Nasopharyngeal Swab Sample-to-Answer Verification Studies Using Simplexa™ Bordetella Direct Assay

Yuan Xie, Huong Mai, Jules Chen, Michelle Tabb
DiaSorin Molecular LLC, Cypress, CA, USA
Correspondence: yuan.xie@diasorin.com

AMP 2016 ID33
Charlotte, NC I November 10-12, 2016

Revised Abstract

Background: *Bordetella pertussis* is the main cause of whooping cough, however other Bordetella species, such as *Bordetella parapertussis*, can cause similar symptoms. The Simplexa™ Bordetella Direct assay is in development as a sample-to-answer assay performed on the Integrated Cycler instrument. Nasopharyngeal swab specimens collected in transport media are loaded directly onto a Direct Amplification Disc without extraction or other specimen preparation. The Simplexa Bordetella Direct assay was developed to detect and differentiate *B. pertussis* and *B. parapertussis*. The goal of this verification study was to evaluate the performance of the Simplexa Bordetella Direct assay.

Methods: Limit of detection (LoD), analytical reactivity, cross reactivity, reproducibility and substance interference studies were performed to evaluate Simplexa Bordetella Direct performance. Limit of detection (LoD) studies were performed to determine the analytical sensitivity of the assay. 10 additional *B. pertussis* strains were evaluated for analytical reactivity. A reproducibility study was performed with medium and low positive panels. A panel of potentially interfering substances was tested to determine whether any inhibition was observed.

Results: LoD studies showed that the Simplexa Bordetella Direct assay detected *B. pertussis* at ≤200 CFU/ml, *B. parapertussis* at ≤500 CFU/ml. All ten additional *B. pertussis* strains evaluated for analytical sensitivity were detected at 100 CFU/ml. No detection is observed for the seventy cross reactivity pathogens. Inter- and intra-assay reproducibility assays yielded <4.0% coefficient of variation. No inhibition or interference was observed from any of the substances tested.

Conclusion: The Simplexa Bordetella Direct assay was capable of directly detecting and differentiating *B. pertussis* and *B. parapertussis* without up-front nucleic acid extraction from nasopharyngeal swab specimens. The assay and instrumentation provide a compact system for rapid detection directly from nasal swab samples.

Methods

Simplexa Bordetella Direct Assay: 50 µL of Simplexa Bordetella Direct assay reaction mix was loaded into the reaction port and 50 µL of sample was loaded into the sample port on the Direct Amplification Disc (DAD). All testing was performed using the Integrated Cycler instrument (Focus Diagnostics, Inc., Cypress, CA). Assay time is about 80 minutes.

Data collection and analysis were performed with Integrated Cycler Studio software version 6.0. IS481 (BP), IS1001 (BPP) and internal control were detected with FAM, CFR 610 and Quasar 670 dyes, respectively.

Methods (Continued)

Limit of Detection : The following Bordetella strains were tested for Limit of Detection (LoD). *B. pertussis* BAA-589, *B. pertussis* ATCC 12742, *B. pertussis* A639, *B. parapertussis* A747, *B. parapertussis* E595, (ZeptoMetrix Corp., Buffalo, NY). The LoD for each Bordetella strain was determined as the lowest concentration with ≥95% detection in negative swab matrix .

Analytical Reactivity: Ten additional *B. pertussis* strains were tested for analytical reactivity at 100 CFU/ml.

Cross-Reactivity: The cross-reactivity panel consisted of industry equivalent 10^6 CFU/ml of bacteria or 10^5 $TCID_{50}$/ml of viruses. Seventy potential cross-reactants were spiked into negative swab matrix and assayed using Simplexa Bordetella Direct assay.

Reproducibility: Reproducibility panels were contrived in negative swab matrix: *B. pertussis* Low Positive (ATCC 12742 at 1X LOD), *B. pertussis* Medium Positive (ATCC 12742 at 3X LOD), *B. parapertussis* Low Positive (A747 at 1.5X LOD), *B. parapertussis* Medium Positive (A747 at 3X LOD).

Substance Interference: The interference panel was contrived with *B. pertussis or B. parapertussis* at 3X above LoD. Each interfering substance was spiked with *B. pertussis* or *B. parapertussis* into negative swab matrix and tested using Simplexa Bordetella Direct.

Results

Limit of Detection was ≤500 CFU/ml for Bordetella Strains Tested in Table 1.

Table 1. Limit of Detection

Bacterial Strains	(CFU/ml)	Replicates Detected
***B. pertussis* (BAA-589)**	40	20/20
***B. pertussis* (ATCC 12742)**	40	20/20
***B. pertussis* (A639)**	200	20/20
***B. parapertussis* (A747)**	400	20/20
***B. parapertussis* (E595)**	500	19/20

All Bordetella Strains Tested for Analytical Reactivity were Detected at 100 CFU/ml in Table 2.

