



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT**

I Background Information:

A 510(k) Number

K243753

B Applicant

Roche Molecular Systems, Inc.

C Proprietary and Established Names

cobas liat Bordetella panel nucleic acid test

Common name: cobas liat Bordetella panel

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
OZZ	Class II	21 CFR 866.3980 - Respiratory Viral Panel Multiplex Nucleic Acid Assay	MI - Microbiology
OOI	Class II	21 CFR 862.2570 – Instrumentation for clinical multiplex test systems	CH - Chemistry

II Submission/Device Overview:

A Purpose for Submission:

The purpose of this submission is to obtain a substantial equivalence determination for the cobas liat Bordetella panel nucleic acid test on the cobas Liat System.

B Measurand:

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

C 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.

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

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

The test is meant to be used in conjunction with other clinical and epidemiological information and laboratory findings. When clinical factors suggest that *B. pertussis*, *B. parapertussis* or *B. holmesii* may not be the cause of respiratory infection, other clinically appropriate investigation(s) should be carried out in accordance with published guidelines.

Negative results do not preclude Bp, Bpp, or Bh infection and should not be used as the sole basis for treatment or other patient management decisions. Conversely, positive results do not rule out co-infection with other bacteria or viruses. The agent detected may not be the definite cause of disease.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

For use with the cobas Liat system

IV Device/System Characteristics:

A Device Description:

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.

The cobas liat system is comprised of the cobas liat analyzer (analyzer) hardware with integrated cobas liat system software (analyzer software + liat assay specific package (script)) for running tests and analyzing the results, and a single-use disposable cobas liat assay tube (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 flocced flexible minitip swab

B Principle of Operation:

The test is performed on the cobas liat analyzer which automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in biological samples using real-time PCR assays. The assay targets the IS110-like element IS1663 family transposase of *Bordetella pertussis*, the ISL3-like element IS1001 family transposase of *Bordetella parapertussis*, and the ISL3 family transposase of *Bordetella holmesii*. An Internal Control (IC) is also included. The IC is present to control for adequate processing of the target bacteria through steps of sample purification, nucleic acid amplification, and to monitor the presence of inhibitors in the PCR processes.

C Instrument Description:

The cobas liat system can automatically perform qualitative in vitro nucleic acid amplification tests. The cobas liat system is comprised of the cobas liat analyzer hardware with integrated cobas liat system software and a single-use disposable cobas liat assay tube. The cobas liat analyzer is a system component that consists of one software subsystem and three hardware units: a) Infrastructure unit which consists of the hardware and embedded software (firmware); b) thermal, loading and motion unit which is the processing module that interacts physically with the assay tube during the assay execution, and c) Detection unit consisting of the photodetectors that is used for the fluorescence detection during the PCR reaction. The assay script provides a set of instructions to the analyzer hardware and software for assay tube processing, PCR, result calculation and interpretation and result reporting. The assay script can be installed on the

analyzer independently of the analyzer software. The cobas liat Bordetella panel nucleic acid test is supported with a Liat Assay Specific Package (LASP).

The assay tube holds all reagents needed for sample preparation and PCR processes. Collectively with an assay tube, the analyzer performs reagent preparation, target enrichment, inhibitor removal, nucleic acid extraction, polymerase chain reaction (PCR) amplification, real-time detection and result interpretation to automate the detection of nucleic acid targets in the biological sample.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Verigene Respiratory Pathogens Flex Nucleic Acid Test (RP Flex)

B Predicate 510(k) Number(s):

K143653

C Comparison with Predicate(s):

Device & Predicate Device	K243753	K143653
Device Trade Name	cobas liat Bordetella Panel nucleic acid test	Verigene Respiratory Flex Panel
General Device Characteristic Similarities		
Regulation name	866.3980	866.3980
Product code	OZZ OOI	OZZ, OOI, OCC, OEM, OEP, OOU, OZE
Intended Use	<p>The cobas liat Bordetella panel nucleic acid test (cobas liat Bordetella panel) is an automated real-time polymerase chain reaction (PCR) test intended for the simultaneous qualitative detection and differentiation of <i>Bordetella pertussis</i> (Bp), <i>Bordetella parapertussis</i> (Bpp), and <i>Bordetella holmesii</i> (Bh) nucleic acid in human nasopharyngeal swabs taken from patients with suspected pertussis respiratory infection.</p> <p>The test is meant to be used in conjunction with other clinical and epidemiological</p>	<p>The Verigene Respiratory Pathogens Flex Nucleic Acid Test (RP Flex) is a multiplexed qualitative test intended for the simultaneous detection and identification of multiple viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infection. The test is performed on the automated Verigene System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and microarray hybridization to detect gene sequences of the following organism types and subtypes:</p>

