CLIA Waiver by Application Approval Determination
Decision Summary

I. Document Number

CW240030

II. Parent Document Number

K243753

III. CLIA Waiver Type

Dual 510(k) and CLIA Waiver by Application (Dual Submission)

IV. Applicant

Roche Molecular Systems, Inc.

V. Proprietary and Established Names

cobas liat Bordetella panel nucleic acid test
Common name: cobas liat Bordetella panel

VI. Measurand (analyte)

Bordetella pertussis, Bordetella parapertussis, and *Bordetella holmesii* nucleic acids

VII. Sample Type(s)

Human Nasopharyngeal Swabs

VIII. Type of Test

This assay is a multiplex nucleic acid assay for the qualitative detection and differentiation of *Bordetella pertussis*, *B. parapertussis*, and *B. holmesii* DNA through nucleic acid extraction, amplification, and detection using real-time PCR. All steps of the assay are automated within the cobas Liat System, after scanning the specimen ID barcode, scanning the assay tube barcode, and the manual addition of sample into the assay tube.

IX. Test System Description

A Overview

The cobas liat Bordetella panel nucleic acid test (cobas liat Bordetella panel) is an automated multiplex real-time polymerase chain reaction (PCR) assay for the rapid in vitro qualitative detection and differentiation of *B. pertussis* (Bp), *B. parapertussis* (Bpp), and *B. holmesii* (Bh) DNA in human nasopharyngeal swabs taken from patients with suspected pertussis respiratory infection.

The different fluorescent dye designs enable the specific detection and differentiation of the three microorganisms (Bp, Bpp, and Bh) independently in a multiplex system. The system automates all nucleic acid amplification test sample processing steps, including inhibitor removal, nucleic acid extraction, purification, amplification, real-time detection, and result interpretation in a rapid manner. The test is designed for use in near-patient settings to deliver results in approximately 15 minutes.

B Test System Components

The cobas liat system is comprised of the cobas liat analyzer hardware with integrated cobas liat system software for running tests and analyzing the results, and a single-use disposable cobas liat assay tube.

Reagents and Controls:

- Cobas liat Bordetella panel
- Cobas liat Bordetella panel control kit

Additional materials required but not provided:

- Nasopharyngeal swab collection kit
    - Flexible minitip FLOQSwab with Universal Transport Media from Copan Diagnostics or BD Universal Viral Transport 3-mL collection kit with a flocked flexible minitip swab

X. Specific Contents for CLIA Waiver

A Demonstrating "Simple":

- The cobas liat System automates all nucleic acid test (NAT) processes, including reagent preparation, target enrichment, inhibitor removal, nucleic acid extraction, amplification, real-time detection, and result interpretation in a rapid manner.
- The assay utilizes NPS specimens collected in transport medium without the need for any specimen manipulation. A provided fixed volume pipette is used to transfer the sample to the assay tube. The tube is capped and remains closed for the entire test process. No further materials need to be added or removed from the tube.
- Running the assay requires no reagent manipulation. The assay tube contains all assay reagents pre-packed in tube segments separated by seals.
- The assay tube is designed such that it can only be inserted in the cobas Liat Analyzer in one direction.
- The test does not require any operator intervention during the analysis step.

- The cobas liat analyzer performs automated analysis of test results, which are reported on the cobas liat Analyzer screen as "Detected" or "Not Detected" for Bp, Bpp and Bh targets. "Assay Invalid" is also reported, if appropriate.
- No technical or specialized training is required for troubleshooting or error code interpretation. If an error code is shown, simple on-screen instructions are provided to the operator for next steps.
- The system requires no electronic or mechanical maintenance tasks by the operator. The analyzer performs self-diagnostics during startup (initialization) and utilizes an advanced error diagnostics system to monitor the analyzer's performance during an assay. Under normal operation, the analyzer alerts the operator if a malfunction or error is detected.
- The analyzer requires no adjustment or calibration from the operator.
- The Quick Reference Instructions are written at a 7th grade comprehension level.

B Demonstrating "Insignificant Risk of an Erroneous Result"- Failure Alerts and Fail-Safe Mechanisms

1. Risk Analysis

A risk analysis was performed according to the principles of risk minimization as found in the standard EN ISO 14971 Medical Devices – Application of risk management to medical devices. Device Hazard Analysis and the Failure Mode Effects Analysis (FMEA) methods were used to assess the risk of failure (false positive, false negative or delayed test results) that may occur during use or misuse of the device. 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.

Based on the hazard analysis, all possible sources of error, which might be at an increased likelihood of occurring when the test system is used in CLIA waived settings by untrained users, were evaluated in flex studies to demonstrate the effectiveness of applicable built-in control measures and assess the insensitivity of the test system to variation under stress conditions. The considered risks included operator errors (human factors), sample and device handling and storage, and environmental factors.

