510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K170284

B. Purpose for Submission:

To obtain a substantial equivalence determination for the Great Basin Bordetella Direct Test.

C. Measurand:

Insertion sequence IS481 of *Bordetella pertussis*.

D. Type of Test:

The Bordetella Direct Test is a nucleic acid-based amplification *in vitro* diagnostic test for the qualitative detection *Bordetella pertussis* nucleic acids isolated from nasopharyngeal swab specimens obtained from patients suspected of having respiratory tract infection attributable to *Bordetella pertussis*.

E. Applicant:

Great Basin Corporation

F. Proprietary and Established Names:

Great Basin Bordetella Direct Test

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3980 – Respiratory viral panel multiplex nucleic acid assay

2. Classification:

   Class II

3. Product code:

   OZZ – *Bordetella pertussis* Nucleic Acid Amplification Assay System

4. Panel:

83- Microbiology

H. Intended Use:

1. Intended use(s):

   The Great Basin Bordetella Direct Test is a qualitative in vitro diagnostic test for the detection of *Bordetella* pertussis DNA from nasopharyngeal swab specimens obtained from patients suspected of having a respiratory tract infection attributable to *B. pertussis*.

   The Bordetella Direct Test is performed on the PA500 Portrait Analyzer and utilizes PCR amplification of the insertion sequence IS481. The IS481 sequence is also found in other organisms including *Bordetella holmesii* or *Bordetella bronchiseptica*. Respiratory infection with *B. pertussis, B. holmesii* or *B. bronchiseptica* may yield positive test results with IS481 assays. *B. holmesii* infection may cause clinical illness similar to *B. pertussis*, and mixed outbreaks involving both *B. pertussis* and *B. holmesii* infection have been reported. Additional testing should be performed if necessary to differentiate *B. holmesii* and *B. pertussis. B. bronchiseptica* is a rare cause of infection in humans. When clinical factors suggest that *B. pertussis* may not be the cause of respiratory infection, other clinically appropriate investigation(s) should be carried out in accordance with published guidelines.

   Negative results for the Great Basin Bordetella Direct Test do not preclude *B. pertussis* infection and positive results do not rule out co-infection with other respiratory pathogens. Results from the Great Basin Bordetella Direct Test should be used in conjunction with information obtained during the patient's clinical evaluation as an aid in diagnosis of *Bordetella pertussis* infection and should not be used as the sole basis for treatment or other patient management decisions.

2. Indication(s) for use:

   Same as intended use.

3. Special conditions for use statement(s):

   For *in vitro* diagnostic use only.

   For prescription use only.

4. Special instrument requirements:

   PA500 Portrait Analyzer
   Heat blocks capable of $95^{o}C \pm 2^{o}C$ and $64^{o}C \pm 2^{o}C$

I. Device Description:

The Great Basin Bordetella Direct Test on the PA500 Portrait Analyzer System utilizes automated hot-start PCR technology to target and amplify the IS481 insertion sequence of *B. pertussis*. Genomic DNA is extracted from microbial cells and diluted to reduce potential inhibitors of PCR. During PCR, double-stranded DNA is separated and the target nucleic acid sequence is amplified by thermal cycling using biotin-labeled primers that target the IS481 sequence for identification of *B. pertussis*. Following PCR, biotin-labeled amplicon is hybridized to sequence-specific capture probes immobilized on the silicon chip surface, then incubated with anti-biotin antibody conjugated to the horseradish peroxidase enzyme (HRP). The unbound conjugate is washed away and tetramethylbenzidine (TMB) is added to produce a visible precipitate at the location of the probe/target sequence complex. The resulting signal is detected by the automated Portrait Optical Reader within the PA500 Portrait Analyzer System. The Specimen Processing Control (SPC) undergoes the extraction, amplification, and detection steps to monitor for inhibitory substances as well as process inefficiency due to instrument or reagent failure. No operator intervention is necessary once the clinical sample is loaded into the sample port and the Bordetella Direct Test cartridge is loaded into the Portrait Analyzer.

The PA500 Portrait Analyzer System is a fully automated system that includes: The Portrait Analyzer, single-use Bordetella Direct Test Cartridges, and the Portrait Data Analysis Software Program. The Portrait System is designed to perform automated sample preparation, PCR, and optical chip-based detection with integrated data analysis in less than two hours.

Reagents and Materials Provided:

- Bordetella Direct Test Cartridge Kit. Each test cartridge includes:
  - Blister Pack 1: Dilution Buffer (Salts)
  - Blister Pack 2: Extraction Buffer (Enzymes, salts)
  - Blister Pack 3: Wash Solution (Saline Sodium Citrate (SSC) buffer, surfactant, preservative)
  - Blister Pack 4: Hybridization Buffer (SSC buffer, surfactant, preservative)
  - Blister Pack 5: Conjugate (Sodium citrate buffer, salts, fetal bovine serum (FBS), peroxidase conjugated monoclonal mouse antibody, preservative)
  - Blister Pack 6: Substrate (Tetramethylbenzidine (TMB))
  - Chamber 1 (Lysis): Stir bar
  - Chamber 2 (Dilution): Stir bar
  - Chamber 3 (PCR): Amplification Reagents (lyophilized) (Tris buffer salts, sucrose, surfactant, nucleotides, primers, DNA polymerase)
  - Chamber 4 (Detect): Silicon chip with immobilized DNA probes
  - Chamber 5 (SPC): Lyophilized Specimen Processing Control (SPC) (Bacillus subtilis)

Materials required but not provided:

- PA500 Portrait Analyzer System and Operator Manual
- Heat blocks capable of 95°C ± 2°C and 64°C ± 2°C
- Fixed volume pipette
- Aerosol barrier pipette tips
- Disposable gloves

J. Substantial Equivalence Information:

1. Predicate device name(s):

   illumigene® Pertussis DNA Amplification Assay

2. Predicate 510(k) number(s):

   k133673

3. Comparison with predicate:

Similarities		
Item	**Bordetella Direct Test (k170284)**	**illumigene® Pertussis DNA Amplification Assay (k133673)**
Intended Use	The Great Basin Bordetella Direct Test is a qualitative in vitro diagnostic test for the detection of *Bordetella pertussis* DNA from nasopharyngeal swab specimens obtained from patients suspected of having respiratory tract infection attributable *to B. pertussis.* The Bordetella Direct Test is performed on the PA500 Portrait Analyzer and utilizes PCR amplification of the insertion sequence IS481. The IS481 sequence is also found in other organisms including *Bordetella holmesii* or *Bordetella bronchiseptica.* Respiratory infection with *B. pertussis, B. holmesii* or *B. bronchiseptica* may yield positive test results in IS481 assays. *B. holmesii* infection may cause clinical illness similar to *B. pertussis*, and mixed outbreaks involving both *B. pertussis* and *B. holmesii* infection have been reported. Additional testing should be performed if necessary to differentiate *B. holmesii* and *B. pertussis. B. bronchiseptica* is a rare cause of infection in humans. When	The illumigene Pertussis DNA Amplification Assay, performed on the illumipro-10™, is a qualitative in vitro diagnostic test for the direct detection of *Bordetella pertussis* in human nasopharyngeal swab samples taken from patients suspected of having respiratory tract infection attributable to *Bordetella pertussis.* The illumigene Pertussis assay utilizes loop-mediated isothermal DNA amplification (LAMP) technology to detect B. pertussis by targeting the IS481 insertional element of the *B. pertussis* genome. The IS481 insertional element can also be found in *B. holmesii* and some *B. bronchiseptica* strains. Respiratory infections with *B. pertussis, B. holmesii* or *B. bronchiseptica* may yield positive test results in IS481 assays. *B. holmesii* infection may cause clinical illness similar to *B. pertussis*, and mixed outbreaks involving both *B. pertussis* and *B. holmesii* infection have been reported. Additional testing should be

