placed unprecedented global demand on laboratory supplies required for testing. Sample pooling has been investigated by laboratories as a strategy to preserve testing capacity. We evaluate the performance of Cepheid Xpert® Xpress SARS-CoV-2 RT-PCR assay for testing samples in pools of 4 and 6. Clinical samples containing SARS-CoV-2, and confirmed negative clinical samples were used to create sample pools. Clinical samples had 'neat' Xpert® E gene cycle threshold values ranging between 20 and 28 and all were detected qualitatively when contained in pools of 4 or 6 samples. For these samples, pooling had a median change in cycle threshold value of 2.0 in pools of 4, and of 2.9 in pools of 6. With the use of Cepheid Xpert® Xpress SARS-CoV-2 RT-PCR assay, pooling of 4 or 6 samples may be an effective strategy to increase testing capacity.

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1. Introduction

Rapid and accurate diagnostic testing for SARS-CoV-2 is central to controlling the global COVID-19 pandemic. The Cepheid Xpert® Xpress SARS-CoV-2 assay (Cepheid, Sunnyvale, CA) is a rapid, near-care, reverse-transcriptase PCR assay (RT-PCR), producing results within 45 minutes. Manufacturer instructions for use claim a limit of detection (LoD) of 250 copies/mL; however, recent work has demonstrated high analytical sensitivity of this assay, with a LoD approaching 100 viral copies/mL (Loeffelholz et al., 2020; Moran et al., 2020; Lieberman et al., 2020; Zhen et al., 2020; Wolters et al., 2020). The sheer scale of global demand for laboratory reagents, including the Xpert® SARS-CoV-2 assay, has led many laboratories to investigate alternative strategies for optimizing the use of testing supplies, including sample pooling (Torres et al., 2020; Williams et al., 2020; Wacharapluesadee et al., 2020; Lohse et al., 2020; Perchetti et al., 2020; Hogan et al., 2020). The diagnostic performance of pooling depends on several factors, including assay sensitivity, prevalence of infection in the population being tested and sample types used for

pooling (Wacharapluesadee et al., 2020; Lohse et al., 2020; Perchetti et al., 2020; Hogan et al., 2020). With increased recognition that asymptomatic SARS-CoV-2 infection may contribute to transmission, the populations tested have been expanded to include asymptomatic patients: in this context when prevalence rates are <5% sample pooling can substantially increase testing capacity (Abdhalamid et al., 2020; Cherif et al., 2020; Ben-Ami et al., 2020). On July 18, 2020, the FDA issued its first Emergency Use Authorization for sample pooling in diagnostic testing for SARS-CoV-2 by RT-PCR which applies for the Quest Diagnostics test for use with pooled samples containing up to 4 individual swab specimens (Coronavirus (COVID-19) Update: FDA Issues First Emergency Authorization for Sample Pooling in Diagnostic Testing FDA, 2020). Although pooling has previously been used successfully with the Xpert® MTB/RIF assay, there are limited data on the performance of pooling using the Xpert® SARS-CoV-2 assay (Abdurrahman et al., 2015). Here, we investigated the performance of the Xpert® SARS-CoV-2 assay for detecting SARS-CoV-2 in pooled clinical samples. We chose to study pools of 4 and 6 samples based on the FDA Emergency Use Authorization authorization, available literature for other assays (Wacharapluesadee et al., 2020; Lohse et al., 2020; Perchetti et al., 2020; Hogan et al., 2020; Abdhalamid et al., 2020; Cherif et al., 2020; Ben-Ami et al., 2020) and our own experience with pooling using an in-house RT-PCR for SARS-CoV-2 (Chong et al.).

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2. Methods

Nasopharyngeal swab samples were collected in viral transport media (Kang Jian, catalogue no KJ502-19) and initially tested for SARS-CoV-2 at the Microbiological Diagnostic Unit Public Health Laboratory, University of Melbourne using the Hologic Panther Aptima™ SARS-CoV-2 Assay. The panel consisted of 7 clinical samples containing SARS-CoV-2, and 24 confirmed negative samples used to create sample pools. Each positive sample was tested: (1) neat; (2) in a pool with 3 negative samples (pool of 4) and (3) in a pool with 5 negative samples (pool of 6). Further, the 2 samples with highest and 2 samples with lowest cycle threshold (Ct) values were tested in duplicate in the pool of 6 to study precision of the assay performance on pooled samples.

