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Analytical sensitivity and specificity of the Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV assay

Sir,

The COVID-19 pandemic caused by SARS-CoV-2 is a public health emergency on a global scale, with over 85 million cases worldwide as of 6 January 2021.[1] Along with clear and decisive public health interventions, one of the cornerstones in controlling the pandemic is rapid and accurate diagnostic testing, with reverse transcription polymerase chain reaction (RT-PCR) testing considered the 'gold standard' testing method.[2] The clinical spectrum of COVID-19 is broad, with an overlap between COVID-19 clinical features and symptoms of other common respiratory viral infections such as influenza and respiratory syncytial virus (RSV).[3] Given the importance of early detection of cases of COVID-19, rapid discrimination between SARS-CoV-2 and other respiratory viruses is essential.

The Xpert Xpress SARS-CoV-2 assay (Cepheid, USA) has been used in many countries for the rapid detection of SARS-CoV-2, with high sensitivity and specificity,[4,5] including 89 remote point of care testing sites across Australia.[6] The assay detects both the pan-sarbecovirus E gene and the N2 region of the N gene specific to SARS-CoV-2 in approximately 45 minutes.[4] Recently, the Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV assay has received Emergency Use Authorization (EUA) from the United States Food and Drug Administration (FDA).[7] It is designed to detect and differentiate SARS-CoV-2, influenza A, influenza B and RSV in nasopharyngeal swabs, nasal swabs or nasal washes/aspirates and is used on GeneXpert Systems. Analytical results are available within 36 minutes and provide a single detected or not detected result for each virus type and a matching Ct value result for interpretation.

Here, we undertook a clinical and laboratory validation study to evaluate the analytical sensitivity and specificity of the Xpert Xpress SARS-CoV-2/Flu/RSV assay.

Testing was conducted at the Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL), The University of Melbourne at the Doherty Institute, Melbourne, Australia. In brief, SARS-CoV-2-positive nasopharyngeal or deep nasal swabs were obtained from routine clinical testing at MDU PHL, and stored SARS-CoV-2-negative nasopharyngeal or deep nasal swabs were obtained from the Department of Microbiology, Royal Melbourne Hospital (RMH), Melbourne, Australia.

All clinical samples were previously tested for SARS-CoV-2 using the AusDiagnostics Coronavirus Typing (8-well) panel (AusDiagnostics, Australia) at RMH, as previously described,[8] and using the Aptima SARS-CoV-2 assay (Hologic, USA) at MDU PHL, according to the manufacturer's instructions.

Analytical sensitivity for SARS-CoV-2 detection was assessed using two approaches. First, a 50 µL volume of quantified inactivated whole virus (SARS-CoV-2 Analytical Q Panel; Qnostics, UK) supplied as a standardised dilution series (6.0–1.7 log10 digital copies/mL) was spiked into universal transport media (UTM). Subsequently, 300 µL of spiked UTM was used in the Xpert Xpress SARS-CoV-2/Flu/RSV assay and also in the Xpert Xpress SARS-CoV-2 assay for comparison. The limit of detection (LOD) was determined, and all testing was performed in triplicate. Second, analytical sensitivity was also determined using heat-killed SARS-CoV-2 virus stock quantified at 1.04×10^5 $TCID_{50}$/mL, obtained from previously isolated SARS-CoV-2 in Melbourne.[9] Virus was diluted in saline, and 50 µL was spiked into universal transport media (obtained from the Media Preparation Unit, University of Melbourne). Three replicates at 10×, 1× and 0.1× LOD (LOD determined by Xpert Xpress SARS-CoV-2) were tested. Clinical sensitivity was assessed by testing 46 RT-PCR confirmed positive samples (previously tested on the Panther Fusion SARS-CoV-2 assay; Hologic), spanning a range of cycle Ct values between 18.2 and 36.1 (Table 1).

Cross-reactivity was assessed using a commercial panel of respiratory control organisms (NATRPC2-BIO; Zeptometrix, USA) comprising purified, intact virus particles and bacterial cells suspended in a matrix that mimics the composition of a clinical specimen. Cross-reactivity was also examined using gamma-irradiated influenza virus (A/Victoria/31/2020 and B/Darwin/58/2019), respiratory syncytial virus (RSV) A 16144363 and RSV B 15136810 and two seasonal human coronavirus strains OC43 and 229E obtained from the Victorian Infectious Diseases Reference Laboratory (VIDRL), Melbourne, Australia, and spiked into pooled nasopharyngeal swab samples that tested negative to SARS-CoV-2. Clinical specificity was assessed by testing 50 SARS-CoV-2 RT-PCR-negative samples obtained from patients with respiratory symptoms attending RMH.

