Bordetella Assay (Panther Fusion™ System)

For *in vitro* diagnostic use.

For US export only.

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General Information

Intended Use

The Panther Fusion™ Bordetella assay is a multiplex real-time PCR *in vitro* diagnostic test for the rapid and qualitative detection and differentiation of *Bordetella pertussis* (Bp) and *Bordetella parapertussis* (Bpp). Nucleic acids are isolated and purified from nasopharyngeal (NP) swab specimens obtained from individuals exhibiting signs and symptoms of a respiratory tract infection.

This assay is intended to aid in the differential diagnosis of *Bordetella pertussis* and *Bordetella parapertussis* infections in humans. Negative results do not preclude *Bordetella pertussis* and *Bordetella parapertussis* infections and should not be used as the sole basis for treatment or other management decisions. This assay is designed for use on the Panther Fusion system.

Summary and Explanation of the Test

Bordetella is a genus of small (0.2 - 0.7 µm), Gram-negative coccobacilli of the phylum Proteobacteria, which is difficult to culture. The genus *Bordetella* contains eight species, four of which are known to cause respiratory diseases in humans: *Bordetella bronchiseptica*, *Bordetella holmesii*, *Bordetella parapertussis* and *Bordetella pertussis*. *B. holmesii* does not produce the virulence factors produced by the other three species.

B. pertussis is thought to be a strictly human pathogen while *B. parapertussis* is found in sheep and humans. *B. bronchiseptica* can cause respiratory infections in many animal species and, infrequently, also in humans. An increasing number of pertussis-like cases are attributed to the emergent pathogen *B. holmesii* but it is still unknown whether this species is truly pathogenic for humans[1, 2].

B. pertussis is the bacteria responsible for whooping cough. This respiratory infection is characterised by paroxysmal cough, whoop and post-tussive vomiting. It is spread through air droplets produced by coughs or sneezes. The most severe disease occurs in infants and young children, while adolescents and adults constitute a disease reservoir. *B. pertussis* remains endemic worldwide and tends to be a cyclic disease, peaking every three to five years.

The prevalence of *B. pertussis* and *B. parapertussis* combined is less than 2% and widely depends on the age of the patient[3, 4, 5]. An estimated 16 million cases of pertussis and 195,000 associated deaths occur globally each year[6]. In the European countries, approximately 40,000 cases are reported each year[7].

Principles of the Procedure

The Panther Fusion Bordetella assay involves the following steps: sample lysis, nucleic acid capture and elution transfer, and multiplex real-time PCR in which analytes are simultaneously amplified, detected and differentiated. Nucleic acid capture and elution take place in a single tube on the Panther Fusion system. The eluate is transferred to the Panther Fusion system reaction tube containing the assay reagents. Multiplex real-time PCR is then performed for the eluted nucleic acid on the Panther Fusion system.

Nucleic acid capture and elution: Prior to processing and testing on the Panther Fusion system, specimens are transferred to a Specimen Lysis Tube containing Specimen Transport

Media (STM) that lyses the bacteria, releases target nucleic acid and protects it from degradation during storage.

The Internal Control-S (IC-S) is added to each test specimen and controls via the working Fusion Capture Reagent-S (wFCR-S). The IC-S in the reagent monitors specimen processing, amplification and detection.

Capture oligonucleotides hybridize to nucleic acid in the test specimen. Hybridized nucleic acid is then separated from the specimen in a magnetic field.

Wash steps remove extraneous components from the reaction tube. The elution step elutes purified nucleic acid. During the nucleic acid capture and elution step, total nucleic acid is isolated from specimens.

Elution transfer and real-time PCR: During the elution transfer step, eluted nucleic acid is transferred to a Panther Fusion reaction tube already containing oil and reconstituted mastermix.

For Bp, Bpp and internal control targets, amplification occurs via PCR that generates DNA copies of the target sequence. For all targets, specific forward and reverse primers and probes amplify targets while simultaneously detecting and discriminating multiple target types via multiplex PCR.

The Panther Fusion system uses the fluorescence signal to produce a qualitative result for the presence or absence of the analyte.

The analytes and the channel used for their detection on the Panther Fusion system is summarized in the table below.

Analyte	Gene Targeted	Instrument Channel
Bordetella pertussis	*IS 481*	FAM
Bordetella parapertussis	*IS 1001*	HEX
Internal Control	Not applicable	RED677

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. Carefully read this entire package insert and the *Panther Fusion System Operator's Manual*.

C. The Panther Fusion Enhancer Reagent-S (FER-S) is corrosive, harmful if swallowed and causes severe skin burns and eye damage.

