Cepheid Xpert Xpress Flu/RSV evaluation performed by minimally trained non-laboratory operators in a CLIA-waived environment

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ARTICLE INFO

ABSTRACT

The COVID-19 pandemic highlighted the significance of readily available and easily performed viral testing for surveillance during future infectious pandemics. The objectives of this study were: to assess the performance of the Xpert Xpress Flu and/or RSV test, a multiplex PCR assay for detecting influenza A and B virus and respiratory syncytial virus nucleic acids in respiratory tract specimens, relative to the Quidel Lyra Influenza A+B assay and the Prodesse ProFlu+ assay, and the system's ease of use by minimally trained operators. Overall, the Xpert Xpress Flu/RSV test demonstrated a high positive and negative percent agreement with the comparator assays, and was easy to use and interpret results, based on the operators' feedback. We concluded that the Xpert Xpress Flu/RSV test is sensitive, specific, and easy to use for the diagnosis of influenza and RSV by minimally trained operators and can be a valuable tool in future infectious clusters or pandemics.

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1. Introduction

It has been speculated, even before the current COVID-19 pandemic, that an influenza virus would result in a worldwide pandemic. After the 2009 H1N1 pandemic, there has been heightened anticipation and preparedness for the next influenza pandemic [1]. The burden of influenza viruses and respiratory syncytial virus (RSV) remains substantial, particularly in vulnerable populations. Influenza viruses are major contributors to hospitalizations among young children, and RSV is the most common pathogen identified in young children, particularly those under 5 years of age, with acute lower respiratory infections [2,3]. A recent study found that RSV may account for 10% of acute respiratory infections in adults, and up to 14% of acute respiratory infections in adults with chronic lung disease or history of transplantation [4]. Often, both influenza and RSV exhibit overlapping symptoms in adults. Therefore identification of both provides an opportunity for antiviral stewardship in an outpatient setting at a time when regulatory organizations are beginning to examine out-patient antimicrobial stewardship [5–7]. The rapid

detection of influenza viruses and RSV in acute care visits to clinics, urgent care centers and emergency departments, would improve patient throughput, aid provider decision-making, and lead to reduced transmission of those viruses [8]. Further, rapid detection is crucial during outbreaks and will be essential in any future pandemics, as evidenced by the COVID-19 pandemic.

The Xpert Xpress Flu/RSV test is a rapid multiplex real-time PCR test capable of detecting and differentiating influenza A (FLUAV), influenza B (FLUBV), and RSV nucleic acids from nasal (NS) swabs and nasopharyngeal (NP) swabs [9]. This test was designed for use by non-laboratory personnel in a Clinical Laboratory Improvement Amendments (CLIA)-Waived (CW) setting. The primary objective of this study was to assess the performance of the Xpert Xpress Flu/RSV test relative to the Quidel Lyra Influenza A+B assay (Quidel Corporation, San Diego, CA, USA) and the Prodesse ProFlu+ (Hologic, Inc., Marlborough, MA, USA) assays. The Lyra assay was Food and Drug Administration (FDA) cleared for the qualitative detection and differentiation of FLUAV and FLUBV RNA in NS and NP swabs. The Prodesse assay is cleared for the detection of RSV in NP swabs; therefore, a separate validation study was conducted in order to use it as a comparator assay for NS specimens.

The second objective of this study was to assess the usability and ease of result interpretation of the GeneXpert Xpress system in a CW setting by minimally trained, non-laboratory operators. This would evaluate the system's applicability for use in settings where a rapid diagnosis of RSV or influenza would improve patient throughput and aid in clinical and therapeutic decision-making.

After the comparison study, we analyzed cycle threshold (Ct) values obtained from Xpert Xpress Flu/RSV positive tests to evaluate whether differences exist among specimen types, patient ages, patient sex, and health care settings.

