This Procedural Bulletin is intended to provide a ready outline reference for performance of the assay. These abbreviated directions for use are not intended to replace the complete Package Insert. It is the obligation of every manufacturer of medical devices labeled FOR *IN VITRO* DIAGNOSTIC USE to provide a complete Package Insert in accordance with FDA labeling regulation (21 CFR 809.10).

Quidel Corporation provides CLSI procedures for your use. The procedures are required to include the same information as listed in the Package Insert. Any modifications to this document are the sole responsibility of the Laboratory.

AmpliVue Bordetella Assay

CLIA Complexity: Moderate

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 IS*481* and a self-contained disposable amplification detection device that allows for manual evaluation of assay results. The IS*481* sequence may 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.

SUMMARY AND EXPLANATION

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 transport medium. Fifty microliters (50 µL) of the sample are then transferred to a process buffer that is provided with the kit and mixed. 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.

PRINCIPLE OF THE PROCEDURE

The AmpliVue Bordetella Assay detects *B. pertussis* DNA isolated from nasopharyngeal swab specimens obtained from symptomatic patients. After the swab is transferred to a liquid transport medium, an aliquot of the sample is then transferred into a Process Buffer Tube and undergoes a simple heat lysis treatment. Following heat treatment, an aliquot of the lysed sample is added to a 0.2 mL Reaction Tube containing a lyophilized mix of HDA reagents, dNTPs, primers, and probes. A competitive Process Control is included in the assay to monitor inhibitory substances in samples, sample processing, and confirm the integrity of the assay reagents and the Detection Chamber. The asymmetric HDA reaction results in the generation of excess single-stranded DNA amplicons, which hybridize to target-specific probes. The *B. pertussis* amplicon hybridizes to two specific probes labeled with biotin and 6-carboxyfluorescein (FAM). The Process Control amplicon hybridizes to two specific probes labeled with biotin and dinitrophenyl (DNP).

Following completion of the HDA reaction, the Reaction Tube is transferred to a proprietary Detection Chamber for detection. The Detection Chamber 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 Detection Chamber is closed, a razor blade and plastic pin located at the bottom of the Detection Chamber 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 Detection Chamber 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 Detection Chamber. 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.

BIOLOGICAL PRINCIPLES

Bordetella pertussis is a Gram-negative bacterium that targets the human respiratory tract and is the causative agent of the acute respiratory disease known as whooping cough, or pertussis. Despite the availability and widespread use of pertussis vaccines, pertussis annually affects 16 million people worldwide, is responsible for approximately 195,000 annual deaths, and is one of the leading worldwide causes of vaccine-preventable deaths.[1] The majority of pertussis-related cases and deaths occur in infants too young to receive the vaccine, unvaccinated children or under-vaccinated children. In the pre-vaccine era, the number of pertussis cases in the U.S. peaked at 250,000 and was significantly reduced to 1,010 cases in 1976 after the introduction of the vaccine in the 1940s.[2] However, the incidence of pertussis has since steadily risen over time and as recently as 2012 and 2013, the number of combined reported pertussis cases exceeded 72,000.[3] Factors that have likely contributed to the increased incidence of pertussis include waning vaccine-induced immunity in adolescent and adult populations, as current vaccines do not offer lifelong immunity and members of this population may not receive booster immunizations to maintain immunity, a decline in vaccine use for nonmedical reasons, and continued circulation of *B. pertussis* in the population.[4-5] The incubation period for pertussis is typically 7-10 days but can range from 1-3 weeks. Following the incubation period, a mild onset of symptoms occurs including mild coughing, sneezing, or runny nose. Within one to two weeks, symptoms progress into a paroxysmal cough followed by a high pitched “whoop” sound in infected infants and children or persistent cough in adolescents and adults. The coughing stage can last up to 6 weeks before subsiding. Pertussis is

clinically diagnosed by culture, nucleic acid amplification test (NAAT), or serology. Although culture is considered to be the gold standard diagnostic test method, as it is the only method that can achieve 100% specificity, its sensitivity is low and results can take as long as 7 days. In addition, culture relies on the presence of viable bacteria in the specimen, which declines as the disease progresses, therefore limiting specimen testing to the first 2 weeks post-cough onset. NAAT does not require viable bacteria, is highly sensitive and provides rapid turnaround of results within 24 to 48 hours. Testing should be performed within 4 weeks post-cough onset. Serological testing must be performed at least 2 weeks post-cough onset, and is ideally performed 4-8 weeks post-cough onset, which is useful for late diagnosis or post-antibiotic use.[6]

