CLIA Waiver by Application

Approval Determination Decision Summary

A. Document Number

CW180002

B. Parent Document Number

K180218

C. Purpose of the Submission

This submission is a Dual 510(k) and CLIA Waiver by Application (Dual Submission) tracked as K180218 and CW180002. CW180002 was submitted to obtain CLIA Waiver for the Xpert Xpress Flu/RSV Assay performed on the Cepheid Gene Xpert Xpress System for nasal swab and nasopharyngeal swab specimens.

D. Measurands (analytes)

Influenza A, influenza B and respiratory syncytial virus (RSV) viral RNA

E. Sample Type

Nasal and nasopharyngeal swabs

F. Type of Test

This assay is a multiplex nucleic acid assay that detects and differentiates influenza A, influenza B, and RSV through nucleic acid extraction, amplification, and detection using qualitative real-time RT-PCR. All steps of the assay are automated, after sample addition, and performed in a single container.

G. Applicant

Cepheid

H. Proprietary and Established Names

Xpert Xpress Flu/RSV
Xpert Xpress Flu/RSV Assay

I. Test System Description

1. Overview

This assay uses nasal or nasopharyngeal swab specimens from patients with signs and symptoms of respiratory infection. Viral nucleic acid is extracted from the sample and the influenza A, influenza B, and/or RSV viral RNA is amplified and detected through real-time reverse transcription polymerase chain reaction (RT-PCR). Detection and differentiation of influenza A, influenza B, and RSV is reported to the user. The user has the ability to choose one of the following assays to run: Xpert Xpress Flu-RSV, Xpert Xpress_Flu , or Xpert Xpress_RSV. Depending on the selection, the assay proceeds as normal, but only the selected assay results are reported.

The assay uses a single use disposable cartridge that has a separate section for specimen loading. The cartridge contains all PCR reagents, all PCR reactions take place in the cartridge. The GeneXpert Xpress System performs all assay steps from clinical sample to the reporting of assay results. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge. The SPC is present to control for adequate processing of the target viruses and to monitor for the presence of inhibitors in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The GeneXpert Xpress System, comprised of the GeneXpert Dx System GX-II, has two modules capable of performing separate sample preparation, real-time PCR, and RT-PCR tests, the GX-IV has four modules. Each module contains a syringe drive for dispensing fluids (i.e., the syringe drive activates the plunger that works in concert with the rotary valve in the cartridge to move fluids between chambers), an ultrasonic horn for lysing cells, and a proprietary thermocycler for performing real-time PCR and RT-PCR and detection.

Turnaround time for analysis of a sample is approximately 30 minutes or less. The assay results are automatically generated at the end of the process and provided in a report that can be viewed and printed.

2. Results Interpretation

A positive result for either analyte is determined by detection of a fluorescent signal generated from sequence specific probes to influenza A, influenza B, RSV, and signal processing control targets at levels above a signal threshold and within a defined cycle range. The algorithm in the assay software contains all the fixed criteria to determine the correct result. These criteria cannot be modified by the end user. At the end of the assay, the result is reported on the tablet screen to the user.

There are ten possible results when the user selects theXpert Xpress Flu-RSV assay: (1) positive for influenza A; (2) positive for influenza A and influenza B; (3) positive for influenza A and RSV; (4) positive for influenza A, influenza B and RSV; (5) positive for influenza B; (6) positive for RSV; (7) positive for influenza B and RSV (8) negative for influenza A, influenza B, and RSV; (9) Instrument Error-Repeat Test and (10) No Result-

Repeat Test. If an “Instrument Error” or “No-Result” (both considered indeterminate results) test result is reported, the test should be repeated with a new patient sample and a new test cartridge.

Should the user select either the FLU only or RSV only assay, the results reported would be more limited and are shown in Tables 1b and 1c, respectively.

Table 1a. Results Reported for Xpert Xpress Flu/RSV Assay

Result	Interpretation
Flu A POSITIVE; Flu B NEGATIVE; RSV NEGATIVE	Flu A target RNA is detected; Flu B target RNA is not detected; RSV target RNA is not detected.
Flu A POSITIVE; Flu B POSITIVE; RSV NEGATIVE**	Flu A target RNA is detected; Flu B target RNA is detected; RSV target RNA is not detected. Repeat test according to the instructions in Section 16.2, Retest Procedure.
Flu A POSITIVE; Flu B NEGATIVE; RSV POSITIVE**	Flu A target RNA is detected; Flu B target RNA is not detected; RSV target RNA is detected. Repeat test according to the instructions in Section 16.2, Retest Procedure.
Flu A POSITIVE; Flu B POSITIVE; RSV POSITIVE**	Flu A target RNA is detected; Flu B target RNA is detected; RSV target RNA is detected. Repeat test according to the instructions in Section 16.2, Retest Procedure.
Flu A NEGATIVE; Flu B POSITIVE; RSV NEGATIVE	Flu A target RNA is not detected; Flu B target RNA is detected; RSV target RNA is not detected.
Flu A NEGATIVE; Flu B NEGATIVE; RSV POSITIVE	Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is detected.
Flu A NEGATIVE; Flu B POSITIVE; RSV POSITIVE**	Flu A target RNA is not detected; Flu B target RNA is detected; RSV target RNA is detected. Repeat test according to the instructions in Section 16.2, Retest Procedure.
Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE	Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is not detected.
INSTRUMENT ERROR	If result is “INSTRUMENT ERROR”, touch **CLEAR ERROR** and follow the on-screen instructions. When the Home screen appears, repeat the test using a new cartridge.
NO RESULT – REPEAT TEST	If result is “NO RESULT - REPEAT TEST”, retest with a new cartridge. If retest is “NO RESULT - REPEAT TEST”, call for assistance at 888-838-3222.

