DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

March 31, 2017

Great Basin Scientific, Inc.
Suzette Chance
2441 S. 3850 West Suite 100
Salt Lake City, UT 84120

Re: K170284
Trade/Device Name: Great Basin Bordetella Direct Test
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory viral panel multiplex nucleic acid assay
Regulatory Class: Class II
Product Code: OZZ
Dated: December January 26, 2017
Received: January 30, 2017

Dear Ms. Chance:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the

electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely,

Steven R. Gitterman -S

for Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics and
Radiological Health
Center for Devices and
Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K170284

Device Name
Great Basin Bordetella Direct Test

Indications for Use *(Describe)*

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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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5.0 510(k) Summary – Bordetella Direct Test

A. Submitted by:

Great Basin Corporation
2441 South 3850 West
Salt Lake City, Utah 84120

Contact Information

Suzette Chance, PhD
Senior Director of Clinical Affairs
Great Basin Scientific
2441 S. 3850 West
Salt Lake City, Utah 84120
Phone: 385-215-3369
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B. Name of Device

Proprietary Name: Great Basin Bordetella Direct Test
Common or Usual Names: Bordetella Test

C. Regulatory Information:

a.	Regulation Section:	21 CFR 866.3980, Respiratory viral panel Multiplex Nucleic Acid Assay 21 CFR 862.2570 – Instrumentation for clinical multiplex test systems
b.	Classification:	Class II (Bordetella Direct Test) Class II (PA500 Portrait Analyzer System)
c.	Classification panel: Product Code:	Microbiology Devices, OIVD (83) Microbiology OZZ *Bordetella pertussis* Nucleic Acid Amplification Assay System OOI Real-Time Nucleic Amplification System

D. Intended use(s)/Indications for Use:

The Great Basin *Bordetella* Direct Test is a qualitative *in vit*ro 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 IS*481*. The IS*481* 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 IS*481* 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.

E. Device Description:

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 IS*481* 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 IS*481* 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.

Test Device:

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. The Portrait System was granted 510(k) clearance for the Portrait Toxigenic *C. difficile* Assay (K113358), Portrait GBS Assay (K143312), Staph ID/R Blood Culture Panel (K152470) and the Shiga Toxin Direct Test (K152955).

F. Substantial Equivalence Information:

Predicate Device: *illumigene*® Pertussis DNA Amplification Assay (K133673)
The following table provides a comparison of the Bordetella Direct Test and the predicate device:

Feature/Characteristic	Bordetella Direct Test (K170284)	Predicate (K133673) Illumigene® Pertussis Assay
Manufacturer	Great Basin Scientific, Inc.	Meridian Biosciences, Inc.
Trade Name	Great Basin Bordetella Direct Test	*illumigene*® Pertussis DNA Amplification Assay
510(k) Number		K133673
Classification	Class II	Same
Intended Use/ Indications for Use	The Great Basin Bordetella Direct Test is a qualitative *in vit*ro 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 IS*481*. The IS*481* 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 IS*481* 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.	The ***illumi****gene* Pertussis DNA Amplification Assay, performed on the ***illumi****pro-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 ***illumi****gene* 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 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 ***illumi***gene 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 ***illumi****gene* 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. ***illumi****gene* Pertussis is intended for use in hospital, reference or state laboratory settings. The device is not intended for point-of-care use.

Feature/Characteristic	Bordetella Direct Test	Predicate (K133673) Illumigene® Pertussis Assay
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
Calibration	Not required	Same
Target Sequence Detected	IS*481*	Same
Sample Lysis and DNA Extraction	Automated	Manual
DNA Amplification Technology	PCR	Loop-mediated amplification (LAMP)
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

The following summarizes the differences and similarities between the Bordetella Direct Test and the predicate device:

The Bordetella Direct Test is similar to the *illumigene*® Pertussis Assay in the following ways:

- The Bordetella Direct Test and the *illumigene*® Pertussis Assay have similar Intended Uses and both detect the *B. pertussis* analyte.
- The Bordetella Direct Test and the *illumigene*® Pertussis Assay are both qualitative, automated nucleic acid based tests.
- The Bordetella Direct Test and the *illumigene*® Pertussis Assay both target the IS*481* DNA sequence of the *B. pertussis* genome.
- The Bordetella Direct Test and the *illumigene*® Pertussis Assay use the same sample type (nasopharyngeal swab – rayon, nylon or flocked).