2. Materials and methods

2.1. Study design

During the 2016-2017 respiratory virus season (October 2016-March 2017), a study was conducted in CW-intended user environments, including emergency departments, urgent care centers, and walk-in clinics in the United States. A total of 16 sites participated: 13 were specimen collection and GeneXpert Xpress testing sites; 2 sites served solely as reference laboratories for comparator method testing; and 1 site collected specimens and performed both GeneXpert Xpress and comparator method testing. Institutional Review Board (IRB) approval or waiver was obtained at collection sites prior to study initiation.

The Xpert Xpress Flu/RSV test was performed by non-laboratory health care personnel, who had no prior experience using the GeneXpert or other moderately complex testing methods. The CW operators were not specifically trained on the operation of the GeneXpert Xpress System or the Xpert Xpress Flu/RSV test. The operators were instead provided with the reference guides that were included with the testing systems and test kits. The operators were responsible for setting up the Xpert Xpress Flu/RSV tests, testing the assay's controls and proceeding with study testing on their own. Additionally, operators were instructed not to discuss the test with any other operators or otherwise train or supervise each other.

Quality control assays for the Xpert Xpress Flu/RSV test consisted of 1 negative and 1 positive external control sample with all the targets for each FLUAV, FLUBV, and RSV sample (Zeptometrix, Buffalo, NY, USA). Study specimens were not tested until the correct control results were determined to be acceptable. Controls were tested on each instrument daily, by the operator performing the testing that day, and were required when a new lot of Xpert Xpress Flu/RSV test reagents were used.

2.2. Assay comparison

Nasal and NP swab specimens were prospectively collected from consented study participants who presented with signs or symptoms of respiratory infection. Each participant provided either a NS or NP swab. Study inclusion criteria consisted of signs or symptoms of respiratory infection (e.g., rhinorrhea, cough, etc.), documentation of informed consent from patients (or from a parent or legal guardian for minors younger than 18 years of age, who also provided assent based on IRB requirements). The patient or guardians were provided their Bill of Rights where applicable. Patients previously enrolled in the study were excluded. Nasal swabs were collected from both nostrils of the participant and NP swabs were collected through 1 nostril. Specimens were stored at 2-8°C following collection until all Xpert Xpress Flu/RSV testing was completed. Xpert Xpress Flu/RSV testing, including repeat testing, was performed within 24 hours of specimen collection. If the initial Xpert Xpress Flu/RSV test result was indeterminate, a single retest was performed using a new aliquot from the original specimen tube and a new Xpert Xpress Flu/RSV cartridge. If another indeterminate result was obtained by the repeat testing, no additional testing was performed, and the result was reported as "indeterminate" and excluded from the final analysis.

The Lyra comparator testing was performed within 72 hours of specimen collection. The Prodesse comparator testing was performed on specimens with sufficient volume which had been frozen after Xpert Xpress Flu/RSV testing was completed. Prodesse testing was initiated within 72 hours of the specimen thawing. For specimens with discordant results between the Xpert Xpress Flu/RSV test and Lyra and Prodesse assays, bi-directional gene sequencing was performed. Sequencing was performed using different primers from those used in the Xpert Xpress Flu/RSV test (AGCT, Inc., Wheeling, IL, USA), which target the FLUAV matrix gene, polymerase B2, and polymerase A gene; FLUBV matrix, and non-structural genes; and RSV nucleocapsid gene. While the Lyra assay used FLUAV matrix gene and FLUBV neuraminidase gene, and Prodesse used RSV polymerase gene.

Positive percent agreement (PPA) and negative percent agreement (NPA) of the Xpert Xpress Flu/RSV test was determined relative to the Lyra and Prodesse assays. Positive and negative agreement for influenza was defined as both the Xpert Xpress Flu/RSV and Lyra assay yielding the same positive or negative over the total number of results. Classification of a positive result was based solely on the comparator assay. The positive and negative agreements were similar for RSV using Prodesse assay. Any results for influenza reported on the Prodesse assay were neither collected nor analyzed. We used PPA and NPA to report the analysis results based on the FDA guidance for reporting results from studies evaluating diagnostics tests [10]. Based on the same guidance, we used a Wilson Score to determine the 95% CI.