**Note: Because the incidence of co-infection with two or more viruses (influenza A, influenza B, or RSV) is low, it is recommended that repeat testing is performed according to the instructions in the Retest Procedure to confirm dual infection.

Table 1b. Results Reported for Xpert Xpress FLU Assay Only

Result	Interpretation
Flu A POSITIVE; Flu B NEGATIVE	Flu A target RNA is detected; Flu B target RNA is not detected.
Flu A NEGATIVE Flu B POSITIVE	Flu A target RNA is not detected; Flu B target RNA is detected.
Flu A POSITIVE Flu B POSITIVE**	Flu A target RNA is detected; Flu B target RNA is detected. Repeat test according to the instructions in Section 16.2, Retest Procedure.
Flu A NEGATIVE Flu B NEGATIVE	Flu A target RNA is not detected; Flu B target RNA is not detected.
INSTRUMENT ERROR	If result is "INSTRUMENT ERROR", touch CLEAR ERROR and follow the on-screen instructions. When the Home screen appears, repeat the test using a new cartridge.
NO RESULT – REPEAT TEST	If result is "NO RESULT - REPEAT TEST", retest with a new cartridge. If retest is "NO RESULT - REPEAT TEST", call for assistance at 888-838-3222.

** Note: Because the incidence of co-infection with two or more influenza viruses (Influenza A and Influenza B) is low, it is recommended that repeat testing is performed according to the instructions in the Retest Procedure to confirm dual infection.

Table 1c. Results Reported for Xpert Xpress RSV Assay Only

Result	Interpretation
RSV POSITIVE	RSV target RNA is detected.
RSV NEGATIVE	RSV target RNA is not detected.
INSTRUMENT ERROR	If result is "INSTRUMENT ERROR", touch CLEAR ERROR and follow the on-screen instructions. When the Home screen appears, repeat the test using a new cartridge.
NO RESULT – REPEAT TEST	If result is "NO RESULT - REPEAT TEST", retest with a new cartridge. If retest is "NO RESULT - REPEAT TEST", call for assistance at 888-838-3222.

The results are displayed on the instrument screen. The results can also be printed on an integrated printer if this option is selected.

The kit contains the following test components:

- 10 individually packaged Xpert Xpress Flu/RSV Test Cartridges with Integrated Reaction Tubes,
- 12 disposable 300 µL fixed volume Transfer Pipettes
- CD containing assay Software

Sample collection kits, Gene Xpert Xpress System and Instruments, and Positive and Negative controls are sold separately.

J. Demonstrating "Simple"

The Xpert Xpress Flu/RSV Assay on the GeneXpert Xpress System was designed to be simple and easy to use. The following features are built-in to the device design to make it simple to use with minimal risk of erroneous results.

Table 2. Features that support the determination that Xpert Xpress Flu/RSV Assay on the GeneXpert Xpress System is 'Simple'

Item	Description	Comment
1	Uses direct unprocessed specimens	The test uses direct nasal and nasopharyngeal swab specimens.
2	Needs only basic, non-technique-dependent specimen manipulation, including any for decontamination.	The collected nasal or nasopharyngeal swab is placed in the collection medium tube, then mixed by inversion. Using the provided fixed volume pipette, a portion of the liquid is transferred to the test cartridge.
3	Needs only basic, non-technique-dependent reagent manipulation, such as "mix reagent A and reagent B."	The reagents are preloaded and automatically processed within the GeneXpert cartridges. There is no reagent handling, all reagents are inside the single use cartridge. The test cartridges are keyed and can be inserted into the analyzer only in one direction.
4	Needs no operator intervention during the analysis steps.	The analysis is performed automatically by the GeneXpert Xpress System.
5	Needs no technical or specialized training with respect to troubleshooting or interpretation of multiple or complex error codes.	Technical or specialized training is not required for troubleshooting or error code interpretation. If an error code is shown, simple on-screen instructions are provided to the operator.
6	Needs no electronic or mechanical maintenance beyond simple tasks, *e.g.*, changing a battery or power cord.	There is no maintenance required other than basic cleaning of the external surfaces of the analyzer. There are no required electronic or mechanical maintenance tasks. System Control Checks for temperature are built-in to ensure the instrument is operating within validated heating and cooling specifications.
7	Produces results that require no operator calibration, interpretation, or calculation.	The GeneXpert Xpress System performs automated analysis of test results and eliminates subjectivity associated with visual reading of results by the end-user. An annual performance check should be performed using Xpert Check kit provided separately from Cepheid. If an error code is