2. Fail-Safe and Failure Alert Mechanisms

i. Internal Control

The built-in Internal Control monitors all processing steps (sample purification, amplification and detections) of targets. It also monitors for processing failure, compromised assay tube or the presence of inhibitors.

ii. External Control

Before using a new lot of cobas liat Bordetella panel, the "Lot Validation" procedure must be performed on the analyzer to validate the cobas liat Bordetella panel assay tube lot. The procedure includes running a negative control and a positive control in separate runs. After processing is completed for each control, the system will inform the user that the control result has been accepted. The user can now use that specific lot of assay tubes for processing samples.

iii. cobas liat system

The following safety features are implemented in the cobas liat system.

- The analyzer has a built-in auto monitoring system to ensure that it is functioning optimally at all times. When the analyzer is starting up, a series of initialization diagnostic tests are performed automatically which helps to maintain the health of the analyzer.
- Optical system surveillance that monitors status of detection optics and electronics.
- System prompts and instructs users on what to scan and when to load the assay tube.
- All the items a user needs to load are barcoded. System checks barcode coding and prevents use of off label assay tubes by verifying barcode validity.
- Assay tube is self-contained which reduces the risk of cross-contamination between samples.
- Assay tubes are barcoded and allowed to be processed only once on the system.
- System checks assay tube position if the assay tube was loaded correctly and aborts the run if not loaded correctly.
- System flags and invalidates runs automatically where processing errors are detected.
- System outputs results as a protected file so that results cannot be altered.
- Software installations are protected by cryptographic methods that verify the origin and integrity.

3. Flex Studies

The cobas Bordetella on the cobas Liat System is performed according to the same workflow as the previously cleared and CLIA waived tests on the Liat System (e.g., cobas Influenza A/B Nucleic acid test (K223591/CW220014), cobas Influenza A/B & RSV Nucleic acid test (K153544/CW150018), cobas SARS-CoV-2 Nucleic acid test (K223783/CW220015, and the cobas liat CT/NG/MG nucleic acid test (K240197/CW240002). The physical properties of the Liat Analyzer are shared between all assays. Therefore, some of the previously conducted flex studies, considered not to be assay specific, demonstrating operational robustness of the cobas Liat System, were not repeated and the previously generated data were leveraged for this application.

Verifying the effectiveness of built-in controls, lock-out features and failure alert mechanisms have been tested as part of the instrument/software and the system level verification and shown to be effective as shown in the table below.

Table 1: Fail-Safe and Failure Alert Mechanisms

Fail Safe /Failure Alert mechanism		Fail Safe/Failure Alert mechanism	Descriptions
Extreme Temperature	Outside the operating range [4°C to 40°C]; temperature <4°C or >40°C	Yes	cobas liat analyzer has a built-in sensor; checks the internal temperature during the power-on-self test that prevents assays from being run when the system is at a temperature <4°C or >40°C.
Non-level Surface	cobas liat analyzer placed on a surface > 10°tilt angle in transverse (x-axis) or sagittal axis (y-axis)	Yes	If the system is at a tilt angle exceeding 10° in either the transverse (x) or sagittal (y) axis it prevents assay run, displays an error message and aborts the run. The tilt sensor control measure is effective at monitoring the tilt angle failure conditions of the cobas liat analyzer.
Reagents and hardware integrity	cobas liat analyzer to prevent execution of runs with expired assay tubes	Yes	During the workflow of the sample processing, a customer is required to scan the barcode of the assay tube to be able to start the run. This barcode has the information about the assay, such as unique assay ID and expiration date. The system warns the user if barcode has expired and does not allow an expired assay tube to be loaded.

The following system-based or assay-independent flex studies were conducted previously and are thus only referenced in the CLIA Waiver Decision Summary of CW240002:

a. Variation in operating temperature (environmental temperature above/below specified range).
b. Variation in humidity levels (environmental humidity above/below specified range).
c. Variation in altitude and atmospheric pressure (operation above specified altitude).
d. Operation of the instrument on non-level surface (instrument tilt (x-, y-tilt)).
e. Sunlight exposure
f. Movement of the Liat Analyzer during analysis.
g. Poor ventilation
h. Drafty conditions
i. Use by multiple operators
j. Use of expired reagents
k. Variable Sample Volume (input sample above/below the specified volume).
l. Assay tube orientation post sample addition.
m. Broken or compromised seals within the Liat tubes (due to mishandling prior to testing)
n. Inadequate temperature equilibration of sample and reagents.