2.3. Ct values analysis

Ct values are the number of cycles needed to yield a positive value in qualitative real-time PCR assays and are inversely proportional to the relative amount of target present in a given sample. A positive reaction is triggered by accumulation of a fluorescent signal. Ct values can be used to infer viral load, and previous studies have demonstrated that several factors, including age and disease severity may influence influenza virus and RSV viral loads [11−14]. As of this writing, the Xpert Xpress Flu/RSV test is not cleared by the U.S. Food and Drug Administration or any other regulatory agency for quantitative use or estimation of viral load.

Ct values were obtained from 1192 positive test result from the Xpert Xpress Flu/RSV test. For detection of FLUAV, the assay uses 2 fluorescence channels, Flu A1, and Flu A2, with primers targeting multiple regions of the FLUAV genome to improve coverage across virus strains. In this analysis, the Ct values from Flu

Table 1
The number and percent of corresponding positive results for the Xpert Xpress Flu/RSV test and the comparator assays using nasal and nasopharyngeal swabs stratified by patient age. Only the number of samples testing positive by the comparator assay, with a corresponding positive result on the Xpert Xpress Flu/RSV test, are included in this table.

Age (years)	n	% of Total	FLUAV		FLUBV	
			Number of Positives	Positivity Rate	Number of Positives	Positivity Rate
≤5	1284	39.3%	137	10.7%	57	4.4%
6-21	516	15.8%	132	25.6%	53	10.3%
22-59	1141	34.9%	122	10.7%	37	3.2%
≥60	324	9.9%	56	17.3%	5	1.5%
Total	3265	100%	447	13.7%	152	4.7%

			RSV	
			Number of Positives	Positivity Rate
≤5	1212	39.1%	483	39.9%
6-21	483	15.6%	21	4.3%
22-59	1090	35.1%	39	3.6%
≥60	318	10.2%	32	10.1%
Total	3103	100%	575	18.5%

Nasal (NS) and nasopharyngeal (NP) specimens by age range of Xpert Flu/RSV compared to Lyra for Flu A and Flu B and Prodesse for RSV.

A2 were used since it has fewer primers that may confound the analysis than Flu A1. Analysis of Variance (ANOVA) was used to analyze the differences among the group mean Ct values for: Specimen type (NP swab, nasal swab), Sex (male, female), Age group (≤5, 6-21, 22-59, ≥60), and Health care setting (Emergency Department, non-ED). Non-ED categories included Extended Care Facilities, Hospitalized, Outpatient, Urgent Care, and Other, and were combined due to small sample sizes for each category. A P value of < 0.05 was considered statistically significant using RStudio Version 1.4.1103 (RStudio Team. 2021; Integrated Development for R. RStudio, Inc., Boston, MA).

2.4. Operator analysis

The usability of the Xpert Xpress Flu/RSV test was evaluated when used by operators with no laboratory experience, representing the CW-intended users. The operators at the clinical sites in this study, as well as operators at sites conducting a separate reproducibility study, were included in this analysis. No other elements of the reproducibility study were included in this study. To minimize bias, operators who performed the Xpert Xpress Flu/RSV test were blinded to the comparator results prior to testing. Similarly, the laboratory personnel performing the Lyra and Prodesse assays were blinded to the Xpert Xpress Flu/RSV test results. Upon completion of the study, each CW operator was asked to complete a 23-question survey about their experience with, and understanding of, the system's operations, and results. The questionnaire asked the operators to rate statements on a Likert scale of 1 (strongly agree) to 5 (strongly disagree), or yes/no, regarding the set-up, use, and interpretation of the GeneXpert Xpress System and the results provided. The questionnaire also presented 5 different screenshots of Xpert Xpress Flu/RSV test results and asked the operators to interpret the results. The same question applied to all 5 screenshots, "Please read the test results below and circle 1 answer (a, b, c or d)." For purposes of this study, users were considered inexperienced. Analyses were not performed on the spectrum of users performing testing in this study to compare 1 "type" of user to another. The information collected for CW users included (a) education and education level relative to all employees at the site (b) employment status at the location (full time, part time, PRN, etc.) (c) title at location (d) years of employment at location at current title and previous titles, if applicable and summary of daily duties at location. Responses were analyzed using descriptive statistics.