Similarities		
Item	**Bordetella Direct Test (k170284)**	**illumigene® Pertussis DNA Amplification Assay (k133673)**
	clinical factors suggest that *B. pertussis* may not be the cause of respiratory infection, other clinically appropriate investigation(s) should be carried out in accordance with published guidelines. Negative results for the Great Basin Bordetella Direct Test do not preclude *B. pertussis* infection and positive results do not rule out co-infection with other respiratory pathogens. Results from the Great Basin Bordetella Direct Test should be used in conjunction with information obtained during the patient's clinical evaluation as an aid in diagnosis of *Bordetella pertussis* infection and should not be used as the sole basis for treatment or other patient management decisions.	performed if necessary to differentiate *B. holmesii* and *B. pertussis*. *B. bronchiseptica* is a rare cause of infection in humans. When clinical factors suggest that *B. pertussis* may not be the cause of respiratory infection, other clinically appropriate investigation(s) should be carried out in accordance with published guidelines. Negative results for the illumigene Pertussis DNA Amplification Assay do not preclude *Bordetella pertussis* infection and positive results do not rule out co-infection with other respiratory pathogens. Results from the illumigene Pertussis assay should be used in conjunction with information obtained during the patient's clinical evaluation as an aid in diagnosis of *B. pertussis* infection and should not be used as the sole basis for treatment or other patient management decisions. illumigene Pertussis is intended for use in hospital, reference or state laboratory settings. The device is not intended for point-of-care use.
Qualitative/ Quantitative	Qualitative	Same
Test Cartridge	Disposable, single-use, self-contained fluidic cartridge	illumigene® Test Device with TEST and CONTROL chambers
Specimen Type	Nasopharyngeal swab (rayon, nylon or flocked)	Same
Organism Detection	*B. pertussis*	Same
Target Sequence Detected	*Bordetella pertussis* IS481 insertion element	Same

Differences		
Item	**Bordetella Direct Test (k170284)**	**illumigene® Pertussis DNA Amplification Assay (k133673)**
Sample Lysis and DNA Extraction	Automated	Manual
DNA Amplification Technology	PCR	Loop-Mediated Isothermal Amplification (LAMP); self-contained and automated
Detection Technology	Colorimetric target specific hybridization to probe on a chip surface, optical reader, automated software with built-in result interpretation	Measures the change in light transmission due to turbidity formation which is a by-product of the amplification reaction.
Controls	One internal assay control - SPC (whole organism). The SPC controls for all analytical steps in the procedure, including DNA extraction from organisms present in the specimen, amplification of target DNA sequences, hybridization, and detection on the chip surface.	One internal control (S. aureus DNA) to monitor amplification inhibition, assay reagent performance and sample processing effectiveness.
Instrument	PA500 Portrait Analyzer System	*illumipro-10*™
Testing Time	~100 minutes	60 – 70 minutes

K. Standard/Guidance Document Referenced (if applicable):

Not applicable.

L. Test Principle:

The Great Basin Bordetella Direct Test on the PA500 Portrait Analyzer System utilizes automated hot-start PCR technology to target and amplify the IS481 insertion sequence of *B. pertussis*. Genomic DNA is extracted from microbial cells and diluted to reduce potential inhibitors of PCR. During PCR, double-stranded DNA is separated and the target nucleic acid sequence is amplified by thermal cycling using biotin-labeled primers that target the IS481 sequence for identification of *B. pertussis*. Following PCR, biotin-labeled amplicon is hybridized to sequence-specific capture probes immobilized on the silicon chip surface, then incubated with anti-biotin antibody conjugated to the horseradish peroxidase enzyme (HRP). The unbound conjugate is washed away and tetramethylbenzidine (TMB) is added to produce a visible precipitate at the location of the probe/target sequence complex. The resulting signal is detected by the automated Portrait Optical Reader within the PA500 Portrait Analyzer System. The Specimen Processing Control (SPC) undergoes the extraction, amplification, and detection steps to monitor for inhibitory substances as well as process inefficiency due to instrument or reagent failure. No operator intervention is necessary once the clinical sample

is loaded into the sample port and the Bordetella Direct Test cartridge is loaded into the Portrait Analyzer.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Reproducibility:

The reproducibility of the Bordetella Direct Test was evaluated at three (3) laboratory sites (two external, one in-house). Reproducibility was assessed using a panel of three (3) simulated samples that include moderate positive and low positive (3.8x and 1.9x LoD), and *Bordetella pertussis* negative sample (negative clinical matrix collected in VTM). The panels and controls were processed and tested on the Bordetella Direct Test at each site by two (2) operators for five (5) non-consecutive days (2 operators x 3 replicates x 5 days x 3 sites = 90 results per concentration). The LoD values were based on the values obtained in the LoD study. The results are shown in Table 1 below.

Table 1: Reproducibility Study Results

Category	Site	Operator	#expected results/# tested	Agreement	#expected results/# tested	Agreement	Overall Percent Agreement
Bordetella pertussis Low Positive (1.9x LoD)	4	1	15/15	100%	30/30	100%	90/90 100%
		2	15/15*†	100%			
	5	3	15/15	100%	30/30	100%	
		4	15/15*	100%			
	6	5	15/15	100%	30/30	100%	
		6	15/15	100%			
Bordetella pertussis Moderate Positive (3.8x LoD)	4	1	15/15	100%	30/30	100%	90/90 100%
		2	15/15	100%			
	5	3	15/15	100%	30/30	100%	
		4	15/15*	100%			
	6	5	15/15	100%	30/30	100%	
		6	15/15	100%			
Bordetella pertussis Negative	4	1	15/15	100%	30/30	100%	90/90 100%
		2	15/15*	100%			
	5	3	15/15	100%	30/30	100%	
		4	15/15	100%			
	6	5	15/15	100%	30/30	100%	
		6	15/15	100%			

*This set of test runs also contained one 'Test Incomplete' run.
†This set of test runs also contained one 'Invalid' run.

The results suggest that there are no significant differences between different users and different sites on different days. Reproducibility studies are acceptable

b. *Linearity/assay reportable range:*

Not applicable – This assay is qualitative.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability:

Not applicable. This assay is qualitative.

Sample Stability and Storage:

Sample stability studies were conducted to support the storage conditions stated in the Bordetella Direct Test product labeling.

The *B. pertussis* positive and negative samples prepared in the second Swab, Transport Media and Elution Buffer Equivalency Study were also used to evaluate sample stability at room temperature (20 - 25°C) and at 2-8°C. Aliquots of each sample type were tested at the time of preparation (T_0), then stored at room temperature (RT) for 24, 48 and 72 hrs or refrigerated (2-8°C) for 24, 48, 72, 96 and 168 hrs. Samples were tested in triplicate at each time point. Results from the room temperature and refrigerated stability studies are listed in Tables 2 and 3 below, respectively.

