510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K143206

B. Purpose for Submission:

To obtain a substantial equivalence determination for the Amplivue® Bordetella Assay.

C. Measurand:

Insertion sequence IS481 of *Bordetella pertussis*.

D. Type of Test:

The Amplivue® Bordetella Assay is a helicase-dependent 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:

Quidel Corporation

F. Proprietary and Established Names:

Amplivue® Bordetella Assay

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 AmpliVue® Bordetella Assay is an in vitro diagnostic test for the qualitative detection of *Bordetella pertussis* nucleic acids isolated from nasopharyngeal swab specimens obtained from patients suspected of having respiratory tract infection attributable to *Bordetella pertussis.*

   The AmpliVue® Bordetella Assay utilizes helicase-dependent amplification (HDA) of the insertion sequence IS481 and a self-contained disposable amplification detection device that allows for manual evaluation of assay results. The IS481 sequence can also be found in strains of other organisms (i.e., *B. holmesii* and *B. bronchiseptica*). *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 AmpliVue® Bordetella Assay do not preclude *B. pertussis* infection and positive results do not rule out co-infection with other respiratory pathogens. Results from the AmpliVue® Bordetella Assay 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 AmpliVue® Bordetella Assay is intended for use in hospital, reference or state laboratory settings. The device is not intended for point-of-care use.

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:

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

I. Device Description:

The AmpliVue® Bordetella Assay combines simple sample processing, an isothermal amplification technology named helicase-dependent amplification (HDA), and a self- contained disposable amplicon detection device, for the detection of *Bordetella pertussis* from nasopharyngeal swabs.

Patient samples are collected using a nasopharyngeal swab and placed into a liquid medium. Fifty microliters (50 µL) of the sample are then transferred to a process buffer that is provided with the kit and mixed. The Process Buffer tubes are heated at 95 °C for 10 minutes. Fifty microliters (50 µL) of the Process Buffer containing sample is added to a reaction tube containing lyophilized mix of HDA reagents. Included in the reaction mix are the isothermal polymerase, helicase and single stranded binding protein. After completion of the HDA reaction the reaction tube is transferred to the amplicon cartridge containing the running buffer. The amplicon cartridge is closed and inserted into the detection chamber. The detection chamber is activated by depressing the detection chamber handle. Upon activation, the reservoir containing the running buffer and the 0.2 mL tube containing the amplicon is punctured and the solutions are wicked to the lateral flow strip.

Materials Provided:

- 16 Tests per Kit

Symbol	Component	Quantity	Storage
1	Detection Cassettes	16/kit	2° to 30°C
2	Process Buffer	16 tubes/kit 1.45mL	2° to 30°C
3	Reaction Tubes	16 tubes/kit	2° to 8°C
4	Amplicon Cartridge	16/kit	2° to 30°C

Materials required but not provided:

- External controls for *Bordetella pertussis* (e.g. Quidel Molecular Bordetella Control Set #M117, which contains positive and negative controls, serves as an external processing and extraction control)
- Sterile DNAse-free filter-blocked or positive displacement micropipettor tips
- Micropipettor
- Stopwatch or timer
- Scissors or a blade
- Micro tube tray
- Heat block capable of 95° C ± 2° C temperature
- Heat block with heated lid capable of 64° ± 2° C temperature
- Thermometer

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	**AmpliVue® Bordetella Assay (k143206)**	**illumigene® Pertussis DNA Amplification Assay (k133673)**
Intended Use	The AmpliVue® Bordetella Assay is an in vitro diagnostic test for the qualitative detection of *Bordetella pertussis* nucleic acids isolated from nasopharyngeal swab specimens obtained from patients suspected of having respiratory tract infection attributable to *Bordetella pertussis*. The AmpliVue® Bordetella Assay utilizes helicase-dependent amplification (HDA) of the insertion sequence IS481 and a self-contained disposable amplification detection device that allows for manual evaluation of assay results. The IS481 sequence can also be found in strains of other organisms (i.e., *B. holmesii* and *B. bronchiseptica*). *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	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 *Bordetella pertussis* by targeting the IS481 insertional element of the *Bordetella pertussis* genome. The IS481 insertional element can also be found in *Bordetella holmesii* and