3. Results

3.1. Assay comparison analysis

Most patients were enrolled in emergency departments (~79%) and were under the age of 21 years (~55%). A total of 3265 patients from 6 categories of health care settings were included in the FLUAV and FLUBV comparisons relative to the Lyra assay. A total of 3103 patients, from 6 categories of health care settings, were included in the RSV comparisons relative to the Prodesse assay. The distribution of patients by age, along with the number and percentage of positive cases using the Xpert Xpress Flu/RSV test, Lyra and Prodesse are shown in Table 1. Five subjects had multi-infections by the Xpert Xpress Flu/RSV Assay and are therefore counted more than once for influenza results. Of the 5 subjects with Xpert multi-infections, 1 sample was Flu A and Flu B positive by comparator method; 1 sample was Flu A positive by comparator method; 1 sample was Flu B positive by comparator method; 2 samples were negative for both Flu A and Flu B targets by comparator method.

For FLUAV detection, the Xpert Xpress Flu/RSV test yielded a PPA of 98.9% (95% CI: 96.2%-99.7%) and an NPA of 97.5% (95% CI: 96.6%-98.2%) with the Lyra assay using nasal swab specimens ($n = 1598$). Using NP swab, ($n = 1667$), specimens for FLUAV detection, the Xpert Xpress Flu/RSV assay demonstrated a PPA of 97.6% (95% CI: 94.4%-99.0%) and an NPA of 98.2% (95% CI: 97.4%-98.8%) with the Lyra assay. Using the nasal and NP swab specimens combined dataset, the Xpert Xpress Flu/RSV test yielded a PPA and NPA with the Lyra comparator method of 98.2% (95% CI: 96.4%-99.1%) and 97.9% (95% CI: 97.3%-98.3%) respectively for FLUAV (Table 2).

For FLUBV detection, the Xpert Xpress Flu/RSV test yielded a PPA of 98.4% (95% CI: 91.7%-99.7%) and an NPA of 99.3% (95% CI: 98.7%-99.6%) with the Lyra assay using nasal swab specimens ($n = 1598$). Using the NP swab specimens ($n = 1667$) for FLUBV detection, Xpert Xpress Flu/RSV test yielded a PPA of 97.3% (95% CI: 90.6%-99.2%) and an NPA of 99.6% (95% CI: 99.1%-99.8%) with the Lyra assay. Using the nasal and NP swab specimens combined dataset, the Xpert Xpress Flu/RSV test yielded a PPA and an NPA with the Lyra comparator method of 97.8% (95% CI: 93.8%-99.3%) and 99.4% (95% CI: 99.1%-99.6%), respectively, for FLUBV (Table 2).

For detection of RSV, the Xpert Xpress Flu/RSV test for yielded a PPA of 98.2% (95% CI: 95.8%-99.2%) and an NPA of 99.1% (95% CI: 98.4%-99.5%) with the Prodesse assay using nasal specimens ($n = 1543$). Using the NP swab specimens ($n = 1560$), the Xpert Xpress Flu/RSV test yielded a PPA of 98.2% (95% CI: 95.9%-99.2%) and an NPA

Table 2
Overall performance of Xpert Xpress Flu/RSV test compared to Lyra and Prodesse.

