
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Clinical Virology 131 (2020) 104593

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Short communication

# Evaluation on testing of deep throat saliva and lower respiratory tract specimens with Xpert Xpress SARS-CoV-2 assay

River Chun-Wai Wong *, Ann Han Wong, Yolanda Iok-Ieng Ho, Eddie Chi-Man Leung, Raymond Wai-Man Lai

*Department of Microbiology, Prince of Wales Hospital, Hong Kong, China*

ARTICLE INFO

*Keywords:*
SARS-CoV-2
Xpert Xpress SARS-CoV-2 assay
Deep throat saliva
Lower respiratory tract specimens
Non-validated specimen types

ABSTRACT

*Background:* Xpert® Xpress SARS-CoV-2 assay is only validated on nasopharyngeal specimens for detection of SARS-CoV-2. Other specimen types such as deep throat saliva (DTS), also known as posterior oropharyngeal saliva and lower-respiratorytract specimens (LRT) including sputum, tracheal aspirate and bronchoalveolar lavage are not validated. These non-validated specimen types, however, do have significant diagnostic value.
*Objective:* Evaluate the performance of Xpert Xpress SARS-CoV-2 assay for detection of SARS-CoV-2 from DTS and LRT specimens.
*Methods:* 162 specimens from 158 patients with suspected COVID-19 disease were tested with Xpert Xpress SARS-CoV-2 assay. These included 120 DTS and 42 LRT specimens i.e. 35 sputum, 6 tracheal aspirate and one bronchoalveolar lavage. Results were compared to those by the TIB-Molbiol LightMix® SarbecoV E-gene assay.
*Results:* Xpert Xpress SARS-CoV-2 assay has satisfactory performance when compared with reference method. The positive percent agreement (PPA) of DTS and LRT specimens were 98.86 % & 100 % respectively while the negative percent agreement (NPA) was 100 % for both DTS and LRT specimens.
*Conclusions:* This study demonstrated with appropriate sample pre-treatment, Xpert Xpress SARS-CoV-2 assay can be used to test on non-validated specimen types including DTS & LRT specimens.

## 1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus responsible for cluster of atypical pneumonia outbreak in Wuhan, China in December of 2019. With its spread globally, World health Organization (WHO) declared pandemic of Coronavirus disease 2019 (COVID-19) in March 2020. It is crucial to confine the spread of COVID-19 in densely populated cities like Hong Kong as outbreak will potentially overwhelm the healthcare system. Provision of diagnostic test for rapid detection of SARS-CoV-2 is the mainstay for early diagnosis, prompt implementation of infection control measures and epidemiological tracking in both hospital setting and community. Nasopharyngeal specimens are recommended by WHO for detection of SARS-CoV-2. Collection of nasopharyngeal samples require trained personnel wearing full personal protective equipment and conduct in airborne isolation facility to minimize the risk of disease transmission. Previous studies have shown that deep throat saliva (DTS) can be considered as an alternative specimen for detection of SARS-CoV-2 [1–5]. Lower-Respiratory-Tract (LRT) specimens including sputum, tracheal aspirate and bronchoalveolar lavage are considered better than upper respiratory tract specimens for detection of SARS-CoV-2 in patients with lower respiratory symptoms [6]. However, most commercial assays including Cepheid Xpert Xpress SARS-CoV-2 assay (Cepheid, Sunnyale, CA, USA) have not been validated for sample types other than nasopharyngeal specimens. Mucus and high viscosity of DTS and LRT specimens render them difficult to be processed by automated sample-to-answer platform.

Cepheid Xpert Xpress SARS-CoV-2 assay is a fully automated *in vitro* diagnostic test performed on GeneXpert platform (Cepheid, Sunnyale, CA, USA). Two targets are included: envelope gene (E gene) and nucleocapsid gene (N2 gene). Results will be interpreted as positive if both targets or N2 gene alone are detected while if E gene alone was detected, result will be interpreted as presumptive positive. Recently, approval for Emergency Use Authorization (EUA) has been granted by Food and Drug Administration (FDA). Previous studies shown that it has satisfactory performance in detection of SARS-CoV-2 [6–9]. In this

* Corresponding author at: Department of Microbiology, Prince of Wales Hospital, Ngan Shing St, Shatin, N T, Hong Kong, China.
*E-mail address:* wcw372@ha.org.hk (R.C.-W. Wong).

https://doi.org/10.1016/j.jcv.2020.104593
Received 7 August 2020; Received in revised form 10 August 2020; Accepted 14 August 2020
Available online 16 August 2020
1386-6532/

study, we aimed to evaluate the performance of Xpert Xpress SARS-CoV-2 assay for detection of SARS-CoV-2 from DTS and LRT specimens with sample pre-treatment before testing.