Using the Qnostics SARS-CoV-2 panel, the LoD for SARS-CoV-2 with the Xpert Xpress SARS-CoV-2/Flu/RSV assay was 8.3 copies/mL, and for the Xpert Xpress SARS-CoV-2, assay was 8.3 copies/mL. Using dilutions of heat inactivated SARS-CoV-2, the LoD for the Xpert Xpress SARS-CoV-2/Flu/RSV assay was 0.002 $TCID_{50}$/mL, and for the Xpert Xpress, SARS-CoV-2 assay was 0.002 $TCID_{50}$/

Table 1 Sensitivity and specificity of the Xpert Xpress SARS-CoV-2/Flu/RSV test with the comparator SARS-CoV-2 assay (using the Aptima SARS-CoV-2 assay)

GeneXpert Ct[a]	GeneXpert[a]	RT-PCR[b]	Concordance (%)
Positive samples			
<20	21	21	100
20–25	10	10	100
26–30	9	9	100
>30	6	6	100
Negative samples			
N/A	50	50	100

[a] Tested on the Xpert Xpress SARS-CoV-2/Flu/RSV assay.
[b] Tested on the Aptima SARS-CoV-2 assay.

Table 2 Evaluation of limit of detection of the Xpert Xpress SARS-CoV-2/Flu/RSV assay using the Qnostics SARS-CoV-2 Analytical Q Panel and dilutions of heat-inactivated virus

Virus/Strain	Xpert Xpress SARS-CoV-2/Flu/RSV assay		Xpert SARS-CoV-2 assay	
	Limit of detection	Ct value[a]	Limit of detection	Ct value[a]
Qnostics panel				
SARS-CoV-2	8.33 copies/mL	39.6	8.33 copies/mL	N2 region 40.4 E gene 39.9
Inactivated virus				
SARS-CoV-2 (VIC01)	0.002 TCID50/mL	30.3	0.002 TCID50/mL	N2 region 41.0 E gene 33.8
Influenza A/Vic/31/2020	0.042 TCID50/ml	37.5	–	–
Influenza B/Darwin/58 2019	0.0004 TCID50/ml	37.6	–	–
RSV A 16144363	0.043 TCID50/ml	34.2	–	–
RSV B 15136810	0.022 TCID50/ml	34.9	–	–

[a] Cycle threshold, mean of three replicates.

mL. Agreement of the Xpert Xpress SARS-CoV-2/Flu/RSV assay with the Panther Fusion SARS-CoV-2 assay was 100%, across a range of Ct values (Table 1). Inactivated influenza A B and RSV were serially diluted in UTM; 10 µL, 25 µL and 50 µL of diluted virus were spiked into pooled negative swab matrix at approx 1×, 2× and 5× LOD (previously determined by MDU). Further limiting dilutions were prepared from the above spike preparations and diluted in negative swab matrix to determine LOD and were tested in triplicate. The LoD values for influenza and RSV strains are described in Table 2.

Using the Zeptometrix respiratory panel, all expected targets were detected using the Xpert Xpress SARS-CoV-2/Flu/RSV assay. In addition, no cross-reactivity was observed across any of the four assay channels in the Xpert Xpress SARS-CoV-2/Flu/RSV assay (SARS-CoV-2; influenza A; influenza B; RSV). Further, the Xpert Xpress SARS-CoV-2 assay did not display any cross-reactivity with the Zeptometrix panels.

Using heat-inactivated virus spiked into negative swab samples, no cross-reactivity was observed, and all expected positive samples were detected (Table 3).

Moreover, all 50 clinical samples that tested negative for SARS-CoV-2 at RMH also tested negative using the Xpert Xpress SARS-CoV-2/Flu/RSV assay, giving a negative percentage agreement of 100%.