Target[a]	Specimen Type	N	TP	FN	TN	FP	PPA (95% CI)	NPA (95% CI)
FLUAV	NS	1598	186	2[b]	1375	35[c]	98.9 (96.2-99.7)	97.5% (96.6-98.2)
	NP	1667	200	5[d]	1436	26[e]	97.6% (94.4-99.0)	98.2% (97.4-98.8)
	Overall	3265	386	7[f]	2811	61[g]	98.2% (96.4-99.1)	97.9% (97.3-98.3)
FLUBV	NS	1598	63	1[h]	1523	11[i]	98.4% (91.7-99.7)	99.3% (98.7-99.6)
	NP	1667	71	2[j]	1587	7[k]	97.3% (90.6-99.2)	99.6% (99.1-99.8)
	Overall	3265	134	3[l]	3110	18[m]	97.8% (93.8-99.3)	99.4% (99.1-99.6)
RSV	NS	1543	269	5[n]	1257	12[o]	98.2% (95.8-99.2)	99.1% (98.4-99.5)
	NP	1560	275	5[p]	1261	19[q]	98.2% (95.9-99.2)	98.5% (97.7-99.0)
	Overall	3103	544	10[r]	2518	31[s]	98.2% (96.7-99.0)	98.8% (98.3-99.1)

TP = true positive; FP = false positive; TN = true negative; FN = false negative; NS = nasal; NP = nasopharyngeal

[a] Five specimens were positive for both Flu A and Flu B
[b] Discrepant Testing: 1 of 2 Flu A NEG; 1 of 2 Flu A POS
[c] Discrepant Testing: 17 of 35 Flu A NEG; 11 of 35 Flu A POS; 7 of 35 inconclusive
[d] Discrepant Testing: 3 of 5 Flu A NEG; 2 of 5 Flu A POS
[e] Discrepant Testing: 11 of 26 Flu A NEG; 9 of 26 Flu A POS; 6 of 26 inconclusive
[f] Discrepant Testing: 4 of 7 Flu A NEG; 3 of 7 Flu A POS
[g] Discrepant Testing: 26 of 61 Flu A NEG; 22 of 61 Flu A POS; 13 of 61 inconclusive
[h] Discrepant Testing: 1 of 1 inconclusive
[i] Discrepant Testing: 5 of 11 Flu B POS; 6 of 11 inconclusive
[j] Discrepant Testing: 1 of 2 Flu B POS; 1 of 2 inconclusive
[k] Discrepant Testing: 1 of 7 Flu B NEG; 5 of 7 Flu B POS; 1 of 7 inconclusive
[l] Discrepant Testing: 1 of 3 Flu B POS; 2 of 3 inconclusive
[m] Discrepant Testing: 1 of 18 Flu B NEG; 10 of 18 Flu B POS; 7 of 18 inconclusive
[n] Discrepant Testing: 3 of 5 RSV NEG; 1 of 5 inconclusive; 1 of 5 not done
[o] Discrepant Testing: 5 of 12 RSV NEG; 3 of 12 RSV POS, 4 of 12 inconclusive
[p] Discrepant Testing: 2 of 5 RSV NEG; 2 of 5 inconclusive; 1 of 5 not done
[q] Discrepant Testing: 6 of 19 RSV NEG; 2 of 19 RSV POS, 6 of 19 inconclusive; 5 of 19 not done
[r] Discrepant Testing: 5 of 10 RSV NEG; 3 of 10 inconclusive; 2 of 10 not done
[s] Discrepant Testing: 11 of 31 RSV NEG; 5 of 31 RSV POS; 10 of 31 inconclusive; 5 of 31 not done

of 98.5% (95% CI: 97.7%-99.0%) with the Prodesse assay. Using the nasal and NP swab specimens combined data set, the Xpert Xpress Flu/RSV test yielded a PPA and an NPA of 98.2% (95% CI: 96.7%-99.0%) and 98.8% (95% CI: 98.3%-99.1%), respectively, for detection of RSV with the Prodesse assay (Table 2).

3.2. Ct values analysis

Ct values were obtained from a total of 1192 positive test results, including 578 (48.6%) RSV positive, 447 (37.4%) FLUAV-positive, and 152 (12.8%) FLUBV-positive results. Four positive test results were missing associated demographic data and were not included in the final analysis. An additional 15 positive test results had co-infections with 1 other respiratory virus, which included 9 (0.76%) FLUAV-RSV-positive results, 5 (0.42%) FLUAV-FLUBV-positive results, and 1 (0.08%) FLUBV-RSV-positive result. For the co-infections, the Ct value for each positive target was treated individually. Positive results were evenly split by sample type (50.7% NP, 49.3% nasal) and sex (50.3% female). The majority of positive results were from the youngest age group (57.2% ≤5 years) and from the Emergency Department (78.5%). The mean Ct values by group are shown in Table 3.

