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LETTER TO THE EDITOR

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Comparison of three commercial SARS-CoV-2 assays for pooled testing of deep throat saliva for surveillance of patients attending general outpatient clinics

To the Editor,

Various studies have demonstrated that deep throat saliva (DTS) is comparable with nasopharyngeal (NP) specimens for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by real-time polymerase chain reaction.[1–3] In Hong Kong, DTS is widely used to enhance surveillance on coronavirus disease 19 (COVID-19) because of its ease of collection. Recently, we have demonstrated that both the Xpert Xpress SARS-CoV-2 assay (Cepheid) and the Panther Fusion (PF) SARS-CoV-2 assay have excellent agreement with the LightMix SarbecoV E-gene assay (TIB MolBiol) when tested on DTS.[4,5] During the COVID-19 third wave which began in early July 2020, the daily number of the general outpatient clinic (GOPC) DTS tested by our laboratory surged up to 700. A review of the LightMix E-gene results within June 28, 2020 to July 28, 2020 revealed that out of 5642 GOPC DTS tested, the positive rate was 0.39% with C_t values ranged from 12.60 to 31.63. These data suggested that pooled testing on DTS from GOPC is favorable.

Potential shortage of diagnostic kits and assay failure as a result of continuous virus mutation pose a challenge. To prepare for the fourth wave, we assessed the suitability of pooled testing on DTS from GOPC with our existing diagnostic platforms. Food and Drug Administration (FDA) has issued pooling guidance for SARS-CoV-2 pooled sample testing.[6] Sample pooling protocol for PF assay on its validated specimen types has recently been approved by FDA[7] and pool testing with the Xpert Xpress SARS-CoV-2 assay on NP swab has also been reported.[8,9] However, the performance of all three assays on pooled DTS has not been reported and was therefore compared in this study.

From July 31, 2020 to August 7, 2020, 11 positive DTS received from patients attending GOPC were detected with our routine LightMix E-gene assay. All DTS tested in this study were pretreated with methods stated earlier.[4] Using a pool size of 5 as recommended by FDA, 11 pools were generated manually by mixing 0.25 ml of one positive sample with 0.25 ml each of four known negative samples. The final volume was 1.25 ml. These pools, together with the individual positive sample in each pool, were tested in parallel with the LightMix E-gene, Xpert Xpress assay, and PF assay by following the manufacturer's instructions. Nucleic acids for the LightMix E-gene were extracted with the MagMax 96 Viral RNA Kit (Thermo Fisher Scientific) as described previously.[1]

All 11 pools were tested positive by the LightMix E-gene and Xpert Xpress assay (Table 1). For the PF assay which targeted at ORF 1ab, the weak positive sample in pool 4 was tested negative but SARS-CoV-2 RNA in the original sample was detected with C_t = 35.6 (Table 1). The theoretical C_t shift (ΔC_t) is $\text{Log}_2(n)$ for a pool size of n.[6] ΔC_t of most of the pools tested with the LigthtMix E-gene and the Xpert Xpress assay were close to this expected shift of 2.3 with mean ΔCt = 2.16 and 2.5, respectively (Table 1). The ΔC_t was up to 5.06 and 4.9 for the LightMix E-gene and Xpert Xpress assay respectively (Table 1). Such findings were comparable with those reported by others who had tested on NP samples.[8–11] Interesting results were observed for the PF assay. It showed great variation in ΔC_t (up to 8.5, Table 1) and despite dilution by pooling, C_t values of some pooled samples were even lower. Unlike other automated liquid handlers with liquid level sensing near the liquid surface, the pipettor of the PF analyzer penetrates through the cap of the lysis tube and starts aspiration from near its bottom. The possibility of aspirating the relatively viscous part of the sample near the bottom of the lysis tube might account for this. If the positive sample was mixed with less viscous samples in the pool, a reduction in sample viscosity would lead to more accurate aspiration, and, hence, a lower Ct in the pool. To further investigate specimen matrix related variability, we applied the same pooling protocol to test three positive NP specimens. PF assay Ct of these samples were 19.9, 25.2, and 34.5, respectively. ΔCt variability was much lower (−0.1, 2.9, and 3.6, respectively) and agreed with those reported by Das et al.[12] Precision of each assay for pooled DTS testing was not tested as sample volume was inadequate. According to the manufacturer, the sample in the lysis tube is stable at 2–8°C for 3 months. When samples in the PF lysis tube stored at this temperature range were retested, results were reproducible. Since the three assays were tested in parallel with the same pooled DTS, our results suggested that for surveillance of SARS-CoV-2 infection by pooled testing on DTS among

TABLE 1 Comparison of the original C_t for positive DTS tested with three commercial platforms with their corresponding C_t in pooled samples

	LightMix E-gene assay			Xpert Xpress SARS-CoV-2 assay			Panther Fusion SARS-CoV-2 assay		
Pool No.	C_t of pool	C_t of original	ΔC_t	C_t of pool (E-gene/N2 gene)	C_t of original (E-gene/N2 gene)	ΔC_t (E-gene/N2 gene)	C_t of pool (ORF 1ab)	C_t of original (ORF 1ab)	ΔC_t
1	18.08	15.55	2.53	17.8/20.5	15.3/18.1	2.5/2.4	25.0	16.5	8.5
2	21.81	19.32	2.49	22.2/25.0	22.5/26.7	−0.3/−1.7	26.5	29.2	−2.7
3	29.55	27.11	2.44	29.2/31.9	26.7/29.0	2.5/2.9	30.9	27.9	3.0
4	35.00	35.00	0.00	37.3/40.3	Negative/37.4	−/2.9	Negative	35.6	–
5	30.83	28.62	2.21	32.9/36.1	28.5/31.4	4.4/4.7	32.7	38.1	−5.4
6	22.04	20.62	1.42	21.5/23.7	20.5/22.7	1.0/1.0	22.9	22.2	0.7
7	34.49	29.43	5.06	34.3/38.0	30.5/33.1	3.8/4.9	35.5	38.1	−2.6
8	25.47	23.05	2.42	23.8/26.8	21.2/24.1	2.6/2.7	24.4	21.8	2.6
9	28.50	27.51	0.99	26.5/29.3	24.4//26.9	2.1/2.4	30.4	30.2	0.2
10	29.65	29.03	0.62	28.6/31.6	26.2/29.1	2.4/2.5	35.9	36.6	−0.7
11	25.59	22.03	3.56	24.7/27.3	21.2/24.1	3.5/3.2	30.2	34.1	−3.9
ΔC_t mean (range)	2.16 (0.00–5.06)			2.5/2.5 (−0.3 to 4.4/−1.7 to 4.9)			0.0 (−5.4 to 8.5)		

Abbreviations: DTS, deep throat saliva; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Note: Remark: $\Delta C_t = C_t$ of pool − C_t of original

patients attending GOPC, the LightMix E-gene and Xpert Xpress SARS-CoV-2 assays are more suitable while testing by PF assay requires further study.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Planned and conducted experiments, performed data analysis, and drafted the manuscript: Yolanda I.I. Ho. *Conceptualized the comparison and co-writing the manuscript*: Ann H. Wong. *Data curation*: Kevin P. S. Tang. *Methodology validation*: River C. W. Wong. *Project coordination*: Eddie C. M. Leung. *Conceptualization, review, and final approval of the submitted manuscript*: Raymond W. M. Lai. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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