The Bordetella Direct Test differs from the *illumigene*® Pertussis Assay in the following:

- The Bordetella Direct Test uses automated sample lysis and DNA extraction whereas these are manual steps in the *illumigene*® Pertussis Assay.
- The Bordetella Direct Test uses hot start PCR for nucleic acid amplification whereas the *illumigene*® Pertussis Assay used Isothermal Loop-mediated amplification (LAMP).

G. Performance Summary – Analytical Studies

a. Analytical Sensitivity

The Analytical Sensitivity as defined by the limit of detection (LoD) of the Bordetella Direct Test was assessed by testing three (3) *B. pertussis* 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 LoDs for each strain tested is shown in Table 1.

Table 1. Bordetella Direct Test Limit of Detection (LoD)

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

Conclusion: 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.

b. Analytical Reactivity (Inclusivity)

The Bordetella Direct Test Analytical Reactivity (Inclusivity) was confirmed by testing an additional eight (8) *B. pertussis* strains beyond the three (3) tested in the Analytical Sensitivity Study.

B. pertussis cultures were prepared and enumerated 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 2.

Table 2. 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

Conclusion: The Bordetella Direct Test correctly identified the additional eight (8) organisms tested in the Inclusivity Study indicating that the Bordetella Direct Test can detect additional strains of *B. pertussis*.

c. Analytical Specificity (Exclusivity)

The potential for cross-reactivity was evaluated in an Exclusivity Study, by testing non-target organisms commonly found in the human respiratory system in the Bordetella Direct Test. The study included 90 organisms including 48 bacteria, 20 viruses, 2 yeast, 19 *Bordetella* species (non-*B. pertussis*) and human genomic DNA. For those organisms that were classified as Biosafety level III, unavailable as intact organisms or difficult to culture via standard clinical microbiology techniques, genomic DNA was tested in place of whole organism.

Whole organism stocks were prepared at high concentrations in liquid media appropriate for each organism. The liquid media stocks were enumerated and frozen at ≤-20°C until testing. On the day of testing, each organism or nucleic acid stock was thawed and spiked into a natural negative NP matrix collected in VTM.

All bacterial and yeast strains were tested at concentrations ≥ 1 x 10^6 CFU/mL. Genomic DNA and viral strains were tested at ≥ 1.4 x 10^6 genomic copies/mL and ≥ 1.6 x 10^6 TCID50 respectively. A minimum of three (3) replicates were tested for each organism evaluated for cross-reactivity. The results from the testing, including specific concentration at which each organism was tested are shown in Tables 3 and 4.

Table 3. Analytical Specificity (Exclusivity) Study Results

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

Organism	Strain ID	Concentration Tested	Expected Negative Results /Total
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	3/3
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	1.6×10^7 CFU/mL	3/3
		5.3×10^7 CFU/mL	3/3
		5.3×10^7 CFU/mL	10/10
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
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 equisimilis	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	5/5*[†]
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
Parainfluenza virus 3	ATCC VR-93D	7.9×10^8 copies/mL	3/3

Organism	Strain ID	Concentration Tested	Expected Negative Results /Total
Respiratory Syncytial Virus	ATCC VR-1540D	3.0×10^9 copies/mL	3/3
Rhinovirus	ATCC MBC091	1.4×10^6 copies/mL	3/3
Varicella Zoster Virus	VR-1367D	1.0×10^9 copies/mL	3/3
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. †This set of test runs also contained one 'Invalid' run.			