Additional flex studies, deemed as assay specific, were performed to evaluate the robustness (i.e., risk of erroneous results) of the cobas liat Bordetella Panel when subjected to potential variations in workflow and control effectiveness that may reasonably be expected to occur with untrained operators in the intended use CLIA waived setting. To perform the assay-specific flex studies, both co-formulated positive samples in negative clinical matrix were contrived at the ~3x LoD concentration and negative samples were used. Studies were acceptable if they either generated no false result or properly trigger a fail-safe condition or failure alert associated with the engineering control design. Test conditions were designed based on a risk analysis of the complete test system and included conditions intended to verify the effectiveness of built-in controls. Each study used two operators across multiple Liat analyzers. These studies are described below.

i. Human Factors

a. Improper Tube Storage

The objective of this flex study was to test the effect of improper storage of the cobas liat Bordetella assay tube both stored within the sealed foil pouch and removed. The study used both negative and co-formulated positive samples contrived in negative clinical matrix. Five replicates of each test samples per test condition were tested. Conditions and results are shown in Table 2 below.

Table 2: Improper Assay Tube Storage – Flex Study Results

Storage Condition			Bp/Bpp/Bh Negative Test Sample			Bp/Bpp/Bh Positive Test Sample		
Foil Pouch	**Temperature**	**Time (Days)**	**Bp Not detected**	**Bpp Not detected**	**Bh Not detected**	**Bp Detected**	**Bpp Detected**	**Bh Detected**
Enclosed	2-8°C	1	5/5	5/5	5/5	5/5	5/5	5/5
	-20°C	7	4*/5	4*/5	4*/5	5/5	5/5	5/5
	30°C	14	5/5	5/5	5/5	5/5	5/5	5/5
	37°C	14	5/5	5/5	5/5	5/5	5/5	5/5
Removed	-20°C	1	5/5	5/5	5/5	5/5	5/5	5/5
	2-8°C	1	5/5	5/5	5/5	5/5	5/5	5/5
	30°C	1	5/5	5/5	5/5	5/5	5/5	5/5
	37°C	1	5/5	5/5	5/5	5/5	5/5	5/5

* For the assay tubes enclosed in the foil pouch, the -20 °C storage temp condition resulted in one invalid run (IC invalid).

Results of this flex study observed a single invalid result for a negative sample stored at -20°C while all other negative and positive samples performed as expected. The invalid result did not cause increased risk or have a negative performance impact on the overall expected results. The results demonstrate that the reagents are robust against such user errors and support the Instructions for Use statement of storing assay tubes at 2-8°C and not freezing reagents.

b. Assay Tube Hold Time

The purpose of this study was to test the effect of hold time between sample addition to the cobas liat Bordetella panel assay tube and initiation of the test run. All studies were

conducted at 20-25°C using five co-formulated ~3x LoD concentration positive and negative samples in negative clinical matrix. All samples generated valid and expected results (see Table 3).

Table 3: Assay Tube Hold Time - Flex Study Results

	Bp/Bpp/Bh Negative Test Sample			Bp/Bpp/Bh Positive Test Sample		
Assay Tube Hold time (hours)	**Bp Not detected**	**Bpp Not detected**	**Bh Not detected**	**Bp Detected**	**Bpp Detected**	**Bh Detected**
0	5/5	5/5	5/5	5/5	5/5	5/5
2	5/5	5/5	5/5	5/5	5/5	5/5
4	5/5	5/5	5/5	5/5	5/5	5/5
6	5/5	5/5	5/5	5/5	5/5	5/5

Based on the results of this study, there was no observed performance impact with delays in test initiation after the specimen is added to the assay tube at room temperature conditions for up to 6 hours. The results demonstrate that the test is robust against user errors.

c. Improper Specimen Storage

Five replicates each of either ~3x LoD co-formulated positive and negative contrived samples in negative clinical matrix were stored at various temperatures and durations to simulate improper storage conditions. Based on results of this study, there was no observed performance impact with improper specimen storage conditions based on Table 4. The results demonstrate that the test is robust against user specimen storage errors.

Table 4: Improper Specimen Storage - Flex Studies Results

Storage Condition		Bp/Bpp/Bh Negative Test Sample			Bp/Bpp/Bh Positive Test Sample		
Temperature	**Days**	**Bp Not detected**	**Bpp Not detected**	**Bh Not detected**	**Bp Detected**	**Bpp Detected**	**Bh Detected**
4 °C	1 day	5/5	5/5	5/5	5/5	5/5	5/5
25 °C	1 day	5/5	5/5	5/5	5/5	5/5	5/5
37 °C	1 day	5/5	5/5	5/5	5/5	5/5	5/5
-80 °C	1 day	5/5	5/5	5/5	5/5	5/5	5/5
-20 °C	1 day	5/5	5/5	5/5	5/5	5/5	5/5
25 °C	2 days	5/5	5/5	5/5	5/5	5/5	5/5
4 °C	4 days	5/5	5/5	5/5	5/5	5/5	5/5

d. Bubbles in Sample Chamber

This study aims to test the effect of accidental introduction of bubbles to the sample aliquot during transfer of the specimen using the included sample pipet. Five replicates of co-formulated positive or negative samples contrived in negative clinical matrix were tested with bubbles and without (control) bubbles. All samples tested in both conditions generated

expected results and demonstrate that the test is robust against accidental introduction of bubbles into the specimen during the transfer process.