D. Only personnel adequately trained on the use of this assay and in handling potentially infectious materials should perform these procedures. If a spill occurs, immediately disinfect using appropriate site procedures.

E. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure[8].

F. Use only supplied or specified disposable laboratory ware.

G. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and reagents.

H. Dispose of all material that has come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

I. Expiration dates listed on the Panther Fusion Specimen Lysis Tubes pertain to the transfer of sample into the tube and not to testing of the sample. Specimens collected/transferred any time prior to these expiration dates are valid for testing provided they are transported and stored in accordance with the appropriate package insert, even if these expiration dates have passed.

J. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

K. Avoid cross-contamination during the specimen handling steps. Specimens can contain high levels of bacteria or other organisms. Ensure that specimen containers do not come in contact with one another, and discard used materials without passing them over any open containers. Change gloves if they come in contact with specimens.

L. Do not use the reagents and controls after the expiration date.

M. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements* and *Panther Fusion System Test Procedure* for more information.

N. Do not combine any assay reagents or fluids. Do not top off reagents or fluids; the Panther Fusion system verifies reagent levels.

O. Avoid microbial and ribonuclease contamination of reagents.

P. Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures.

Q. Do not use the assay cartridge if the storage pouch has lost its seal or if the assay cartridge foil is not intact. Contact Hologic Technical Support if either occurs.

R. Do not use the fluid packs that are damaged or leaking. Contact Hologic Technical Support if this occurs.

S. Handle the assay cartridges with care. Do not drop or invert assay cartridges. Avoid prolonged exposure to ambient light.

T. Some reagents used with the Panther Fusion Bordetella assay are labeled with risk and safety symbols.

Note: *Hazard Communication reflects the EU Safety Data Sheets (SDS) classifications. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com. For more information on the symbols, refer to the symbol legend on www.hologic.com/package-inserts.*

EU Hazard Information
 **Panther Fusion Oil** ***Polydimethylsiloxane 100%*** **Warning** H315 - Causes skin irritation H319 - Causes serious eye irritation
    **Panther Fusion Enhancer Reagent-S** ***Lithium Hydroxide Monohydrate 5-10%*** **Danger** H302 - Harmful if swallowed H314 - Causes severe skin burns and eye damage P260 - Do not breathe dust/fume/gas/mist/vapours/spray P280 - Wear protective gloves/protective clothing/eye protection/face protection P303 + P361 + P353 - IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing P310 - Immediately call a POISON CENTER or doctor/physician P280 - Wear eye protection/ face protection

Reagent Storage and Handling Requirements

A. The following table provides storage and handling requirements for this assay.

Reagent	Unopened Storage	On Board/ Open Stability[a]	Opened Storage
Panther Fusion Bordetella assay Cartridge	2°C to 8°C	60 days	2°C to 8°C [b]
Panther Fusion Capture Reagent-S (FCR-S)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Enhancer Reagent-S (FER-S)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Internal Control-S (IC-S)	2°C to 8°C	(In wFCR-S)	Not applicable
Panther Fusion Elution Buffer	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Oil	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Reconstitution Buffer I	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Bordetella Positive Control	2°C to 8°C	Single use vial	Not applicable-single use
Panther Fusion Negative Control	2°C to 8°C	Single use vial	Not applicable-single use

When reagents are removed from the Panther Fusion system, return them immediately to their appropriate storage temperatures.

[a] On board stability starts at the time the reagent is placed on the Panther Fusion system for the Panther Fusion Bordetella assay cartridge, FCR-S, FER-S and IC-S. The on board stability for the Panther Fusion Reconstitution Buffer I, Panther Fusion Elution Buffer and Panther Fusion Oil Reagent starts when the reagent pack is first used.

[b] If removed from the Panther Fusion System, store the assay cartridge in an air-tight container with desiccant at the recommended storage temperature.

B. wFCR-S and FER-S are stable for 60 days when capped and stored at 15ºC to 30ºC. Do not refrigerate.

C. Discard any unused reagents that have surpassed their on board stability.

D. Controls are stable until the date indicated on the vials.

E. Avoid cross-contamination during reagent handling and storage.

F. **Do not freeze reagents.**

Specimen Collection and Storage

Specimens - Clinical material collected from patient and placed in an appropriate transport system. For the Panther Fusion Bordetella assay this includes NP swab specimens in transport medium.

Samples - Represents a more generic term to describe any material for testing on the Panther Fusion System including specimens, specimens transferred into a Panther Fusion Specimen Lysis Tube and controls.