MATERIALS PROVIDED

Cat. #M209
16 Tests per Kit

Component	Quantity	Storage
Detection Chambers	16/kit	2°C to 30°C
Process Buffer	16 tubes/kit 1.45 mL	2°C to 30°C
Reaction Tubes	16 tubes/kit	2°C to 8°C
Amplicon Cartridge	16/kit	2°C to 30°C

MATERIALS REQUIRED BUT NOT PROVIDED

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

WARNINGS AND PRECAUTIONS

- Treat all specimens/samples as potentially infectious. Follow universal precautions when handling samples, this kit and its contents.
- Proper sample collection, storage and transport are essential for correct results.
- Store assay reagents as indicated on their individual labels.
- Reagents are not interchangeable between lots.
- Never pool reagents from different tubes even if they are from the same lot.
- Do not use the reagents after their expiration date.
- Do not interchange caps among reagents as contamination may occur and compromise test results.
- Only open the tubes when adding aliquots into tubes or removing aliquots from tubes. Keep the tubes closed at any other time to avoid contamination.
- To avoid contamination of the environment with *B. pertussis* amplicons, do not open the reaction tubes post-amplification.
- Avoid microbial and deoxyribonuclease (DNase) contamination of reagents when removing aliquots from tubes. The use of sterile DNase-free disposable filter-blocked or positive displacement pipettor tips is recommended.
- Use a new pipette tip for each specimen or reagents.
- Performing the assay outside of the recommended time ranges can produce invalid results. Assays not completed within specified time ranges should be repeated.

- Additional controls may be tested according to guidelines or requirements of Local, State, Provincial and/or Federal regulations or accrediting organizations.
- In cases where open-tube PCR tests are conducted in the same general area by the laboratory, separated or segregated working areas should be used for specimen preparation and amplification/detection activities. Supplies and equipment should be dedicated to each area and should not be moved from one area to another. Gloves must always be worn and must be changed before going from one area to another. Gloves must be changed before manipulating the reagents.
- Do not pipette by mouth.
- Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
- For accurate results, pipette carefully using only calibrated equipment.
- Maintenance and decontamination of workspace and equipment should follow and be performed according to established laboratory protocols and schedules.
- Use micropipettes with an aerosol barrier or positive displacement tips for all procedures.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

STORAGE AND HANDLING OF KIT REAGENTS

Store assay reagents and Detection Chambers as indicated on their individual labels.

SPECIMEN COLLECTION, STORAGE AND HANDLING

Specimen Type: Nasopharyngeal swabs.

Nasopharyngeal swabs used for the validation of the AmpliVue Bordetella Assay were obtained using standard techniques from patients suspected of having respiratory tract infection attributable to *Bordetella pertussis*. These specimens were collected, transported, and stored at 2°C to 8°C for up to 72 hours before processing. Any additional leftover specimen should be stored ≤–70°C.

The swabs were eluted in either saline (0.85%), Tris EDTA, Molecular Grade Water, Amies liquid media (i.e. E-Swab), or viral transport media. A series of studies were performed evaluating a number of routinely used transport media at a volume of 3 mL: M4, M4-RT, M5, and UTM. No significant difference in assay performance was seen between the four different types of viral transport media, saline (0.85%), Tris EDTA, Molecular Grade Water, or Amies.

ASSAY PROCEDURE

Heat Lysis

1. Warm the heat block to 95°C, 25 minutes prior to Heat Lysis Step 2.
2. Transfer 50 µL of specimen-containing medium to a labeled Process Buffer tube and vortex for 10 seconds.
   Note: Processed clinical samples are stable in Process Buffer for up to 96 hours between 2°C and 31°C.
3. Heat the Process Buffer tube at 95°C ± 2°C for 10 minutes, then vortex for 10 seconds.
   Note: Begin the 10 minute countdown for the heat lysis procedure after the tubes have been placed into the heat block and the heat block temperature has returned to 95°C.