Table 2: Sample Stability Study: Room Temperature Storage

Strain Tested and Concentration	**Media**	**Time Point (hrs), Expected Results/Total**			
		T_0	**T_{24}**	**T_{48}**	**T_{72}**
ATCC 8467 (6.9×10^3 CFU/mL)	M5 VTM	3/3	3/3	3/3	3/3
	ESwab	3/3	3/3	3/3	3/3
	Liquid Stuart	3/3	3/3	3/3	3/3
ATCC 9797 (7.3×10^3 CFU/mL)	M5 VTM	3/3	3/3	3/3	3/3
	ESwab	3/3	3/3	3/3	3/3
	Liquid Stuart	3/3	3/3	3/3	3/3
ATCC BAA-589 (4.3×10^3 CFU/mL)	M5 VTM	3/3	3/3	3/3	3/3
	ESwab	3/3	3/3	3/3	3/3
	Liquid Stuart	3/3	3/3	3/3	3/3
Negative	M5 VTM	3/3	3/3	3/3	3/3*
	ESwab	3/3	3/3	3/3	3/3

	Liquid Stuart	3/3	3/3*†	3/3	3/3

*This set of test runs also contains one 'Test incomplete' run.
†This set of test runs also contains one 'Invalid' run.

Table 3: Sample Stability Study: 2-8°C Storage

Strain Tested and Concentration	Media	Time Point (hrs), Expected Results/Total					
		T_0	T_{24}	T_{48}	T_{72}	T_{96}	T_{168}
ATCC 8467 (6.9×10^3 CFU/mL)	M5 VTM	3/3	3/3	3/3	3/3	3/3	3/3
	ESwab	3/3	3/3	3/3	3/3	3/3	3/3
	Liquid Stuart	3/3	3/3	3/3	3/3	3/3	3/3
ATCC 9797 (7.3×10^3 CFU/mL)	M5 VTM	3/3	3/3	3/3	3/3	3/3	3/3
	ESwab	3/3	3/3	3/3	3/3	3/3	3/3
	Liquid Stuart	3/3	3/3	3/3	3/3	3/3	3/3
ATCC BAA-589 (4.3×10^3 CFU/mL)	M5 VTM	3/3	3/3	3/3	3/3	3/3	3/3
	ESwab	3/3	3/3	3/3	3/3	3/3	3/3
	Liquid Stuart	3/3	3/3	3/3	3/3†	3/3	3/3
Negative	M5 VTM	3/3	3/3	3/3	3/3	3/3	3/3*
	ESwab	3/3	3/3	3/3	3/3	3/3	3/3
	Liquid Stuart	3/3	3/3	3/3	3/3	2/2†	3/3

*This set of test runs also contains one 'Test incomplete' run.
†This set of test runs also contains one 'Invalid' run.

The results demonstrated 100% agreement with the expected results for all samples tested at each time point. These results support the specimen storage claims in the Product Insert of 48 hours at room temperature or ≤120 hours at 2-8°C.

Controls:

Controls were run on the Bordetella Direct Test each day of testing. All *Bordetella pertussis* positive controls were detected accurately (100%, 47/47). All *Bordetella pertussis* negative controls were detected accurately (100%, 47/47) in the clinical study. The daily QC panel consisted of 2 samples to control for all assay outcomes:

- QC1: B. pertussis (ATCC 9797) contrived in natural negative NP matrix collected in VTM. Expected Bordetella Direct Test result: 'B. pertussis DETECTED'.

- QC3: Natural negative NP matrix collected in VTM; screened and pooled. Expected Bordetella Direct Test result: 'B. pertussis NOT DETECTED'.

d. Detection limit:

The analytical sensitivity (limit of detection or LoD) of the Bordetella Direct Test was determined using quantified (CFU/mL) cultures of three (3) *Bordetella pertussis* bacterial strains: ATCC 8467, ATCC 9797 and ATCC BAA-589. The limit of detection (LoD) is defined as the lowest number of colony forming units (CFU) that can be reproducibly distinguished from negative samples with 95% confidence, or the lowest concentration at which 19/20 replicates are positive.

The *B. pertussis* strains were prepared using fresh isolates from Bordet-Gengou (BG) agar plates incubated for 3-5 days at 37°C until colony isolates were visible. The colony isolates were suspended and vortexed in Mueller Hinton II Broth (MH II) to create a uniform cell suspension with an optical density (OD600 nm) of 0.05, then serially diluted in MH II to a targeted cell concentration based on the OD600 nm value. The targeted cell concentration was spiked into natural negative nasopharyngeal (NP) matrix collected in Viral Transport Media (VTM) for testing. The tested sample cell concentrations (CFU/mL) were determined by plating and enumerating on BG agar plates.

The established LoD for the Bordetella Direct Test for the detection of B. pertussis is between 1.6×10^3 and 3.3×10^3 CFU/mL with an average LoD of 2.4×10^3 CFU/mL. The LoD study results are shown in Table 4 below.

Table 4: Bordetella Direct Test Analytical Sensitivity (LoD) Results

B. pertussis ATCC Strain	***Bordetella pertussis* DETECTED**	**LoD (CFU/mL)**
8467	20/20	3.3×10^3
9797	20/20	1.6×10^3
BAA-589	19/20	2.3×10^3

These study results are acceptable.

e. Analytical Reactivity (Inclusivity):

The Bordetella Direct Test Analytical Reactivity (Inclusivity) was confirmed by testing an additional eight (8) *B. pertussis* strains.

B. pertussis cultures were prepared and quanntified as described in the LoD Study. All testing was conducted in a natural negative NP matrix collected in VTM. Each strain was tested in triplicate at a targeted range of approximately 2-3X LoD based on the 2.4×10^3 CFU/mL average LoD obtained in the LoD study. The concentrations tested along with the results from the Inclusivity Study are shown in Table 5 below.

Table 5: Analytical Reactivity (Inclusivity) Panel Results.		
B. pertussis Strain	Concentration tested (CFU/mL)	*Bordetella pertussis* DETECTED
ATCC 8478	5.5×10^3	3/3
ATCC 9340	2.6×10^3	3/3
ATCC 10380	5.8×10^3	3/3
ATCC 12742	4.9×10^3	3/3
ATCC 51445	5.3×10^3	3/3
ATCC 53894	3.2×10^3	3/3
ATCC BAA-1335	4.3×10^3	3/3
Zeptometrix A639	1.8×10^4	3/3
	3.6×10^3	3/3

f. Analytical Specificity (Exclusivity):

A study was performed to evaluate the cross-reactivity of the Bordetella Direct Test with ninety (90) microorganisms including forty-eight (48) bacteria, twenty (20) viruses, two (2) yeast, nineteen (19) *Bordetella* species (non-*B. pertussis*) and human genomic DNA potentially found in specimens collected to test for *Bordetella pertussis* (BP) infection. All bacterial and yeast strains were tested at concentrations ≥ 1×10^6 CFU/mL. Genomic DNA and viral strains were tested at ≥ 1.4×10^6 genomic copies/mL and ≥ 1.6×10^6 $TCID_{50}$ respectively Cross-reactive microorganism was tested at clinically relevant levels of viruses (10^5 PFU/mL) and bacteria (10^6 CFU/mL) in the device. A minimum of three (3) replicates were tested for each organism evaluated for cross-reactivity The organisms and their concentrations included in the cross-reactivity study are shown in Table 6 below.