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during specimen handling steps. For example, discard used material without passing over open tubes.*

A. Specimen types include NP swab specimens.

Collect NP swab specimens according to standard technique using a polyester-, rayon-, or nylon-tipped swab. Immediately place the swab specimen into dedicated transport medium.

The following types of transport media were verified for use.

- Copan ESwab Transport Medium and Universal Transport Medium (UTM)
- Remel MicroTest M4, M4RT, M5 and M6 formulations
- BD Universal Viral Transport (UVT) Medium

B. Specimen processing

1. Prior to testing on the Panther Fusion system, transfer specimen* to a Panther Fusion Specimen Lysis Tube.
   - Transfer 500 µL of the NP swab specimens to a Panther Fusion Specimen Lysis Tube.

     Note: *When testing frozen specimen, allow specimen to reach room temperature prior to processing.*

2. Storing specimens before testing
   a. After collection, specimens can be stored at 2°C to 8°C up to 96 hours before transfer to the Panther Fusion Specimen Lysis Tube. Remaining specimen volumes can be stored at ≤-70°C.

   b. Specimens in the Panther Fusion Specimen Lysis Tube may be stored under one of the following conditions:
      - 15°C to 30°C up to 6 days or
      - 2°C to 8°C up to 3 months

      Note: *It is recommended that specimens transferred to the Panther Fusion Specimen Lysis Tube are stored capped and upright in a rack.*

C. Specimens on board the Panther Fusion system may be archived for additional testing at a later time.

D. Storing samples after testing

1. Samples that have been assayed should be stored upright in the rack under one of the following conditions:
   - 15°C to 30°C up to 6 days or
   - 2°C to 8°C up to 3 months
2. The samples should be covered with a new, clean plastic film or foil barrier.
3. If assayed samples need to be frozen or shipped, remove the penetrable cap and place a new non-penetrable cap on the specimen tubes. If samples need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping previously tested and recapped samples, specimen transport tubes must be centrifuged for 5 minutes at 420 Relative Centrifugal Force (RCF) to bring all of the liquid down to the bottom of the tube. Avoid splashing and cross-contamination.

Specimen Transport

Maintain specimen storage conditions as described in the *Specimen Collection and Storage*.

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Reagents and Materials Provided

Assay Packaging

Components[a]	Cat. No.	Storage
Panther Fusion Bordetella Assay Cartridges 96 Tests Panther Fusion Bordetella Assay cartridge, 12 tests, 8 per box	PRD-04868	2°C to 8°C
Panther Fusion Bordetella Assay Controls Panther Fusion Bordetella Positive Control tube, 5 per box Panther Fusion Negative Control tube, 5 per box	PRD-04869	2°C to 8°C
Panther Fusion Internal Control-S 960 Tests Panther Fusion Internal Control-S tube, 4 per box	PRD-04332	2°C to 8°C
Panther Fusion Extraction Reagent-S 960 Tests Panther Fusion Capture Reagent-S bottle, 240 tests, 4 per box Panther Fusion Enhancer Reagent-S bottle, 240 tests, 4 per box	PRD-04331	15°C to 30°C
Panther Fusion Elution Buffer 2400 Tests Panther Fusion Elution Buffer pack, 1200 tests, 2 per box	PRD-04334	15°C to 30°C
Panther Fusion Reconstitution Buffer I 1920 Tests Panther Fusion Reconstitution Buffer I pack, 960 tests, 2 per box	PRD-04333	15°C to 30°C
Panther Fusion Oil Reagent 1920 Tests Panther Fusion Oil Reagent pack, 960 tests, 2 per box	PRD-04335	15°C to 30°C

[a] Components can also be ordered in the following bundles:
Panther Fusion Universal Fluids Kit, PRD-04430, contains 1 each Panther Fusion Oil and Panther Fusion Elution Buffer.
Panther Fusion Assay Fluids I-S, PRD-04431, contains 2 Panther Fusion Extraction Reagents-S, 2 Panther Fusion Internal Control-S, and 1 Panther Fusion Reconstitution Buffer I.