Amplification

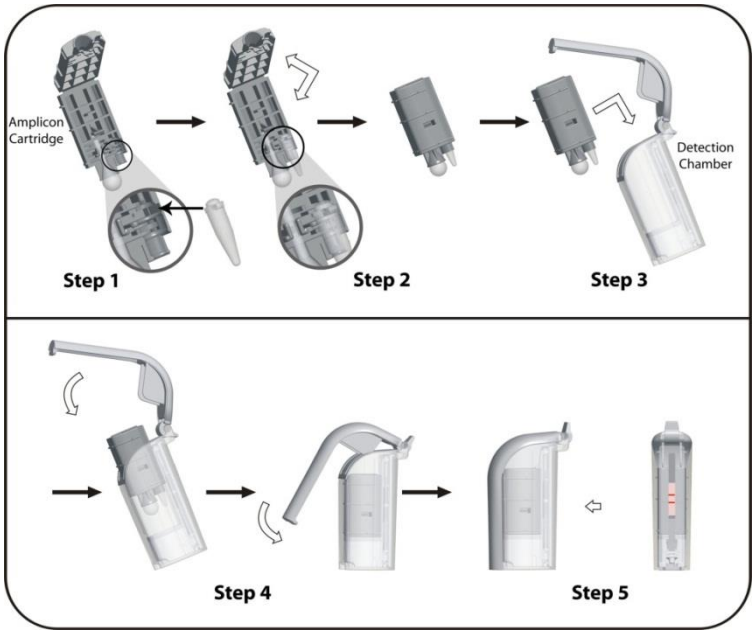
1. Warm a heating block with a heated lid to 64°C, 15 minutes prior Amplification Step 2.
2. Transfer 50 µL of Process Buffer-containing sample to a labeled Reaction Tube and rehydrate lyophilized reagents by pipetting up and down 3 to 5 times to mix. Close the lid tightly and proceed to the next step.

3. Incubate the Reaction Tube at 64°C ± 2°C for 60 minutes in a heating block with a heated lid.
   Note: To avoid laboratory contamination, **DO NOT** open the Reaction Tube once the tube has been closed and the amplification reaction has started.

Detection

1. Tear open a new Detection Chamber package. Label the Detection Chamber appropriately.
   Make sure a buffer bulb is attached to the Amplicon Cartridge.
2. Place the Reaction Tube into the Amplicon Cartridge (Figure 1, Step 1).
   Note: Be sure to insert the **hinge** of the Reaction Tube cap into the largest slot adjacent to the buffer bulb.
3. Close the Amplicon Cartridge (Figure 1, Step 2) and ensure that it snaps shut.
   If the cartridge does not snap shut, reposition the tube within the cartridge.
4. Insert the closed Amplicon Cartridge into the Detection Chamber (Figure 1, Step 3).
   Ensure that the arrow faces the detection strip (Reaction Tube should face the razor blade and the buffer bulb should face the pin). Label the Detection Chamber on the top and/or side of the outer casing.
5. Keep the device upright and press the handle of the outer casing to close the device (Figure 1, Step 4). The handle will lock into place when closed completely (Figure 1, Step 5).

Figure 1



6. Results are read at 10 minutes. **Note:** The results are stable for up to 60 minutes.
7. Discard the used Detection Chambers in sealed bags and as appropriate for your laboratory.

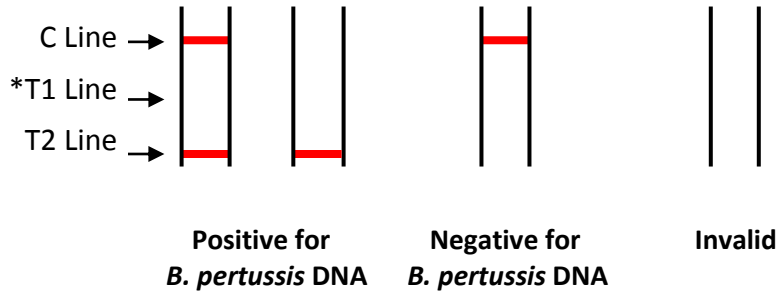
Warning

1. **DO NOT** open the AmpliVue Detection Chamber after use. Opening the Detection Chamber after use may result in amplicon contamination of the test area.
2. Remove the required number of reaction tubes from the protective pouch, remove the excess air and reseal the bag.

