Comparison of Molecular Assays for the Diagnosis of Pertussis

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Background

Pertussis (whooping cough) is a vaccine preventable disease caused by *Bordetella pertussis* with highest mortality seen in infants. Virulence factors for *Bordetella* include toxins and components that mediate adherence to ciliated cells of the respiratory tract. Infection with *Bordetella parapertussis* can mimic that caused by *Bordetella pertussis*, but symptoms tend to be milder. Pertussis infection results from contact with aerosolized droplets from infected coughing individuals. In infants and children, typical pertussis presentation follows catarrhal, paroxysmal, and convalescent stages (Graph 1). In older children and adults, pertussis infection tends to be atypical and may be unrecognized. Despite gains in vaccination, pertussis is endemic worldwide with sporadic outbreaks. In 2008, 16 million pertussis cases were reported worldwide resulting in 195,000 deaths. Culturing nasopharyngeal (NP) swabs is the gold standard for detecting pertussis, but has a long turnaround time and recovery is poor if samples are not collected during the catarrhal stage. We compared the workflow and performance characteristics of two molecular assays for diagnosing pertussis from NP swabs in universal transport media.

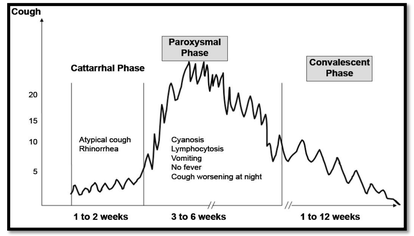
Methods

At the Cleveland Clinic, NP swabs for pertussis diagnosis are routinely tested by AmpliVue® Bordetella Assay (Quidel, Figure 1). The AmpliVue Bordetella Assay is a helicase-dependent amplification assay targeting the insertion sequence IS481 followed by detection in a lateral flow device.

Remnant samples (total = 112; 76 frozen at -70°C, 36 fresh) were tested using DiaSorin Molecular's Simplexa™ Bordetella Direct PCR assay targeting IS481 and IS1001 for the detection *B. pertussis* and *B. parapertussis* respectively.

For Simplexa Bordetella Direct testing, samples were brought to room temperature and briefly vortexed. 50µL of Reaction Mix and 50µL of test sample were pipetted into respective wells on the 8 well Direct Amplification Disc (DAD) (Figure 2). After re-sealing wells and tearing off tabs, the DAD was loaded onto the LIAISON® MDX (Figure 3) for analysis. Quality Control was performed on each day of testing following manufacturer recommendations. Testing and set-up areas were tested weekly for contamination.

The Simplexa Bordetella Direct assay and AmpliVue Bordetella Assay results were compared, and discordant *B. pertussis* results or positive results for *B. parapertussis* (a target not included in the AmpliVue Bordetella Assay) were arbitrated by sequencing performed by DiaSorin Molecular. Sensitivity and specificity were determined for each assay's detection of *B. pertussis* based on sequencing as the reference method for discordant samples.



Graph 1: Pertussis symptoms

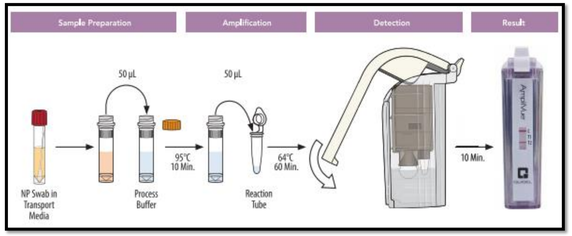


Figure 1: AmpliVue® Bordetella Assay

Figure 2: Direct Amplification Disc (DAD)

Figure 3: LIAISON® MDX workstation

Results

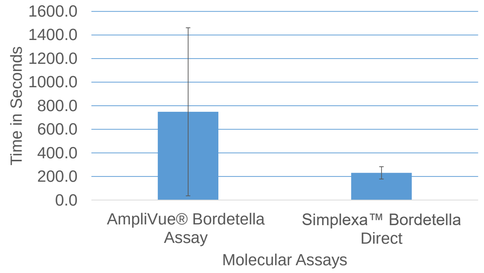
Positive results for *B. pertussis* were detected for 14 specimens by AmpliVue Bordetella Assay and 18 specimens by Simplexa Bordetella Direct (Table 1). Discrepancy analysis by sequencing confirmed 4 *B. pertussis* positive specimens detected only by Simplexa Bordetella Direct and one false positive result for each assay. The sensitivity of AmpliVue Bordetella Assay was 76.5% while that of Simplexa Bordetella Direct was 100.0%. The specificity of both assays was 98.9%.

	Simplexa™ Bordetella Direct	AmpliVue® Bordetella Assay
True Positive	17	13
True Negative	94	94
False Positive	1	1
False Negative	0	4
Total	112	112
Sensitivity %	100.0%	76.5%
Specificity %	98.9%	98.9%
Positive Predictive Value %	94.4%	92.9%
Negative Predictive Value %	100.0%	95.9%

Table 1: Performance characteristics of the molecular assays

Positivity rates were 27% for 48 children ≥1 year, 8% for 39 adults (≥ 18 years), and 4% for 25 infants tested. *B. parapertussis* was detected in one (1) sample from a child and was confirmed by sequencing.

Chart 1: Mean Hands-on-Time (sec/sample) with ± 1 Standard Deviation



For each assay, distinct procedural steps were determined and used to calculate Hands-on-Time (HOT) in seconds (s) per sample as a proxy for assessing workflow. The HOT for AmpliVue Bordetella Assay averaged 748.7 s (~12.5 minutes) per sample while that of Simplexa Bordetella Direct averaged 231 s (~4 minutes) per sample (Chart 1).

The standard deviation for the HOT assessment did not show statistical significance because only 3 assessments were done for each assay.

The average result turnaround time for AmpliVue Bordetella Assay was 6944 s (~2 hours) while that for the Simplexa Bordetella Direct was 5404 s (1.50 hours).

Conclusions

Compared to the AmpliVue Bordetella Assay, the Simplexa Bordetella Direct assay required less hands on time and provided detection of more specimens containing *B. pertussis.*

The Simplexa Bordetella Direct assay was also able to detect one *B. parapertussis* specimen which was not possible with the AmpliVue Bordetella Assay since this target is not included in the AmpliVue Bordetella assay.

Acknowledgements

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