Device & Predicate Device	K243753	K143653																														
	<p>information and laboratory findings. When clinical factors suggest that <i>B. pertussis</i>, <i>B. parapertussis</i> or <i>B. holmesii</i> may not be the cause of respiratory infection, other clinically appropriate investigation(s) should be carried out in accordance with published guidelines.</p> <p>Negative results do not preclude Bp, Bpp, or Bh infection and should not be used as the sole basis for treatment or other patient management decisions. Conversely, positive results do not rule out co-infection with other bacteria or viruses. The agent detected may not be the definite cause of disease.</p>	<table><tr><td>Viruses:</td><td>Bacteria:</td></tr><tr><td>Adenovirus</td><td>Bordetella</td></tr><tr><td>Human</td><td>parapertussis/br</td></tr><tr><td>Metapneumovirus</td><td>onchiseptica,</td></tr><tr><td>Influenza A</td><td>Bordetella</td></tr><tr><td>Influenza A (Subtype H1)</td><td>holmesii,</td></tr><tr><td>Influenza A (Subtype H3)</td><td>Bordetella</td></tr><tr><td>Influenza B</td><td>pertussis</td></tr><tr><td>Parainfluenza 1</td><td></td></tr><tr><td>Parainfluenza 2</td><td></td></tr><tr><td>Parainfluenza 3</td><td></td></tr><tr><td>Parainfluenza 4</td><td></td></tr><tr><td>Respiratory Syncytial Virus A</td><td></td></tr><tr><td>Respiratory Syncytial Virus B</td><td></td></tr><tr><td>Rhinovirus</td><td></td></tr></table> <p>Detecting and identifying specific viral and bacterial nucleic acids from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory infection, if used in conjunction with other clinical and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or patient management decisions. Negative results in the presence of a respiratory illness do not preclude respiratory infection and may be due to infection with pathogens that are not detected by this test or lower respiratory tract infection that is not detected by an NPS specimen. Conversely, positive results do not rule-out infection or co-infection with organisms not detected by RP Flex. The agent(s) detected may not be the definite cause of disease. The use of additional laboratory testing and clinical presentation may be necessary to establish a final diagnosis of respiratory infection. Clinical evaluation indicates a lower sensitivity specific to RP Flex for the</p>	Viruses:	Bacteria:	Adenovirus	Bordetella	Human	parapertussis/br	Metapneumovirus	onchiseptica,	Influenza A	Bordetella	Influenza A (Subtype H1)	holmesii,	Influenza A (Subtype H3)	Bordetella	Influenza B	pertussis	Parainfluenza 1		Parainfluenza 2		Parainfluenza 3		Parainfluenza 4		Respiratory Syncytial Virus A		Respiratory Syncytial Virus B		Rhinovirus	
Viruses:	Bacteria:																															
Adenovirus	Bordetella																															
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Device & Predicate Device	K243753	K143653
		detection of Rhinovirus. If infection with Rhinovirus is suspected, negative samples should be confirmed using an alternative method. Performance characteristics for Influenza A were established when Influenza A/H1 (2009 Pandemic) and A/H3 were the predominant Influenza A viruses in circulation. RP Flex may not detect novel Influenza A strains. If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions used specifically for novel virulent influenza viruses and sent to appropriate health authorities for testing. Viral culture should not be attempted in these cases unless a biosafety level (BSL) 3+ facility is available to receive and culture specimens.
Sample Type	Nasopharyngeal swab (NPS)	Same
Ancillary Collection Kits	Flocked swab with Universal Transport Media (UTM)	Same
Amplification Technology	Real-time PCR	Same
Controls Used	Sample processing control (IC) and Positive and negative control	Same
General Device Characteristic Differences		
Analyte Targets	<i>Bordetella pertussis</i> (BP) <i>Bordetella parapertussis</i> (BPP) <i>Bordetella holmesii</i> (BH)	Viruses: Adenovirus Human Metapneumovirus Influenza A Influenza A (Subtype H1) Influenza A (Subtype H3) Influenza B Parainfluenza 1 Parainfluenza 2 Bacteria: <i>Bordetella parapertussis/b</i> <i>ronchiseptica</i> , <i>Bordetella holmesii</i> , <i>Bordetella pertussis</i>

Device & Predicate Device	K243753	K143653
		Parainfluenza 3 Parainfluenza 4 Respiratory Syncytial Virus A Respiratory Syncytial Virus B Rhinovirus
Time to result	~15 minutes	~ 2 hours
Detection chemistry	TaqMan probes with fluorescent dyes	Microarray hybridization, Gold nanoparticles probe with light scattering
Instrumentation	cobas liat system	Verigene System

VI Standards/Guidance Documents Referenced:

ISO 14971 – Application of risk management to medical devices.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

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 1.