Table 6: Analytical Specificity (Exclusivity) Panel Results			
Organism	**Strain ID**	**Concentration Tested**	**Expected Negative Results /Total**
Bacteria			
Acinetobacter baumannii	ATCC 19606	1.2×10^7 CFU/mL	6/6
Acinetobacter calcoaceticus	ATCC 23055	1.5×10^7 CFU/mL	3/3
Acinetobacter haemolyticus	ATCC 19002	2.2×10^7 CFU/mL	3/3
Actinomyces odontolyticus	ATCC 17929	1.5×10^6 CFU/mL	3/3
Arcanobacterium haemolyticum	ATCC BAA-1784	2.4×10^7 CFU/mL	3/3
Bacteroides fragilis	ATCC 23745	4.6×10^7 CFU/mL	3/3
Bordetella avium	ATCC 35086	2.7×10^7 CFU/mL	1/3
		1.2×10^7 CFU/mL	3/3
		1.2×10^7 CFU/mL	10/10
Bordetella parapertussis	ATCC 9305	1.2×10^7 CFU/mL	3/3
Bordetella parapertussis	ATCC 15237	1.7×10^7 CFU/mL	3/3
Bordetella parapertussis	ATCC 15311	2.4×10^7 CFU/mL	3/3*

Table 6: Analytical Specificity (Exclusivity) Panel Results			
Organism	**Strain ID**	**Concentration Tested**	**Expected Negative Results /Total**
Bordetella parapertussis	ATCC 15989	3.2 x 10^7 CFU/mL	2/3
		3.2 x 10^7 CFU/mL	10/10
Bordetella parapertussis	ATCC 53892	1.4 x 10^7 CFU/mL	3/3
Bordetella parapertussis	ATCC 53893	3.2 x 10^7 CFU/mL	3/3
Bordetella parapertussis	ATCC BAA-587	3.8 x 10^7 CFU/mL	3/3
Bordetella parapertussis	Zeptometrix A747	2.8 x 10^7 CFU/mL	3/3
Bordetella petrii	ATCC BAA-461	2.7 x 10^7 CFU/mL	1/3
		1.2 x 10^7 CFU/mL	3/3
		1.2 x 10^7 CFU/mL	10/10*
Bordetella trematum	ATCC 700309	2.0 x 10^7 CFU/mL	0/3
		1.3 x 10^7CFU/mL	3/3
		1.3 x 10^7 CFU/mL	10/10
Burkholderia cepacia	ATCC 25416	3.1 x 10^7 CFU/mL	3/3
Chlamydia trachomatis	ATCC VR-879D	1.5 x 10^8 copies/mL	3/3
Citrobacter freundii	ATCC 8090	1.7 x 10^7 CFU/mL	3/3
Clostridium difficile	ATCC 43255	1.5 x 10^7 CFU/mL	3/3
Corynebacterium diptheriae	ATCC 13812	9.3 x 10^6 CFU/mL	3/3
Enterobacter aerogenes	ATCC 15038	1.5 x 10^7 CFU/mL	3/3
Enterobacter cloacae	ATCC 13047	1.6 x 10^7 CFU/mL	3/3
Enterococcus faecalis	ATCC 29212	8.6 x 10^6 CFU/mL	3/3
Escherichia coli	ATCC 43895	1.4 x 10^7 CFU/mL	3/3
Haemophilus influenza	ATCC 9006	3.0 x 10^7 CFU/mL	3/3
Haemophilus parainfluenza	ATCC 33392	3.6 x 10^7 CFU/mL	3/3
Klebsiella pneumoniae	ATCC BAA-1705	2.1 x 10^7 CFU/mL	3/3
Lactobacillus acidophilus	ATCC 4356	1.1 x 10^6 CFU/mL	3/3
Lactobacillus plantarum	ATCC 8014	4.6 x 10^7 CFU/mL	3/3
Legionella pneumophila	ATCC 33152D-5	1.3 x 10^8 copies/mL	3/3
Moraxella catarrhalis	ATCC 8176	9.8 x 10^6 CFU/mL	3/3
Morganella morganii	ATCC 25829	7.3 x 10^7 CFU/mL	3/3
Mycobacterium tuberculosis	ATCC 25177D-2	4.1 x 10^7 copies/mL	3/3
Mycoplasma pneumoniae	ATCC 15531D	2.0 x 10^7 copies/mL	3/3
Neisseria gonorrhoeae	ATCC 19424	2.2 x 10^7 CFU/mL	3/3
Organism	**Strain ID**	**Concentration Tested**	**Expected Negative Results /Total**
Neisseria meningitidis	ATCC 13077	4.2 x 10^6 CFU/mL	3/3
Pandoraea oxalativorans	DSM-23570	1.6 x 10^7 CFU/mL	3/3
		5.3 x 10^7 CFU/mL	3/3
		5.3 x 10^7 CFU/mL	10/10
Peptostreptococcus anaerobius	ATCC 27337	5.9 x 10^6 CFU/mL	3/3
Proteus mirabilis	ATCC 25933	8.4 x 10^7 CFU/mL	3/3
Proteus vulgaris	ATCC 6896	2.2 x 10^7 CFU/mL	3/3
Pseudomonas aeruginosa	ATCC 10145	4.3 x 10^7 CFU/mL	3/3
Pseudomonas flourescens	ATCC 13525	1.8 x 10^7 CFU/mL	3/3
Serratia liquefaciens	ATCC 35551	7.3 x 10^6 CFU/mL	3/3
Staphylococcus aureus (MRSA)	ATCC 33591	1.6 x 10^7 CFU/mL	3/3
Staphylococcus aureus (MSSA)	ATCC 25923	3.6 x 10^7 CFU/mL	3/3
Staphylococcus epidermidis (MS)	ATCC 12228	2.4 x 10^6 CFU/mL	3/3
Stenotrophomonas maltophilia	ATCC 13637	2.0 x 10^7 CFU/mL	3/3
Streptococcus anginosus	ATCC 33397	9.2 x 10^6 CFU/mL	3/3
Streptococcus bovis	ATCC 33317	1.2 x 10^7 CFU/mL	3/3

Table 6: Analytical Specificity (Exclusivity) Panel Results			
Organism	**Strain ID**	**Concentration Tested**	**Expected Negative Results /Total**
Streptococcus dysgalactiae ssp dysgalactiae	ATCC 43078	1.5 x 10^7 CFU/mL	3/3
Streptococcus dysgalactiae ssp equisimilis	ATCC 35666 ATCC 35666	5.5 x 10^7 CFU/mL	3/3
Streptococcus intermedius	ATCC 27335	3.4 x 10^7 CFU/mL	3/3
Streptococcus mitis	ATCC 13770	2.3 x 10^6 CFU/mL	3/3
Streptococcus mutans	ATCC 25175	2.5 x 10^7 CFU/mL	3/3
Streptococcus pneumoniae	ATCC 6303	1.3 x 10^6 CFU/mL	3/3
Streptococcus pyogenes	ATCC 49399	1.4 x 10^7 CFU/mL	3/3
Streptococcus salivarius	ATCC BAA-2593	1.6 x 10^7 CFU/mL	3/3
Virus			
Adenovirus	ATCC VR-846D	8.5 x 10^9 copies/mL	3/3
Coronavirus	ATCC VR-740D	7.2 x 10^8 copies/mL	3/3
Coxsackievirus	ATCC VR-169	1.0 x 10^7 copies/mL	3/3
Cytomegalovirus	ATCC VR-538D	2.5 x 10^9 copies/mL	3/3
Echovirus	ATCC VR-1734D	4.2 x 10^9 copies/mL	3/3
Epstein-Barr Virus	ATCC VR-3247SD	5.3 x 10^7 copies/mL	3/3
Herpes Simplex Virus 1	ATCC VR-539D	1.0 x 10^9 copies/mL	3/3*
Herpes Simplex Virus 2	ATCC VR-540D	1.6 x 10^6 TCID50	3/3
Human Bocavirus	ATCC VR-3251SD	4.8 x 10^7 copies/mL	3/3
Human Metapneumovirus	ATCC VR-3250SD	6.2 x 10^7 copies/mL	3/3*
Influenza A	ATCC VR-1738D	3.9 x 10^9 copies/mL	5/5*†
Influenza B	ATCC VR-1813D	1.2 x 10^{10} copies/mL	3/3
Measles virus	ATCC VR-24D	1.6 x 10^{10} copies/mL	3/3
Mumps virus	ATCC VR-106D	1.3 x 10^{10} copies/mL	3/3
Parainfluenza virus 1	ATCC VR-94D	1.8 x 10^9 copies/mL	3/3
Parainfluenza virus 2	ATCC VR-92D	3.4 x 10^9 copies/mL	3/3
Parainfluenza virus 3	ATCC VR-93D	7.9 x 10^8 copies/mL	3/3
Respiratory Syncytial Virus	ATCC VR-1540D	3.0 x 10^9 copies/mL	3/3
Rhinovirus	ATCC MBC091	1.4 x 10^6 copies/mL	3/3
Varicella Zoster Virus	VR-1367D	1.0 x 10^9 copies/mL	3/3
Yeast			
Candida albicans	ATCC 18804	1.1 x 10^7 CFU/mL	3/3
Candida glabrata	ATCC 66032	1.3 x 10^7 CFU/mL	3/3†
Human Genomic DNA			
Human Genomic DNA	ATCC HTB-20D	3.8 x 10^7 copies/mL	3/3