Mean Ct values for FLUAV and FLUBV were significantly lower in NP swabs, while mean Ct values for RSV were similar in nasal and NP swabs (Table 3; Fig. 1). There was no observed difference in Ct values by age group for FLUAV and FLUBV; however, RSV Ct values were significantly associated with age, with the lowest Ct values (highest RSV RNA concentrations) in specimens from patients ≤5 years of age and ≥60 years of age (Table 3). There

was no statistically significant Ct value association between sex or care setting (ED vs. non-ED) on (Table 3).

3.3. Operators analysis

A total of 44 operators at 14 sample collection clinical study sites, 2 reference laboratory sites, and 3 reproducibility sites were included in the study. Most CW operators on the GeneXpert Xpress System were research assistants and clinical research coordinators and most had a bachelor's degree. A total of 34 of the 35 operators at clinicals and 8 of 9s operators at the reference laboratory sites completed the questionnaire. Two of the operators left employment at the sites prior to completion of the study. Users included in this study had titles of Research Coordinator, Research Intern, Pharmacy Technician, Patient Care Technician, Clinical Research Coordinator, Associate Clinical Research Coordinator, Clinical Trials Coordinator, Specialty registered nurse (RN), Research Assistant, Patient Relations Coordinator, Laboratory Technician (Lab Tech), Medical Assistant (MA), Lab Tech/MA, Research Nurse Coordinator, and Program Assistant.

Twenty-six of the 44 operators (61.9%) had no prior experience working in any laboratory environment. Sixteen of the 44 operators (38.1%) had prior experience working in a laboratory environment; however, none had any experience working in a moderately complex laboratory environment. The average and median operator responses to questions regarding the Xpress system's set-up and use showed that the test system is easy to set up and operate (Table 4). All operators interpreted the 5 different screenshots of the Xpert Xpress Flu/RSV test results correctly (100%).

Table 3
Mean RT-PCR cycle threshold values for FLUAV, FLUBV and RSV targets by group.

	FLUAV		FLUBV		RSV	
	Mean Ct	P value	Mean Ct	P value	Mean Ct	P value
Specimen Type						
Nasopharyngeal swab (NP)	27.4	0.003	24.4	0.03	27.1	0.13
Nasal swab (NS)	29.1		26.6		27.8	
Age Category (in years)						
≤5	26.0	0.09	25.5	0.14	27.0	<0.0001
6-21	25.6		24.5		30.1	
22-59	27.3		27.2		30.3	
≥60	27.2		23.0		28.7	
Sex						
Female	26.9	0.06	25.5	0.91	27.3	0.6
Male	25.8		25.4		27.6	
Setting						
Emergency Department (ED)	26.3	0.44	25.6	0.62	27.6	0.12
Non-ED	26.8		25.1		26.8	

Ct = cycle threshold
Significance based on results from ANOVA within each of the 4 categories.

4. Discussion

Point-of-care testing should have a high degree of sensitivity, specificity and be easy to perform and interpret by non-laboratory personnel [15]. This study showed that Xpert Xpress Flu/RSV is an accurate test for detection of FLUAV, FLUBV, and RSV, and is easily performed and interpreted by minimally trained operators in a variety of CW environments. Our findings support the use of this system for point-of-care respiratory pathogen testing, which would be a critical tool during infectious clusters or pandemics.

Currently, antigen-based influenza tests are often used for the rapid diagnosis of Influenza and RSV infection [16]. In general, these tests suffer from poor performance in terms of analytical and clinical sensitivity and low negative predictive values compared to molecular detection methods. In a previous study, the pooled antigen test sensitivity for influenza was calculated to be 62.3% and the pooled specificity was 98.2% [15]. A previous meta-analysis on the sensitivity of

RSV rapid antigen detection tests (RADT) in children found it to be 80% [17]. The Xpert Flu+RSV Xpress test has previously been compared to the Prodesse ProFlu+ and yielded high sensitivities and specificities for FLUAV and FLUBV, and RSV when used in the CW setting [18]. Therefore, a rapid molecular assay, such as the Xpert Xpress Flu/RSV, may have larger impact on patient care due to the improved sensitivity over rapid antigen assays.