Table 1: 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)	<i>B. pertussis</i>	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
	<i>B. parapertussis</i>	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
	<i>B. holmesii</i>	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)	<i>B. pertussis</i>	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
	<i>B. parapertussis</i>	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
	<i>B. holmesii</i>	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 summarized in Table 2.

Table 2: 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 (1-2x LoD)	<i>B. pertussis</i>	258	100.0 (85/85)	100.0 (85/85)	98.9 (87/88)	99.6 (257/258) [97.8, 99.9]
	<i>B. parapertussis</i>	258	100.0 (85/85)	100.0 (85/85)	98.9 (87/88)	99.6 (257/258) [97.8, 99.9]
	<i>B. holmesii</i>	258	100.0 (85/85)	100.0 (85/85)	98.9 (87/88)	99.6 (257/258) [97.8, 99.9]
Moderate Positive (3-5x LoD)	<i>B. pertussis</i>	265	100.0 (88/88)	100.0 (89/89)	100.0 (88/88)	100.0 (265/265) [98.6, 100.0]
	<i>B. parapertussis</i>	265	100.0 (88/88)	100.0 (89/89)	100.0 (88/88)	100.0 (265/265) [98.6, 100.0]
	<i>B. holmesii</i>	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 2 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 LoD is expected to yield a $\geq 95\%$ detection rate.

2. Linearity:

Not applicable; this is a qualitative assay.

3. Analytical Specificity/Interference:

i. *Cross-reactivity and Microbial Interference*

A cross-reactivity and microbial interference study was conducted to verify the analytical specificity of the cobas liat Bordetella panel by demonstrating that microorganisms closely related to Bp, Bpp and Bh as well as common respiratory flora, pathogens and other clinically relevant microorganisms that could be present in nasopharyngeal swab specimens, do not cause false positive results due to cross-reactivity or false negative results due to interference. Forty-four different microorganisms listed in Table 3 representing closely related or found in the upper respiratory tract were individually mixed with pooled negative clinical matrix with and without co-formulated Bp, Bpp and Bh isolates at ~3x LoD concentration. Samples that contained co-formulated Bp, Bpp and Bh assessed potential interference with detection of one or all of the target analytes, while samples without Bp, Bpp and Bh assessed potential cross-reactivity.

Each potentially interfering microorganism was tested at 1E+06 CFU/mL for bacterial and yeast organisms and 1.0E+05 TCID₅₀ of copies/mL for viruses or at the highest concentration possible. Results from the study indicated that none of the non-target organisms generated false positive or false negative results due to cross-reactivity or interference.

The in-silico analysis for possible cross-reactivity was conducted by mapping binding regions of the primers and probes in the cobas assay to sequences available from the NCBI database. In silico analysis revealed two potential cross-reactants for the Bpp target, *B. bronchiseptica* and *A. denitrificans* (i.e., greater than or equal to 80% homology for the Bpp primers and probe). However, no cross-reactivity was observed in wet testing using three different strains of these organisms listed in Table 3.

Table 3: List of Microorganisms tested for Analytical Specificity or Interference

Micro-organism name, Bacteria/Fungi		Micro-organism name/Virus
<i>Bordetella bronchiseptica</i> *	<i>Legionella pneumophila</i>	Cytomegalovirus ^a
<i>Bordetella avium</i>	<i>Moraxella catarrhalis</i>	Epstein-Barr Virus
<i>Bordetella hinzii</i>	<i>Mycobacterium tuberculosis</i>	Influenza A
<i>Bordetella petrii</i>	<i>Mycoplasma pneumoniae</i>	Influenza B
<i>Bordetella trematum</i>	<i>Neisseria gonorrhoeae</i>	Human Rhinovirus
<i>Achromobacter denitrificans</i> **	<i>Neisseria meningitidis</i>	Respiratory Syncytial Virus Type A
<i>Chlamydia pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	Adenovirus
<i>Chlamydia trachomatis</i>	Methicillin-resistant <i>Staphylococcus aureus</i>	Human Coronavirus
<i>Corynebacterium diphtheria</i>	<i>Staphylococcus epidermidis</i>	Herpes Simplex Virus Type 1