Similarities		
Item	**AmpliVue® Bordetella Assay (k143206)**	**illumigene® Pertussis DNA Amplification Assay (k133673)**
	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 AmpliVue® Bordetella Assay do not preclude *B. pertussis* infection and positive results do not rule out co-infection with other respiratory pathogens. Results from the AmpliVue® Bordetella Assay 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.	*Bordetella bronchiseptica* strains. Respiratory infection with *Bordetella pertussis*, *Bordetella holmesii* or *Bordetella 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

Similarities		
Item	**AmpliVue® Bordetella Assay (k143206)**	**illumigene® Pertussis DNA Amplification Assay (k133673)**
		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 *Bordetella 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

Similarities		
Item	**AmpliVue® Bordetella Assay (k143206)**	**illumigene® Pertussis DNA Amplification Assay (k133673)**
		state laboratory settings. The device is not intended for point-of-care use.
Sample Types	Nasopharyngeal swab specimens obtained from patients suspected of having respiratory tract infection attributable to *Bordetella pertussis*	Same
Sample Heat Lysis	Manual	Same
Target Sequence Detected	*Bordetella pertussis* IS481 insertion element	Same

Differences		
Item	**AmpliVue® Bordetella Assay (k143206)**	**illumigene® Pertussis DNA Amplification Assay (k133673)**
DNA Amplification Technology	Helicase-dependent amplification (HDA); self- contained	Loop-Mediated Isothermal Amplification (LAMP); self- contained and automated
Self-Contained System Assay after sample preparation	No	Yes
Detection Technique	Manual	Automated
Instrument	None	*illumipro-10*™

Differences		
Item	**AmpliVue® Bordetella Assay (k143206)**	**illumigene® Pertussis DNA Amplification Assay (k133673)**
Testing Time	85 - 90 minutes	60 -70 minutes

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

Not applicable.

L. Test Principle:

The AmpliVue® Bordetella Assay detects *Bordetella pertussis* DNA isolated from nasopharyngeal swab specimens obtained from symptomatic patients suspected of having respiratory tract infection attributable to *Bordetella pertussis*. The assay consists of three major steps: 1) specimen preparation, 2) isothermal Helicase-Dependent Amplification (HDA) of a target sequence of B. pertussis, and 3) detection of the amplified DNA by target-specific hybridization probes via a colorimetric reaction on a lateral-flow strip which is embedded in a self-contained disposable cassette to prevent amplicon contamination.

Patient samples are collected using a nasopharyngeal swab and placed into a liquid medium. Fifty microliters (50 µL) of the sample are then transferred to a process buffer that is provided with the kit and mixed. The Process Buffer tubes are heated at 95 °C for 10 minutes. Fifty microliters (50 µL) of the Process Buffer containing sample is added to a reaction tube containing lyophilized mix of HDA reagents.

A HDA reaction is carried out in the Reaction Tube which contains lyophilized HDA reagents, dNTPs, primers and probes. Incubation at 64°C for 60 minutes results in isothermal amplification of the target sequence by *B. pertussis* specific primers. The amplified DNA is detected by a set of specific detection probes included in the Reaction Tube: *B. pertussis* target hybridizes to two specific probes labeled with Biotin (BioTEG) and 6-carboxyfluorescein (FAM). A competitive process control (PRC) is included in the Lysis Tube to monitor specimen processing and inhibitory substances in clinical samples, reagent failure or device failure. The PRC target is amplified and hybridizes to the PRC specific probes labeled with Biotin (BioTEG) and 2,4-dinitrophenyl (DNP).