Table 2. Bordetella Strains tested for Analytical Reactivity

Bordetella pertussis BAA-1335	*Bordetella pertussis ATCC 53894*
Bordetella pertussis ATCC 8467	*Bordetella pertussis ATCC 8478*
Bordetella pertussis ATCC 12742	*Bordetella pertussis ATCC 12743*
Bordetella pertussis ATCC 51445	*Bordetella pertussis ATCC 9340*
Bordetella pertussis ATCC 10380	*Bordetella pertussis ATCC 9797*

Results (Continued)

No Detection was Observed for the Cross Reactivity Pathogens Listed in Table 3.

Table 3. Cross Reactivity Pathogens

Acinetobacter baumannii	*Legionella pneumophila*	*Streptococcus mutans*
Acinetobacter lwoffi	*Listeria monocytogenes*	*Streptococcus pneumoniae*
Arcanobacterium haemolyticum	*Moraxella catarrhalis*	*Streptococcus pyogenes*
Bacillus cereus	*Mycobacterium tuberculosis (DNA)*	*Streptococcus salivarius*
Bacteroides fragilis	*Mycoplasma hominis*	*Ureaplasma urealyticum*
Bordetella bronchiseptica	*Mycoplasma pneumoniae M129*	*Adenovirus*
Burkholderia cepacia	*Neisseria elongota*	*Coronavirus 229E*
Candida albicans	*Neisseria gonorrhoeae*	*Coxsackievirus A16*
Candida glabrata	*Neisseria meningitidis*	*Cytomegalovirus*
Citrobacter freundii	*Peptostreptococcus anaerobius*	*HSV-1*
Clostridium difficile	*Proteus mirabilis Z050*	*HSV-2*
Corynebacterium diptheriae	*Proteus Vulgaris*	*Influenza A Swine H1N1*
Enterobacter aerogenes Z052	*Pseudomonas aeruginosa*	*Influenza B Malaysia*
Enterobacter cloacae	*Pseudomonas fluorescens*	*Measles*
Enterococcus faecalis vanB	*Serratia marcescens*	*Metapneumovirus-9*
Epstein Barr Virus	*Serratia liquefaciens*	*Mumps*
Escherichia coli	*Staph. aureus (MRSA)*	*Parainfluenza 1*
Haemophilus influenzae	*Staph. epidermidis (MRSE)*	*Parainfluenza 2*
Haemophilus parainfluenzae	*Stenotrophomonas maltophilia*	*Parainfluenza 3*
Klebsiella oxytoca	*Streptococcus anginosus*	*Rhinovirus 1A*
Klebsiella pneumoniae	*Streptococcus canis*	*RSV A*
Lactobacillus acidophilus	*Streptococcus dysgalactiae*	*RSV B*
Lactobacillus plantarum	*Streptococcus intermedius*	
Legionella longbeachae	*Streptococcus mitis*	

Results (continued)

<4% Coefficient of Variation for the Reproducibility Studies Listed in Table 4.

Table 4. Reproducibility

Target	Sample Name	N	Between Instrument		Between Operator		Between Run		Within Run		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
B. pertussis	LP	48	0.00	0.0	0.00	0.0	0.57	1.6	0.98	2.7	1.13	3.2
	MP	48	0.00	0.0	0.00	0.0	0.00	0.0	0.90	2.7	0.90	2.7
B. parapertussis	LP	48	0.00	0.0	0.25	0.7	0.00	0.0	0.83	2.5	0.87	2.6
	MP	48	0.12	0.4	0.00	0.0	0.26	0.8	0.55	1.7	0.62	1.9

LP=Low Positive, MP=Medium Positive, CV=Coefficient of Variation, SD=Standard Deviation.

No Interference was Observed with the Substances Tested in Table 5.

Table 5. Potential Interference Substances Tested

Interfering Substance	Concentration Tested
Mucin	10 mg/ml
Ampicillin powder	10 mg/ml
Azithromycin powder	10 mg/ml
Ciprofloxacin	10 mg/ml
Erythromycin	10 mg/ml
Mupirocin	10 mg/ml
Rifampicin	10 mg/ml
Beclomethasone dipropionate	10 mg/ml
Sudafed PE	10 mg/ml
Robitussin DM	10% (v/v)
Zicam 12 hrs spray	10% (v/v)
FLONASE Nasal Spray	10% (v/v)
Chloraseptic sore throat spray	10% (v/v)
Saline Nasal spray-Sodium chloride	10% (v/v)
Whole blood (with EDTA)	10% (v/v)

Conclusions

- Simplexa™ Bordetella Direct is a simple and rapid molecular test, without requiring a separate extraction step.
- Simplexa™ Bordetella Direct was capable of directly detecting and differentiating *Bordetella pertussis* and *Bordetella parapertussis* from nasopharyngeal swab specimens.

FOCUS Diagnostics
Focus Diagnostics is now operating as DiaSorin Molecular
DiaSorin Molecular

NOTE: SIMPLEXA BORDETELLA DIRECT ASSAY IS UNDER DEVELOPMENT AND IS NOT AVAILABLE FOR COMMERCIAL SALE IN THE U.S.