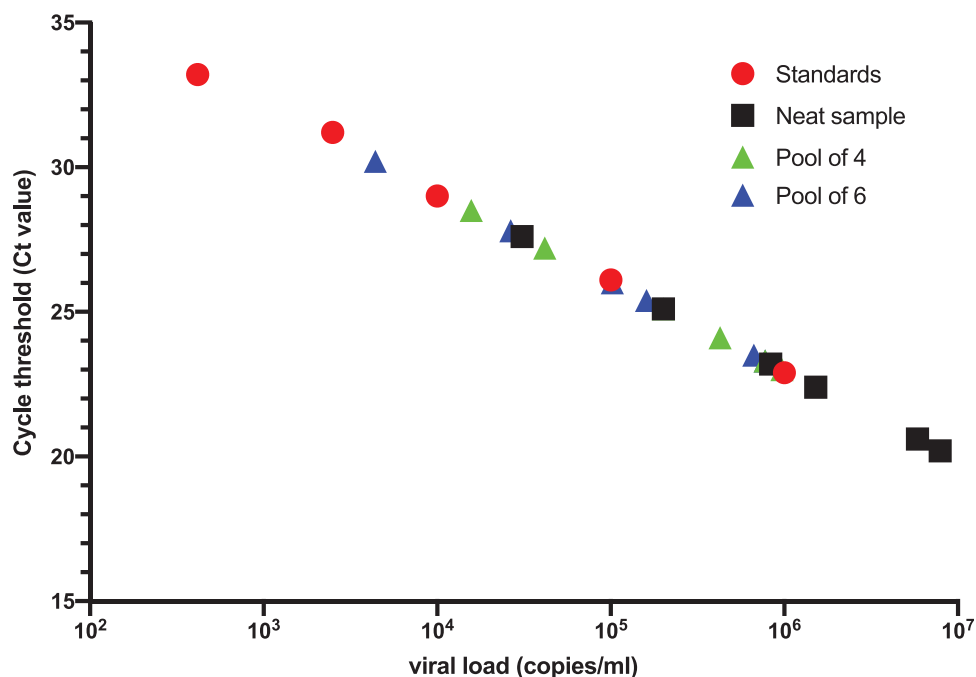
Pools were constructed by transferring 100 μL of each positive and negative sample into a sterile secondary tube and mixing this by inverting rapidly 5 times: 300 μL of this was aliquoted into the Xpert® cartridge and tested within 30 minutes of specimen addition.

In addition, serial dilutions of gamma-irradiated SARS-CoV-2 virus were prepared in viral transport media to allow correlation of viral concentration with Xpert® Ct values (Table 1). These serial dilutions of inactivated virus were used to create standards for a standard curve (Fig. 1) from which the neat clinical sample extrapolated viral load was calculated.

All Xpert® testing was performed by 1 investigator (E.W.), blinded to previous testing results and pooling details.

3. Results

Clinical samples with 'neat' Xpert® E gene Ct values ranging between 20 and 28 were detected qualitatively when contained in pools of 4 or 6 samples (Table 2). For these samples, pooling had a median change in E gene Ct (ΔCt) value of 2.0 in pools of 4, and a ΔCt 2.9 in pools of 6. All 24 negative samples were negative for SARS-CoV-2 by Xpert® when tested in 4 pools of 6 samples to confirm a specificity of pool testing of 100%.



$$R^2 = 0.995 \text{ (value calculated by method of least squares)}$$

Fig. 1. Standard curve of Xpert E gene Ct value and viral load.

Table 1

Xpert® cycle threshold values of serial dilutions of inactivated high-titer SARS-CoV-2 virus

Dilution	E gene	N gene	SPC
1×10^6 copies/mL	22.9	25.3	27.5
1×10^5 copies/mL	26.1	28.4	27.5
1×10^4 copies/mL	29	31.6	27.4
2.5×10^3 copies/mL	31.2	34.8	27.7
4.17×10^2 copies/mL	33.2	35.8	27.8
4.17×10^2 copies/mL in pool of 4 (104 copies/mL)*	0	0	28.1
4.17×10^2 copies/mL in pool of 6 (70 copies/mL)*	0	42.8	28

E = envelope; N = nucleocapsid; SPC = sample processing control.

These serial dilutions of inactivated virus were used to create standards for standard curve in Fig. 1.

* Note: Manufacturer claimed LOD for Xpert® is 250 copies/mL (lowest concentration that can be reproducibly distinguished from a negative sample $\geq 95\%$ of the time with 95% confidence).

Serial dilutions of inactivated SARS-CoV-2 virus between 4.17×10^2 copies/mL and 1×10^6 copies/mL were reliably detected by the Xpert® assay. Virus was detected when testing the lowest dilution of 4.17×10^2 copies/mL in a pool of 6 but not when tested in a pool of 4 negative samples.