INTERPRETATION OF RESULTS

- Any pink- to red-colored visible line should be recorded as positive (+) and no line should be recorded as (–); for example, “T2+” = Visible T2 line and “T2–" = No T2 line (See diagram below).
- The T2 line detects *B. pertussis* DNA.

- The C line detects the process control DNA.
- The control line intensity may vary with each test. Any pink to red colored visible line in the control signifies a valid test.



The assay results are interpreted according to the following criteria:

Test line (T)Reading	Control line (C) Reading	Interpretation of result
T2+	C+	***B. pertussis*** DNA detected (Positive)
T2+	C–	***B. pertussis*** DNA detected (Positive)
T2–	C+	No ***B. pertussis*** DNA detected (Negative)
T2–	C–	Invalid: failure due to inhibitory specimen, reagent failure, or device failure. Repeat test with original specimen.

***Note 1:** The T1 line is not used on this assay. The presence of a T1 line should be considered invalid for this assay. Repeat test with original specimen.

QUALITY CONTROL

The AmpliVue Bordetella Assay incorporates several controls to monitor assay performance.

1. The Process Control is used to monitor sample processing, detect HDA inhibitory specimens and confirm the integrity of assay reagents and chamber detection. The Process Control is included in the Process Buffer Tube.
2. External positive controls may be treated as a patient specimen. The control should be sampled and tested as if it were a sample in Transport Medium. The external positive control is intended to monitor substantial reagent and Detection Chamber failure.
3. External negative controls may be treated as a patient specimen. The control should be sampled and tested as if it were a sample in Transport Medium. The external negative control is used to detect reagent or environmental contamination (or carry-over) by *B. pertussis* DNA or amplicon.

LIMITATIONS

- Although there is no need for reagent preparation, the main laboratory technique required is pipetting. Good laboratory technique is essential for the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of all reagents, especially in cases where multiple aliquots are taken from a tube.
- The AmpliVue Bordetella Assay procedure must be carried out in an environment that does not exceed 30°C.

- The IS481 sequence used in the AmpliVue Bordetella Assay 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.
- This device has not been evaluated for monitoring treatment of Bordetella pertussis infections.
- As with all molecular based diagnostic tests, (A) False-negative results may occur from the presence of inhibitors, technical error, sample mix-up or low numbers of organisms in the clinical specimen; (B) False-positive results may occur from the presence of cross-contamination by target organisms, their nucleic acids or amplified product, and from non-specific amplification signals.
- Organism nucleic acids may persist *in vivo*, independent of organism viability. The AmpliVue Bordetella assay does not distinguish between viable and nonviable organisms.
- Environmental contamination of an exam room from a prior patient or a recent pertussis vaccination administration may result in false-positive test results.
- Respiratory infections can be caused by Bordetella pertussis as well as other pathogens. Positive results do not preclude coinfection with other respiratory pathogens. False-negative Bordetella pertussis results are more likely if patients are tested later in the disease course (more than two weeks after symptom onset), due to declining concentrations of Bordetella DNA. False-negative results may also be increased in patients treated with antibiotic therapy.
- Prevalence of Bordetella pertussis will affect the positive and negative predictive values for the assay.
- Bordetella parapertussis which causes a pertussis-like illness is not detected by the AmpliVue Bordetella Assay.
- Illness caused by B. parapertussis is generally milder than illness caused by B. pertussis because the bacteria do not produce pertussis toxin.
- Results from this test must be correlated with the clinical history, epidemiological data, and any other data available to the clinician.
- This test has not been evaluated for specimens other than nasopharyngeal swab specimens, for immunocompromised individuals or from patients not suspected of infection with Bordetella pertussis.

EXPECTED VALUE

Performance characteristics of the AmpliVue Bordetella Assay were established during a prospective study conducted April to August 2014. Eight hundred forty two (842) fresh nasopharyngeal swab specimens were collected at five distinct geographical sites across the United States from female and male patients suspected of having respiratory tract infection attributable to *Bordetella pertussis*. A single specimen was collected per patient and were collected and transported to each laboratory for testing with the AmpliVue Bordetella Assay.