Item	Description	Comment
		shown, the operator should contact Cepheid for technical support. Interpretation of results is automated. Results are displayed on the instrument screen and may be printed. No calculation by the operator is required.
8	Produces results that are easy to determine, such as "positive" or "negative," a direct readout of numerical values, the clear presence or absence of a line, or obvious color gradations.	The Gene Xpert Xpress System screen is designed for ease of use and features a color display that facilitates easy-to-read messages. The results are reported on a screen for each of the 3 analytes for every test. Each analyte is reported as either "Analyte POSITIVE" or "Analyte NEGATIVE". Non-reportable results are displayed as "NO RESULT-REPEAT TEST" or "INSTRUMENT ERROR" and there is no interpretation required by the end-user. Error messages are unambiguous and include easy-to-interpret solutions.
9	Contains a quick reference instruction sheet that is written at no higher than a 7th grade reading level.	The Quick Reference instructions and Getting Started Guide are written at a 7th grade comprehension level. In addition, the GeneXpert Xpress System software includes an instructional video that the operator can watch on the GeneXpert Xpress System that demonstrates how to prepare a sample, add the sample to the cartridge, and load the cartridge into the instrument.

K. Demonstrating "Insignificant Risk of an Erroneous Result"- Failure Alerts and Fail-safe Mechanisms

1. Risk Assessment

A risk analysis for the Xpert Xpress Flu Assay and GeneXpert Xpress System for risks associated with hazards and hazardous situations due to user skills, human factors and foreseeable misuse has been performed. The risk management has been performed per ISO 14971 and Cepheid's internal procedure for risk management. The sponsor utilized the Device Hazard Analysis and the Failure Mode Effects Analysis (FMEA) methods to assess the risks of failure that may occur during use or misuse of the device. The FMEA includes potential failure modes and effect of the failure, potential causes, built in design controls and evaluation of severity, frequency of occurrence, and ability to detect the failure. The elements considered include the intended user, environment, human factors/potential human errors, and historical field data from similar devices.

Potential sources of errors that could adversely affect system performance were identified and mitigated first through system design and then through additional cautions in the labeling. The identified risks which could result in erroneous test results were evaluated in flex studies that stressed the functional limits of the test system (see below).

The sponsor provided detailed software validation and verification documentation, including requirements related to assay performance when the Xpert Xpress Flu/RSV Assay is run on the GeneXpert Xpress System. The instrument software was reviewed under the parent 510(k) submissions (K162456 and K180218).

2. Fail-safe and Failure Alert Mechanisms

The Xpert Xpress Flu/RSV Assay and the GeneXpert Xpress System was designed to include numerous features and "lockouts" built into the hardware and software to prevent erroneous results.

Table 3. Summary of Fail-Safe and Failure Alert Mechanisms

Design Feature	Description
Operator Lockout	• Module door will not latch if tube is incorrectly positioned. Incorrect position can also be detected by force increase and assay will not run. The assay will also not run without a cartridge inserted. • Module door closes before assay start to block external light; there is also a signal check for light leak. • Module door would not close if an attempt to run a test on a GeneXpert Xpress module was made without a blinking green light. Only a GeneXpert module with a blinking green light can be used to start a new test. • Only one assay can be started at a time.
Instrument Self-Test	• The GeneXpert Xpress has an internal function of on-going internal performance monitoring and if the data indicate that maintenance is required the operator will be instructed to contact Cepheid Technical Support, in which case the company will send a support technician to the operator. • Self-check performed by software before assay includes: thermal checks for temperature out of range, checks of the heating rate and cooling rate, check of the force sensor for cartridge loading, optics check, syringe drive and valve checks.
GeneXpert Instrument	• The GeneXpert instrument has an ambient temperature sensor that monitors the internal operating temperature and is designed to prevent the test from proceeding when the ambient temperature of the module is above 55°C. • The system should be checked for proper calibration on annual basis using the Xpert Check kit. If an error code is shown, the operator contacts Cepheid for technical support.

Design Feature	Description
Consumable Design	The assay barcode is read once the cartridge is placed inside the instrument: o The instrument will not start if the assay cartridge has previously been used on the same instrument. o The instrument will not start if the assay selected does not match the cartridge being used or the wrong assay cartridge is used. o The instrument will not start if the assay cartridge is expired.

External Controls

External controls are available but are distributed separately from the kit. The device labeling includes information regarding the availability of commercially prepared External Flu A/B and RSV Positive and Negative Controls. The Negative Control consists of Coxsackie virus; catalog number NATCXVA9-6C-C (ZeptoMetrix, Buffalo, New York, USA). The external Positive Control consists of inactivated influenza A, influenza B and RSV viruses, catalog number NATFLURSV-6C (NATtrol control, ZeptoMetrix, Buffalo, New York, USA). The package insert and Quick Reference Instructions recommend that these controls are tested each time a new lot or new shipment of Xpert Xpress Flu/RSV Assay reagents is received or any time a new operator is introduced.

In-test Controls

- The assay includes a Sample Processing Control (SPC) which confirms if the sample was processed correctly. It is composed of Armored RNA and verifies the release of RNA during the extraction procedure and can detect inhibition in the RT-PCR and PCR reactions.
- The assay also includes a Probe Check Control (PCC) which checks the fluorescence signal from the probes before the PCR reaction is started. This monitors bead rehydration, reaction tube filling, probe integrity and dye stability.