Micro-organism name, Bacteria/Fungi		Micro-organism name/Virus
<i>Escherichia coli</i>	<i>Stenotrophomonas maltophilia</i>	Herpes Simplex Virus Type 2
<i>Haemophilus influenzae</i>	<i>Streptococcus pneumoniae</i>	Measles
<i>Klebsiella pneumoniae</i>	<i>Streptococcus pyogenes</i>	Mumps
<i>Lactobacillus acidophilus</i>	<i>Streptococcus salivarius</i>	SARS-WA1
<i>Lactiplantibacillus plantarum</i>	<i>Candida albicans (fungus)</i>	Human Metapneumovirus
--	--	Human Bocavirus ^a
--	--	Enterovirus***

* Three strains of *B. bronchiseptica* were tested: ATCC 785, Z339 (NCTC 452), Z341 (NCTC 13252)

** Three strains of *A. denitrificans* were tested: ATCC 337, ATCC 13138, ATCC 15173

*** Three strains of Enterovirus were tested: ATCC 836, ATCC 1824, ATCC 1432

^aTested at highest concentration possible per stock concentration

ii. Exogenous/endogenous Interference

Potentially interfering exogenous/endogenous substances that are commonly encountered in nasopharyngeal swab specimens were tested at the concentrations found in Table 4. Each substance was tested in replicates of five both with and without co-formulated Bp, Bpp and Bh bacterial culture (each at 3x LoD) spiked into negative clinical matrix. Internal controls remained valid for all samples and replicates. One false negative result was reported with Bpp and 10% Flonase; however, reduction to 5% Flonase resolved the interference. All other substances tested did not interfere with the performance of the cobas liat Bordetella panel assay.

Table 4: Exo/Endogenous Substances Interference Results Summary

Interferent	Concentration	Positive Sample # Positive/ # Total (%Agreement)			Negative Sample # Positive/ # Total (% Agreement)		
		Bp	Bpp	Bh	Bp	Bpp	Bh
Human cell (PBMC)	1E+06 cell/mL	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Mucin Bovine Type 1-S	10 mg/mL	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Human Whole Blood	10% (v/v)	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Afrin Pump Mist	10.0% (v/v)	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Flonase	5.0% (v/v)	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
	10% (v/v)	5/5 (100%)	4/5 (80%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Chloraseptic Sore Throat Lozenges	10.0 mg/mL	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Mupirocin	10.0 mg/mL	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)

Interferent	Concentration	Positive Sample # Positive/ # Total (%Agreement)			Negative Sample # Positive/ # Total (% Agreement)		
		Bp	Bpp	Bh	Bp	Bpp	Bh
Relenza	10.0 mg/mL	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Tamiflu	7.5 mg/mL	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Tobramycin	0.6 mg/mL	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Albuterol Sulfate	5.0% (w/v)	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Sucrets Sore Throat & Cough – Vapor Cherry	5.0% (w/v)	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Ampicillin powder	10.0 mg/mL	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Azithromycin tablet	0.25 mg/mL	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Beconase AQ Nasal Spray	8.5 mg/mL	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Children's Dimetapp Cold and Cough	5.0% (v/v)	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Ciprofloxacin	1.3 mg/mL	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Erythromycin	0.47 mg/mL	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Neo-Syneprine Nasal Spray	15.0% (v/v)	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Rifampicin	3.75 mg/mL	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Robitussin Cough + Chest Congestion DM liquid gel capsule	10.0% (v/v)	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Ocean Saline Nasal Spray (sodium chloride)	10.0 % (v/v)	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)
Flumist	6.3% (v/v)	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (100%)	0/5 (100%)	0/5 (100%)

iii. Competitive Inhibition

A competitive interference study was conducted to evaluate the potential interference of on-panel organisms when one or more targets are present at high concentrations relative to the other targets. Contrived mixtures of low (3x LoD) and high (1.0E+07 CFU/mL) concentration targets in negative clinical matrix were tested with the cobas liat Bordetella panel assay to assess competitive inhibition between Bp, Bpp and Bh analytes. Five replicates of each co-formulated sample were utilized. Test results as shown in Table 5 indicate that when two target microorganisms were present at high concentrations, no interference was observed for microorganisms that were present at low concentrations.

