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Dear Editor,

Large-scale surveillance for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the pathogen for coronavirus disease 19 (COVID-19) is an important strategy for preventing the second wave of the disease [1]. Group testing of pooled specimens to detect for SARS-CoV-2 has been shown to be an effective strategy to conserve resources and to substantially increase testing capacity [2,3]. In order to reveal the importance of group testing for COVID-19, the FDA issued a statement on facilitating diagnostic test availability for asymptomatic testing and pool testing on June 16, 2020 [4]. To our knowledge, this is the first study to determine the cost effectiveness of SARS-CoV-2 sample pooling. A total of 4,630 nasopharyngeal specimens were collected from both asymptomatic and symptomatic patients suspected to have COVID-19. Based on our proof-of-concept study [2], these specimens were randomly pooled into groups of 5 specimens per pool for a total of 926 pools. RNA from each pool was extracted using the QIAGEN EZ1 Virus Mini Kit v2.0 (QIAGEN, Germantown, MD) with subsequent RT-PCR testing with the CDC EUA nCoV-2019 assay. RT-PCR assays were performed using Applied Biosystems 7500 Fast Dx Real-time PCR analyzer as recommended by the manufacturer. Specimens within positive pools were individually re-tested using the same procedure. Costs for reagents, consumables, and labor were subsequently compared between pool testing and individual testing using standard costs for supplies, reagents, and per hour labor plus benefits (Table 1). A total of 303 pools (representing 1,515 patients) were positive that contained 455 specimens with SARS-CoV-2 (represented a positive rate of 9.8%). All positive pools had at least one individual specimen that was identified as positive. In this pooling process, 2,441 tests were performed (926 represented the original pools and 1,515 represented individuals from split pools). This compares with 4,630 tests if all were tested individually, resulting in a saving of 2,189 tests (47.3%).

The cost of pool testing to include repeat testing of individual specimens from positive pools was $45,787 compared to $80,921 for individual tests resulting in a cost savings of $35,134 (43.4%) (Table 1). The total labor cost required for pooling tests was $10,124 compared to $13,277 for individual testing with saving of $3,153 (23.7%). No additional technologists were required to conduct SARS-CoV-2 testing when a pooling approach was implemented. All reporting of pool test results and backtracking positive pools to split for individual testing were done manually.

These data showed that specimen pooling for SARS-CoV-2 at a positive rate of 9.8% resulted in a savings of 47.3% on the use of reagents and 23.7% on labor. In a low prevalence population where large numbers of asymptomatic individuals are tested for surveillance, the cost savings would even be greater [2]. For example, with a positive rate of 1% and an optimal pooling size of 11 samples, there would be an approximate 80% reduction in test volume with a similar cost and time savings [2]. Anticipations are that large-scale screening of asymptomatic populations such as students, athletes, and military personnel, will be a common practice to monitor for COVID-19. The

optimal group size, which is dependent on the COVID-19 prevalence rate, the sensitivity and specificity of the assay, and on the pooling algorithm (a 2-stage or 3-stage algorithm), will be important to determine [2,5]. The cost and time saving of pooling will also vary based on the extraction and RT-PCR methods used to detect SARS-CoV-2. In addition, savings will also depend on the reporting process used and whether the tracking of positive pools for individual retesting is automated or manual.

In conclusion, diagnostic testing to identify people with COVID-19 for quarantine and contact tracing will continue to be needed to control future outbreaks of SARS-CoV-2 in the population. Large-scale surveillance will require increased amounts of reagents and supplies, which currently are limited. This study was conducted as a proof-of-concept to show the savings of group testing at one institution in a localized geographical location. Further studies are needed to evaluate the impact of group testing using different RNA extraction methods and PCR platforms for optimal diagnostics. In addition, other issues will need to be considered such as changes required to the laboratory information management system (LIMS) for reporting, the cost involved in performing verification testing as defined by the FDA to add a pooling procedure to laboratory testing to meet regulatory standards, and changes to the work flow in the laboratory.

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Table 1. Comparison of costs between individual compared to pooled specimens for the detection of SARS-CoV-2.

Category	Total cost	
	Individual [a]	Pooled [b]
Reagents and Consumables		
RNA extraction [c]	$ 42,503	$ 22,408
RT-PCR [d]	$ 25,141	$ 13.255
Labor [e]		
Set-up for original pools [f]	$ 0	$ 1,128
RNA extraction [g]	$ 6,035	$ 3,181
RT-PCR [h]	$ 3,017	$ 1,590
Reporting [i]	$ 4,225	$ 4,225
(Total costs for labor)	($13,277)	($10,124)
Total cost	**$ 80,921**	**$ 45,787**

[a] Based-on testing 4,630 individual specimens; [b] Based-on testing 2,441 individual specimens (926 original pools and 1515 individuals from split pools); [c] Based on an average kit/unit cost of $9.18 for RNA extraction kit, AVL buffer, and consumables; [d] Based on an average cost of $5.43 for RT-PCR mix, primers/probes, and consumables; [e] The average technologist salary plus benefits used for this analysis was $36.50 per hour; [f] Time required to prepare 926 pools (5 specimens per pool) was calculated at 30.9 hours; [g] Time required was based on 14 samples per extraction run at 0.5 hours; [h] Time required was based on 28 samples per PCR run at 0.5 hours; [i] Time required for reporting was determined to be 1.5 minutes per specimen.