Table 3 Cross-reactivity and specificity across reactivity testing of the Xpert Xpress SARS-CoV-2/Flu/RSV

Panel/Virus	Xpert Xpress SARS-CoV-2/Flu/RSV				Xpert SARS-CoV-2 assay
	SARS-CoV-2	Influenza A	Influenza B	RSV	
NATRPC2-BIO, Zeptometrix, pool 1					
Adenovirus Type 1	–	+	–	–	–
Adenovirus Type 3					
Adenovirus Type 31					
C. pneumoniae (CWL-029)					
Influenza A 2009 H1N1 (A/NY/02/2009)					
Influenza A H3N2 (A/Brisbane/10/07)					
Human metapneumovirus Type 8 (Peru6-2003)					
M. pneumoniae (M129)					
Parainfluenza Type 1					
Parainfluenza Type 4					
Rhinovirus Type 1A					
NATRPC2-BIO, Zeptometrix, pool 2					
B. parapertussis (A747)	–	+	+	+	–
B. pertussis (A639)					
Coronavirus (229E)					
Coronavirus HKU-1 recombinant					
Coronavirus (NL63)					
Coronavirus (OC43)					
Influenza A H1N1 (A/New Cal/20/99)					
Influenza B (B/Florida/02/06)					
Parainfluenza Type 2					
Parainfluenza Type 3					
RSV A (2006 isolate)					
Inactivated virus					
SARS-CoV-2 (VIC01)	+	–	–	–	+
Coronavirus OC43	–	–	–	–	–
Coronavirus 229E	–	–	–	–	–
Influenza A/Vic/31/2020	–	+	–	–	–
Influenza B/Darwin/58 2019	–	–	+	–	–
RSV A 16144363	–	–	–	+	–

Here, we provide a comprehensive evaluation of the performance of the new Xpert Xpress SARS-CoV-2/Flu/RSV assay. We demonstrate that the performance characteristics of this assay are comparable to the existing Xpert Xpress SARS-CoV-2 assay for the detection of SARS-CoV-2, with similar LoD and specificity for both assays. Further, our results are in keeping with a recent study by Mostafa *et al.* that demonstrated an overall positive percentage agreement for the SARS-CoV-2 target of 98.7% when compared to a range of other RT-PCR platforms (including the Xpert Xpress SARS-CoV-2 and Hologic Panther Fusion SARS-CoV-2 assays), and a negative percentage agreement of 100% with other targets (influenza A/B and RSV) showing 100% total agreement.[6] More recently, Lueng *et al.* also reported high concordance between the Xpert Xpress SARS-CoV-2 and Xpert Xpress SARS-CoV-2/Flu/RSV assay.[10] For end-users, one notable difference exists between the two Xpert assay types. The single Xpert Xpress SARS-CoV-2 assay reports N2 and E cycle threshold values independently within the analyte results for each sample. The test also calls out three result types: positive, presumptive positive and not detected. In contrast, the Xpert Xpress SARS-CoV-2/Flu/RSV assay reports a single Ct value for each of the four pathogens and each result is reported as detected or undetected. While some purchase cost differential is likely between the single Xpert SARS-CoV-2 assay and its 4plex cousin, commercial pricing had not been finalised at the time of writing.

More broadly, rapid and reliable testing for SARS-CoV-2 has been critical to the COVID-19 response. Given the overlap in clinical symptoms between COVID-19 and other respiratory illnesses such as influenza, rapid differentiation of causative pathogens is essential in ensuring appropriate clinical and public health control measures. This is particularly important for responses to COVID-19, i.e., rapid isolation, treatment intervention and contact tracing. Moreover, as COVID-19 vaccination is gradually implemented globally, and national and international movement resumes over the next few years, inevitably, the circulation of other respiratory viruses such as influenza and RSV will increase.

There is both an immediate and ongoing need for rapid multiplex testing, particularly testing that can be performed near or at the point of care, such as the Xpert Xpress SARS-CoV-2/Flu/RSV assay which requires minimal sample preparation steps. Use of this assay in combination with a validated viral inactivating transport medium[11] also reduces pathogenic exposure risk for test operators at that point of care or in laboratory settings. Possible uses include testing in aged care and emergency hospital settings, where rapid diagnosis will enable triage of patients to appropriate treatment, isolation rooms, and prompt implementation of infection control measures. Remote or low resource locations could also benefit from either Xpert COVID molecular point of care test given they may reduce the need for laboratory reflex testing and shorten clinical decision-making time. In addition, the presence of such a test could avert unnecessary isolation and costly evacuation of some individuals to larger health facilities.

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