Individually Packaged Items

Items	Cat. No.
Panther Fusion Specimen Lysis Tubes, 100 per bag	PRD-04339

Materials Required and Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

Material	Cat. No.
Panther™ System	303095
Panther Fusion™ System	PRD-04172
Panther™ System, Continuous Fluid and Waste (Panther Plus)	PRD-06067
Aptima™ Assay Fluids Kit (Aptima™ Wash Solution, Aptima™ Buffer for Deactivation Fluid, and Aptima™ Oil Reagent)	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther System Run Kit for Real Time Assays contains MTUs, waste bags, waste bin covers, and assay fluids	PRD-03455 (5000 tests)
Or Panther System Run Kit (when running TMA assays in parallel with real-time TMA assays) contains MTUs, waste bags, waste bin covers, auto detect[a], and assay	303096 (5000 tests)
Panther Fusion™ Tube Trays, 1008 tests, 18 trays per box	PRD-04000
Aptima™ penetrable caps (optional)	105668
Replacement non-penetrable caps (optional)	103036A
Replacement Hologic Solid Caps (single-use tube cap)	PRD-06720 (100 caps per bag)
Replacement extraction reagent bottle caps	CL0040
P1000 pipettor and tips with hydrophobic plugs	_
Tips, 1000µL, filtered, liquid-sensing, conductive, and disposable: *Not all products are available in all regions. Contact your representative for region-specific information*	901121 (1061513 Tecan) 903031 (10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128
Bleach, 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution	_
Disposable powderless gloves	_

[a] Needed only for Panther Aptima TMA assays.

Panther Fusion System Test Procedure

Note: *Refer to the Panther Fusion System Operator's Manual for additional procedural information.*

A. Work Area Preparation

1. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and follow with a deionized (DI) water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface with clean, plastic-backed absorbent laboratory bench covers.
2. Clean a separate work surface where samples will be prepared using the procedure described in step A.1.

B. Reagent Preparation

1. Remove the IC-S, FCR-S and FER-S bottles from storage.
2. Open the IC-S, FCR-S and FER-S bottles, and discard the caps. Open the TCR door on the upper bay of the Panther Fusion system.
3. Place the IC-S, FCR-S and FER-S bottles in the appropriate positions on the TCR carousel.
4. Close the TCR door.

Note: *The Panther Fusion system adds the IC-S to the FCR-S. After the IC-S is added to the FCR-S, it is referred to as wFCR-S (working FCR-S). If the FCR-S and FER-S are removed from the system, use new caps and immediately store according to the proper storage conditions.*

C. Specimen Handling

Note: *Prepare specimens per the Specimen Processing instructions in the Specimen Collection and Storage section before loading specimens onto the Panther Fusion system.*

1. **Do not vortex samples.**
2. Inspect sample tubes before loading into the rack. If a sample tube contains bubbles or has a lower volume than is typically observed, gently tap the bottom of the tube to bring contents to the bottom.

Note: *To avoid a processing error, ensure adequate specimen volume is added to the Panther Fusion Specimen Lysis Tube. When 500 µL of NP swab specimen is added to the Panther Fusion Specimen Lysis Tube, there is sufficient volume to perform 3 nucleic acid extractions.*

D. System Preparation

For instructions on setting up the Panther Fusion system including loading samples, assay cartridges and universal fluids, refer to the *Panther Fusion System Operator's Manual.*

Procedural Notes

A. Controls

1. The Panther Fusion Bordetella Positive Control and Panther Fusion Negative Control can be loaded in any rack position, in any Sample Bay lane on the Panther Fusion system.
2. Once the control tubes are pipetted and are processed for the Panther Fusion Bordetella assay, they are active for up to 30 days (control frequency configured by an administrator) unless control results are invalid or a new assay cartridge lot is loaded.
3. Each control tube can be tested once.
4. Patient specimen pipetting begins when one of the following two conditions is met:
   a. Valid results for the controls are registered on the system.
   b. A pair of controls is currently in process on the system.

Quality Control

A run or specimen result may be invalidated by the Panther Fusion system if problems occur while performing the assay. Specimens with invalid results must be retested.

Negative and Positive Controls

To generate valid results, a set of assay controls must be tested. One replicate of the negative assay control and positive assay control must be tested each time a new lot of assay cartridges is loaded on the Panther Fusion system or when the current set of valid controls for an active cartridge lot has expired.

The Panther Fusion system is configured to require assay controls run at an administrator-specified interval of up to 30 days. Software on the Panther Fusion system alerts the operator when assay controls are required and does not start new tests until the assay controls are loaded and have started processing.

During processing, criteria for acceptance of the assay controls are automatically verified by the Panther Fusion system. To generate valid results, the assay controls must pass a series of validity checks performed by the Panther Fusion system.

If the assay controls pass all validity checks, they are considered valid for the administrator-specified time interval. When the time interval has passed, the assay controls are expired by the Panther Fusion system and a new set of assay controls is required prior to starting any new samples.

If any one of the assay controls fails the validity checks, the Panther Fusion system automatically invalidates the affected samples and requires a new set of assay controls be tested prior to starting any new samples.

