R-Biopharm AG

Evaluation of the RIDA®GENE Bordetella real-time PCR assay for the laboratory diagnosis of Bordetella infections

L.Kastl[1], *A. Simons*[1], *A. Veldenzer*[2], *U. Eigner*[2], *M. Holfelder*[2]
[1] R-Biopharm AG, Darmstadt, Germany
[2] Limbach Laboratory, Department of Microbiology, Heidelberg, Germany

Objectives

Bordetella pertussis is the major cause of pertussis (whooping cough). Pertussis can cause a serious illness in people of all age groups, which can be life-threatening particular in infants. Other species, including *Bordetella holmesii* and *Bordetella parapertussis* cause a milder whooping cough-like illness, however it is estimated that 3 - 35 % of the Bordetella infections are caused by *B. parapertussis* and also *B. holmesii* is frequently detected upon Bordetella infections.[1,2,3] Transmission of pertussis still occurs frequently because protection from vaccination lasts for 5 - 10 years and protection after natural infection wanes after 10 to 15 years. In addition, pertussis vaccination is described to lack cross-protection against *Bordetella holmesii*.[4]

After the incubation time, the clinical course of a Bordetella infection can be divided into three stages: catarrhal stage (1 - 2 weeks), paroxysmal stage (1 - 2 weeks) and convalescent stage (6 - 10 weeks).[5] Whereas culture is only appropriate in the first two weeks and serological diagnosis at earliest 2 weeks after infection, real-time PCR allows rapid and sensitive detection in the first four weeks after cough onset. This study aimed to evaluate the performance of the RIDA®GENE Bordetella assay for the direct detection of Bordetella infections. The results were compared to the GenoQuick® Bordetella assay (Hain Lifescience, Nehren, Germany).

Methods

The RIDA®GENE Bordetella multiplex real-time PCR assay is a qualitative assay using fluorogenic target-specific hydrolysis probes for the differential detection of *Bordetella pertussis*, *Bordetella parapertussis* and *Bordetella holmesii* (Table 1). The GenoQuick® Bordetella assay allows direct detection and differentiation of Bordetella pertussis and Bordetella parapertussis.

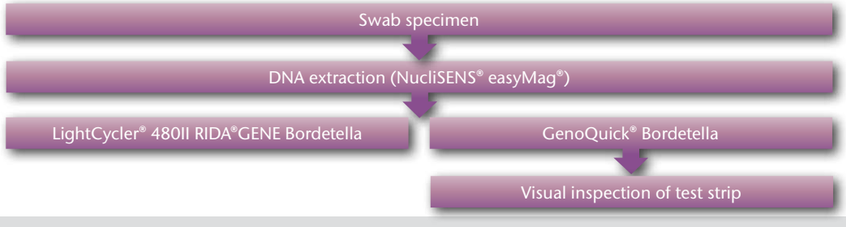
212 swab specimens from patients with symptoms of a bacterial respiratory infection were isolated with the NucliSENS® easyMag® automated extraction platform (bioMérieux). DNA was analysed with the RIDA®GENE Bordetella assay on the LightCycler® 480II (Roche) and by visual inspection of the GenoQuick test strip according to the manufacturer's instructions (Figure 1).

Table 1 Analyte target gene and detection channel overview

Analyte	Target Gene	Detection Channel Light® Cycler 480II
B. pertussis/B. holmesii	IS481	465/510
B. parapertussis	IS1001	533/610
B. holmesii	IS1001	618/660
ICD	Synthetic target DNA sequence	533/580

Picture 1 RIDA®GENE Bordetella multiplex real-time PCR

Figure 1 Study design



Results

Of the 212 patient samples, 210 were concordant (99 %). 187 samples were negative for a Bordetella infection.

Both tests identified 22 positive samples for *Bordetella pertussis* (10.43 %) and 1 positive sample for *Bordetella parapertussis* (0.47 %). There was a discrepancy in the result for 2 samples between both assays (Figure 2). Those samples were weak positive for *B. parapertussis* with the GenoQuick® Bordetella assay and were not detected by the RIDA®GENE Bordetella multiplex real-time PCR assay. The discrepant samples were sent to the national reference center which stated those 2 samples as negative for *Bordetella spp.*, confirming the result obtained by the RIDA®GENE Bordetella multiplex real-time PCR assay.

Figure 2 RIDA®GENE Bordetella *vs.* Geno®Quick Bordetella

		GenoQuick® Bordetella +	GenoQuick® Bordetella -	Total	
RIDA®GENE Bordetella	+	23	0	23	Pos. agreement 92 %
	-	2*	187	189	Neg. agreement 100 %
	Total	25	187	212	Overall agreement 99 %

*two discrepant samples were sent to the national reference center for Bordetella, Germany. Both samples were tested negative for a Bordetella infection

Figure 3 Example of *B. pertussis/B. holmesii* run on the LightCycler® 480II

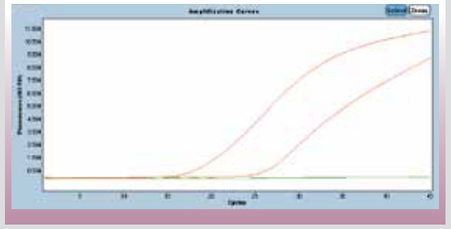


Figure 4 Example of *B. parapertussis* run on the LightCycler® 480II

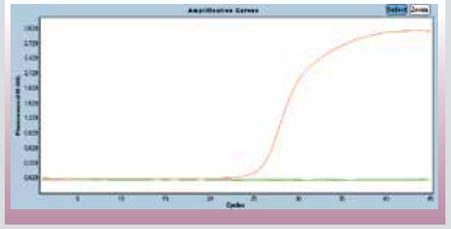
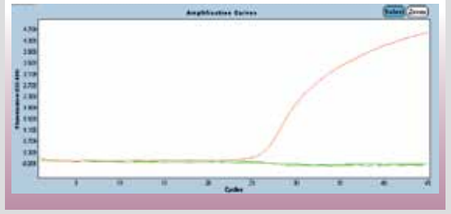


Figure 5 Example of *B. holmesii* run on the LightCycler® 480II



Acknowledgment
We thank Dr. Marion Riffelmann and Dr. Nicole Kennerknecht from the German consulting laboratory for Bordetella pertussis, Krefeld for analysis of discrepant results.

References:

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Conclusion

The RIDA®GENE Bordetella assay is a highly sensitive and specific multiplex real-time PCR assay for diagnosis of *Bordetella pertussis*, *Bordetella parapertussis* and *Bordetella holmesii*.

The RIDA®GENE Bordetella multiplex real-time PCR assay allows detection and differentiation of *B. pertussis*, *B. parapertussis* and *B. holmesii*.

The real-time PCR format reduces the time to result to 2 hours. The RIDA®GENE Bordetella real-time PCR kit contains an internal control DNA that can be used as an amplification control or additionally as an extraction control.

R-Biopharm AG
An der neuen Bergstraße 17, 64297 Darmstadt, Germany
Phone: +49 (0) 61 51 - 81 02-0, Fax: +49 (0) 61 51 - 81 02-40
www.r-biopharm.com, E-mail: info@r-biopharm.de