*This set of test runs also contained one 'Test incomplete' run.
†This set of test runs also contained one 'Invalid' run.

The Cross Reactivity study tested a panel of 90 microorganisms. Unexpected '*Bordetella pertussis* DETECTED' results were initially observed for one (1) of two (2) replicates for *B. parapertussis* (ATCC 15989), two (2) of three (3) replicates for *B. avium* (ATCC 35086) and *B. petrii* (ATCC BAA-461), and three (3) of three (3) replicates for *B. trematum* (ATCC 700309). For these unexpected positives, an additional three (3) replicates were tested with a new preparation to test the veracity of the positive results. When the retesting gave the expected negative result for all three (3) replicates instead of the positive result seen with the first test, a minimum of ten (10) more replicates were tested to confirm the negative result. All testing done with new preparations yielded '*Bordetella pertussis* NOT DETECTED' results.

Testing for analytical specificity was also performed on three *Bordetella* (non-B. pertussis) species identified as potentially cross-reacting: *Bordetella bronchiseptica, Bordetella holmesii*, and *Bordetella hinzii*. Species were defined as potentially cross-reacting based on the presence of the IS*481* insertion sequence reported in a subset of strains for these species. The results are summarized in Table 7 below.

Table 7: Analytical Specificity (Exclusivity) Results for Potential Cross-Reacting Strains

Potential Cross-Reacting Organism	Strain ID	Concentration Tested	Expected Positive Results /Total
Bordetella bronchiseptica	ATCC 19395	2.1×10^7 CFU/mL	0/3
Bordetella bronchiseptica	ATCC 4617	1.2×10^7 CFU/mL	3/3
		2.8×10^7 CFU/mL	3/3
Bordetella bronchiseptica	ATCC BAA-588	3.1×10^7 CFU/mL	2/3
		3.0×10^7 CFU/mL	0/3
		3.0×10^7 CFU/mL	0/10
Bordetella holmesii	ATCC 700052	1.4×10^7 CFU/mL	3/3*
		2.1×10^7 CFU/mL	3/3
Bordetella holmesii	ATCC 700053	2.0×10^7 CFU/mL	3/3
		1.1×10^7 CFU/mL	3/3
Bordetella holmesii	ATCC 51541	2.1×10^7 CFU/mL	3/3
Bordetella hinzii	ATCC 51730	3.0×10^7 CFU/mL	0/3
Bordetella hinzii	ATCC 51784	3.3×10^7 CFU/mL	3/3
		3.6×10^7 CFU/mL	3/3

*This set of test runs also contained one 'Test incomplete' run.

For *B. bronchiseptica*, three strains were tested: ATCC 19395, ATCC 4617, and ATCC BAA-588. For ATCC 19395, all three (3) replicates gave a '*B. pertussis* NOT DETECTED' result, demonstrating no cross-reactivity. For ATCC 4617, all three (3) replicates gave a result of '*B. pertussis* DETECTED.' A second preparation was tested and cross-reactivity of ATCC 4617 was confirmed. For ATCC BAA-588, two (2) out of three (3) replicates gave a result of '*B. pertussis* DETECTED.' A second preparation was tested, and all three (3) results were '*B. pertussis* NOT DETECTED.' Due to the confounding results, another ten replicates were tested, and '*B. pertussis* NOT DETECTED' results were obtained for all replicates confirming no cross-reactivity for ATCC BAA-588. In total, cross-reactivity was observed in one (1) of three (3) *B. bronchiseptica* strains.

For *B. holmesii*, three strains were tested: ATCC 700052, ATCC 700053, and ATCC 51541 (Table 4). For both ATCC 700052 and ATCC 700053, all three (3) replicates gave a result of '*B. pertussis* DETECTED.' Cross-reactivity was confirmed upon retest for both ATCC 700052 and 700053 with three (3) replicates each. For ATCC 51541, the result for all three (3) replicates was also '*B. pertussis* DETECTED," agreeing with the previous *B. holmesii* results. In total, cross-reactivity was observed for all three (3) *B. holmesii* strains.

For *B. hinzii*, two strains were tested: ATCC 51730 and ATCC 51784 (Table 7). For ATCC 51730, all three (3) replicates gave a '*B. pertussis* NOT DETECTED' result, as expected from the *in silico* analysis. For ATCC 51784, the first test triplicate gave

'*B. pertussis* DETECTED' results. Repeat testing was performed in triplicate on ATCC 51784, and the retest results confirmed the '*B. pertussis* DETECTED' results. Further investigation revealed that this particular *B. hinzii* strain (ATCC 51784), isolated from chickens and a known avian pathogen (Register, KB, Kunkle RA, Avian Diseases. 53:50-54, 2009), has been reported in the literature to have a weak IS*481*-positive signal (Roorda et. al., BMC Res Notes. 4:11, 2011), Therefore, the observed cross-reactivity is consistent with the presence of the IS*481* insertion sequence. Specific *in sil*ico analysis on this strain could not be performed because an accession number does not exist in the NCBI database.

In summary, cross-reactivity was observed for one (1) of three (3) *B. bronchiseptica* strains (ATCC 4617), three (3) of three (3) *B. holmesii* strains (ATCC 700052, 700053, and 51541), and one (1) of two (2) *B. hinzii* strains (ATCC 51784). These results are likely due to the presence of the IS481 insertion sequence reported in a subset of strains for these species.

These cross-reactive results are noted in the intended use and limitation sections.

g. Microbial Interference:

A study was performed to evaluate the potential for cross-reactivity in a mixed infection of the Bordetella Direct Test with ninety (90) microorganisms including forty-eight (48) bacteria, twenty (20) viruses, two (2) yeast, nineteen (19) Bordetella species (non-*B. pertussis*) and human genomic DNA potentially found in specimens collected to test for Bordetella pertussis (BP) infection. All bacterial and yeast strains were tested at concentrations

The potential for cross-reactivity in a mixed infection was evaluated in a Microbial Interference Study by testing a subset of the organisms used in the Exclusivity Study in the Bordetella Direct Test in the presence of *B. pertussis*.