4. Discussion

We have found that testing samples in pools of 4 or 6 using Xpert® assay can increase testing capacity and that virus from samples with neat Ct values of between 20 and 28 can be reliably detected during pooling. Consistent with other studies, we have found that samples with viral load around the LoD for the assay used may be missed when testing in pools (Ben-Ami et al., 2020). Since the required input into the Xpert® cartridge is fixed at 300 μL , when testing a pool size of 4, 75 μL of each original sample is tested. In comparison, for assays where RNA extraction of the pool can be

Table 2
Cycle threshold (Ct) results for clinical samples with pools of 4 and 6 samples

Neat sample extrapolated viral load* (copies/mL)	Neat sample Ct (Xpert®)			Four sample pool Ct (Xpert®)			Six sample pool Ct & replicate result (Xpert®)		
	E gene	N gene	SPC	E gene	N gene	SPC	E gene	N gene	SPC
7.91 × 10 ⁶	20.2	23.3	26.9	23.3	25.9	27.3	23.3	25.6	27.3
5.86 × 10 ⁶	20.6	23.2	27	23	25.4	27.1	23.1	25.5	27.6
							23.5	26.3	27.4
							23.1	25.8	27.2
1.52 × 10 ⁶	22.4	24.5	27.1	24.1	26.2	27.7	26	28.3	27.4
8.35 × 10 ⁵	23.2	25.5	27.4	25.1	26.9	27.3	25.4	28.1	27
4.26 × 10 ⁵	24.1	26.5	27.2	26.1	28.8	27.2	28.3	29.8	27.8
2.01 × 10 ⁵	25.1	27.7	27	27.2	29.7	27.3	27.8	30.7	27.4
3.09 × 10 ⁴	27.6	30.1	27.6	28.5	31.3	27.4	28.1	31.3	27.3
							30.2	33.9	27.3
							30.6	34.5	27.4

E = envelope; N = nucleocapsid; SPC = sample processing control.

* Based on Xpert® E gene Ct value: viral load for clinical samples was extrapolated from standard curve (Fig. 1).

performed as a separate step greater possible original sample input volumes can be accommodated to increase sensitivity. However, use of more sensitive assays such as the Xpert® to test sample pools is less likely to miss samples with low viral load when using lower sample input volumes. Furthermore, samples with low viral load, particularly in asymptomatic persons, may indicate the presence of noninfectious virus since studies have found that samples with higher Ct values are less likely to yield culturable virus (La Scola et al., 2020).

Strategies to optimize the performance of pooling include limiting its use to low prevalence situations such as testing of asymptomatic populations. By implementing pooling in low prevalence settings, the work of 'de-coupling' of pools for individual testing in the event of a positive pool is minimized. For a test with assumed sensitivity and specificity of 99% and a SARS-CoV-2 prevalence of under 5%, the expected number of PCR reactions required for testing of 1000 samples in pools of 4 is under 500, inclusive of initial pool testing and deconstruction of positive pools (Chong et al.). The Xpert® assay is authorized to be used in patient care settings outside of the clinical laboratory environment and it has been widely used in low-resource settings particularly for the diagnosis of tuberculosis (Abdurrahman et al., 2015). Its ease of use, including for pool testing, makes it an ideal assay for use in settings where expertise for SARS-CoV-2 testing may not be readily available. However, in view of high global demand for test kits the supply of cartridges does not always meet demand in some settings, which further supports the use of pooling with this assay.

During our study, we developed laboratory strategies to mitigate against errors during the pooling process. These included: (1) oversight of the pool assembly process by a second staff member; (2) checking of manual transcriptions by a second staff member; (3) holding back reporting of negative pooled samples until individual testing of positive pools is complete; (4) use of standardized worksheets for recording 2 identifiers (specimen number and patient name) of each specimen in the pool, and (5) requirement for operator signatures at each step of the testing process.

Here, we demonstrate that with the use of a sensitive and specific molecular assay that is easy to use, pooling of 4 or 6 samples is an effective strategy to increase testing capacity. With limited testing resources, sample pooling may preserve testing capacity; findings of our study may be particularly valuable for low-resource settings.

Authors' contribution

Maryza Graham: Data curation; Formal analysis; Investigation; Methodology; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing.

Elaine Williams: Data curation; Formal analysis; Investigation; Writing - review & editing.

Nicole Isles: Data curation; Formal analysis; Investigation; Methodology; Validation; Writing - review & editing.

Eka Buadromo: Conceptualization; Methodology; Resources; Writing - review & editing.

Tebuka Toatu: Conceptualization; Methodology; Resources; Writing - review & editing.

Julian Druce: Methodology; Supervision; Validation; Visualization; Writing - review & editing.

Mike Catton: Formal analysis; Methodology; Supervision; Validation; Writing - review & editing.

Chantel Lin: Funding acquisition; Project administration; Resources; Writing - review & editing.

Benjamin P. Howden: Conceptualization; Funding acquisition; Supervision; Writing - review & editing.

Deborah A. Williamson: Conceptualization; Formal analysis; Funding acquisition; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing - review & editing.

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Declarations of interest

None.

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