Internal Control

An internal control is added to each sample during the extraction process. During processing, the internal control acceptance criteria is automatically verified by the Panther Fusion system software. Detection of the internal control is not required for samples that are positive for Bp and/or Bpp. The internal control must be detected in all samples that are negative for Bp and Bpp targets; samples that fail to meet that criteria will be reported as Invalid. Each sample with an Invalid result must be retested.

The Panther Fusion system is designed to accurately verify processes when procedures are performed following the instructions provided in this package insert and the *Panther Fusion System Operator's Manual.*

Interpretation of Results

The Panther Fusion system automatically determines the test results for samples and controls. Results for Bp and Bpp detection are reported separately. A test result may be negative, positive, or invalid.

Table 1 shows the possible results reported in a valid run with result interpretations.

Table 1: Result Interpretation

Pertussis Result	Parapertussis Result	IC Result	Interpretation
Neg	Neg	Valid	Bp and Bpp not detected.
POS	Neg	Valid	Bp detected. Bpp not detected.
Neg	POS	Valid	Bpp detected. Bp not detected.
POS	POS	Valid	Bp and Bpp detected. Retest to confirm result.
Invalid	Invalid	Invalid	Invalid. There was an error in the generation of the result; retest sample.

IC = internal control, Neg = negative, POS = positive.

Note: POS result will be accompanied by cycle threshold (Ct) values.

Limitations

A. Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

B. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

C. Avoid contamination by adhering to good laboratory practices and to the procedures specified in this package insert.

D. Negative results do not preclude *B. pertussis* or *B. parapertussis* infections and should not be used as the sole basis for treatment or other management decisions.

E. A positive result indicates the detection of nucleic acid from the relevant bacteria. Nucleic acid may persist even after the bacteria is no longer viable.

F. The Panther Fusion Bordetella assay does not differentiate *Bordetella* species other than *B. pertussis* and *B. parapertussis* (i.e., *B. holmesii*, *B. bronchiseptica*, *B. bronchialis*). An additional testing step is required to differentiate any specific *Bordetella* species or strains, in consultation with local public health departments.

Panther Fusion Bordetella Assay Performance

Reproducibility

Panther Fusion Bordetella assay precision was evaluated with a 5-member panel. The panel was tested by three operators in two separate runs per day, using three reagent lots on three Panther Fusion systems over 12 non-consecutive days.

The panel members are described in Table 2, along with a summary of the agreement with expected results for each target. Table 3 presents the mean and variability analysis between instruments, between reagent lots, between operators, between days, between runs and within runs, and overall (total) for Ct.

Table 2: Panel Description and % Agreement

Target	Panel Member	% Positive	% Total Agreement (95% CI)
B. pertussis	Bp 1-2X LoD	100 (180/180)	100 (97.9 - 100%)
	Bp 5X LoD	100 (180/180)	100 (97.9 - 100%)
	Negative	0 (0/180)	100 (97.9 - 100%)
B. parapertussis	Bpp 1-2X LoD	100 (180/180)	100 (97.9 - 100%)
	Bpp 5X LoD	100 (180/180)	100 (97.9 - 100%)
	Negative	0 (0/180)	100 (97.9 - 100%)

CI = confidence interval, LoD = limit of detection.

Table 3: Signal Variability

Target	Panel Member	Mean Ct	Between Instruments		Between Reagent Lots		Between Operators		Between Days		Between Runs		Within Runs		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Bp	Bp 1-2X LoD	36.9	0.3	0.9	0.3	0.9	0.2	0.5	0.2	0.5	0.6	1.6	0.8	2.2	1.0	2.6
	Bp 5X LoD	35.5	0.3	0.9	0.3	0.9	0.2	0.7	0.2	0.5	0.4	1.2	0.5	1.3	0.6	1.7
Bpp	Bpp 1-2X LoD	38.1	0.4	1.0	0.4	1.0	0.2	0.6	0.2	0.6	0.7	1.8	0.7	1.7	0.9	2.4
	Bpp 5X LoD	37.3	0.3	0.8	0.3	0.8	0.2	0.5	0.2	0.5	0.6	1.5	0.6	1.6	0.8	2.2
IC	Negative	30.5	0.2	0.6	0.2	0.6	0.2	0.8	0.1	0.3	0.3	1.1	0.2	0.8	0.4	1.3

Ct = threshold cycle, CV = coefficient of variation, IC = internal control, LoD = limit of detection, SD = standard deviation.