The gender and age demographics for each category are listed below.

Combined Study – Age and Gender Distribution		
Gender	Female	Male
Total	464	378
Age		
≤ 2 years	70	67
3 to 12 years	132	146
13 to 21 years	68	77
≥ 22 years	194	88

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. The table below presents the data for the remaining eight hundred forty two (836) specimens.

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

CLINICAL PERFORMANCE

Performance characteristics of the AmpliVue Bordetella Assay was established in the Spring to Summer of 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* which 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 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.

Combined Data

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.

Combined Sites – Composite Reference Method versus AmpliVue Bordetella Assay			
	Composite Reference Method		
AmpliVue Bordetella Assay	Positive	Negative	Total
Positive	64	15	79
Negative	2	755	757
Total	66	770	836
			95% CI
Positive Percent Agreement	64/66	97.0%	89.6% to 99.2%
Negative Percent Agreement	755/770	98.1%	96.8% to 98.8%

ANALYTICAL PERFORMANCE

Limit of Detection

The analytical sensitivity (limit of detection or LOD) of the AmpliVue Bordetella Assay was determined quantified (CFU/mL) cultures of two (2) *Bordetella pertussis* (BP) bacterial stocks, BP A639 and E431 serially diluted in negative nasal matrix.

Bacterial Strain	Concentration CFU/mL	Concentration CFU/Assay
A639	2,358	3.93
E431	761	1.27

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.

Analytical Reactivity (Inclusivity)

The reactivity of the AmpliVue Bordetella Assay was evaluated against an additional six (6) additional strains of *Bordetella pertussis* (BP) tested in triplicate at concentrations near the level of detection of the assay (2,358 CFU/mL). All six (6) strains were detected by the AmpliVue Bordetella Assay in this study at a LOD of 2,358 CFU/mL.

Bacterial Strain	Concentration CFU/mL	Strain Detected (Yes/No)
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

Repeatability Study

The Precision/Within Laboratory Repeatability was determined via a study, where a three-member panel (5x, 2x, and a negative sample) was tested by two (2) operators, twice a day (2X) for twelve (12) days.

The AmpliVue Bordetella Assay produces results that are reproducible. This observation is based on the following findings:

- All negative samples generated negative results for BP.
- The percentage of positive of the Low Positive (2x LOD) samples was 100%.
- The percentage of positive of the Moderate Positive (5x LOD) samples was 100%.

Reproducibility Study

In order to confirm the reproducibility of the AmpliVue Bordetella Assay a blinded and randomized study panel containing *Bordetella pertussis* (BP) negative and positive samples (5x and 2x,) were tested at three (3) test sites (one in-house laboratory and two (2) clinical sites). Each site tested a reproducibility panel and Assay Controls for five (5) days in triplicate. Testing was done by two operators at each site. Each operator ran the panel once a day using one lot of AmpliVue Bordetella Assay. A total of four hundred fifty (450) specimens were tested (including controls). The AmpliVue Bordetella Assay generated reproducible results in this study.

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	100%	95.9% to 100%
BP Moderate Positive (11,790 cfu/mL)	30/30	100%	30/30	100%	30/30	100%	90/90	100%	95.9% to 100%
BP Negative	30/30	100%	30/30	100%	30/30	100%	90/90	100%	95.9% to 100%
BP Positive Control	30/30	100%	30/30	100%	30/30	100%	90/90	100%	95.9% to 100%
BP Negative Control	30/30	100%	30/30	100%	30/30	100%	90/90	100%	95.9% to 100%

Analytical Specificity – Microbial Interference

A study was performed to evaluate the performance of the AmpliVue Bordetella Assay in the presence of seventy-nine (79) other microorganisms potentially found in specimens collected to test for *Bordetella pertussis* (BP) infection. Each potentially interfering microorganism was tested in the presence of 2x LOD BP in the presence of clinically relevant levels of viruses (10^5 pfu/mL) and bacteria (10^6 cfu/mL) or higher. The organisms and their concentrations included in the interference study are shown in the table below.