Following completion of the HDA reaction, the Reaction Tube is transferred to a proprietary Cassette for detection. The Cassette is comprised of two components: 1) an Amplicon Cartridge that holds the running buffer and the 0.2 mL Reaction Tube and 2) the Detection Chamber which houses the Amplicon Cartridge and an embedded vertical-flow DNA detection strip. The DNA detection strip is coated with anti-FAM and anti-DNP antibodies. Once the Cassette is closed, a razor blade and plastic pin located at the bottom of the Cassette opens the Reaction Tube and running buffer bulb, resulting in the release of their

contents. The contents flows through a fiberglass paper connected to the DNA detection strip that is attached to a fiberglass pad pre-loaded with streptavidin-conjugated color particles. The B. pertussis amplicon with biotin- and FAM-labeled probes is captured by the anti-FAM antibodies at the test (T2) line, and the Process Control amplicon with biotin- and DNP-labeled probes is captured by the anti-DNP antibodies at the control (C) line. The streptavidin-conjugated color particles bind to the biotin in the probe-amplicon hybrid and the test results are displayed in the Cassette window as colored T2 and/or C lines that are visible to the naked eye.

Detection of *B. pertussis* is reported when the T2 line is visible through the detection window of the Cassette. No detection of B. pertussis is reported when only the C line is displayed. The assay is regarded as invalid when none of the lines are displayed.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision studies for the AmpliVue® Bordetella Assay were conducted by two (2) operators three times (3x) per day for twelve (12) days with a panel of three (3) simulated samples that include moderate positive (5x LoD) and low positive (2x LoD), and *Bordetella pertussis* (BP) negative. The study results are acceptable. The results are shown in the Table I below.

TABLE I: PRECISION

Category	Operator #1		Operator #2		Combined	
	#expected results/# tested	% Agreement	#expected results/# tested	% Agreement	#expected results/# tested	% Agreement
BP Low Positive (4,716 cfu/mL)	36/36	100%	36/36	100%	72/72	100%
BP Moderate Positive (11,790 cfu/mL)	36/36	100%	36/36	100%	72/72	100%
BP Negative	36/36	100%	36/36	100%	72/72	100%

The reproducibility of the AmpliVue® Bordetella Assay 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 (5x and 2x LoD), and *Bordetella pertussis* (BP) negative. The panels and controls were processed and tested on the AmpliVue® Bordetella Assay at each site by 2 operators for 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 the Table II below.

TABLE II: REPRODUCIBILITY								
Category	SITE						Overall Percent Agreement	95% Confidence Interval
	Site #1		Site #2		Site #3			
	#expected results/# tested	% Agreement	#expected results/# tested	% Agreement	#expected results/# tested	% Agreement		
BP Low Positive (4,716 cfu/mL)	30/30	100%	30/30	100%	30/30	100%	90/90	95.9% to 100%
BP Moderate Positive (11,790 cfu/mL)	30/30	100%	30/30	100%	30/30	100%	90/90	95.9% to 100%
BP Negative	30/30	100%	30/30	100%	30/30	100%	90/90	95.9% to 100%
BP Positive Control	30/30	100%	30/30	100%	30/30	100%	90/90	95.9% to 100%
BP Negative Control	30/30	100%	30/30	100%	30/30	100%	90/90	95.9% to 100%

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.

Stability:

A study was performed to determine the stability of samples in nasal matrix that are used in the AmpliVue® Bordetella Assay. A contrived positive sample was prepared by spiking a pooled negative clinical sample with a bacterial stock of *Bordetella pertussis* that was prepared and frozen during the LoD study at a concentration of 2x LoD. The contrived positive sample was tested with AmpliVue Bordetella Validation Lot reagents using the AmpliVue Bordetella assay workflow, to establish a 0 hour (h) time point. The contrived positive sample was aliquoted into four tubes and stored at 2°C to 8°C. An aliquot of the specimen was tested at 24 h, 48 h, 72 h, and 96 h time points. A total of 15 positive samples were assayed for each time point along with a positive and negative control.

Based on this study, clinical samples are expected to be stable between 2°C to 8°C for

up to 96 hours.