ii. Control Effectiveness

a. Internal Control Effectiveness

The objective of this flex study is to evaluate Internal Control (IC) results under process and reagent failure conditions to verify the ability of the IC to monitor for performance of sample preparation and PCR amplification and detection. Control and test conditions are described in Table 5 with results presented in Table 6 below.

Table 5: Internal Control Effectiveness Test Conditions

Test Condition	Performance of	Failure Description
0	Control	Normal Conditions
1	Systematic Error in Sample Preparation	Process Failure: Failure to capture magnetic glass particles during nucleic acid extraction
2	Assay Tube Lot in Sample Preparation	Reagent Failure: Break off frangible seal between the assay tube Sample Preparation segments
3	Systematic Error in PCR amplification and detection	Process Failure: Deviation in PCR temperature
4	Assay Tube Lot in PCR amplification and detection	Reagent Failure: Break off frangible seal between the assay tube PCR segments

Invalid or aborted results were observed for each of the conditions 1-4 above, with negative and positive results generated as expected for control conditions. Aborted results were only found in 2/5 negative samples in condition 4 and did not present concerns or risk of false results. Overall, the results demonstrate that the IC is effective to monitor the performance of sample preparation and PCR amplification and amplicon detection.

Table 6: IC Effectiveness Study Results

Condition	Negative Test Sample		Positive Test Sample	
	Invalid	Bp/Bpp/Bh Not detected	Invalid	Bp/Bpp/Bh Detected
0. Normal process (no simulated failures)	0/5	5/5	0/5	5/5
1. Failure to capture magnetic glass particles during nucleic acid extraction	5/5	0/5	5/5	0/5

Condition	Negative Test Sample		Positive Test Sample	
	Invalid	Bp/Bpp/Bh Not detected	Invalid	Bp/Bpp/Bh Detected
2. Frangible seal break between the assay tube sample preparation segments (adjacent tube segments containing Lysis Buffer and Wash Buffer)	5/5	0/5	5/5	0/5
3. Deviation in PCR temperature from set point	5/5	0/5	5/5	0/5
4. Frangible seal break between assay tube PCR segments (adjacent tube segments containing Lysis Buffer and Wash Buffer and Elution Buffer)	3*/5	0/5	5/5	0/5

*Of the 5 replicates, there were 2 abort runs.

a. External Control Effectiveness

This study evaluates the external control results under process and reagent (assay tube) failures to determine the ability of the Positive Control (PC) and Negative Control (NC) to monitor performance of an assay tube lot or systemic errors. Conditions of testing are listed in Table 7. For conditions 1 and 3, the assay script was altered to simulate process failure while for conditions 2 and 4, seal breaks were done to simulate reagent and assay tube failure. The design of condition 5 emulates potential low-level contamination of an external NC by spiking a co-formulated positive sample at ~3x LoD concentration into a NC sample. Condition 5 was only performed using a contaminated negative sample.

Table 7: External Control Effectiveness Test Conditions

Test Condition	Performance of	Failure Description
0	Control	Normal process (no simulated failures)
1	Systematic Error in Sample Preparation	Failure to capture magnetic glass particles during nucleic acid extraction
2	Assay Tube Lot in Sample Preparation	Break off frangible seal between the assay tube Sample Preparation segments
3	Systematic Error in PCR amplification and detection	Deviation in PCR temperature
4	Assay Tube Lot in PCR amplification and detection	Break off frangible seal between the assay tube PCR segment
5	NC to detect low level contamination	Failure of external NC and invalid result reporting

Results are shown in Table 8 below. The NC and PC responded to all test conditions as expected. Conditions 1-5 resulted in either invalid or aborted runs while the control (condition 0) produced valid results for both external controls, as expected. These results demonstrate the external control robustness to monitor the performance of the assay tube lot or systematic errors.