Self-checks

The GeneXpert Xpress has an internal function for on-going internal performance monitoring and if the data indicate that maintenance is required the user will be instructed to contact Cepheid Technical Support, in which case the company will send a support technician to the user.

The functionality of Fail-Safe mechanisms built into the software of the Xpert Xpress Flu/RSV Assay on the GeneXpert Xpress System was tested and demonstrated as described below.

Table 4. Fail-Safe Mechanisms for Xpert Xpress Flu/RSV Assay and GeneXpert Xpress Instrument

Item	Operator Action	Expected Results
1	Ambient temperature of the module is above 55°C.	Error message: The testing module icon will be greyed out and cannot be selected, indicating that tests cannot be performed. Testing does not proceed. The Getting Started Guide informs the user to contact Cepheid Technical Support.
2	Test was stopped before results were obtained.	Test results were reported as “ERROR”.
3	Operator turned off instrument before test was completed and tried to resume the test once the instrument was back on.	First Error Message: Code 2123: Module A2 lost communication while test was running, attempting recovery. Please follow the instructions below. 1) Press Start 2) Keep hands clear of instrument until operation completes 3) If you need more help, contact Technical Support. Second Error message: “Cartridge serial number[xxxxxx] for assay with product code[xxx] lot [xx] has already been used. Cartridges can only be used once. Select a new cartridge”. Testing does not proceed.
4	Operator attempts to run cartridges beyond the expiration date.	Test cannot be started. All test modules are greyed out so they cannot be selected.
5	Cartridge reaction tube is missing.	Testing proceeds but “NO RESULT-REPEAT TEST” is obtained. The package insert and QRI contain a warning to the operator that cartridge should not be used if cartridge reaction tube is missing.
6	Cartridge reaction tube is damaged	Testing proceeds but “NO RESULT-REPEAT TEST” is obtained. The package insert and QRI contain a warning to the operator that the cartridge should not be used if cartridge reaction tube is damaged.
7	Non CLIA waived Flu/RSV ADF tested with Non-CLIA-Waived cartridge is used on the Xpress instrument.	Error message: “The Xpert Xpress Flu/RSV assay is not CLIA-Waived and cannot be run on this system. Select a correct CLIA-Waived cartridge”.
8	CLIA-waived cartridge run with non-CLIA waived/incorrect assay definition file (ADF)	Error Message: “No assays found for product code [xxx]. Import assay definition file”. Test could not be started.

Item	Operator Action	Expected Results
9	Try to start a test using a cartridge that has already been used.	Error message: Cartridge serial number [xxxxxxxxx] for assay with product code [xxx] lot [xx] has already been used. Cartridges can only be used once. Select a new cartridge. Test cannot be started.

Studies either produced a valid result as expected confirming that there is no need for further mitigations for that feature or generated the expected error messages confirming the effectiveness of the fail-safe mechanisms built into the analyzer's software.

3. Flex Studies

The flex studies have been designed to evaluate the tolerance of the test system and to challenge it with conditions of stress that were identified in the Risk Analysis. The goal is to identify potential operator-related failures and verify the effectiveness of associated control measures/mitigations or the need for additional measures to enhance robustness.

Contrived test samples were used in the flex studies for the Xpert Xpress Flu/RSV Assay. Negative samples consisted of one nasal swab added to the Cepheid Transport Medium. Each positive sample consisted of a combination of Flu A (A/Victoria/361/2011), Flu B (B/Wisconsin/01/11) and RSV (A/Long/MD/56) viruses spiked at 2X LoD spiked into pooled negative nasal swab specimen. Negative and positive controls were also tested in all flex studies and returned expected results. All test samples (Negative, Low Positive Flu A, Low Positive Flu B, Low Positive RSV) were blinded and randomized and tested in five replicates per condition.

All data was collected using the GeneXpert Xpress software on the GeneXpert Xpress II and IV instruments.

Potential sources of error that were evaluated in the flex studies with the Xpert Xpress Flu/RSV on the GeneXpert Xpress instrument are outlined in Table 5 below.

Table 5. Potential Sources of Error Evaluated in the Flex Studies

Error Source	Flex Study
Human Factors/Operator Errors	a. Incorrect Handling of Test Cartridge
	b. Incorrect Pipette/Incorrect Sample Volume
	c. Incorrect Handling (Mixing) of Sample and Transport Medium and External Controls
	d. Incorrect Timing of Cartridge Preparation
	e. Screen functionality
	f. Testing by multiple operators with multiple assays on one GeneXpert Xpress Instrument
Specimen Integrity and Handling	Incorrect Storage of Sample
Environmental Factors	a. Non-level positioning of the instrument
	b. Instrument environmental conditions (temperature and humidity)
	c. Reducing ventilation of the instrument
	d. Effects of Vibration

The effect of the following conditions on the performance of the assay was evaluated:

Human Factors/Operator Errors

a. Incorrect Handling of Test Cartridge

   This study evaluated the potential of invisible damage to the test cartridge when inadvertently dropped or knocked over during the procedure both before and after sample addition to the cartridge.