Controls:

Controls (Quidel Molecular Bordetella Control Set #M117, which contains positive and negative controls, serves as an external processing and extraction control) were run on the AmpliVue® Bordetella Assay each day of testing. All *Bordetella pertussis* positive controls were detected accurately (100%, 111/111). All *Bordetella pertussis* negative controls were detected accurately (100%, 111/111) in the clinical study. These controls are described as follows:

a. The *process control* is used to monitor sample processing, to detect HDA inhibitory specimens and to confirm the integrity of assay reagents and cassette detection. The process control is included in the Lysis Buffer tube.

b. The *external positive control* may be treated as a patient specimen. The control should be sampled and tested as if it were a swab specimen and processed as described in the Assay Procedure. The external positive control is intended to monitor substantial reagent and cassette failure.

c. The *external negative control* may be treated as a patient specimen. The control should be sampled and tested as if it were a swab specimen and processed as described in the Assay Procedure. The external negative control is used to detect reagent or environmental contamination (or carry-over) by *B. pertussis* DNA or amplicon.

d. Detection limit:

The analytical sensitivity (limit of detection or LoD) of the AmpliVue® Bordetella Assay was determined using quantified (CFU/mL) cultures of two (2) *Bordetella pertussis* bacterial stocks, BP A639 and E431 serially diluted in negative nasal matrix. The LoD is defined as the lowest concentration at which 95% of all replicates tested positive.

The two bacterial strains of *Bordetella pertussis* (BP) were freshly grown. The organisms were cultured onto Bordet-Gengou (BG) Blood Agar plates from commercially obtained glycerol stock. The resulting colonies were harvested and collected in Phosphate Buffered Saline (PBS), and the density of the bacterial cell suspension was determined using the McFarland assay. After a cell suspension of 0.25 McFarland units was established, the bacteria were serially diluted in PBS. BP CFU/mL concentrations were confirmed by plating onto fresh BG Blood Agar and colony counts.

After the LoD was determined, 20 replicates of the LoD dilutions of BP A639 and E431were tested using two additional validation lots of buffers and amplification reagents. If 19 or 20 of 20 replicates of the dilution showed positive results for BP, the three lots were deemed equivalent. For the three validation lots of reagents to be

deemed equivalent, the LoD across the three reagent lots must not exceed three halving concentrations (i.e. must not exceed 8 fold).

The assay LoD for *Bordetella pertussis* is 3.93 CFU/assay or 2,358 CFU/mL (sample input), which was demonstrated on three Validation Lots. The LoD study results are shown in Table III below.

Table III: LoD for *Bordetella pertussis*

Strain	Strain ID	CFU/mL
Bordetella pertussis strain 1	A639	2,358
Bordetella pertussis strain 2	E431	760.8

These study results are acceptable.

e. Analytical Reactivity (Inclusivity):

The reactivity of the AmpliVue® Bordetella Assay was evaluated against an additional six (6) strains of *Bordetella pertussis.* The testing was performed at concentrations near the level of detection (LoD).

Each strain was tested as three replicates in the AmpliVue® Bordetella Assay. The study was performed in multiple experiments of no more than 14 assays per experiment. For each experiment, three replicates of up to four strains were performed, along with a positive and a negative run control. All six (6) strains were detected by the AmpliVue® Bordetella Assay in this study at the 1x LoD concentration (3.93 CFU/assay or 2,358 CFU/mL). The inclusivity study results and the final organism concentrations tested are shown in Table IV below.

Table IV: *Bordetella* pertussis Inclusivity

Bacterial Strain	Concentration CFU/mL	Strain Detected
9797	2,358	Yes
9340	2,358	Yes
BAA-1335	2,358	Yes
BAA-589	2,358	Yes
51445	2,358	Yes
10380	2,358	Yes

An *in silico* BLAST analysis of primers used in the AmpliVue® Bordetella Assay against twenty-three (23) *Bordetella* strains in the NCBI database was done to further demonstrate inclusivity. The *Bordetella* primers matched 1068 *Bordetella* sequences, including eight (8) *B. pertussis* complete genome/WGS sequences and 25 gene/CDS

sequences. The primers and probes all have 3-4 mismatches in less than 10% of all sequences, indicating that the region is well conserved. The primers, however, did not match one WGS strain, *B. pertussis* B1917. In addition, the primers will also detect *B. bronchiseptica* and *B. holmesii* strains. The primers did not show evidence of cross-reactivity with other organisms.

f. Cross Reactivity:

A study was performed to evaluate the cross-reactivity of the AmpliVue® Bordetella Assay with seventy-nine (79) other microorganisms potentially found in specimens collected to test for *Bordetella pertussis* (BP) infection. Cross-reactive microorganism was tested at clinically relevant levels of viruses (10^5 PFU/mL) and bacteria (10^6 CFU/mL) in the device. The organisms and their concentrations included in the cross-reactivity study are shown in Table V below.