The Bordetella Direct Test correctly reported '*Bordetella pertussis* NOT DETECTED' for all replicates reported in Table 3 as expected, with the following exceptions:

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. The negative results from new preparations suggest original positive results were likely due to sample contamination. These data demonstrate that *B. avium, B. petrii, B. trematum,* and *B. parapertussis* do not cross-react in the Bordetella Direct Test.

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 4.

Table 4. Analytical Specificity (Exclusivity) Results for Potential Cross-Reacting Strains

Potential Cross-Reacting Organism	Strain ID	Concentration Tested	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 (Table 4). 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 4). 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. Since this was unexpected, 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 silico* analysis on this strain could not be performed because an accession number does not exist in the NCBI database.

Further investigation of the negative-resulting *B. hinzii* strain (ATCC 51730) revealed that this a clinical isolate from a blood sample of an AIDS patient (isolated from human blood, AIDS patient; Vandamme et al., Int J. Syst. Bacteriol. 45: 37- 45, 1995). The entire sequenced genome of this isolate is found in the NCBI database (strain F582, accession number CP012076), allowing for specific *in silico* analysis. *In silico* analysis demonstrated partial homology with the reverse primer, but no homology with the forward primer, consistent with the negative result.

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 IS*481* insertion sequence reported in a subset of strains for these species. This is noted in Section 11 for *B. bronchiseptica* and *B. holmesii* (*in silico Analysis for Bordetella Direct Test*) and in the literature for *B. hinzii* ATCC 51784, a pathogen which mainly infects avian species.

Conclusion: All organisms shown in Table 3 gave the expected '*B. pertussis* NOT DETECTED' result indicating that there was no cross-reactivity in the Bordetella Direct Test. Organisms known to potentially contain the IS*481* sequence showed some cross-reactivity in the Bordetella Direct Test (Table 4), including *B. bronchiseptica, B. holmesii,* and *B. hinzii*. This cross-reactivity is listed as a limitation in the product labeling (Section 13), with the exception of *B. hinzii* which is mainly an avian pathogen.

d. Microbial Interference

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 5.

Table 5. Microbial Interference Study Results: Organisms Tested in the Presence of 2.5X LoD *B. pertussis*

Organism	Strain ID	Non-Target Microbe Input Tested	Expected Positive Results/Total
Bacteria			
Acinetobacter baumannii	ATCC 19606	1.2 x 10^7 CFU/mL	3/3
Acinetobacter calcoaceticus	ATCC 23055	1.5 x 10^7 CFU/mL	3/3
Acinetobacter haemolyticus	ATCC 19002	2.2 x 10^7 CFU/mL	3/3
Actinomyces odontolyticus	ATCC 17929	1.5 x 10^6 CFU/mL	3/3
Arcanobacterium haemolyticum	ATCC BAA-1784	2.4 x 10^7 CFU/mL	3/3
Bacteroides fragilis	ATCC 23745	4.6 x 10^7 CFU/mL	3/3
Bordetella avium	ATCC 35086	1.2 x 10^7 CFU/mL	3/3
Bordetella bronchiseptica	ATCC BAA-588	3.0 x 10^7 CFU/mL	3/3
Bordetella bronchiseptica	ATCC 19395	2.1 x 10^7 CFU/mL	3/3
Bordetella hinzii	ATCC 51730	3.0 x 10^7 CFU/mL	3/3*
Bordetella parapertussis	ATCC 9305	1.2 x 10^7 CFU/mL	3/3
Bordetella parapertussis	ATCC 15237	1.7 x 10^7 CFU/mL	3/3
Bordetella parapertussis	ATCC 15311	2.4 x 10^7 CFU/mL	3/3
Bordetella parapertussis	ATCC 15989	3.2 x 10^7 CFU/mL	3/3
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	1.2 x 10^7 CFU/mL	3/3
Bordetella trematum	ATCC 700309	1.3 x 10^7 CFU/mL	3/3
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	13/14
Mycoplasma pneumoniae	ATCC 15531D	2.0 x 10^7 copies/mL	3/3

Organism	Strain ID	Non-Target Microbe Input Tested	Expected Positive Results/Total
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
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 equismilis	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	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.			