In our cohort, the mean Xpert Xpress Flu/RSV Ct values were indicative of abundant genomic target detection across all viruses. For FLUAV, the mean Ct value in NP swabs was 1.7 cycles lower than nasal swabs, and the mean Ct value for FLUBV was approximately 2.2 cycles lower in NP swabs. While these represent significant differences between sample type, this corresponds to less than 10-fold difference in RNA concentration and did not translate into lower clinical sensitivity. Mean Ct values for RSV were similar in nasal and NP swabs, which was an unexpected and novel finding based on previous observations [19]. Ct values for RSV were significantly lower in
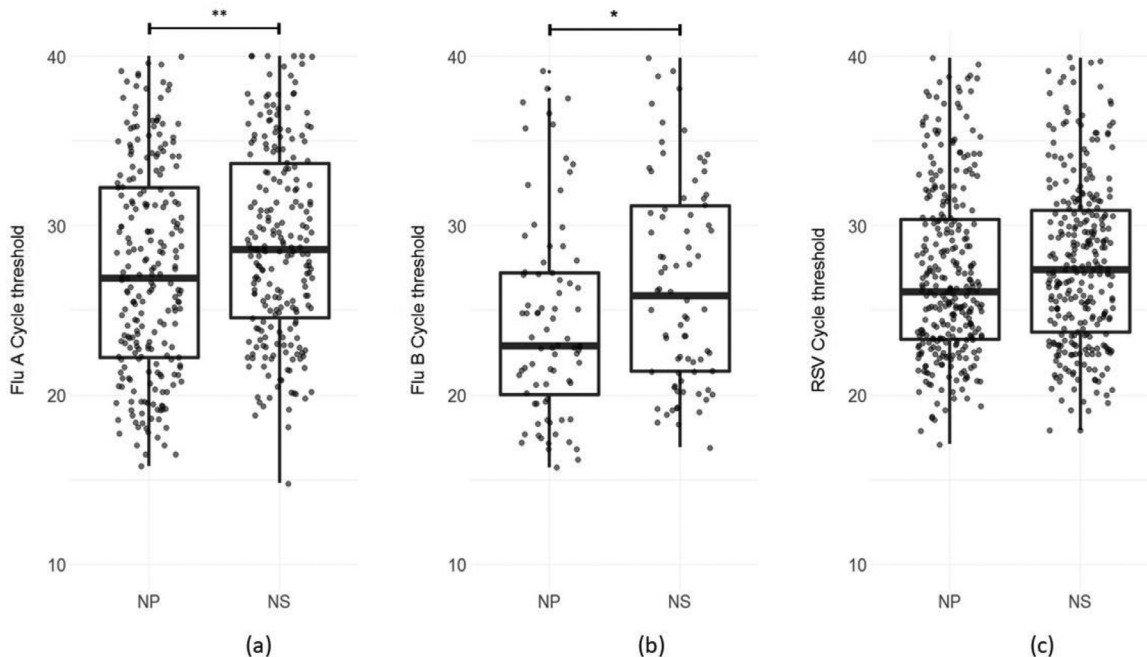

Fig. 1. Distribution of cycle threshold values by specimen types, for FLUAV (A), FLUBV (B), and RSV (C). NP: nasopharyngeal swab; NS: nasal swab. NP swabs resulted in significantly lower Ct values for FLUAV and FLUBV, but not RSV. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 4
Xpert Flu/RSV test operator questionnaire responses evaluating the system.