Organism	**Test Concentration**	
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 (ATCC 780)	9.45 x10^6	cfu/mL
Bordetella bronchiseptica (ZeptoMetrix	1.17 x10^6	cfu/mL
Bordetella bronchiseptica (ATCC 4617)	7.74 x10^6	cfu/mL
Bordetella bronchiseptica (ATCC 10580)	1.97 x10^6	cfu/mL
Bordetella hinzii	1.40 x10^6	cfu/mL
Bordetella holmesii (ZeptoMetrix F061)	3.83 x10^6	cfu/mL

Organism	**Test Concentration**	
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 (ZeptoMetrix 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	DNA copies/mL
Corynebacterium diptheriae	4.00 x10^6	cfu/mL

Enterobacter aerogenes	1.31 x10^6	cfu/mL
Enterococcus faecalis	3.45 x10^6	cfu/mL
Escherichia coli	8.42 x10^6	cfu/mL
Fusobacterium necrophorum	3.10 x10^6	cfu/mL
Haemophilus influenzae	2.13 x10^6	cfu/mL
Klebsiella pneumoniae	1.61 x10^6	cfu/mL
Lactobacillus acidophilus	2.00 x10^6	cfu/mL
Lactobacillus plantarum	7.97 x10^6	cfu/mL
Legionella pneumophilia	1.76 x10^6	cfu/mL
Moraxella catarrhalis	9.90 x10^6	cfu/mL
Morganella morganii	1.57 x10^6	cfu/mL
Mycobacterium avium	1.84 x10^6	cfu/mL
Mycobacterium tuberculosis (avirulent)	1.80 x10^6	cfu/mL
Mycoplasma pneumoniae	3.16 x10^6	cfu/mL
Neisseria gonorrhoeae	2.45 x10^6	cfu/mL
Neisseria meningitidis	7.07 x10^6	cfu/mL
Neisseria mucosa	1.66 x10^6	cfu/mL
Parvimonas micra	1.55 x10^6	cfu/mL
Proteus mirabilis	1.06 x10^6	cfu/mL
Proteus vulgaris	3.40 x10^6	cfu/mL
Pseudomonas aeruginosa	2.60 x10^6	cfu/mL
Staphylococcus aureus (MRSA)	7.10 x10^6	cfu/mL
Staphylococcus epidermidis	2.14 x10^6	cfu/mL
Stenotrophomonas maltophilia	1.90 x10^6	cfu/mL
Streptococcus pneumoniae	1.00 x10^6	cfu/mL
Streptococcus pyogenes	1.29 x10^6	cfu/mL
Streptococcus salivarius	1.70 x10^6	cfu/mL
Candida albicans	3.00 x10^6	cfu/mL
Adenovirus 31	3.55 x10^5	$TCID_{50}$/mL
Adenovirus 31	2.74 x10^7	DNA copies/mL
Coronavirus 229E	1.51 x10^6	$TCID_{50}$/mL
Coronavirus NL63	1.41 x10^5	$TCID_{50}$/mL
Coronavirus OC43	8.51 x10^6	$TCID_{50}$/mL
Organism	**Test Concentration**	
Coxsackievirus B4	1.08 x10^5	$TCID_{50}$/mL
Coxsackievirus B5/10/2006	1.02 x10^5	$TCID_{50}$/mL
Echovirus 6	1.02 x10^6	$TCID_{50}$/mL
Echovirus 7	1.05 x10^5	$TCID_{50}$/mL
Echovirus 9	1.41 x10^5	$TCID_{50}$/mL
Echovirus 11	1.51 x10^6	$TCID_{50}$/mL
Enterovirus 70	1.78 x10^6	$TCID_{50}$/mL
Enterovirus 71	4.17 x10^5	$TCID_{50}$/mL
Epstein-Barr Virus	1.34 x10^6	Virus particles/mL
HSV Type 1 (MacInytre)	6.65 x10^6	$TCID_{50}$/mL
HSV Type 2 (G)	2.27 x10^6	$TCID_{50}$/mL
Influenza A/Mexico/4108/2009	2.88 x10^6	Virus particles/mL
Influenza B/Florida/04/2006	2.82 x10^6	Virus particles/mL