Table 5: Competitive Inhibition Result Summary

Concentration			# Detected (%)		
Bp	Bpp	Bh	Bp	Bpp	Bh
Low	Low	Low	5/5 (100%)	5/5 (100%)	5/5 (100%)
High	High	Low	5/5 (100%)	5/5 (100%)	5/5 (100%)

Concentration			# Detected (%)		
Bp	Bpp	Bh	Bp	Bpp	Bh
High	Low	High	5/5 (100%)	5/5 (100%)	5/5 (100%)
Low	High	High	5/5 (100%)	5/5 (100%)	5/5 (100%)

4. Assay Reportable Range:

Not applicable; this is a qualitative assay

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

i. *Specimen Stability*

Pooled nasopharyngeal swab (NPS) clinical specimens were stored at various times at each of three storage temperatures: $\leq -70^{\circ}\text{C}$, $2-8^{\circ}\text{C}$, and 30°C using three different media types: UTM, M4RT and eSwab. For each media type, two replicates each of five unique specimen pools negative for all assay targets (Bp/Bpp/Bh) and five unique specimen pools positive for all assay targets at 3x LoD were tested, giving a total of 10 positive and 10 negative results for each media at each storage temperature and at each time point as shown in Table 6 below.

Table 6: Conditions Evaluated for Specimen Stability

Sample*	Temperature	Timepoint	No. of Replicates
Negative	30°C, Sample	4hr	10
		5hr	10
	30°C, Tube	4hr	10
		5hr	10
	2-8°C	0	10
		1d	10
		2d	10
		3d	10
		4d	10
	-80°C, 2 F/T	3d	10
		4d	10
	-80°C, 3 F/T	3d	10
		4d	10
		103d [#]	10
Positive	30°C, Sample	4hr	10
		5hr	10
	30°C, Tube	4hr	10
		5hr	10
	2-8°C	0 (Baseline)	10
		1d	10
		2d	10
		3d	10
		4d	10
	-80°C, 2 F/T	3d	10
		4d	10
	-80°C, 3 F/T	3d	10
		4d	10
		103d [#]	10

*Positive and negative Samples were tested with UTM, M4RT and ESwab media types

[#]This condition was tested only for samples in UTM media.

The data supported specimen stability for use with cobas liat Bordetella panel is as follows:

- NPS specimens (UTM, M4RT and ESwab) are stable for up to 3 days when stored at 2-8°C
- NPS specimens (UTM, M4RT and ESwab) are stable for up to 4 hours when stored at 30°C in both the specimen collection tube and the assay tube.
- NPS specimens (M4RT and ESwab) are stable for up to 3 days when stored at $\leq -70^{\circ}\text{C}$ with three freeze thaw cycles.
- NPS specimens (UTM) are stable for up to 4 days when stored at $\leq -70^{\circ}\text{C}$ with three freeze thaw cycles.

ii. Assay Controls

The cobas liat Bordetella panel nucleic acid test for use on the cobas Liat System contains two sets of controls: an internal process control and an external assay quality control. The Internal Process Control (IPC) monitors the performance of the cobas Bordetella panel assay sample processing and PCR amplification/detection. The external positive and negative controls monitor the assay process and potential reagent/assay tube failure.

6. Detection Limit

The limit of detection of the cobas liat Bordetella panel nucleic acid test was determined by analyzing a dilution series of two representative strains of *B. pertussis* (Strain A639 and E431), *B. parapertussis* (Strain E838 and A747), and *B. holmesii* (Strain F061 and ATCC 51541). The culture stocks were co-formulated to an intermediate level and serially diluted in pooled negative nasopharyngeal swab (NPS) clinical specimens collected in UTM to seven (7) concentration levels. All levels were tested with at least 20 replicates per concentration tested across three unique lots of reagents, over the course of three days. A summary of the LoD as determined by the hit rate for each specimen type, lot and strain is shown below in Table 7. The highest concentration detected at $\geq 95\%$ from testing of the two strains across three lots of the assay is the final LoD and is highlighted in the below table.