Clinical Performance

This study was performed to demonstrate the clinical performance of the Panther Fusion Bordetella assay. NP swab specimen collected from symptomatic patients were used for a retrospective evaluation. Each NP swab specimen was diluted into a Panther Fusion Specimen Lysis Tube containing Specimen Transport Media (STM). A single replicate of each sample was tested with the Panther Fusion Bordetella assay. The result was compared to the result obtained with a CE-marked nucleic acid (NAT) test. Positive percentage agreement (PPA) and negative percentage agreement (NPA) for the detection of Bp and Bpp nucleic acid were determined.

A total of 290 NP specimens including 50 contrived Bpp positive specimens were tested with the Panther Fusion Bordetella assay and with the Diagenode Diagnostics R-DiaBorM™ assay. PPA and NPA for detection of Bp and Bpp are shown in Table 4 and Table 5 respectively.

Table 4: Bp Assay Performance Compared to Diagenode Diagnostics R-DiaBorM™ Assay

Specimen Type	N	Bp+		Bp-		PPA (%) 95% CI	NPA (%) 95% CI	Overall Agreement (%) 95% CI
		Fusion Bp +	Fusion Bp -	Fusion Bp +	Fusion Bp -			
Retrospective NP Swab	290	72	0	7	211	100	96.8	97.6
						94.9 - 100%	93.5 - 98.4%	95.1 - 98.8%

CI = confidence interval, NPA = negative percentage agreement, PPA = positive percentage agreement.

Table 5: Bpp Assay Performance Compared to Diagenode Diagnostics R-DiaBorM™ Assay

Specimen Type	N	Bpp+		Bpp-		PPA (%) 95% CI	NPA (%) 95% CI	Overall Agreement (%) 95% CI
		Fusion Bpp +	Fusion Bpp -	Fusion Bpp +	Fusion Bpp -			
Retrospective NP Swab	140	18	0[a]	1	121	100	99.2	99.3
						82.4 - 100%	95.5 - 99.9%	96.1 - 99.9%
Contrived NP Swab	150[b]	50	0	0	100	100	100	100
						92.9 - 100%	96.3 - 100%	97.5 - 100%
Total	290	68	0	1	221	100	99.6	99.7
						94.7 - 100%	97.5 - 99.9%	98.1 - 99.9%

CI = confidence interval, NPA = negative percentage agreement, PPA = positive percentage agreement.

[a] Two specimens gave a late emergence Ct with low and flat fluorescence curves after testing with the R-DiaBorM™ assay. Per protocol, these samples were retested and yielded a negative result.

[b] To minimize bias, 50 contrived specimens were prepared by spiking clinically relevant concentrations of Bpp target (between 3X and 100X LoD) and tested along with an equal number of unique negative specimens and 50 retrospective Bp positive/Bpp negative specimens in blinded and randomized fashion.

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the Panther Fusion Bordetella assay was determined by testing serial dilutions of quantified cultures (CFU/mL) of *B. pertussis* and *B. parapertussis* spiked separately in negative clinical NP specimens. Twenty replicates of each dilution were tested with each of the three reagent lots for a combined total of 60 replicates per dilution. Probit analysis was performed for each reagent lot with the reported 95% LoD based upon the worst reagent lot estimate, as shown in Table 6. Target specific LoD concentrations were verified by testing an additional 20 replicates with one reagent lot.

Table 6: B. pertussis and B. parapertussis Limit of Detection

Target	LoD Concentration (95%CI)
B. pertussis	20.1 CFU/mL (13.6 - 42.1 CFU/mL)
B. parapertussis	162.5 CFU/mL (92.9 - 441.4 CFU/mL)

CI = confidence interval, CFU = colony-forming units, LoD = limit of detection.

Reactivity

The reactivity of the Panther Fusion Bordetella assay was evaluated by testing distinct isolates of *B. pertussis and B. parapertussis*. *Bordetella* isolates were tested in triplicate with one reagent lot as presented in Table 7.

Table 7: Reactivity Results

Target	Description	Concentration	Bp	Bpp
B. pertussis	LMG14454	25 CFU/mL	+	-
	LMG14455	25 CFU/mL	+	-
	LMG15140	25 CFU/mL	+	-
	LMG15585	25 CFU/mL	+	-
B. parapertussis	LMG14449	202 CFU/mL	-	+
	LMG1833	202 CFU/mL	-	+
	LMG1818	202 CFU/mL	-	+

CFU = colony-forming units

Analytical Specificity

The analytical specificity of the Panther Fusion Bordetella assay was assessed with a total of 68 microorganisms (Table 8), consisting of 25 viral, 42 bacterial and 1 yeast strains representing common respiratory pathogens commonly found in the nasopharynx area. Bacteria and yeast were tested at concentrations of 10^6 CFU/mL, CCU/mL or IFU/mL, except where noted. Viruses were tested at concentrations of 10^5 to 10^6 TCID50/mL or CEID50/mL, except where noted. Organisms were tested with and without *B. pertussis* and *B. parapertussis* analytes spiked at a concentration of 3X LoD. All of the non-*Bordetella* microorganisms tested were found to have no impact on the performance or analytical specificity of the Panther Fusion Bordetella assay.