Table V: Strains Included in Cross-Reactivity

Strain	Strain ID	CFU/mL
Acinetobacter baumanii	2.90 x10^6	CFU/mL
Arcanobacterium haemolyticum	1.15 x10^6	CFU/mL
Bacteroides fragilis	1.19 x10^6	CFU/mL
Bordetella avium	3.85 x10^6	CFU/mL
Bordetella bronchiseptica	9.45 x10^6	CFU/mL
Bordetella bronchiseptica	1.17 x10^6	CFU/mL
Bordetella bronchiseptica (ATCC 4617)	7.74 x10^6	CFU/mL
Bordetella bronchiseptica	1.97 x10^6	CFU/mL
Bordetella hinzii	1.40 x10^6	CFU/mL
Bordetella holmesii (ZeptoMetrix	3.83 x10^6	CFU/mL
Bordetella holmesii (ATCC 51541)	4.10 x10^6	CFU/mL
Bordetella holmesii (ATCC 700053)	4.70 x10^6	CFU/mL
Bordetella holmesii (ATCC 700052)	4.00 x10^6	CFU/mL
Bordetella parapertussis A747	1.00 x10^6	CFU/mL
Bordetella petrii	6.26 x10^6	CFU/mL
Bordetella trematum	9.24 x10^6	CFU/mL
Burkholderia cenocepacia	2.35 x10^6	CFU/mL
Burkholderia cepacia	2.52 x10^6	CFU/mL
Burkholderia multivorans	1.95 x10^6	CFU/mL
Burkholderia thailandensis	3.95 x10^6	CFU/mL
Chlamydia trachomatis	7.83 x10^6	CFU/mL
Chlamydophila pneumoniae	2.10 x10^6	CFU/mL
Corynebacterium diptheriae	4.00 x10^6	CFU/mL

Enterobacter aerogenes	1.31×10^6	CFU/mL
Enterococcus faecalis	3.45×10^6	CFU/mL
Escherichia coli	8.42×10^6	CFU/mL
Fusobacterium necrophorum	3.10×10^6	CFU/mL
Haemophilus influenzae	2.13×10^6	CFU/mL
Klebsiella pneumoniae	1.61×10^6	CFU/mL
Lactobacillus acidophilus	2.00×10^6	CFU/mL
Lactobacillus plantarum	7.97×10^6	CFU/mL
Legionella pneumophilia	1.76×10^6	CFU/mL
Moraxella catarrhalis	9.90×10^6	CFU/mL
Morganella morganii	1.57×10^6	CFU/mL
Mycobacterium avium	1.84×10^6	CFU/mL
Mycobacterium tuberculosis	1.80×10^6	CFU/mL
Mycoplasma pneumoniae	3.16×10^6	CFU/mL
Neisseria gonorrhoeae	2.45×10^6	CFU/mL
Neisseria meningitidis	7.07×10^6	CFU/mL
Neisseria mucosa	1.66×10^6	CFU/mL
Parvimonas micra	1.55×10^6	CFU/mL
Proteus mirabilis	1.06×10^6	CFU/mL
Proteus vulgaris	3.40×10^6	CFU/mL
Pseudomonas aeruginosa	2.60×10^6	CFU/mL
Staphylococcus aureus (MRSA)	7.10×10^6	CFU/mL
Staphylococcus epidermidis	2.14×10^6	CFU/mL
Stenotrophomonas maltophilia	1.90×10^6	CFU/mL
Streptococcus pneumoniae	1.00×10^6	CFU/mL
Streptococcus pyogenes	1.29×10^6	CFU/mL
Streptococcus salivarius	1.70×10^6	CFU/mL
Candida albicans	3.00×10^6	CFU/mL
Adenovirus 31	3.55×10^5	$TCID_{50}$/mL
Adenovirus 31	2.74×10^7	DNA copies/mL
Coronavirus 229E	1.51×10^6	$TCID_{50}$/mL
Coronavirus NL63	1.41×10^5	$TCID_{50}$/mL
Coronavirus OC43	8.51×10^6	$TCID_{50}$/mL
Coxsackievirus B4	1.08×10^5	$TCID_{50}$/mL
Coxsackievirus B5/10/2006	1.02×10^5	$TCID_{50}$/mL
Echovirus 6	1.02×10^6	$TCID_{50}$/mL
Echovirus 7	1.05×10^5	$TCID_{50}$/mL