## 2. Methods

### 2.1. Study design

A total of 162 samples (119 positive and 43 negative specimens) collected from 158 patients with suspected COVID-19 were tested. These included 120 DTS and 42 LRT i.e. 35 sputum, 6 tracheal aspirate and one bronchoalveolar lavage. The median age of patients was 46 (Interquartile Range (IQR) of 35 (28–63)) with 56.3 % (89/158) was female. Among them, 58.9 % (93/158) were from Accident & Emergency Department (AED), 29.7 % (47/158) were in-patients and 11.4 % (18/158) were out-patients. Out of the 162 samples, 74 of them were archived samples kept frozen at -70℃ and the remaining samples (n = 88) were prospective samples. Upon receipt, all samples were screened with our standard-of-care (SOC) nucleic acid amplification test (NAAT) which is the TIB-Molbiol LightMix® SarbecoV E-gene assay (formerly named as Modular SARS and Wuhan CoV E-gene, Berlin, Germany) as published previously [2]. All positive cases were confirmed by the reference laboratory of Hong Kong (Public Health Laboratory Service Branch, PHLSB).

### 2.2. Sample collection and selection

Both DTS and LRT specimens were collected in plain sterile container, transported to laboratory on the same day and tested promptly. The 119 samples tested positive by the SOC NAAT span the entire range of cycle threshold (Ct) scores with Ct value ranged from 11 to 38 (in which 26 % with Ct <20, 50 % with Ct 20–30 and 24 % with Ct >30).

### 2.3. Sample preparation and test procedures

#### 2.3.1. DTS specimens

Sterile Phosphate-Buffered Saline (PBS) (pH 7.2–7.4) was added into neat saliva specimens in the ratio of 1:1. Sample was then vortexed for homogenization and allowed to settle for 5−10 min. Two mL of the homogenized sample was transferred to another vial for centrifugation at 2000 *g* for 5 min.

#### 2.3.2. LRT specimens

One mL of the specimen was added to 3 mL of in-house prepared Maintenance Medium (MM) (10X Minimum Essential Medium (MEM), 200 mM glutamine, 1 M HEPES, 7.5 % NaHCO3, 12 mg gentamicin, 0.5 mg amphotericin B, 10,000 units penicillin, 10 mg streptomycin, pH 7.1–7.4) and the mixture was emulsified by pipetting up and down, followed by centrifugation at 2000 *g* for 5 min.

For each sample, supernatant was used for testing with both the SOC NAAT and Xpert Xpress SARS-CoV-2 assay according to manufacturer's instruction. Samples were loaded on GeneXpert Dx system with running time around 45 min.

### 2.4. Statistical analysis

Agreement statistics in comparison with SOC NAAT was applied. PPA, NPA and Weighted Kappa were determined by Inter-rater agreement and diagnostic test (2 × 2 table) by using MedCalc 19.4.1 (Ostend, Belgium). Values for Cohen's Kappa coefficient (κ) of <0.20, 0.21–0.40, 0.41–0.60, 0.61–0.80 and 0.81–1.00 were characterized as poor, fair, moderate, good and very good agreement respectively.

## 3. Results

Xpert Xpress SARS-CoV-2 assay has very good agreement with SOC NAAT. The weighted Kappa values were 0.98 and 1.00 for DTS and LRT specimens. The overall performance on both non-validated specimen types has weighted Kappa value 0.98, PPA of 99.16 % and NPA of 100 % (Table 1). Discrepancy was only observed in one archived DTS specimen (Table 1).

## 4. Discussion

The SOC NAAT only allows batch testing. With the use of Xpert Xpress SARS-CoV-2 assay as a complementary test, it will allow rapid testing of ad-hoc samples received from AED & intensive care unit and provide round-the-clock service for samples received after batch testing cut-time. Similar testing algorithm has been adopted in our laboratory for testing of influenza [10].

The overall performance of Xpert Xpress SARS-CoV-2 assay was satisfactory when tested with DTS and LRT specimens. Review of the sample with discrepancy showed that it was sent from a known positive COVID-19 patient for disease monitoring. Such discrepancy might be attributed to the low viral load in this sample (Ct = 34.47) as well as potential RNA degradation due to repeated freeze and thaw.

With the use of Exact Diagnostics SARS-CoV-2 reference standard (BioRad, USA), the analytical 95 % lower limit of detection of Xpert Xpress SARS-CoV-2 assay and TIB-Molbiol LightMix® SarbecoV E-gene assay was determined as 50 and 100 copies/mL respectively.

To our knowledge, this is the first report to evaluate the use of PBS for sample homogenization of DTS prior to testing with Xpert Xpress SARS-CoV-2 assay. Previous study used viral transport medium (VTM) for sample homogenization [1]. One study recommended direct transfer of the liquid, non-viscous part of neat sample into the cartridge without pre-treatment [3]. Our previous experience with Xpert Xpress Flu/RSV assay showed that direct transfer of LRT samples, in particular sputum, into the cartridge resulted in a high error frequency. This study was the first to evaluate the testing of LRT specimens (mainly sputum) with pre-treatment to minimize potential invalid results or instrument error. These procedures can minimize the mucus and viscous substances among non-validated specimen types and broaden the testing scope of Xpert Xpress SARS-CoV-2 assay.