Table 8: External Negative Control Effectiveness

		Results Interpretation		Run Status	Results Interpretation		Run Status
Condition	**Failure Description**	**Negative Control Invalid**	**Negative Control Valid**	**Negative Control Aborted**	**Positive Control Invalid**	**Positive Control Valid**	**Positive Control Aborted**
0	Normal process (no simulated failures)	0/5	5/5	0/5	0/5	5/5	0/5
1	Failure to capture magnetic glass particles during nucleic acid extraction	5/5	0/5	0/5	5/5	0/5	0/5
2	Break off frangible seal between the assay tube Sample Preparation segments*	4/5	0/5	1/5	4/5	0/5	1/5
3	Deviation in PCR temperature	5/5	0/5	0/5	5/5	0/5	0/5
4	Break off frangible seal between the assay tube PCR segment**	5/5	0/5	0/5	4/5	0/5	1/5
5	Run NC to simulate low level contamination	5/5	0/5	0/5	NA	NA	NA

NA: Not Applicable
*There was 1 aborted run for the NC and 1 aborted run for the PC. Aborted or invalid runs are acceptable for this condition.
**There was 1 aborted run for the PC. Aborted or invalid runs are acceptable for this condition.

The risk mitigations for the flex studies performed and leveraged are shown in Table 9 below.

Table 9: Summary of Flex studies and Risk Mitigations

Flex Studies	Test Conditions	Acceptance Criteria Met		Fail Safe mechanism/Failure Alert or Labeling Mitigation
		Negative Test Samples	Positive Test Samples	
Assay tube seal breakage	Deliberate seal break between each segment of the Liat tube.	Yes	Yes	Yes, built-in effective Internal Control (abort or invalid) when selected seal is broken between segments. *[Labeling]* cobas liat Bordetella panel IFU advises the operator not to use assay tubes if the assay tube is punctured.
Input sample volume (incorrect sample volume)	Sample volumes tested at maximum system volume, low volume within threshold, and low volume below threshold.	Yes	Yes	Yes, built-in fail-safe mechanism or failure alert. When sample input volume was too low, the Internal Control was effective.
Assay tube orientation post-sample-addition (incorrect cartridge handling)	After adding sample, assay tubes were held in vertical, horizontal, or inverted orientation, vigorously shaken, or dropped from a height of ~3 feet.	Yes	Yes	Yes, built-in fail-safe mechanism or failure alert. For dropped tubes, the Internal Control and System fail-safe measures were effective. *[Labeling]* cobas liat Bordetella panel IFU advises the operator not to use an assay tube that has been dropped after removal from its foil pouch.
Sample and reagent temperature (inadequate temperature equilibration)	Run assay tubes immediately after removing from 2-8 °C storage	Yes	Yes	No built-in fail-safe mechanism or failure alert.
Improper Tube Storage	Assay tube incorrectly stored in freezer, or extended storage in room temperature or incorrect storage at high temperature before and after opening the foil.	Yes (except one invalid run at -20°C for 7 days)	Yes	No built-in fail-safe mechanism or failure alert. *[Labeling]* cobas liat Bordetella panel IFU recommends the tube storage at 2-8°C
Assay Tube Hold Time	Assay tube was held for 2, 4 and 6 hours after addition of the sample and before initiation of the run on the cobas liat analyzer	Yes	Yes	No built-in fail-safe mechanism or failure alert. *[Labeling]* cobas liat Bordetella panel IFU recommends to start the run as soon as possible but no later than 4 hours with storage at room temperature.

Flex Studies	Test Conditions	Acceptance Criteria Met		Fail Safe mechanism/Failure Alert or Labeling Mitigation
		Negative Test Samples	Positive Test Samples	
Improper Specimen Storage	Specimens were stored at different temperatures for extended time (4°C for 4 days, 25°C for 2 days, 37°C, -20°C and -80°C for 1 day)	Yes	Yes	No built-in fail-safe mechanism or failure alert. *[Labeling]* cobas liat Bordetella panel IFU recommends the specimen storage at 15-30°C for upto 4 hours after collection, or at 2-8°C for up to 72 hours.
Bubbles in sample chamber	Bubbles introduced in the sample chamber	Yes	Yes	No built-in fail-safe mechanism or failure alert.

B Demonstrating "Insignificant Risk of an Erroneous Result" - Accuracy

1. Clinical Study:

   The clinical performance of the cobas liat Bordetella panel was evaluated in a multi-site prospective study in the U.S. between July 2023 - February 2024. The study enrolled 823 subjects suspected of pertussis respiratory infection and nasopharyngeal specimens were prospectively collected from these subjects presenting to point-of-care settings (e.g., emergency rooms, outpatient clinics, etc.). Out of 823 subjects, 54 subjects were excluded due to protocol deviations or indeterminate or invalid comparator results. Twenty-six operators representative of CLIA-waived users at eight different external study sites were involved in sample collection and testing with the cobas liat Bordetella panel. The demographic summary of the prospective clinical subjects is shown in Table 10 below.