Question Number	Question	Mean Operator Score[a]	Median Operator Score[a]
01	I have experience working in a lab environment (Yes or No)	-	-
02	It was easy to remove the GeneXpert and all parts from the packaging.	4.4	4
03	It was easy to set up the GeneXpert instrument.	4.0	4
04	It was easy to understand and use the Getting Started Guide for assembling the Xpress system.	4.2	4
05	It was easy to understand and use the Quick Reference Instructions for performing the test and external controls.	4.2	4
06	It was easy to use the computer to perform a test from the video.	4.5	5
07	It was easy to understand the instructions to invert the tube.	4.6	5
08	It was easy to understand the instructions on how to transfer the sample from the tube to the cartridge.	4.5	5
09	It was easy to use the pipette to transfer the sample from the tube to the cartridge.	4.6	5
10	It was easy to understand the instructions for placing the cartridge into the instrument.	4.7	5
11	It was easy to understand the results of the tests performed.	4.6	5
12	It was easy to understand what to do with the cartridge after the test was complete.	4.5	5

[a] Respondents' rated the statements, were applicable, ranged from 1 (strongly disagree) to 5 (strongly agree). The remaining questions were answered with "yes" or "no".

patients ≤5 years, as well as patients ≥60 years, who are at high risk of complications [2,4]. The mean Ct value in patients ≤5 years was 3 cycles lower compared with those between the ages of 6 to 59 years for RSV, indicating an approximately 10-fold increase in RNA concentration in this youngest age group.

Importantly, the Xpress Flu/RSV test was easily used, and testing results were readily interpreted by operators with a wide range of backgrounds and minimal training. Successful rapid point-of-care testing by minimally trained operators would result in a more generalizable practice in multiple different clinical settings [20]. Considering staffing shortages during the current COVID-19 pandemic, the ability to have a test that could be performed rapidly by any available staff is crucial. The definition of an assay's clinical utility is that the results lead to clinical decisions that improve patient outcomes [21]. Further, these results can lead to improved preventative care both in the health care settings, and for patients and families. If the need arises, it could also improve tracing efforts in the community [22].

Our study is not without limitations. There was no data available on alternate viral etiologies. As only symptomatic patients were included, we cannot comment on test characteristics in asymptomatic patients who may be asymptomatic carriers or have prolonged shedding after a recent acute illness. However, previous studies found that detection of respiratory viruses in asymptomatic patients by PCR is uncommon [23]. We did not assess disease severity; therefore, we cannot comment on the test results in patients with possibly variable viral loads. However, we were able to compare samples from patients in the Emergency Department to other hospitalized and outpatient clinics and observed no differences in mean Ct value or assay sensitivity between different testing environments. In the Ct analysis, the Flu A2 channel was chosen, as it has fewer primers. This may have missed any differences in Ct values in cases of coinfection, but the overall number of coinfections in our study was relatively small. Finally, we did not collect data on test turnaround times that would enable commentary on the potential impact on patient throughput. However, the Xpert Xpress Flu/RSV test was found in a previous study to have significantly shorter turnaround times compared to other molecular devices [24]. The limitations identified in this study could be included in further implementation studies of the Xpert Xpress Flu/RSV test.

5. Conclusion

Our study found the Xpert Xpress Flu/RSV test to have a high clinical sensitivity and specificity for detection of FLUAV, FLUBV, and RSV in a CW setting by minimally trained operators. Xpert Xpress Flu/RSV

Ct values indicate that the Xpert Xpress Flu/RSV assay is robust across health care settings, where sampling variability may exist, as well as between different sexes, sample types and age groups. The instrument was rated as easy to set-up, use, and interpret by minimally trained operators for diagnosis of influenza and RSV infections. The results and ease of use for point-of-care testing can improve patient throughput and inform clinical decision-making in diverse clinical environments.

Authors' contributions

All authors contributed equally to all aspects of this study, as well as manuscript preparation and revision. I would like to acknowledge that 2 of the authors (Michael Loeffelholz and Fiona Strouts) are employees of Cepheid. All other authors have no conflicts of interest to disclose.

Declaration of competing interest

We would like to acknowledge that Dr. Strouts and Dr. Loeffelholz are employees of Cepheid.

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