All organisms shown in Table 5 gave the expected '*Bordetella pertussis* DETECTED' result demonstrating that there was no interference in the Bordetella Direct Test, with one exception. One (1) of three (3) replicates tested with *M. tuberculosis* reported '*Bordetella pertussis* as 'NOT DETECTED'. Additional testing with 11 replicates yielded the expected '*Bordetella pertussis* DETECTED' results, demonstrating that *M. tuberculosis* does not interfere with the Bordetella Direct Test.

Conclusion: No interference in the Bordetella Direct Test was observed with the organisms tested in Table 5 at the concentrations indicated.

e. Interfering Substances

A panel of 19 chemical substances commonly used or present in patients with upper respiratory conditions were evaluated for interference with the Bordetella Direct Test.

Samples were prepared by mixing *B. pertussis* (ATCC 9797) at 4.0 x10^3 CFU/mL (2.5X LoD) contrived in a natural negative NP matrix collected in VTM with each substance. A clinical negative sample was also tested as a control to evaluate potential interference of each substance with the internal Sample Processing Control (SPC) in the absence of analyte. Each sample was tested in triplicate. The chemical substances, test concentration (mg/mL or %), and results are listed in Table 6.

Table 6. Interfering Substances Study Results

		Expected Results/Total	
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.			

Conclusion: No interference in the Bordetella Direct Test was observed for the 19 substances tested at the concentrations shown in Table 6.

f. Carry-over/Cross-Contamination

The Bordetella Direct Test was evaluated for carry-over/cross-contamination by testing a series of alternating high positive and negative samples for 20 consecutive runs on 2 separate Analyzers for a total of 40 runs. The high positive and negative samples were prepared as follows:

- High Positive: *B. pertussis* 1.55 x10^7 CFU/mL in natural negative NP matrix collected in VTM
- Negative: natural negative NP matrix collected in VTM

The results are shown in Table 7.

Table 7. Carry-over/Cross-contamination Study Results

		***Bordetella pertussis* Result**	
Runs	**Sample**	**Portrait Analyzer 5.075**	**Portrait Analyzer 5.435**
1	High Positive	DETECTED	DETECTED
2	Negative	NOT DETECTED	NOT DETECTED
3	High Positive	DETECTED	DETECTED
4	Negative	NOT DETECTED	NOT DETECTED
5	High Positive	DETECTED	DETECTED
6	Negative	NOT DETECTED	NOT DETECTED
7	High Positive	DETECTED	DETECTED
8	Negative	NOT DETECTED	NOT DETECTED
9	High Positive	DETECTED	DETECTED
10	Negative	NOT DETECTED	NOT DETECTED
11	High Positive	DETECTED	DETECTED
12	Negative	NOT DETECTED	NOT DETECTED
13	High Positive	DETECTED	DETECTED
14	Negative	NOT DETECTED	NOT DETECTED
15	High Positive	DETECTED	DETECTED
16	Negative	NOT DETECTED	NOT DETECTED
17	High Positive	DETECTED	DETECTED
18	Negative	NOT DETECTED	NOT DETECTED
19	High Positive	DETECTED	DETECTED
20	Negative	NOT DETECTED	NOT DETECTED
Total Runs Per Device		20	20
Total Runs for Study		**40**	

Conclusion: The expected results were obtained in each run. No carry-over or cross- contamination was observed in the Bordetella Direct Test.

g. 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 8. Note: Copan UTM is the same transport media as BD Universal VTM and therefore was not also tested.