Table 7: Limit of Detection Reported Based on 95% Hit Rate

Target	Strain	Lot 1	Lot 2	Lot 3
		CFU/mL		
Bp	A639	43	43	43
	E431	18	36	36
Bpp	E838	32	32	32
	A747	9	36	18
Bh	F061	5	34	9
	51541	21	21	11

To demonstrate that presence of all three *Bordetella* strains did not impact the LoD for each individual strain, the LoD for single analyte samples were compared to the co-formulated samples containing all on-panel *Bordetella* strains. The LoDs were considered equal if the single LoD and co-formulated LoD was no more than 3-fold different between results. Co-formulated samples were derived from culture stocks and spiked into clinical negative nasopharyngeal swab matrix. Three dilution levels were prepared for each co-formulated panel, one above, one below and the LoD set at the hit rate of $\geq 95\%$. Twenty replicates of

each concentration were tested using one lot of the cobas liat Bordetella assay tubes, across 21 cobas liat analyzers. The results of this analysis demonstrate that the co-formulated LoD is comparable to the single LoD (see Table 8).

Table 8: Limit of Detection Comparison for Single and Co-formulated Panels

Analyte	Target Concentration (CFU/mL)	Formulation	
		Co-formulated	Single
		Hit Rate	Hit Rate
Bp	126	100%	100%
	63	85%	100%
	21	60%	65%
Bpp	120	100%	100%
	60	100%	100%
	20	75%	90%
Bh	30	100%	100%
	15	100%	100%
	5	95%	85%

The results of the study met the acceptance criteria, verifying that the LoD with co-formulated target panels is comparable to single formulated target panels when tested with the cobas liat Bordetella panel. The results demonstrate that co-formulated samples are representative of single-formulated samples with respect to assay performance. Further analytical studies were conducted using co-formulated samples, unless otherwise specified.

7. Analytical Reactivity:

An inclusivity study was conducted using various strains of Bp, Bpp and Bh by diluting these cultures into negative clinical matrix pool using one reagent lot. Three replicates were tested for each dilution level for each strain. The strains tested and the lowest concentration detected are listed in Table 9.

Table 9: Inclusivity Testing Result Summary

Analyte	Strains	Detection Level (CFU/mL)
Bp	ATCC 8467	0.12
	ATCC BAA-589	0.006
	ATCC 51445	0.12
	ATCC 53894	0.05
	ATCC 9340	0.04
	ATCC 10380	0.48
	ATCC BAA-1335	0.3
	ATCC 9306	0.3

Analyte	Strains	Detection Level (CFU/mL)
	ATCC 12743	0.12
	ATCC 9797	0.12
	ATCC 12742	5.0
	ATCC 8478	40.0
Bpp	ATCC 15311	2.5
	E595	40.0
	ATCC 15237	10.0
	ATCC BAA-587	15.0
	ATCC 9305	11.9
	C510	47.6
Bh	ATCC 700053	1.75
	ATCC 700052	0.48
	C690	12.5

8. Assay Cut-Off:

The assay cutoffs for Bp, Bpp and Bh and internal control were initially evaluated using past remnant samples from other clinical study samples, contrived cultures into clinical negative nasopharyngeal matrix, and neat UTM. This data (3,843 valid results, including 1,350 neat UTM samples) were used to confirm adequate assay cut-off and separation between the latest Ct value observed in the analyte positive specimens and negative samples. Assay cut-off values were verified during clinical performance evaluation.

The final Ct cutoffs were set at 40 for Bp, 34.8 for Bpp and 38 for Bh.

9. Accuracy (Instrument):

N/A

10. Carry-Over:

A carry-over study evaluating the cobas liat system was conducted with the first assay cleared for use with the cobas liat system (K153544) and demonstrated that there was no carry over or cross-contamination observed. The cobas liat Bordetella panel utilizes the same instrumentation. Therefore, additional no carry-over studies were conducted in this submission.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not Applicable

2. Matrix Comparison:

Not Applicable

C Clinical Studies:

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 (PCR-BDS) 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 respective comparator results 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%

Abbreviations: 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)
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

Strain	LoD	BP Target Percent Agreement (n / N)	BPP Target Percent Agreement (n / N)	BH Target Percent Agreement (n / N)
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.

D Clinical Cut-Off:

Not Applicable

E Expected Values/Reference Range:

The observed positivity for *Bordetella* in the prospective population during the cobas liat *Bordetella* panel clinical studies conducted between July 2023 – February 2024 is 0% (0/1) for *Bordetella parapertussis* and not calculable for *Bordetella pertussis* and *Bordetella holmesii* due to the lack of any positive prospective samples.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.