   i. Cartridges were dropped from workbench height onto the floor prior to sample addition. After dropping, test samples were added and each cartridge was tested. All positive and negative samples generated expected results. There were no false positive or false negative results observed.

   ii. Cartridges were dropped from workbench height onto the floor after sample addition. After dropping, each cartridge was tested. All positive and negative samples generated expected results. There were no false positive or false negative results observed.

b. Incorrect Pipette/Incorrect Sample Volume

   This study evaluated the effect of varied sample volume (outside of the 300 µL delivered with the fixed-volume transfer pipette) on the performance of the assay. Seven different volumes were evaluated, ranging from 50 µL to 900 µL. The data showed that 300 µL to 900 µL sample volumes generate expected results. At the low volume of 50 µL no result was reported and an error message was displayed. For positive samples, when tested at 100 µL volume in 5 replicates, the following results were obtained: 1 negative, 2 positive and 2 indeterminate results; all negative swabs

were indeterminate. At 150 µL, for the positive swabs tested in 5 replicates, the following results were obtained:1 negative, 2 positive and 2 indeterminate results; all negative swabs were negative. All other sample volumes generated expected results. The likelihood of this error is minimized by the fixed volume pipette that is included with the kit.

c. Incorrect Handling (Mixing) of Sample and Transport Medium and External Controls

This study evaluated the effect of improper mixing on the performance of the assay. The proper mixing procedure is inversion of the viral transport media tube 5 times. Six different improper mixing techniques were tested; no mixing of the tube, shaking the tube 1, 3, 7, and 10 times and vortexing the tube for 5 seconds at maximum speed. All samples generated expected results.

d. Incorrect timing of cartridge preparation

The test directions instruct the user to start the test within 30 minutes of adding the sample. Testing was conducted at 60 minutes after adding the sample. All samples generated expected results.

e. Screen functionality

Operators attempted to conduct testing and use the instrument touchscreen with no gloves, single or triple layers of either nitrile or latex gloves. All users could easily use the touchscreen and all tests generated expected results.

f. Testing by multiple operators with multiple assays on one GeneXpert Xpress Instrument

A separate field study was conducted using a 4-module instrument with four untrained, inexperienced operators and two different assays. The purpose of this test was to determine that multiple operators conducting different Xpert Xpress assays (Xpert Xpress Flu Only and Xpert Xpress Flu/RSV) could all use one instrument without generating unnecessary errors. Testing was performed by alternating operators who followed the assay instructions provided in the quick reference instructions. Each operator could run all tests assigned to them and all test runs obtained the expected results.

<u>Specimen Integrity and Handling</u>

a. Incorrect sample storage

Testing was conducted with samples stored outside the specified temperature conditions and for a longer than recommended storage time (15-30°C for 24 hours or 2-8°C for 7 days). The following conditions were tested: 14 days at -20°C, 48 hours at 35°C, 14 days at 2-8°C, 48 hours at room temperature, 7 days at -20°C and 24 hours at 35°C. All samples generated expected results.

Environmental Factors

a. Non-level positioning of the GeneXpert Xpress instrument

Three instruments were tilted on a benchtop at 15° angle and each sample was tested in five individual replicates at each position. No failures were observed and all samples generated expected results.

b. Operational temperature and humidity

The study was designed to evaluate the effect of temperature and humidity outside of the expected normal conditions of use (15-30°C and 20%-80% relative humidity). Temperatures of 20°C and 36°C with humidity up to 95% RH (relative humidity) were examined. The cartridges and instruments were equilibrated to the specified conditions for 2 hours before testing for conditions a and b below. The instrument was abruptly transitioned to the conditions for c and d. The effect of the temperature and humidity was evaluated in different combinations:

a. 95% Relative humidity (RH) at 20°C, 2 hour acclimation
b. 95% Relative humidity (RH) at 36°C, 2 hour acclimation
c. 95% Relative humidity (RH) at 20°C, abrupt transition
d. 95% Relative humidity (RH) at 36°C, abrupt transition

When the instrument could stabilize for 2 hours before testing, all expected results were obtained although there was a 17% increase in time to result. Abrupt transition or exposure to high humidity produced an instrument error where the user could not select a module to start testing; this is part of the Fail-safe mechanisms for this instrument. Once the instrument acclimates, the module will be available for testing.

c. Reducing ventilation of the GeneXpert Xpress instrument

Two GeneXpert Xpress instruments were placed back to back of each other, thus reducing the ventilation area. No failures were observed and all test samples generated expected results.

d. Effects of Vibration:

The following study was conducted during testing for submission CW170014, Xpert Xpress Strep A assay with the GeneXpert Xpress instrument and were not repeated with the Xpert Xpress Flu/RSV assay.

The purpose of this study was to evaluate the effect of vibrations produced by nearby laboratory equipment on the performance of the Xpert Xpress Strep A assay when run on the GeneXpert Xpress instrument. Cartridge was prepared with sample and tested on the GeneXpert Xpress instrument at ambient temperature, placed in close proximity (within 1 foot) to a centrifuge running at 9,500 rpm. No failure modes were observed in the study, and all testing conditions yielded expected results. Vibrations coming from a centrifuge at the rpm tested had no impact on the performance of the

Xpert Xpress Strep A assay with GeneXpert Xpress instrument. No additional risk mitigation was therefore required.