Table 10: Demographics of Evaluable Subjects from Prospective Clinical Study

Age Category	No. of Specimens	Percentage
< 1	27	3.51%
1 to <5	89	11.57%
5 to <12	69	8.97%
12 to <18	32	4.16%
18 to <40	219	28.48%
40 to <65	270	35.11%
>=65	63	8.19%
Sex at Birth	**No. of specimens**	**Percentage**
Male	338	43.95%
Female	431	56.05%
Total	**769**	**100%**

Archived clinical specimens and contrived samples were also evaluated to establish device performance due to the very low prevalence observed in the prospective clinical study. The archived positive specimens previously collected from symptomatic patients were characterized using an FDA-cleared NAAT (Nucleic Acid Amplification Test) and included in the study along with additional negative archived specimens to avoid potential bias. Contrived samples were prepared in pooled negative clinical matrix by spiking 2x, 3x, 5x, 10x and 20x LoD of each of the target. All archived and contrived specimens were randomized and distributed to the study site for testing on the cobas liat Bordetella panel. Prospective and archived specimens were then sent to a laboratory for comparator method testing per respective IFU and if required a validated sequencing method was preformed to confirm the comparator result.

The clinical performance of the cobas liat Bordetella panel was assessed by comparing results to FDA-cleared target-specific NAAT. For Bp, the reference method was a composite of an FDA cleared NAAT and a validated bi-directional sequencing method to confirm the presence of Bp. For Bpp and Bh, NAAT was used as the reference comparator method.

The clinical performance of the cobas liat Bordetella panel in terms of Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) versus the comparator reference method is shown in Table 11 below. For prospective and archived specimens, the reference method is the patient infected status and for contrived specimens the reference method is the expected result.

Table 11: Overall Clinical Performance Summary of cobas liat Bordetella Panel

Target	Specimen Type	Total (N)	PPA	PPA 95% CI	NPA	NPA 95% CI
Bp	Prospective	743	NC	NC	100.0% (743/743)	99.5 – 100%
	Archived	160	100% (42/42)	91.6 – 100%	99.2% (117/118)	95.4 – 99.9%
	Contrived	327	98.8% (80/81)	93.3 – 99.8%	99.6% (245/246)	97.7 – 99.9%
	Overall	1230	99.2% (122/123)	95.5 – 99.9%	99.8% (1105/1107)	99.3 - 100%
Bpp	Prospective	743	0.0% (0/1)	0.0 –79.3%	100.0% (742/742)	99.5 - 100%
	Archived	170	100.0% (28/28)	87.9 – 100%	100.0% (142/142)	97.4 - 100%
	Contrived	327	100.0% (108/108)	96.6 – 100%	99.5% (218/219)	97.5 – 99.9%
	Overall	1240	99.3% (136/137)	96.0 – 99.9%	99.9% (1102/1103)	99.5 - 100%
Bh	Prospective	702	NC	NC	99.9% (701/702)	99.2 - 100%
	Contrived	328	100.0% (139/139)	97.3 – 100%	98.9% (187/189)	96.2 – 99.7%
	Overall	1030	100.0% (139/139)	97.3 – 100%	99.7% (888/891)	99.0 – 99.9%

NC- Not Calculable; CI: Confidence Interval

The percent agreement of contrived positive clinical specimens included in the study are provided in Table 12 by strain and LoD level for Bp, Bpp and Bh targets.

Table 12: Contrived Specimens Percent Agreement for Bp, Bpp and Bh

Strain	LoD	BP Target Percent Agreement (n / N)	BPP Target Percent Agreement (n / N)	BH Target Percent Agreement (n / N)
A	2x	100.0% (4/4)	100.0% (5/5)	100.0% (9/9)
A	3x	100.0% (4/4)	100.0% (5/5)	100.0% (9/9)
A	5x	100.0% (3/3)	100.0% (3/3)	100.0% (8/8)
A	10x	100.0% (2/2)	100.0% (3/3)	100.0% (5/5)