Similar to the Exclusivity Study, bacteria, virus, yeast and human genomic DNA were prepared at high concentrations (> 1 x 10^6 CFU/mL, genomic copies/mL or TCID50) in a natural negative NP matrix collected in VTM. Samples were prepared by spiking B. pertussis (ATCC 9797) to a final concentration of 4.0 x 10^3 CFU/mL (2.5X LoD) in the presence of each high concentration non-*B. pertussis* organism or genomic DNA. A total of 84 organisms (48 bacteria, 19 viruses, 2 yeast, 14 *Bordetella* strains (non-*B. pertussis*) and human genomic DNA were evaluated.

A minimum of three (3) replicates of each sample were tested. The specific concentrations at which each organism was evaluated along with the results are shown in Table 8 below.

Table 8: Microbial Interference Panel Results with 2.5X LoD *B. pertussis* in high background of non-target microbe			
Organism	**Strain ID**	**Non-Target Microbe Concentration**	**Expected Positive Results /Total**
Bacteria			
Acinetobacter baumannii	ATCC 19606	1.2×10^7 CFU/mL	3/3
Acinetobacter calcoaceticus	ATCC 23055	1.5×10^7 CFU/mL	3/3
Acinetobacter haemolyticus	ATCC 19002	2.2×10^7 CFU/mL	3/3
Actinomyces odontolyticus	ATCC 17929	1.5×10^6 CFU/mL	3/3
Arcanobacterium haemolyticum	ATCC BAA-1784	2.4×10^7 CFU/mL	3/3
Bacteroides fragilis	ATCC 23745	4.6×10^7 CFU/mL	3/3
Bordetella avium	ATCC 35086	1.2×10^7 CFU/mL	3/3
Bordetella bronchiseptica	ATCC BAA-588	3.0×10^7 CFU/mL	3/3
Bordetella bronchiseptica	ATCC 19395	2.1×10^7 CFU/mL	3/3
Bordetella hinzii	ATCC 51730	3.0×10^7 CFU/mL	3/3*
Bordetella parapertussis	ATCC 9305	1.2×10^7 CFU/mL	3/3
Bordetella parapertussis	ATCC 15237	1.7×10^7 CFU/mL	3/3
Bordetella parapertussis	ATCC 15311	2.4×10^7 CFU/mL	3/3
Bordetella parapertussis	ATCC 15989	3.2×10^7 CFU/mL	3/3
Bordetella parapertussis	ATCC 53892	1.4×10^7 CFU/mL	3/3
Bordetella parapertussis	ATCC 53893	3.2×10^7 CFU/mL	3/3
Bordetella parapertussis	ATCC BAA-587	3.8×10^7 CFU/mL	3/3*
Bordetella parapertussis	Zeptometrix A747	2.8×10^7 CFU/mL	3/3
Bordetella petrii	ATCC BAA-461	1.2×10^7 CFU/mL	3/3
Bordetella trematum	ATCC 700309	1.3×10^7 CFU/mL	3/3
Burkholderia cepacia	ATCC 25416	3.1×10^7 CFU/mL	3/3
Chlamydia trachomatis	ATCC VR-879D	1.5×10^8 copies/mL	3/3
Citrobacter freundii	ATCC 8090	1.7×10^7 CFU/mL	3/3
Clostridium difficile	ATCC 43255	1.5×10^7 CFU/mL	3/3
Corynebacterium diptheriae	ATCC 13812	9.3×10^6 CFU/mL	3/3
Enterobacter aerogenes	ATCC 15038	1.5×10^7 CFU/mL	3/3
Enterobacter cloacae	ATCC 13047	1.6×10^7 CFU/mL	3/3
Enterococcus faecalis	ATCC 29212	8.6×10^6 CFU/mL	3/3*
Escherichia coli	ATCC 43895	1.4×10^7 CFU/mL	3/3
Haemophilus influenza	ATCC 9006	3.0×10^7 CFU/mL	3/3
Haemophilus parainfluenza	ATCC 33392	3.6×10^7 CFU/mL	3/3
Klebsiella pneumoniae	ATCC BAA-1705	2.1×10^7 CFU/mL	3/3
Lactobacillus acidophilus	ATCC 4356	1.1×10^6 CFU/mL	3/3
Lactobacillus plantarum	ATCC 8014	4.6×10^7 CFU/mL	3/3
Legionella pneumophila	ATCC 33152D-5	1.3×10^8 copies/mL	3/3
Moraxella catarrhalis	ATCC 8176	9.8×10^6 CFU/mL	3/3
Morganella morganii	ATCC 25829	7.3×10^7 CFU/mL	3/3
Mycobacterium tuberculosis	ATCC 25177D-2	4.1×10^7 copies/mL	2/3
		4.1×10^7 copies/mL	11/11
Mycoplasma pneumoniae	ATCC 15531D	2.0×10^7 copies/mL	3/3
Neisseria gonorrhoeae	ATCC 19424	2.2×10^7 CFU/mL	3/3
Neisseria meningitidis	ATCC 13077	4.2×10^6 CFU/mL	3/3
Pandoraea oxalativorans	DSM-23570	5.3×10^7 CFU/mL	3/3
Peptostreptococcus anaerobius	ATCC 27337	5.9×10^6 CFU/mL	3/3
Proteus mirabilis	ATCC 25933	8.4×10^7 CFU/mL	3/3
Proteus vulgaris	ATCC 6896	2.2×10^7 CFU/mL	3/3
Pseudomonas aeruginosa	ATCC 10145	4.3×10^7 CFU/mL	3/3
Pseudomonas flourescens	ATCC 13525	1.8×10^7 CFU/mL	3/3