The flex studies demonstrated that the system is robust and is not sensitive to user errors or environmental stresses. The combination of built in fail-safe mechanisms and explicit cautions in the labeling provide adequate controls to ensure that improper use of the device is not likely to yield erroneous results.

L. Demonstrating "Insignificant Risk of an Erroneous Result" - Accuracy

1. Clinical Performance of the Xpert Xpress Flu Assay on the GeneXpert Xpress instrument

The clinical study was conducted at 14 CLIA-waived sites. Each location was assessed by Cepheid to confirm that all testing sites saw patients in the same location where the testing for the study would take place. The operators at each site provided their education level, employment status, years of employment, job title and a summary of daily duties. Personnel who were selected to participate in the study had no professional CLIA moderate or high complexity laboratory work experience. The operators had also never used a Cepheid instrument before this study and received no training on how to set up the instrument or perform the assay. Reasons for personnel not selected to participate in the study were provided. There were 35 users participating in the clinical study across 14 testing sites. Of the 35 users, 25 tested at least five positive and five negative samples.

Table 6. Clinical Sites used during the CLIA-Waiver Clinical Study

Site #	Site Type	# of Users at Site
1	Emergency Department	2
2	Emergency Department	2
3	Urgent Care Clinic/Primary Care Office	2
4	Emergency Department	3
5	Emergency Department	1
6	Emergency Department	3
7/8	Emergency Department	3
9	Emergency Department	6
10	Urgent Care Clinic/Primary Care Office	2
11	Emergency Department	2
12	Emergency Department	3
13	Emergency Department	5
14	Emergency Department	1

A total of 3576 specimens were enrolled in this clinical study. The number of specimens included in the analyses differ for Flu A/B and RSV because two different comparator methods were used for these analytes. Each patient provided either a nasal swab (NS) or a nasopharyngeal (NP) swab specimen. For nasal swab specimens, one swab was used to swab both nostrils; for the NP swab specimen, only one nostril was sampled. Specimens

were prospectively collected fresh and tested as soon as possible after collection and within 24 hours. FDA cleared NAAT assay for Flu A/B and FDA cleared NAAT assay for RSV were used as comparator methods testing freshly collected nasopharyngeal swab specimen from each subject.

There were 3545 specimens tested with the Xpert Xpress Flu/RSV assay. There were 67 initially indeterminate Xpert Xpress Flu/RSV results (38 “NO RESULT-REPEAT TEST” and 29 “INSTRUMENT ERROR”); 58 of the 67 initially indeterminate samples were retested to yield 53 valid results from repeat testing. The initial indeterminate rate was 1.9% (67/3545), 95% CI: 1.5%-2.4%. After retesting the final indeterminate rate was 0.4% (14/3545), 95% CI: 0.2%-0.7%.

For Flu A/B a total of 311 samples were excluded. The following samples were excluded due to comparator related issues; 234 unresolved comparator results, 1 not tested with comparator, 6 with invalid comparator assay controls, 17 specimens frozen, 14 shipping problem. Additionally, the following samples were excluded from testing with the Xpert Xpress Flu/RSV and comparator assay; 9 incorrect specimen collection, 8 not tested with GX system, 14 indeterminate Xpert Xpress Flu/RSV result, 4 run on incorrect assay, and 4 not tested within protocol specified time period.

Table 7 below shows the distribution of patients by age group and the number of positives by the Xpert Xpress Flu/RSV for Flu A and Flu B. There were 3265 specimens evaluated for Flu A and Flu B (1598 NS specimens and 1667 NP specimens); 54.8% from female subjects and 45.2% from male subjects).

For RSV, a total of 473 samples were excluded. The following samples were excluded due to comparator related issues; 415 unresolved comparator results, 5 not tested with comparator, 14 shipping problem. Additionally, the following samples were excluded from testing with the Xpert Xpress Flu/RSV and comparator assay 9 incorrect specimen collection, 8 not tested with GX system, 14 indeterminate Xpert Xpress Flu/RSV result, 4 run on incorrect assay, and 4 not tested within protocol specified time period.

The total number of eligible samples with valid results was 3103 (1543 NS specimens and 1560 NP specimens); 55.1% from female subjects and 44.9% from male subjects. Table 8 below shows the distribution of patients by age group and the number of positives for RSV by the Xpert Xpress Flu/RSV assay.

Table 7. Number and Percent of Specimens by Age Range[a] Positive for Flu A/B by Xpert Xpress Flu/RSV Assay

Age Group	Number of Patients	% of Total	Flu A		Flu B	
			Number of Positives	Percent Positive	Number of Positives	Percent Positive
≤5 years	1284	39.3%	137	10.7%	57	4.4%
6-21 years	516	15.8%	132	25.6%	53	10.3%
22-59 years	1141	34.9%	122	10.7%	37	3.2%
≥60 years	324	9.9%	56	17.3%	5	1.5%
Total	3265	100%	447	13.7%	152	4.7%

[a]Six subjects had multi-infections by the Xpert Xpress Flu Assay and are therefore counted more than once in this table. Of the 6 subjects with multi-infections, 1 sample was Flu A and Flu B positive by the comparator assay; 5 samples were negative for both targets by the comparator assay.