Echovirus 9	1.41×10^5	$TCID_{50}$/mL
Echovirus 11	1.51×10^6	$TCID_{50}$/mL
Enterovirus 70	1.78×10^6	$TCID_{50}$/mL
Enterovirus 71	4.17×10^5	$TCID_{50}$/mL
Epstein-Barr Virus	1.34×10^6	Virus particles/mL
HSV Type 1 (Mclnytre)	6.65×10^6	$TCID_{50}$/mL
HSV Type 2 (G)	2.27×10^6	$TCID_{50}$/mL
Influenza A/Mexico/4108/2009	2.88×10^6	Virus particles/mL
Influenza B/Florida/04/2006	2.82×10^6	Virus particles/mL
Measles virus	1.95×10^6	$TCID_{50}$/mL
Metapneumovirus A1	3.80×10^6	$TCID_{50}$/mL
Mumps virus	5.89×10^6	$TCID_{50}$/mL
Parainfluenza Type 1 (#2)	3.97×10^6	$TCID_{50}$/mL
Parainfluenza Type 2 (Greer)	3.15×10^6	$TCID_{50}$/mL
Parainfluenza Type 3 (C234)	2.56×10^6	$TCID_{50}$/mL
Parainfluenza Type 4 (VR-1377)	1.37×10^6	$TCID_{50}$/mL
Respiratory Syncytial Virus	1.15×10^6	$TCID_{50}$/mL
Rhinovirus 1A	1.26×10^6	$TCID_{50}$/mL
Varicella Zoster Virus	1.70×10^6	DNA copies/mL

The Cross Reactivity study tested a panel of 79 microorganisms. This study determined that 1 of 4 *Bordetella bronchiseptica* strains (strain 4617) and 4 of 4 *Bordetella holmesii* strains tested were cross-reactive with the AmpliVue® BordetellaAssay. These results can be expected as 5% of all *Bordetella bronchiseptica* strains and all *Bordetella holmesii* strains are known to carry the IS481 target sequence.

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

g. Interference:

A panel of seventeen (17) chemical and biological substances potentially present in *Bordetella pertussis* infection specimens were evaluated for interference with the AmpliVue® Bordetella Assay. 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 AmpliVue® Bordetella Assay.

None of the substances tested were found to interfere with the AmpliVue® Bordetella Assay. The interference substances and their concentrations included in the interference study are shown in Table VI below.

Table VI: Interference Substances	
Common Name	**Test Concentration**
Cepacol Sore Throat Lozenges	5% w/v
Halls Cherry Menthol-Lyptus Cough Drops	15% w/v
Children's Dimetapp	15% v/v
Chloraseptic Sore Throat Lozenges	10% w/v
Ricola Original Swiss Sugar-Free Herb Cough Suppressant Throat	15% w/v
Sucrets Complete Lozenges - Vapor Cherry	5% w/v
Mucin (Bovine Submaxillary Gland, Type I-S)	5 mg/ml
Blood (human), EDTA anticoagulated	5% v/v
Neo-Synephrine	15% v/v
Afrin Nasal Spray Original	15% v/v
Zicam Non-Drowsy Allergy Relief Nasal Gel	5% v/v
Rite Aid Brand Saline Nasal Spray	15% v/v
Zanamivir (Relenza)	5 mg/ml
Tobramycin	4 µg/ml
Mupirocin	10 mg/ml
Oseltamivir Phosphate (Tamiflu)	10 mg/ml
Ricola Original Swiss Sugar Free Herb Cough Suppressant Throat	15% w/v

h. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable.