Measles virus	1.95 x10^6	$TCID_{50}$/mL
Metapneumovirus A1	3.80 x10^6	$TCID_{50}$/mL
Mumps virus	5.89 x10^6	$TCID_{50}$/mL
Parainfluenza Type 1 (#2)	3.97 x10^6	$TCID_{50}$/mL
Parainfluenza Type 2 (Greer)	3.15 x10^6	$TCID_{50}$/mL
Parainfluenza Type 3 (C234)	2.56 x10^6	$TCID_{50}$/mL
Parainfluenza Type 4 (VR-1377)	1.37 x10^6	$TCID_{50}$/mL
Respiratory Syncytial Virus	1.15 x10^6	$TCID_{50}$/mL
Rhinovirus 1A	1.26 x10^6	$TCID_{50}$/mL
Varicella Zoster Virus	1.70 x10^6	DNA copies/mL

None of the organisms used in the study demonstrated interference with the AmpliVue Bordetella Assay.

Analytical Specificity – 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. Each microorganism was tested at clinically relevant levels of viruses (10^5 pfu/mL) and bacteria (10^6 cfu/mL) in the device. The organisms included in the cross-reactivity study and their tested concentrations are shown in the table below.

Organism	**Test Concentration**	
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 (ATCC 780)	9.45 x10^6	cfu/mL
Bordetella bronchiseptica (ZeptoMetrix	1.17 x10^6	cfu/mL
Bordetella bronchiseptica (ATCC 4617)	7.74 x10^6	cfu/mL
Bordetella bronchiseptica (ATCC 10580)	1.97 x10^6	cfu/mL
Bordetella hinzii	1.40 x10^6	cfu/mL
Bordetella holmesii (ZeptoMetrix F061)	6.40 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 (Zepto Metrix 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	1.60 x10^6	DNA copies/mL
Corynebacterium diptheriae	4.00 x10^6	cfu/mL
Enterobacter aerogenes	1.31 x10^6	cfu/mL
Enterococcus faecalis	3.45 x10^6	cfu/mL
Escherichia coli	8.42 x10^6	cfu/mL
Fusobacterium necrophorum	3.10 x10^6	cfu/mL

Organism	Test Concentration	
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 (avirulent)	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
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Adenovirus 31	3.55×10^5	$TCID_{50}$/mL
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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
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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

Organism	Test Concentration	
Parainfluenza Type 2 (Greer)	3.15 x10^6	$TCID_{50}$/mL
Parainfluenza Type 3 (C234)	2.56 x10^6	$TCID_{50}$/mL
Parainfluenza Type 4 (VR-1377)	1.37 x10^6	$TCID_{50}$/mL
Respiratory Syncytial Virus	1.15 x10^6	$TCID_{50}$/mL
Rhinovirus 1A	1.26 x10^6	$TCID_{50}$/mL
Varicella Zoster Virus	1.70 x10^6	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 Bordetella Assay. These results can be expected as 5% of all *Bordetella bronchiseptica* strains and all *Bordetella holmesii* strains are known to carry the IS*481* target sequence. These cross-reactive results are noted in the intended use and limitation sections.

Analytical Specificity – Interfering Substances

A study was conducted to determine if the AmpliVue Bordetella assay is inhibited in the presence of a panel of sixteen (16) substances potentially present in specimens collected to test for *Bordetella pertussis* (BP) infection. Each of the potential interfering substances was tested in three replicates in the presence and absence of near LOD (2x) levels of BP bacteria in the AmpliVue Bordetella Assay. Substances were introduced into the assay at concentrations which were medically relevant.

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

There was no evidence of interference caused by the substances tested.

Carryover – Cross Contamination

A stock of high positive sample containing greater than 1x 10^6 CFU/mL of *Bordetella pertussis* (BP) was prepared for testing in the assay workflow. In each run of testing, processing of high positive samples in UTM was alternated with negative UTM only samples to assess the risk of cross contamination. In total, three runs consisting of 11 samples positive for BP and 11 negative samples were tested for a total of 33 positive and 33 negative samples.

All positive BP samples were positive and all negative BP samples were negative. No carryover/contamination was seen when performing the AmpliVue Bordetella Assay in accordance with the Package Insert.

CUSTOMER AND TECHNICAL SUPPORT

To place an order or for Technical Support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at 740.592.9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com. For services outside the U.S., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website **quidel.com**.

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