Table 8. Number and Percent of Specimens Positive by Age Range for RSV by Xpert Xpress Flu/RSV Assay

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

Flu A Performance with Nasal Swabs and Nasopharyngeal Swabs compared to an FDA cleared NAAT assay.

Table 9. Clinical Performance for Influenza A, Nasal Swabs

Nasal Swab Specimens (1598 specimens)		Comparator Result	
		Positive	Negative
Xpert Xpress Flu/RSV Assay	Positive	186	35
	Negative	2	1375
Total		188	1410

PPA: 98.9% (186/188); 95% CI: (96.2%-99.7%)
NPA: 97.5% (1375/1410); 95%CI: (96.6%-98.2%)

Table10. Clinical Performance for Influenza A, Nasopharyngeal Swabs

Nasopharyngeal Swab Specimens (1667 specimens)		Comparator Result	
		Positive	Negative
Xpert Xpress Flu/RSV Assay	Positive	200	26
	Negative	5	1436
Total		205	1462

PPA: 97.6% (200/205); 95% CI: (94.4%-99.0%)
NPA: 98.2% (1436/1462); 95%CI: (97.4%-98.8%)

Flu B Performance with Nasal Swabs and Nasopharyngeal Swabs compared to an FDA cleared NAAT assay.

Table 11. Clinical Performance for Influenza B, Nasal Swabs

Nasal Swab Specimens (1598 specimens)		Comparator Result	
		Positive	Negative
Xpert Xpress Flu/RSV Assay	Positive	63	11
	Negative	1	1523
Total		64	1534

PPA: 98.4% (63/64); 95% CI: (91.7%-99.7%)
NPA: 99.3% (1523/1534); 95%CI: (98.7%-99.6%)

Table 12. Clinical Performance for Influenza B, Nasopharyngeal Swabs

Nasopharyngeal Swab Specimens (1667 specimens)		Comparator Result	
		Positive	Negative
Xpert Xpress Flu/RSV Assay	Positive	71	7
	Negative	2	1587
Total		73	1594

PPA: 97.3% (71/73); 95% CI: (90.6%-99.2%)
NPA: 99.6% (1587/1594); 95%CI: (99.1%-99.8%)

RSV Performance with Nasal Swabs and Nasopharyngeal Swabs compared to an FDA cleared NAAT assay.

Table 13. Clinical Performance for RSV, Nasal Swabs

Nasal Swab Specimens (1543 specimens)		Comparator Result	
		Positive	Negative
Xpert Xpress Flu/RSV Assay	Positive	269	12
	Negative	5	1257
Total		274	1269

PPA: 98.2% (269/274); 95% CI: (95.8%-99.2%)
NPA: 99.1% (1257/1269); 95%CI: (98.4%-99.5%)

Table 14. Clinical Performance for RSV, Nasopharyngeal Swabs

Nasopharyngeal Swab Specimens (1560 specimens)		Comparator Result	
		Positive	Negative
Xpert Xpress Flu/RSV Assay	Positive	275	19
	Negative	5	1261
Total		280	1280

PPA: 98.2% (275/280); 95% CI: (95.9%-99.2%)
NPA: 98.5% (1261/1280); 95%CI: (97.7%-99.0%)

Table 15. Clinical Performance for Influenza A, All Swabs Combined

Combined Swabs (3265 specimens)		Comparator Result	
		Positive	Negative
Xpert Xpress Flu/RSV Assay	Positive	386	61
	Negative	7	2811
Total		393	2872

PPA: 98.2% (386/393); 95% CI: (96.4% -99.1%)
NPA: 97.9% (2811/2872); 95% CI: (97.31% -98.3%)

Table 16. Clinical Performance for Influenza B, All Swabs Combined

Combined Swabs (3265 specimens)		Comparator Result	
		Positive	Negative
Xpert Xpress Flu/RSV Assay	Positive	134	18
	Negative	3	3110
Total		137	3128

PPA: 97.8% (134/137); 95% CI: (93.8% -99.3%)
NPA: 99.4% (3110/3128); 95% CI: (99.1% -99.6%)

Table 17. Clinical Performance for RSV, All Swabs Combined

Combined Swabs (3103 specimens)		Comparator Result	
		Positive	Negative
Xpert Xpress Flu/RSV Assay	Positive	544	31
	Negative	10	2518
Total		554	2549

PPA: 98.2% (544/554); 95% CI: (96.7% -99.0%)
NPA: 98.8% (2518/2549); 95% CI: (98.3% -99.1%)

2. Performance with Analyte Concentrations Near the Assay Cutoff:

A study was conducted at three geographically diverse CLIA waived healthcare provider sites. The study was designed to evaluate the ability of untrained operators in CLIA waived settings to obtain accurate results with low positive samples when testing with the Xpert Xpress Flu/RSV Assay on the GeneXpert Xpress instrument. A total of 9 operators participated in the study (3 operators each at three sites) none of which had previous laboratory experience. The study was conducted over a period of eleven days.

