Molecular detection of *Bordetella pertussis/parapertussis* and prevalence of *B. holmesii*

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Background

Bordetella pertussis (Bp) is an important agent responsible for severe respiratory infections in children and in adults. Rapid and accurate laboratory diagnosis is important to start antibiotic therapy as early as possible and prevent transmission.

We compared three commercial assays and one laboratory developed test (LDT) for molecular detection of *Bp* and *B. parapertussis (Bpp)* in nasopharyngeal specimens. Additionally, the prevalence of *B. holmesii (Bh)*, a species cross-reacting with *Bp* in tests using IS*481* as a target, was determined in our study group.

Materials & Methods

Prospective Part

Specimens: 121 consecutive nasopharyngeal specimens (eSwab, Copan; stored for max. 72h at +4 ˚C) were enrolled in the study.

Methods: All specimens were analyzed in parallel with Simplexa™ Bordetella Direct Kit (DiaSorin; *Bp*/IS*481* and *Bpp*), FilmArray® Respiratory Panel Version 1.7 (N=62) or 2*plus* (N=59) (both Biofire®, *Bp/ptx* only), *illumigene*® Pertussis (Meridian; *Bp*/IS*481* only) and a laboratory developed test (LDT, *Bp*/IS*481* and *Bpp*).

Final result definition: A true positive result was defined as two or more assays being positive.

Retrospective Part

Due to the low number of positives in the prospective study, 30 *Bp* and five *Bpp* LDT-positive frozen specimens (stored max. 6 years at -80 ˚C) in eSwab (Copan) were tested using Simplexa™ Bordetella Direct Kit (DiaSorin).

Prevalence of *B. holmesii*

The prevalence of *Bh* was determined by using the Bordetella Speciation Plus Toxin – OSR kit for the BD MAX™ System (BioGX; PCR only procedure) with 223 DNA extracts previously LDT-positive for *Bp* and stored up to 18 months at -80 ˚C. Positive *Bh* results were confirmed in an independent laboratory.

Results

Prospective Part

The inhibition/unresolved rates for FilmArray®, *illumigene*® and Simplexa™ were 0%/0.8%, 0%/0.8% and 1.6%/1.6%, respectively.

Table 1. Results obtained with different assay systems for *Bp* and *Bpp*.

B. pertussis 12 pos / 109 neg

N	FilmArray®	Simplexa™	illumigene®	LDT	Final result
10	+	+	+	+	+
2	-	+ C_t = 36, 37	-	+ C_t = 36, 34	+
2	-	-	-	+ C_t = 38, 37	-
107	-	-	-	-	-

B. parapertussis 2 pos / 119 neg

N	FilmArray® 1.7	FilmArray® 2*plus*	Simplexa™	LDT	Final result
1	n.a.		+	+	+
1		+	+	+	+
61	n.a.		-	-	-
58		-	-	-	-

Table 2. Performance characteristics of assays.

	B. pertussis				***B. parapertussis***		
%	FA® 1.7 + 2*plus*	Simplexa™	illumigene®	LDT	FA® 2*plus*	LDT	Simplexa™
Sens	83.3	100	83.3	100	100	100	100
Spec	100	100	100	98.2	100	100	100
PPV	100	100	100	84.6	100	100	100
NPV	98.2	100	98.2	100	100	100	100

Retrospective Part

All 35 LDT-positive frozen specimens were also positive by Simplexa™ with C_t-values of 19-37 for *B. pertussis* (N=30) and 17-28 for *B. parapertussis* (N=5). Neither inhibitions nor unresolved results were observed.

Table 3. Results obtained with Simplexa™ for *Bp* and *Bpp*.

B. pertussis		***B. parapertussis***	
N	Simplexa™ IS*481*	N	Simplexa™ IS*1001*
30	+	5	+

Prevalence of *B. holmesii*

Of the 223 frozen specimens analysed with the BioGX assay on BD MAX™ System, 175 (78.5%) were positive. Among these, *B. holmesii* was found in two cases (C_t=20.4, C_t=36.8) indicating a very low prevalence (1.2%) of this organism in our study group. Both positive *B. holmesii* results were confirmed in an independent laboratory by LDT (C_t=20.5, C_t=27).

Conclusions

- Among the commercial systems tested, Simplexa™ was the most sensitive one (100%). It also detected *Bordetella pertussis* DNA in all 30 retrospectively analyzed specimens.
- Cross-reactivity with *Bordetella holmesii* might be an issue for Simplexa™ and *illumigene*® (multi-copy target IS*481*) but not for FilmArray® (single-copy target *ptx*).
- However, the very low prevalence of *Bordetella holmesii* confirms that more specific tests – at the expense of reduced sensitivity – are currently not needed.

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