Strain	LoD	BP Target Percent Agreement (n / N)	BPP Target Percent Agreement (n / N)	BH Target Percent Agreement (n / N)
A	20x	100.0% (1/1)	100.0% (2/2)	100.0% (4/4)
B	2x	100.0% (4/4)	100.0% (5/5)	100.0% (9/9)
B	3x	75.0% (3/4)	100.0% (4/4)	100.0% (9/9)
B	5x	100.0% (3/3)	100.0% (3/3)	100.0% (8/8)
B	10x	100.0% (2/2)	100.0% (3/3)	100.0% (5/5)
B	20x	100.0% (1/1)	100.0% (2/2)	100.0% (4/4)
C	2x	100.0% (4/4)	100.0% (5/5)	100.0% (9/9)
C	3x	100.0% (4/4)	100.0% (5/5)	100.0% (9/9)
C	5x	100.0% (3/3)	100.0% (3/3)	100.0% (8/8)
C	10x	100.0% (2/2)	100.0% (3/3)	100.0% (5/5)
C	20x	100.0% (1/1)	100.0% (2/2)	100.0% (4/4)
D	2x	100.0% (4/4)	100.0% (5/5)	100.0% (9/9)
D	3x	100.0% (4/4)	100.0% (6/6)	100.0% (9/9)
D	5x	100.0% (3/3)	100.0% (3/3)	100.0% (8/8)
D	10x	100.0% (2/2)	100.0% (3/3)	100.0% (5/5)
D	20x	100.0% (1/1)	100.0% (2/2)	100.0% (3/3)
E	2x	100.0% (4/4)	100.0% (5/5)	N/A
E	3x	100.0% (3/3)	100.0% (5/5)	N/A
E	5x	100.0% (3/3)	100.0% (3/3)	N/A
E	10x	100.0% (2/2)	100.0% (3/3)	N/A
E	20x	100.0% (1/1)	100.0% (2/2)	N/A
F	2x	100.0% (4/4)	100.0% (5/5)	N/A
F	3x	100.0% (3/3)	100.0% (5/5)	N/A
F	5x	100.0% (3/3)	100.0% (3/3)	N/A
F	10x	100.0% (1/1)	100.0% (3/3)	N/A
F	20x	100.0% (1/1)	100.0% (2/2)	N/A
Positive Percent Agreement (95% CI)		98.8% (80/81: 93.3%, 99.8%)	100.0% (108/108: 96.6%, 100.0%)	100.0% (139/139: 97.3%, 100.0%)

Note: n is the number of positive results and N is the number of valid results.
Strains used for BP contrived specimen: A=A639, B=E431, C=ATCC 51445, D=ATCC 9797, E=ATCC 8467, F=ATCC 9306; for BPP: A=E838, B=A747, C=ATCC 15311, D=ATCC 15237, E=BAA-587, F=E595; for BH: A=F061, B=ATCC 51541, C=ATCC 700053, D=ATCC 700052.

2. Device Performance with Analyte Concentrations Near the Cut-Off:

A reproducibility study was conducted to assess the total variability of the cobas liat Bordetella panel assay across operators, study sites, testing days, cobas liat analyzers, and cobas liat assay tube lots. The reproducibility study was conducted across three CLIA waived sites using a testing panel of three sample types: low positive (1-2x LoD), moderate positive (3-5x LoD), and negative samples. Each sample was run in triplicate on three analyzers across five different days with three different reagent lots. The study was performed by two

operators/site resulting in approximately 270 test results/panel member or 810 total test results (3 panel members × 3 replicates × 2 operators × 5 days × 3 sites × 3 lots).

The reproducibility panel samples were prepared by spiking different concentrations of one strain each of Bp, Bpp and Bh bacteria into a UTM-based human clinical matrix. The panels were provided to the sites with coded sample identification numbers to reduce bias. Each sample was processed according to the cobas liat Bordetella panel instructions for use. Analysis of the Ct signal variability for the positive panel members is presented below in Table 13.

Table 13: Reproducibility Results of Positive Panel Members

Panel Member	Target Analyte	Mean Ct	Between-Site		Between-Lot		Between-Day		Between-Run (Operator)		Repeatability		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
Low Positive (1-2X LoD)	*B. pertussis*	33.2	0.05	0.1	0.35	1.1	0.23	0.7	0.15	0.5	0.59	1.8	0.74	2.2
	B. parapertussis	31.9	0.17	0.5	0.31	1.0	0.15	0.5	0.00	0.0	0.57	1.8	0.68	2.1
	B. holmesii	27.8	0.00	0.0	0.27	1.0	0.14	0.5	0.11	0.4	0.56	2.0	0.65	2.3
Moderate Positive (3-5X LoD)	*B. pertussis*	32.0	0.20	0.6	0.49	1.5	0.00	0.0	0.22	0.7	0.45	1.4	0.73	2.3
	B. parapertussis	30.5	0.21	0.7	0.29	1.0	0.14	0.5	0.05	0.2	0.51	1.7	0.64	2.1
	B. holmesii	26.8	0.14	0.5	0.29	1.1	0.04	0.1	0.14	0.5	0.41	1.5	0.54	2.0

Ct: cycle threshold, CV%: percent coefficient of variation, LoD: limit of detection, SD: standard deviation.

The total Ct CV% ranged from 2.0 – 2.3 across the target panel members tested. These results indicate that the reproducibility of the cobas liat Bordetella panel assay on the liat system is acceptable in NPS samples. For all positive panel members, the repeatability/within-run factor (i.e., random error) followed by the between lot reproducibility was the largest contributor to total variability. Percent agreement across the three testing sites is shown in Table 14.

