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Performance evaluation of Panther Fusion SARS-CoV-2 assay for detection of SARS-CoV-2 from deep throat saliva, nasopharyngeal, and lower-respiratory-tract specimens

To the Editor,

Tremendous increase in workload due to coronavirus disease 2019 (COVID-19) pandemic has caused intense strain on laboratory service. To enhance testing capacity, installation of high-throughput platform with short hands-on time becomes essential. The Panther Fusion severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) assay (PF assay) (Hologic, Inc.) operated in Panther Fusion system (PF system) is one of the options. It is validated with nasopharyngeal (NP), oropharyngeal and bronchoalveolar lavage (BAL) specimens while other sample types like deep throat saliva (DTS) and lower respiratory tract (LRT) specimens besides BAL are not validated. This study aims to evaluate the diagnostic performance of PF assay for detection of SARS-CoV-2 in comparison to the TIB-Molbiol LightMix SarbecoV E-gene assay (TIB-Molbiol assay) (TIB-Molbiol) using DTS, NP, and LRT specimens.

One hundred and fifyt-eight specimens (87 positive and 71 negative specimens) collected from 142 patients with suspected COVID-19 disease were tested. These included 60 NP, 59 DTS, and 39 LRT specimens (including 26 sputum, 12 tracheal aspirate, and 1 BAL). The median age of patients was 49 (interquartile range, 32–70) with 47.2% of female and proportion of in-patients was 75.4%. One hundred and nine of them were prospective samples and the remaining ones were archived samples stored at −70°C. All positive samples were confirmed by reference laboratory as published previously.[1,2] Results of PF assay were compared with TIB-Molbiol assay. Discordant results were resolved with Xpert Xpress SARS-CoV-2 assay (Xpert Xpress assay) (Cepheid).

NP samples were collected in 3 ml viral transport medium. Both DTS and LRT specimens were collected in sterile plain bottles. The 87 samples tested positive by TIB-Molbiol assay spanned the full range of cycle threshold (C_t) scores ranged from 13.57 to 38.58 (n = 21 with C_t < 20, n = 35 with C_t 20–30 and n = 31 with C_t > 30).

DTS and LRT specimens were pretreated as published previously[2] while NP specimens were tested without pretreatment. TIB-Molbiol assay was performed as described previously[1] and PF assay was performed according to manufacturer's instruction except supernatant was used for DTS or LRT specimens.

Positive percent agreement (PPA), negative percent agreement (NPA) and Cohen's kappa were determined by using consensus results as reference method. Statistical analysis was performed by

TABLE 1 Performance of the Panther Fusion SARS-CoV-2 assay by specimen types

Panther Fusion SARS-CoV-2 assay	Consensus result: Detected	Consensus result: Not detected	PPA (95% CI)	NPA (95% CI)	Cohen's kappa value (95% CI)
Nasopharyngeal					
Detected	27	0	96.43%	100%	0.97
Not detected	1	32	(81.65%–99.91%)	(89.11%–100%)	(0.90–1.00)
Lower respiratory tract					
Detected	28	0	100%	100%	1.00
Not detected	0	11	(87.66%–100%)	(71.51%–100%)	(1.00–1.00)
Deep throat saliva					
Detected	24	0	96.00%	100%	0.97
Not detected	1	34	(79.65%–99.90%)	(89.72%–100%)	(0.90–1.00)
Overall sample types					
Detected	79	0	97.53%	100%	0.97
Not detected	2	77	(91.36%–99.70%)	(95.32%–100%)	(0.94–1.00)

Abbreviations: CI, confidence interval; NPA, negative percent agreement; PPA, positive percent agreement; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

MedCalc 19.4.1. Cohen's kappa value greater than 0.81 was interpreted as very good agreement.

Results of PF assay were compared to those of TIB-Molbiol assay, there were eight samples with discrepancy and were resolved by testing with Xpert Xpress assay. Using consensus results as gold standard, PPA of NP, LRT, and DTS specimens were 96.43%, 100%, and 96.00%, respectively. The overall PPA and NPA were 97.53% and 100%, respectively and overall Cohen's kappa value was 0.97 (Table 1).

Among the eight samples with discrepancy, six of them initially tested positive by TIB Miobiol assay were tested negative by both PF and Xpert Xpress assay. These samples were hence regarded as false-positive. Despite the manufacturer of TIB Miobiol regarded results with $C_t < 36$ as positive, when use as an initial screening test in our laboratory, sample with any C_t value will be tested supplementary with Xpert Xpress assay. The high C_t values of two discrepant samples (36.49 and 37.90, respectively) suggested that their viral loads were low.

Studies have verified the LoD of PF assay ranged from 62.5 to 100 copies/ml.[3–5] When tested with SARS-CoV-2 synthetic quantified standard from Exact Diagnostics (BioRad), we found that the LoD of both TIB-Molbiol and PF assays were 100 copies/ml while that of Xpert Xpress assay was 50 copies/ml.[2]

Detection of other respiratory viruses with the respiratory panels on PF System for upper and LRT samples including BAL have been evaluated in various studies.[6–8] For SARS-CoV-2, several studies have assessed the diagnostic performance of PF assay with NP specimens.[4,9–11] Performance of PF assay with DTS and LRT samples other than BAL has not been reported. In this study, we demonstrated that in addition to NP specimens, performance of PF assay for DTS and various LRT specimens was good and comparable to reference method.

Presence of mucus in the lysis tube will lead to pipetting errors and invalid results. Similar problem has been reported by Szymczak et al.[3] To resolve such problem, before adding sample into the lysis tube, we recommend to centrifuge the homogenized DTS & LRT specimens according to the pretreatment protocol reported in our previous study.[2]

In conclusion, the Panther Fusion SARS-CoV-2 assay has comparable performance with the reference method. With its capability of random access and high-throughput, it maximizes the flexibility of SARS-CoV-2 testing in clinical laboratories.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBTUONS

River Chun-Wai Wong: planned and conducted experiments, performed data analysis, and drafted the manuscript. Ann Han Wong: supervision, review, and editing the manuscript. Yolanda Iok-Ieng Ho: methodology, review, and editing the manuscript. Eddie Chi-Man Leung: coordination, review, and editing the manuscript. Raymond Wai-Man Lai: project administration and supervision. All authors discussed the results and contributed to the final manuscript.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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Abbreviations: CI, confidence interval; NPA, negative percent agreement; PPA, positive percent agreement; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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