b. Matrix comparison:

The compatibility of eight (8) different types of media with the AmpliVue Bordetella assay was determined testing four (4) strains each of pre-titered BP bacterial strains at 1x LoD concentrations. A diluted bacterial strain and one of the eight types of media (Tris EDTA, Molecular Grade Water, E-swab, M4, M4-RT, M5, 0.85% Saline) were added to three Process Buffer tubes and were tested in the AmpliVue Bordetella assay according to the established workflow using Validation Lot reagents

The study was performed in multiple assay runs, with up to four strains tested per assay run. Each experiment included a positive and a negative run control. Any media that resulted in BP- positive detection for 3 of 3 replicates was considered to be compatible with the AmpliVue Bordetella assay.

All four *Bordetella pertussis* strains were determined to be compatible with the 8 different media types when tested at the assay LoD. Based on these results, Tris

EDTA, Molecular Grade Water, E-swab, M4, M4-RT, M5, 0.85% Saline will be considered to be compatible with the AmpliVue Bordetella assay.

3. Clinical studies:

a. Clinical Sensitivity:

Performance characteristics of the AmpliVue® Bordetella Assay was established in 2014 (April to August 2014) at four locations in the United States. Eight hundred forty two (842) fresh nasopharyngeal swab specimens were obtained from female and male patients suspected of having respiratory tract infection attributable to *Bordetella pertussis.* Specimens were collected and transported to each laboratory for testing with the AmpliVue® Bordetella Assay.

Clinical performance was based on comparison of the AmpliVue Bordetella Assay results to those obtained by Composite Reference Method that included two manufacturer validated, IS481-targeted real-time PCR assays (PCR1 and PCR2) followed by bi-directional sequencing from PCR positive specimens. The PCR1 and PCR2 assay protocols included 37 amplification cycles. Bi-directional sequencing was performed for all specimens producing amplicon prior to the end of 37-cycle amplification. Specimens were considered positive when bi-directional sequence sequencing results from either comparator PCR assay confirmed the presence of *Bordetella pertussis* amplicon. Specimens were considered negative when neither comparator PCR assay produced *Bordetella pertussis* amplicon at the end of the 37-cycles.

Eight hundred forty two (842) fresh nasopharyngeal swab specimens were tested as described above. Six (6) specimens (0.7%) were invalid (in both the initial and repeat test neither the T2 or control lines were detected) and have been removed from additional analysis. The table below details the comparison data of the AmpliVue Bordetella Assay and the Composite Reference Method for the remaining eight hundred thirty six (836) specimens.

Table VII: Clinical Performance Data for the Amplivue® Bordetella Assay vs. Composite Reference Method

All Sites			
Amplivue® Bordetella Assay	**Composite Reference Culture**		
	Positive	**Negative**	**Total**
Positive	64	15	79
Negative	2	755	757
Total	66	770	836
Sensitivity: 97.0% (64/66) 95% CI (89.6%-99.2%) **Specificity:** 98.1% (755/770) 95% CI (96.8%-98.8%)			

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:

The prevalence of *Bordetella pertussis* detected with the AmpliVue® Bordetella Assay has been calculated for the combined sites based on the age of the patient. Six (6) specimens (0.7%) were invalid (in both the initial and repeat test neither the T2 or control lines were detected) and have been removed from the Expected Values table. All clinical specimens collected during this study were collected between April, 2014 and August 2014. Table VIII below presents the data for the remaining eight hundred forty two (842) specimens.

Table VIII: Combined Study – Expected Values (N=836)

Bordetella pertussis			
Age	**Total #**	**Total Positive**	**Prevalence**
≤ 2 years	137	8	5.8%
3 to 12 years	274*	27	9.9%
13 to 21 years	145	30	20.7%
≥ 22 years	280**	14	5.0%

* Four (4) specimens were invalid
** Two (2) specimens were invalid

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.