Table 8. Swab, Transport Media and Elution Buffer Interference Study Results

	Expected Results/Total	
Swab Type	***B. pertussis* DETECTED (2.5X LoD)**	**Negative**
Flocked Nylon	3/3	3/3
Polyester	3/3	3/3
Rayon	3/3	3/3
Transport Media Type	***B. pertussis* DETECTED (2.5X LoD)**	**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 (2.5X LoD)**	**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		

Results: None of the swabs, transport media or elution buffers demonstrated interference in the Bordetella Direct Test.

In a separate study the Bordetella Direct Test was evaluated using the following 3 distinct combinations of nasopharyngeal swab types and transport/elution media:

- 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 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 9.

Table 9. Swab, Transport Media and Elution Buffer Equivalence Study Results

		Sample Type, Concentration, Expected Results/Total			
Transport/Elution Media	**Swab**	***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 (1mL)	Rayon	3/3	3/3	3/3	3/3

Results: There was 100% agreement with the expected results demonstrating equivalent performance of the Bordetella Direct Test with various swab and media combinations tested.

Conclusion: 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.

h. Sample Stability and Storage

Sample stability studies were conducted to support the storage conditions stated in the 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 10 and 11, respectively.

Table 10. Sample Stability Study: Room Temperature Storage

		Time Point (hrs), Expected Results/Total			
Strain Tested and Concentration	**Media**	**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 11. Sample Stability Study: 2-8°C Storage

		Time Point (hrs), Expected Results/Total					
Strain Tested and Concentration	**Media**	**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 contained one 'Test incomplete' run.
†This set of test runs also contained one 'Invalid' run.

Conclusion: 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.

i. Reproducibility

A Reproducibility Study was conducted at three clinical study sites. Two of the testing sites were external sites (Site 4: Providence Health Services Oregon and Site 5: Indiana University School of Medicine) and the third site was internal (Site 6: Great Basin Scientific). The study incorporated several variables including 6 different operators at 3 sites (2 operators/site), 33 different Portrait Analyzers and 3 different Cartridge lots.

A panel consisting of three (3) different samples: a low positive (*B. pertussis* at 1.9X LoD) a moderate positive (*B. pertussis* at 3.8X LoD) prepared in negative clinical matrix collected in VTM and a negative sample (negative clinical matrix collected in VTM) were tested in the Bordetella Direct Test. Each operator tested each sample in triplicate over five (5) non-consecutive days. Six (6) different operators performed the testing.

The results were analyzed for percent agreement of positive or negative results, by operator, by site and in total (overall) and are shown in Table 12.

Table 12. Reproducibility Study Results

Analyte	Conc.	Site	Operator	Correct Results	Agreement	Correct Results	Agreement	Total Correct Results
Bordetella pertussis Positive Samples	1.9X LOD (Low Positive)	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%			
	3.8X LOD (Moderate Positive)	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%			
Negative Sample	N/A	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.

Conclusion: There was 100% agreement of results between replicates, runs, operators and sites.

H. Performance Summary – Clinical Studies

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. These studies compared 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*.

The Prospective study was conducted at four external, geographically-diverse U.S. clinical study sites (Northwest, Southwest, Midwest, West) from July, 2016 to January, 2017. 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. A total of 936 samples were collected for all four sites combined. Subsequent to enrollment, 21 samples were excluded from the data set due to improperly stored sample or failed daily QC leaving 915 samples included in the analysis.

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 124 frozen samples were tested. Subsequent to testing, 2 samples were excluded from the data set due to failed daily QC leaving 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. Table 13 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	Fresh	915	85.7% (65.4% - 95.0%) 18/21	99.6% (98.9% - 99.8%) 890/894
	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.

Conclusion:

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%.

I. Overall Conclusion

The submitted information in this 510(k) pre-market notification is complete and supports substantial equivalence for the Great Basin Bordetella Direct Test.