With spiked samples, Rodino et al. demonstrated that saline, PBS or MEM can be used as alternative for VTM in SARS-CoV-2 testing [11].

**Table 1**
Overall agreements between Xpert Xpress SARS-CoV-2 assay and reference method among 162 deep throat saliva and lower–respiratory-tract specimens for detection of SARS-CoV-2.

| Sample type | | Reference method | | (95 % CI) | | |
|---|---|---|---|---|---|---|
| | | SOC NAAT | | | | |
| | Xpert Xpress | Detected | Not detected | Kappa (κ) | PPA | NPA |
| DTS | Detected | 87 | 0 | 0.98 | 98.86 % | 100 % |
| | Not detected | 1 | 32 | (0.94−1.00) | (93.83−99.97%) | (89.11−100%) |
| LRT | Detected | 31 | 0 | 1.00 | 100 % | 100 % |
| | Not detected | 0 | 11 | (1.00−1.00) | (88.78−100%) | (71.51−100%) |
| Overall | Detected | 118 | 0 | 0.98 | 99.16 % | 100 % |
| | Not detected | 1 | 43 | (0.95−1.00) | (95.41−99.98%) | (91.78−100%) |

Another study by Williams et al. showed that liquid Amies medium can be used for homogenization of saliva samples [4]. In this study, we demonstrated that both PBS and MM can be used for sample homogenization. LRT samples were suspended with MM for virus isolation in our routine practice, however, this service was obsoleted in February 2020. As in-house preparation for MM is labour intensive, by using PBS to replace MM for sample homogenization will be an effective way to save time and reduce manpower. Future study on usage of PBS for homogenization of LRT specimens will be conducted.

In conclusion, with appropriate sample pre-treatment before testing, both DTS & LRT specimens can be tested with the Xpert Xpress SARS-CoV-2 assay with results comparable to the SOC NAAT.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## CRediT authorship contribution statement

**River Chun-Wai Wong:** Conceptualization, Data curation, Formal analysis, Validation, Investigation, Writing - original draft, Writing - review & editing. **Ann Han Wong:** Supervision, Writing - review & editing. **Yolanda Iok-Ieng Ho:** Methodology, Writing - review & editing. **Raymond Wai-Man Lai:** Project administration, Supervision.

## Declaration of Competing Interest

The authors report no declarations of interest.

## Acknowledgements

We would like to thank all the technical staff who provide technical assistance and contribute to SARS-CoV-2 testing in our department.

## References

[1] J.H. Chen, et al., Evaluating the use of posterior oropharyngeal saliva in a point-of-care assay for the detection of SARS-CoV-2, Emerg. Microbes Infect. 9 (1) (2020) 1356–1359.
[2] E.C. Leung, et al., Deep throat saliva as an alternative diagnostic specimen type for the detection of SARS-CoV-2, J. Med. Virol. (2020).
[3] C. McCormick-Baw, et al., Saliva as an alternate specimen source for detection of SARS-CoV-2 in symptomatic patients using cepheid xpert xpress SARS-CoV-2, J. Clin. Microbiol. 58 (8) (2020).
[4] E. Williams, et al., Saliva as a noninvasive specimen for detection of SARS-CoV-2, J. Clin. Microbiol. 58 (8) (2020).
[5] S. Cheuk, et al., Posterior oropharyngeal saliva for the detection of SARS-CoV-2, Clin. Infect. Dis. (2020).
[6] M.J. Loeffelholz, et al., Multicenter evaluation of the cepheid Xpert Xpress SARS-CoV-2 test, J. Clin. Microbiol. 58 (8) (2020).
[7] W. Zhen, et al., Clinical evaluation of three sample-to-answer platforms for detection of SARS-CoV-2, J. Clin. Microbiol. 58 (8) (2020).
[8] D. Goldenberger, et al., Brief validation of the novel GeneXpert Xpress SARS-CoV-2 PCR assay, J. Virol. Methods 284 (2020), p. 113925.
[9] J.A. Lieberman, et al., Comparison of commercially available and laboratory-developed assays for in vitro detection of SARS-CoV-2 in clinical laboratories, J. Clin. Microbiol. 58 (8) (2020).
[10] Y.I.I. Ho, A.H. Wong, R.W.M. Lai, Comparison of the cepheid Xpert Xpress Flu/RSV assay to in-house flu/RSV triplex real-time RT-PCR for rapid molecular detection of Influenza A, Influenza B and respiratory syncytial virus in respiratory specimens, J. Med. Microbiol. 67 (11) (2018) 1576–1580.
[11] K.G. Rodino, et al., Evaluation of saline, phosphate-buffered saline, and minimum essential medium as potential alternatives to viral transport media for SARS-CoV-2 testing, J. Clin. Microbiol. 58 (6) (2020).