Table 8: Microbial Interference Panel Results with 2.5X LoD *B. pertussis* in high background of non-target microbe			
Organism	**Strain ID**	**Non-Target Microbe Concentration**	**Expected Positive Results /Total**
Serratia liquefaciens	ATCC 35551	7.3×10^6 CFU/mL	3/3
Staphylococcus aureus (MRSA)	ATCC 33591	1.6×10^7 CFU/mL	3/3
Staphylococcus aureus (MSSA)	ATCC 25923	3.6×10^7 CFU/mL	3/3
Staphylococcus epidermidis (MS)	ATCC 12228	2.4×10^6 CFU/mL	3/3
Stenotrophomonas maltophilia	ATCC 13637	2.0×10^7 CFU/mL	3/3
Streptococcus anginosus	ATCC 33397	9.2×10^6 CFU/mL	3/3*
Streptococcus bovis	ATCC 33317	1.2×10^7 CFU/mL	3/3
Streptococcus dysgalactiae ssp dysgalactiae	ATCC 43078	1.5×10^7 CFU/mL	3/3
Streptococcus dysgalactiae ssp	ATCC 35666	5.5×10^7 CFU/mL	3/3
Streptococcus intermedius	ATCC 27335	3.4×10^7 CFU/mL	3/3
Streptococcus mitis	ATCC 13770	2.3×10^6 CFU/mL	3/3
Streptococcus mutans	ATCC 25175	2.5×10^7 CFU/mL	3/3
Streptococcus pneumoniae	ATCC 6303	1.3×10^6 CFU/mL	3/3*
Streptococcus pyogenes	ATCC 49399	1.4×10^7 CFU/mL	3/3
Streptococcus salivarius	ATCC BAA-2593	1.6×10^7 CFU/mL	3/3
Virus			
Adenovirus	ATCC VR-846D	8.5×10^9 copies/mL	3/3
Coronavirus	ATCC VR-740D	7.2×10^8 copies/mL	3/3
Coxsackievirus	ATCC VR-169	1.0×10^7 copies/mL	3/3
Cytomegalovirus	ATCC VR-538D	2.5×10^9 copies/mL	3/3
Echovirus	ATCC VR-1734D	4.2×10^9 copies/mL	3/3
Epstein-Barr Virus	ATCC VR-3247SD	5.3×10^7 copies/mL	3/3
Herpes Simplex Virus 1	ATCC VR-539D	1.0×10^9 copies/mL	3/3
Herpes Simplex Virus 2	ATCC VR-540D	1.6×10^6 TCID50	3/3
Human Bocavirus	ATCC VR-3251SD	4.8×10^7 copies/mL	3/3
Human Metapneumovirus	ATCC VR-3250SD	6.2×10^7 copies/mL	3/3
Influenza A	ATCC VR-1738D	3.9×10^9 copies/mL	3/3
Influenza B	ATCC VR-1813D	1.2×10^{10} copies/mL	3/3
Measles virus	ATCC VR-24D	1.6×10^{10} copies/mL	3/3
Mumps virus	ATCC VR-106D	1.3×10^{10} copies/mL	3/3
Parainfluenza virus 1	ATCC VR-94D	1.8×10^9 copies/mL	3/3
Parainfluenza virus 2	ATCC VR-92D	3.4×10^9 copies/mL	3/3
Respiratory Syncytial Virus	ATCC VR-1540D	7.9×10^8 copies/mL	3/3
Rhinovirus	ATCC MBC091	3.0×10^9 copies/mL	3/3
Varicella Zoster Virus	VR-1367D	1.4×10^6 copies/mL	6/6
Yeast			
Candida albicans	ATCC 18804	1.1×10^7 CFU/mL	3/3
Candida glabrata	ATCC 66032	1.3×10^7 CFU/mL	3/3
Human Genomic DNA			
Human Genomic DNA	ATCC HTB-20D	3.8×10^7 copies/mL	3/3

* This set of test runs also contained one 'Test incomplete' run.

One (1) of three (3) replicates with *M. tuberculosis* reported '*Bordetella pertussis* NOT DETECTED'. Additional testing with eleven (11) replicates yielded the expected '*Bordetella pertussis* DETECTED' results, demonstrating that *M. tuberculosis* does not interfere with the Bordetella Direct Test.

Testing of specimen with the above described potential interfering non-target organisms at high input concentrations produced the expected positive results, indicating that none of the non-target organisms compete or interfere with obtaining accurate test results with the Bordetella Direct Test.

h. *Interfering Substances*:

A panel of nineteen (19) chemical and biological substances potentially present in *Bordetella pertussis* infection specimens were evaluated for interference with the Bordetella Direct Test. Each substance was tested in three replicates at concentrations which were medically significant in the presence and absence of near LOD (2x) levels of *B. pertussis* in the Bordetella Direct Test. A clinical negative sample was also tested as a control to evaluate potential interference with the internal assay control (SPC) in the absence of analyte.

None of the substances tested were found to interfere with the Bordetella Direct Test. The interference substances and their concentrations included in the interference study are shown in Table 9 below.

Table 9: Interfering Substance Study Results

Interfering Substance	Concentration Tested	*B. pertussis* DETECTED (2.5X LoD)	Negative
Afrin Nasal Spray	15% v/v	3/3	3/3
Cepacol Sore Throat Pain Relief Lozenges	5% w/v	3/3	3/3
Children's Dimetapp Cold & Allergy	15% v/v	3/3	3/3
Chloraseptic Max Sore Throat Lozenges	10% w/v	3/3	3/3
Diphenhydramine HCl, 25mg	1 mg/mL	3/3	3/3
Erythromycin	20 mg/mL	3/3	3/3
Flonase Nasal Spray	15% v/v	3/3	3/3
Mucin (Bovine Submaxillary Gland, Type I-S)	10 mg/mL	3/3	3/3
Mupirocin	10 mg/mL	3/3	3/3
NasalCrom Nasal Spray	15% v/v	3/3	3/3
Vaseline Petroleum Jelly	1% w/v	3/3	3/3
Releev Cold Sore Treatment	15% v/v	3/3	3/3
Robitussin Cough Syrup	5%v/v	3/3	3/3
Saline Nasal Moisturizing Spray	15% v/v	3/3	3/3
Sucrets Complete Lozenges	5% w/v	3/3	3/3
Tobramycin	1 mg/mL	3/3	3/3
Vicks Vapor Rub	1% w/v	3/3	3/3
Blood (human), EDTA anticoagulated	5% v/v	3/3	3/3
Zicam Nasal Gel	5% v/v	3/3*	3/3

*This set of test runs contained one 'Test incomplete' run.

i. Carry-over/Cross-Contamination:

A study was performed to assess the cross-contamination/carry-over of the Bordetella Direct Test by testing a series of alternating high positive and negative samples for twenty (20) consecutive runs on two (2) separate Analyzers for a total of forty (40) runs. The high positive samples were prepared with 1.55 $x10^7$ CFU/mL of B. pertussis in natural negative NP matrix collected in VTM. The negative samples were prepared with natural negative NP matrix collected in VTM.

No false positive results were observed during consecutive testing of high positive samples alternating with negative samples, demonstrating that recommended sample handling and testing practices are effective in preventing false positive results due to carryover or cross-contamination between samples.

j. Swab, Transport Media and Elution Buffer Equivalency Studies:

Two studies were conducted to demonstrate the equivalency between various swab types, transport media types and elution buffers. In the first study, various types of swabs, transport media and elution buffers were tested for potential interference in the Bordetella Direct Test. To test the transport media and elution buffers, samples were prepared by mixing *B. pertussis* (ATCC 9797) at 4.0x10^3 CFU/mL (2.5x LoD) with each transport media or elution buffer. To test for interference of the swabs, each swab type was inserted into natural negative NP matrix that was spiked with B. pertussis (ATCC 9797) at 4.0x10^3 CFU/mL (2.5X LoD). A clinical negative sample was also tested as a control to evaluate potential interference of each substance with the internal assay control (SPC) in the absence of analyte. Each sample was tested in triplicate. The swab types, media and elution buffers tested along with the study results are shown in Table 10 below.

Table 10: Swab, Transport Media and Elution Buffer Interference Study Results		
Swab Type	***B. pertussis* DETECTED**	**Negative**
Flocked Nylon	3/3	3/3
Polyester	3/3	3/3
Rayon	3/3	3/3
Transport Media Type	***B. pertussis* DETECTED**	**Negative**
Remel M4 VTM	3/3	3/3
Remel M4RT VTM	3/3	3/3
Remel M5 VTM	3/3	3/3
Remel M6 VTM	3/3	3/3
BD Universal VTM	3/3	3/3
Elution Buffer Type	***B. pertussis* DETECTED**	**Negative**
Molecular grade water	3/3	3/3
1x Phosphate Buffered Saline (PBS)	3/3	3/3
0.85% Saline	3/3	3/3*
Tris-EDTA (TE) Buffer	3/3	3/3

*This set of test runs contained one 'Test incomplete' run
Note: Copan UTM is the same transport media as BD Universal VTM and therefore was not tested.

None of the swabs, transport media or elution buffers tested was found to interfere with the Bordetella Direct Test.