Table 8: Specificity Results

Organism	Concentration	Bp	Bpp
Achromobacter denitrificans	$1x10^6$ CFU/mL	-	-
Acinetobacter baumanii	$1x10^6$ CFU/ml	-	-
Adenovirus 4	$1x10^5$ TCID50/mL	-	-
Arcanobacterium haemolyticum	$1x10^6$ CFU/mL	-	-
Bordetella avium	$1x10^6$ CFU/mL	-	-
Bordetella bronchialis[a]	$1x10^6$ CFU/mL	+	-
Bordetella bronchiseptica	$1x10^6$ CFU/mL	-	-
Bordetella hinzii	$1x10^6$ CFU/mL	-	-
Bordetella holmesii[a]	$1x10^6$ CFU/mL	+	-
Bordetella petrii	$1x10^6$ CFU/mL	-	-
Bordetella trematum	$1x10^6$ CFU/mL	-	-
Burkholderia cepacia	$1x10^6$ CFU/mL	-	-
Candida albicans	$1x10^6$ CFU/mL	-	-
Chlamydia pneumoniae	$1x10^6$ IFU/mL	-	-
Chlamydia trachomatis	$1x10^6$ copies/mL[b]	-	-
Coronavirus OC43	$1x10^6$ TCID50/mL	-	-
Corynebacterium diphtheriae	$1x10^4$ CFU/mL	-	-
Coxsackievirus B4	$1x10^6$ TCID50/mL	-	-
Coxsackievirus B5	$1x10^6$ TCID50/mL	-	-
Cytomegalovirus AD-169	$1x10^5$ TCID50/mL	-	-
Echovirus 11	$1x10^6$ TCID50/mL	-	-
Echovirus 6	$1x10^6$ TCID50/mL	-	-
Echovirus 7	$1x10^6$ TCID50/mL	-	-
Echovirus 9	$1x10^6$ TCID50/mL	-	-
Enterococcus faecalis	$1x10^6$ CFU/mL	-	-
Enterovirus 71	$1x10^6$ TCID50/mL	-	-
Epstein-Barr virus B95-8	$1x10^6$ copies/mL	-	-
Escherichia coli	$1x10^6$ CFU/mL	-	-
Fusobacterium necrophorum	$1x10^4$ CFU/mL	-	-
Haemophilus influenzae	$1x10^6$ CFU/mL	-	-
Haemophilus parainfluenzae	$1x10^5$ CFU/mL	-	-
Herpes Simplex Virus 1 (HSV-1)	$1x10^6$ TCID50/mL	-	-
Herpes Simplex Virus 2 (HSV-2)	$1x10^6$ TCID50/mL	-	-
Human Rhinovirus A1	$1x10^6$ TCID50/mL	-	-

Table 8: Specificity Results (continued)

Organism	Concentration	Bp	Bpp
Influenza A virus New Jesey/8/76	$1x10^6$ CEID50/mL	-	-
Influenza B/Florida/04/2006	$1x10^5$ TCID50/mL	-	-
Klebsiella pneumoniae	$1x10^6$ CFU/mL	-	-
Lactobacillus acidophilus	$1x10^6$ CFU/mL	-	-
Lactobacillus plantarum	$1x10^6$ CFU/mL	-	-
Legionella longbeachae	$1x10^4$ CFU/mL	-	-
Legionella micdadei	$1x10^5$ CFU/mL	-	-
Legionella pneumophilia	$1x10^4$ CFU/mL	-	-
Measle virus	$1x10^6$ TCID50/mL	-	-
Metapneumovirus 27 Type A2	$1x10^5$ TCID50/mL	-	-
Moraxella catarrhalis	$1x10^6$ CFU/mL	-	-
Mumps virus	$1x10^5$ TCID50/mL	-	-
Mycobacterium tuberculosis	$1x10^6$ copies/mL[b]	-	-
Mycoplasma genitalium	$1x10^3$ CCU/mL	-	-
Mycoplasma hominis	$1x10^6$ CFU/mL	-	-
Mycoplasma pneumoniae	$1x10^6$ CCU/mL	-	-
Neisseria elongata	$1x10^6$ CFU/mL	-	-
Neisseria mucosa	$1x10^6$ CFU/mL	-	-
Parainfluenza Type 1	$1x10^6$ TCID50/mL	-	-
Parainfluenza Type 2	$1x10^6$ TCID50/mL	-	-
Parainfluenza Type 3	$1x10^5$ TCID50/mL	-	-
Parainfluenza Type 4B	$1x10^5$ TCID50/mL	-	-
Proteus mirabilis	$1x10^6$ CFU/mL	-	-
Proteus vulgaris	$1x10^6$ CFU/mL	-	-
Pseudomonas aeruginosa	$1x10^6$ CFU/mL	-	-
Respiratory syncitial virus A	$1x10^5$ TCID50/mL	-	-
Respiratory syncitial virus B	$1x10^4$ TCID50/mL	-	-
Staphylococcus aureus (MRSA)	$1x10^6$ CFU/mL	-	-
Staphylococcus epidermidis	$1x10^6$ CCU/mL	-	-
Stenotrophomonas maltophilia	$1x10^6$ CFU/mL	-	-
Streptococcus pneumoniae	$1x10^6$ CFU/mL	-	-
Streptococcus pyogenes	$1x10^6$ CFU/mL	-	-
Streptococcus salivarius	$1x10^6$ CFU/mL	-	-
Ureaplasma urealyticum	$1x10^6$ CFU/mL	-	-

