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Multicenter Clinical Evaluation of a Real-Time PCR Assay for *Bordetella pertussis*

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Background

Pertussis is a contagious respiratory disease caused by *Bordetella (B.) pertussis* that can lead to life-threatening complications in young children. Early and accurate diagnosis is critical for positive patient outcomes and to aid public health officials in controlling spread during outbreaks. The Great Basin Bordetella Direct Test is a qualitative *in vitro* diagnostic test for the detection of *B. pertussis* DNA from nasopharyngeal (NP) swab specimens obtained from patients suspected of having a respiratory tract infection from *B. pertussis*. The assay was performed on the PA500 Portrait™ Analyzer and utilizes PCR amplification of the insertion sequence IS*481*.

Methods

The performance of the Bordetella Direct Test was evaluated on 1037 NP samples, 915 prospectively collected and 122 retrospective frozen NP samples at 5 US clinical sites from August, 2016 to January, 2017. Results generated by the Bordetella Direct Test were compared to those obtained with the Nanosphere Verigene® RP Flex Test. Discordant samples were adjudicated by testing with the Quidel Amplivue *B. pertussis,* an FDA approved molecular assay. Reproducibility studies were performed at 3 sites with 2 operators at each site. A 3 sample panel consisting of a moderate positive sample (1.9x the limit of detection {LoD}) a high positive sample (3.8x LoD) and a negative sample was tested in triplicate over 5 non-consecutive days by each operator (n=270).

Results

In the combined evaluation of the Bordetella Direct Test (prospective and retrospective samples), the Positive Percent Agreement (PPA) for *B. pertussis* was 92.5% (74/80; 95% confidence interval [CI], 84.6% to 96.5%). The Negative Percent Agreement (NPA) for *B. pertussis* was 99.6% (953/957; 95% CI, 98.9% to 99.8%). Six samples were positive by the RP Flex Test and negative by the Bordetella Direct Test, 4 of the 6 were confirmed positive using the Amplivue assay. Conversely, 4 samples were Bordetella Direct Test positive and RP Flex negative and 2 of these were confirmed positive using the Amplivue assay.

Results Continued

Table 1. Summary of Clinical Study Results

Specimen		n	% Agreement (95% CI)	
			Positive	Negative
Bordetella pertussis	Fresh	915	85.7% (65.4% - 95.0%) 18/21	99.6% (98.9% - 99.8%) 890/894
	Frozen	122	94.6% (86.1% - 98.3%) 56/59	100.0% (94.3% - 100.0%) 63/63
	Combined (Fresh + Frozen)	1037	92.5% (84.6% - 96.5%) 74/80	99.6% (98.9% - 99.8%) 953/957

Table 2. Summary Table – *B. pertussis* False Negative Discrepant Results – Prospective Study

#	Sample ID	Bordetella Direct Test *B. pertussis* Result	Verigene® RP Flex *B. pertussis* Result	Amplivue *B. pertussis* Result
1	TRC-21	Not Detected	Detected	Positive
2	MCL-171	Not Detected	Detected	Positive
3	MCL-182	Not Detected	Detected	Negative

Table 3. Summary Table – *B. pertussis* False Positive Discrepant Results – Prospective Study

#	Sample ID	Bordetella Direct Test *B. pertussis* Result	Verigene® RP Flex *B. pertussis* Result	Amplivue *B. pertussis* Result
1	MCL-5	Detected	Not Detected	Negative
2	MCL-13	Detected	Not Detected	Positive
3	MCL-273	Detected	Not Detected	Negative
4	MCL-477	Detected	Not Detected	Positive

Results Continued

Table 4. Summary Table – *B. pertussis* Discrepant Results – Frozen Archived Sample

#	Sample ID	Bordetella Direct Test *B. pertussis* Result	Verigene® RP Flex *B. pertussis* Result	Amplivue *B. pertussis* Result
1	FR-046	Not Detected	Detected	Positive
2	FR-064	Not Detected	Detected	Negative
3	FR-085	Not Detected	Detected	Positive

% positive agreement after discrepancy analysis was 95% (76/80) and
% negative agreement after discrepancy analysis was 99.8% (955/957)

Table 5. Reproducibility Study Panel

Panel	Sample Type	Sample Composition	Concentration (CFU/mL), LoD
RP-01	Low Positive	Bordetella pertussis (ATCC 9797)	3.1 x 10^3, 1.9X
RP-02	Moderate Positive	Bordetella pertussis (ATCC 9797)	6.2 x 10^3, 3.8X
RP-05	Negative	Negative	N/A

There were no discordant results in the reproducibility study, all 1.9x, 3.8x LoD and negative samples gave the expected results in all testing events (270/270).

Conclusions

The Great Basin Bordetella Direct Test is a sensitive and specific diagnostic assay for detection of *B. pertussis* in NP swabs from subjects with suspected *B. pertussis* infection.

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