Table 14: Reproducibility Result Summary Across Sites

Panel Member	Target Analyte	Total number of valid test runs	% Agreement (n Agreement/N Tested) [95% CI]			
			Site A	Site B	Site C	Overall
Negative	N/A	264	100.0 (87/87)	100.0 (89/89)	100.0 (88/88)	100.0 (264/264) [98.6, 100.0]
Low Positive (1-2× LoD)	*B. pertussis*	258	100.0 (85/85)	100.0 (85/85)	98.9 (87/88)	99.6 (257/258) [97.8, 99.9]
	B. parapertussis	258	100.0 (85/85)	100.0 (85/85)	98.9 (87/88)	99.6 (257/258) [97.8, 99.9]
	B. holmesii	258	100.0 (85/85)	100.0 (85/85)	98.9 (87/88)	99.6 (257/258) [97.8, 99.9]
Moderate Positive (3-5× LoD)	*B. pertussis*	265	100.0 (88/88)	100.0 (89/89)	100.0 (88/88)	100.0 (265/265) [98.6, 100.0]
	B. parapertussis	265	100.0 (88/88)	100.0 (89/89)	100.0 (88/88)	100.0 (265/265) [98.6, 100.0]
	B. holmesii	265	100.0 (88/88)	100.0 (89/89)	100.0 (88/88)	100.0 (265/265) [98.6, 100.0]

The cobas liat Bordetella panel assay demonstrated 100% agreement for the negative panel members and for all target analytes tested at the moderate positive concentration across the three testing sites. For low positive panel members, the assay yielded 99.6% agreement for all target analytes (see Table 14 above). Notably, there was one negative test result for all 3 target analytes when tested at 1-2x LoD, occurring at site C, with one operator on day two. Overall, the total agreement of 99.6% for low positive panel member is acceptable, since the analyte concentration between 1-2x the LoD is expected to yield a ≥95% detection rate.

3. Operator Questionnaire:

Upon completing their participation within the clinical study, operators were provided a questionnaire to assess the ease-of-use. Ease-of- use agreement scores were high, ranging from 4.1 (4 being agree) to 4.7 (5 being strongly agree). The overall score was 4.4 for all operators' answers to all 8 statements, indicating that operators agreed the device was easy-to-use overall (Table 15).

Table 15: Operators Post Study Ease-of Use Questionnaire Results

Statement	Average Agreement with Statement Score[a] (1 = Strongly Disagree, 5 = Strongly Agree)
The instructions to add lot and perform controls were easy to follow.	4.3
The instructions to test specimens were easy to follow.	4.6
It was easy to load the sample into the Liat assay tube.	4.7
It was easy to start the assay on the Liat analyzer.	4.4
It was easy to read the test results.	4.5
It was easy to understand the test results.	4.4
The Instructions For Use and Quick Reference Instructions clearly explain what to do if a test result is invalid.	4.1
I did not need help when I tested samples using the Liat assay.	4.3
Overall Score	4.4

[a] Statements were scored as follows: 1 = Strongly Disagree, 2 = Disagree, 3 = Neutral, 4 = Agree, 5 = Strongly Agree.

Separately, operators were assessed for proficiency for reporting the correct positive result, negative result, and conducting the correct response on targets that cannot be assessed. The overall operator proficiency score (Table 16) across these responses was 99.1% indicating that the test is easy to use, and the written instructions are clear.

Table 16: Overall Study Operator Proficiency Test Result

Site ID	Operator	Score for Correct Response on Bp Target	Score for Correct Response on Bpp Target	Score for Correct Response on Bh Target	Score for Correct Response on Assay Result	Overall Score
Mean Score	Overall	98.6%	99.1%	99.1%	100.0%	99.1%

C Labeling for Waived Devices

The labeling includes the following:

a) Quick Reference Instructions (QRI)

b) Package insert or Instructions for Use (IFU)

c) Package Labeling – Tube pouch, tube sleeve, carton sleeve, carton, Unique Device Identification (UDI), PC pouch, PC vial, NC pouch, NC vial, Control pouch, card for control kit and card for add lot purposes

d) Technical/operator manuals.

The following elements are appropriately present in the labeling documents:

- The test procedures within the QRI are written at 7th grade comprehension level.
- The QRI and the IFU identify the test as CLIA waived.
- The IFU contains a statement that a Certificate of Waiver is required to perform the test in a waived setting.
- The QRI and the IFU contain a statement that laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test.
- The IFU contains a statement that any modification to the test or the manufacturer’s instructions will result in the test being classified as high complexity.
- The IFU and QRI provide instructions for conducting quality control procedures.
- The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

XI. Conclusion

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