CCU = color change unit, CEID = chicken embryo infectious dose, CFU = colony-forming units, IFU = infectious units, TCID= tissue culture infectious dose.

[a] *Bordetella bronchialis* and *Bordetella holmesii* contain the targeted *IS 481* sequence.

[b] Microorganisms evaluated as extracted nucleic acid.

Competitive Interference

Competitive interference of the Panther Fusion Bordetella assay was evaluated using a surrogate clinical matrix with both target bacteria at two different concentrations. One target concentration was near the Limit of Detection (upper CI of the LoD) while the other target concentration was high (10,000X LoD). The presence of the two bacteria at varying concentrations in a single sample had no effect on detection (100% detection for both targets).

Table 9: Competitive Interference

Condition	Target 1		Target 2		Bp Result	Bpp Result
	Description	Concentration	Description	Concentration		
1	Bp	Upper LoD[a]	Bpp	10,000X LoD	+	+
2	Bpp	Upper LoD[a]	Bp	10,000X LoD	+	+

LoD = limit of detection.

[a] Upper CI of the LoD is reported in Table 6.

Interference

Mucin, whole blood, and other potentially interfering substances that may be present in the samples were evaluated in the Panther Fusion Bordetella assay. Clinically relevant amounts of the potentially interfering substances were added to surrogate clinical matrix and tested with or without Bp and Bpp at their respective 3X LoD concentration. The substances consisted of nasal sprays (liquid and powder), ingestible pills, lozenges, and injectable or endogenous substances, as shown in Table 10.

All of the substances tested were found to have no impact on the performance of the Panther Fusion Bordetella assay.

Table 10: Potentially Interfering Substances

Type	Substance Name	Active Ingredient(s)	Concentration
Endogenous	Mucin	Purified mucin protein	60 µg/mL
	Human blood	Blood	2% v/v
Nasal sprays or drops	Neo-Synephrine®	Phenylephrine	15% v/v
	Anefrin	Oxymetazoline	15% v/v
	Saline	Sodium chloride	15% v/v
Nasal corticosteroids	QVAR®, Beconase AQ	Beclomethasone	5% v/v
Nasal gel	Zicam® (Allergy Relief)	Histaminum dihydrochloride, *Luffa opperculata*, *Galphimia glauca*, sulfur	5% v/v
Throat lozenges	Chloraseptic Throat Lozenges	Benzocaine	4.14 mg/mL
		Menthol	6.9 mg/mL
Anti-viral drugs	Relenza®	Zanamivir	3.3 mg/mL
	TamiFlu®	Oseltamivir	2.5% w/v
	Rebitol	Ribavirin	2% w/v
Antibiotic, nasal ointment	Bactroban cream	Mupirocin	6.6 mg/mL
Antibiotic, systemic	Tobramycin	Tobramycin	4.4 µg/mL

v/v = volume/volume, w/v = weight/volume.

Carryover/Contamination

The carryover/cross-contamination study was performed with negative samples alternately placed between high positive samples and tested. High positive samples were prepared by spiking 10^6 CFU/mL (corresponds to > 6,000X LoD) of Bp and Bpp strains in surrogate matrix. Three separate runs with 30 negative samples and 30 positive samples placed in a checkerboard pattern were tested on three different instruments for a combined total of 270 positive and 270 negative samples. The carryover rate was 0.0%.

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