The operators were non-laboratorian personnel and included research assistants, coding specialist and research coordinator. The work experience of the operators ranged from <0.5 years to 5 years and their education level ranged from high school to college; none of the operators had experience with diagnostic testing other than simple CLIA waived tests. The operators performed the testing using the Quick Reference Instructions; no additional training was provided to the operators.

Each operator tested a coded panel of individual samples contrived at virus concentrations near the assay cutoff. The test samples were contrived by spiking inactivated strains of influenza A, influenza B and RSV into negative simulated clinical matrix. The selected virus strains were diluted with the simulated clinical matrix to concentrations targeting the LoD of the assay. Seven samples were prepared: a low positive sample for influenza A, a low positive sample for influenza B, a moderate positive sample for influenza A, a moderate positive sample for influenza B, a low

positive sample for RSV, a moderate positive sample for RSV and a negative sample which consisted of the unspiked simulated clinical matrix.

All samples were coded and the operators at each site tested 30 replicates of each sample (a total of 90 samples per level across the 3 sites). The samples were blinded and randomized and the testing was incorporated into the daily workflow of each testing site.

Testing of 9 samples was repeated due to invalid results; 8 samples generated a valid result on the repeat testing and are included in the calculations of agreement with expected results, 1 sample remained invalid (NO RESULT-REPEAT TEST) .

Table 18: Performance of the Xpert Xpress Flu Assay Testing Samples at Virus Concentrations Near the Assay Cutoff

Percent Agreement with Expected Results					
Sample Type	**Site 1**	**Site 2**	**Site 3**	**Overall**	**Overall 95% CI**
Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.1% - 100%
Influenza A Low Positive	96.7% (29/30)	90% (27/30)	86.2% (25/29)	91.0% (81/89)	83.3% -95.4 %
Influenza A Moderate Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.1% - 100%
Influenza B Low Positive	93.3% (28/30)	96.7% (29/30)	90.0% (27/30)	93.3% (84/90)	86.2% - 96.9%
Influenza B Moderate Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.1% - 100%
RSV Low Positive	90.0% (27/30)	93.3% (28/30)	90.0% (27/30)	91.1% (82/90)	83.4% - 95.4%
RSV Moderate Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)	95.1% - 100%

There were no significant differences in the observed positivity of the device with low positive samples between operators, between sites and between the two operational modes. For this analysis Fisher's Exact test was performed indicating no statistically significant differences. All negative samples yielded expected results at all three sites for all operators. The study results demonstrated that untrained operators could perform the test correctly and the test provided the expected results for samples with virus concentrations near the assay cutoff.

3. Quick Reference Instructions (QRI)

The QRI and Getting Started Guide were reviewed in detail to ensure that the directions are clear and easy to understand and that all precautions are included as appropriate. The QRI for the use of the test with the GeneXpert Xpress instrument and the Getting Started Guide were written in simple language (at 7th grade reading level) and contain pictorial descriptions of the individual steps and of the system components. Additionally, the instrument software gives the operator the option to watch an instructional video on how to prepare the sample and perform the test. The interpretation of results is simple and easy to understand. The results are reported in different colors (red for positive and green for negative) to make the display and results interpretation more operator-friendly.

4. Operator Questionnaire Results:

At the end of the study each operator was given an operator questionnaire to provide feedback on the ease of use of the GeneXpert Xpress instrument and the Xpert Xpress Flu/RSV Assay. Based on the operator feedback, they found the on-screen instructions and initiating a test easy and simple. Users also found the Quick Reference Instructions and package insert easy to understand and follow.

M. Labeling for Waived Devices

The labeling consists of:

a. Package insert,
b. Quick Reference Instructions (QRI),
c. Cepheid User's Getting Started Guide
d. Cepheid GX Xpress Users Guide

The following elements are appropriately present:

- The QRI and the Cepheid User's Getting Started Guide are written at no higher than a 7th grade reading level and, where appropriate, contain graphic representation of system components and procedure steps.
- The package insert and the QRI identify the test as CLIA waived, and contain a statement that a Certificate of Waiver is required to perform the test in a waived setting; information on how operators can obtain a certificate is also provided.
- The package insert and the QRI contain a statement that laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test. 42 CFR 493.15(e)(1).
- Instructions for quality control (QC) are integrated with procedural instructions for performing the test in both the package insert and the QRI.
- Appropriate cautions have been added to the Package Insert and Quick Reference Instructions to ensure safe use of the product.
- The results of a Clinical Study that support the determination of eligibility for CLIA Waiver are included in the Package Insert.
- The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

N. Conclusion:

The sponsor conducted an appropriate clinical study in a CLIA waived healthcare setting. They also conducted a study to evaluate the performance of the assay with contrived samples near the assay cut-off with untrained operators in CLIA waived setting. The sponsor also conducted Hazard Analysis and performed appropriate flex studies to demonstrate that the test system is robust. Appropriate design and labeling mitigations are incorporated into the device to minimize misuse and reporting of erroneous results.

The submitted information in this CLIA waiver application supports a CLIA Waiver approval decision.