In a separate study, the Bordetella Direct Test was evaluated with three (3) distinct combinations of nasopharyngeal swab types and transport/elution media to demonstrate equivalent performance:

- Polyester swab with Viral Transport Media (M5 VTM)
- Flocked nylon NP swab with Liquid Amies transport media (ESwab)
- Rayon swab in Liquid Stuart transport media, eluted in water

Each swab/media combination listed above was collected as clinical negative NP matrix. *B. pertussis* strains (ATCC 8467, ATCC 9797, and ATCC BAA-589) were used to prepare contrived samples in the above three (3) swab/media combinations. Positive samples were prepared by the addition of each *B. pertussis* strain at varying LoDs in clinical negative NP matrix. The negative clinical matrix served as the negative sample. Each unique sample was tested in triplicate in the Bordetella Direct Test. The test conditions and results are shown in Table 11below:

Table 11: Media Equivalency Samples Results					
Transport/ Elution Media	**Swab**	**Sample Type, Input, Expected Results/Total**			
		***B. pertussis* ATCC 8467 2.1x LoD** (6.9×10^3 CFU/mL)	***B. pertussis* ATCC 9797 4.5x LoD** (7.3×10^3 CFU/mL)	***B. pertussis* ATCC BAA-589 1.9x LoD** (4.3×10^3 CFU/mL)	**Negative**
M5 VTM (3mL)	Polyester	3/3	3/3	3/3	3/3
ESwab (1mL)	Flocked nylon	3/3	3/3	3/3	3/3
Liquid Stuart/ Eluted in water	Rayon	3/3	3/3	3/3	3/3

The media equivalency study produced expected positive results, demonstrating equivalent performance of the Bordetella Direct Test with various swab and media combinations tested.

The Bordetella Direct Test is compatible with the following types of swabs: Polyester, Rayon, Flocked Nylon and with the following type of transport media: VTM (M4, M4RT, M5, M6), BD Universal VTM, Copan UTM, ESwab (Liquid Amies) and Liquid Stuart. Samples stored in VTM or ESwab are eluted in the same transport media. Samples stored in Liquid Stuart can be eluted with 1.0 mL of any of the following: Molecular Grade water, PBS, 0.8% Saline or Tris-EDTA (TE) buffer.

k. Fresh vs. Frozen Study:

In order to utilize frozen Reproducibility Panel specimens as well as frozen archived samples in the evaluation of the Bordetella Direct Test, an analytical study was conducted to demonstrate that preservation of samples by freezing at ≤ -70°C does not affect the accuracy of test results compared to freshly collected or freshly prepared samples. All samples were stored at ≤ -70°C and tested in triplicate at the following time points: T = 0 (fresh), 7, 14 and 72 days. The results are presented in Table 12 below.

Table 12: Fresh vs. Frozen Study Results				
Sample (Input)	**Time Point (days), Expected Results/Total**			
	T_0 = Fresh	**T_7 days**	**T_{14} days**	**T_{72} days**
*B. pertussis*ATCC 9797 (7.3 x 103 CFU/mL)	3/3	9/9	9/9†	9/9
*B. pertussis*ATCC 8467 (6.9 x10^3 CFU/mL)	3/3	9/9	9/9	7/7
*B. pertussis*ATCC BAA-589 (4.3 x 10^3 CFU/mL)	3/3	9/9	9/9	6/6
Negative	3/3	9/9	9/9†	9/9

†This set of test runs also contained one 'Invalid' run.

The fresh vs. frozen study results produced expected result for the Bordetella Direct Test, for negative and positive sample types at all time points. The data support the use of frozen samples when stored at -70^{o}C for ≤ 72 days.

l. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable.

3. Clinical studies:

a. Clinical Sensitivity:

The clinical performance of the Bordetella Direct Test was evaluated in a multi-site clinical study. The evaluation included a Prospective and a Frozen Retrospective method comparison study. Performance characteristics of the Bordetella Direct Test was established in 2017 (July 2016 to January 2017) at four locations in the United States. Nine hundred thirty six (936) fresh nasopharyngeal swab specimens were obtained from female and male patients suspected of having respiratory tract infection attributable to *Bordetella pertussis.* The specimens enrolled in the Prospective study were excess, de-identified nasopharyngeal (NP) swab specimens collected in VTM that were submitted for standard of care *B. pertussis* testing and which would have

otherwise been discarded. Specimens were collected and transported to each laboratory for testing with the Bordetella Direct Test.

Clinical performance was based on comparison of the performance of the Bordetella Direct Test to an FDA cleared Nucleic Acid Amplification Test for the detection of *Bordetella pertussis* (Reference NAAT). Discrepant results were investigated by testing in a second FDA cleared NAAT (NAAT 2) which also detects *Bordetella pertussis.*

Nine hundred thirty six (936) fresh nasopharyngeal swab specimens were tested as described above. Twenty one (21) specimens were excluded from the data set due to improperly stored sample or failed daily QC. The table below details the comparison data of the Bordetella Direct Test and the Composite Reference Method for the remaining nine hundred fifteen (915) specimens.

Samples used in the Frozen Retrospective Study were frozen archived NP swab specimens collected in VTM. The specimens were de-identified specimens that were previously characterized as positive or negative by a nucleic acid amplification test used at the particular institution from which they were obtained (historical result).

The historical result for each sample was first confirmed by the Reference NAAT prior to enrolling the sample in the study. A total of one hundred twenty four (124) frozen samples were tested. Subsequent to testing, two (2) samples were excluded from the data set due to failed daily QC leaving one hundred twenty two (122) samples included in the final analysis.

The results from the method comparison studies comparing the Bordetella Direct Test to the Reference NAAT are summarized in Table 13 below. The Table shows the calculated PPA and NPA and the associated 95% confidence intervals for the results obtained in the Prospective (Fresh) and Frozen Retrospective Study (Frozen). The number of true-positive (co-positive) and true-negative (co-negative) samples is also included.

Table 13: Comparison of Bordetella Direct Test to the Reference NAAT

Specimen		***n***	***% Agreement (95% CI)***	
			Positive	***Negative***
Bordetella pertussis	Prospective (Fresh)	915	85.7% (65.4% - 95.0%) 18/21	99.6% (98.9% - 99.8%) 890/894
	Retrospective (Frozen)	122	94.6% (86.1% - 98.3%) 56/59	100.0% (94.3% - 100.0%) 63/63

In total, there were six (6) false negative and four (4) false positive results. Two (2) of the six (6) false negatives were also negative by a second FDA cleared NAAT and two (2) of the four (4) false positives were also positive by the second FDA cleared NAAT.

In the Prospective Study, the results for 915 samples were analyzed comparing the Bordetella Direct Test to the reference NAAT. For all sites combined, the point estimate achieved for PPA was 85.7% with a 95% confidence interval of 65.4% - 95.0%. The point estimate for NPA was 99.6% with a 95% confidence interval of 98.9% - 99.8%.

In the Frozen Retrospective Study, the results for 122 samples were analyzed comparing the Bordetella Direct Test to the reference NAAT. The point estimate achieved for PPA was 94.6% with a 95% confidence interval of 86.1% - 98.3%. The point estimate for NPA was 100.0% with a 95% confidence interval of 94.3% - 100.0%.

*b. Clinical specificity**:***

See table above.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable.

4. Clinical cut-off**:**

Not Applicable.

5. Expected values/Reference range:

Overall incidence of *B. pertussis* as detected by the Bordetella Direct Test in prospectively collected specimens (all comers) during the period of this study was 2.4% (22/915).

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10, 21 CFR 801.109, and special controls.

O. Conclusion:

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