Evaluation of RealStar® Bordetella PCR Kit 1.0 for qualitative detection and differentiation of Bordetella pertussis and Bordetella parapertussis specific DNA in respiratory samples

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Summary

This study aimed to compare the qualitative performance of 2 commercially available real-time PCR kits for detection of Bordetella in routine diagnostics. Respiratory samples from 50 patients were collected and tested with both PCR kits. Additionally, the QCMD Bordetella DNA EQA Programme samples from 2016 were tested in parallel. Both real-time PCR kits demonstrated high agreement in results in detecting Bordetella DNA in respiratory samples and the QCMD EQA Programme samples.

Introduction

Pertussis is an acute respiratory illness caused by the Bordetella pertussis (BP) bacterium. The majority of human illness is caused by Bordetella pertussis, and some is caused by Bordetella parapertussis. Bordetella is a gram-negative, pleomorphic, aerobic coccobacillus[1].

Initial symptoms are usually relatively mild, and can include cough, sneezing, coryza, and slight fever. The disease then progresses to produce more severe, paroxysmal coughing fits. During these episodes, the characteristic "whooping" may be heard as the patient tries to inhale through narrowed upper airways and the larynx. Severe symptoms like prolonged strong cough, attended by vomiting, cyanosis, and apnoea can occur[2].

For infants and young children, complications from pertussis may be life-threatening, and hospitalization may be warranted[2].

The goal of this study was the evaluation of the performance of RealStar® Bordetella PCR PCR Kit 1.0 (altona Diagnostics, Germany) for the qualitative detection and differentiation of Bordetella pertussis and Bordetella parapertussis specific DNA in respiratory samples. The evaluation is based on the comparison of performance data of RealStar® Bordetella PCR Kit 1.0 and RIDA® GENE Bordetella real-time PCR Kit (r-biopharm).

Material and Methods

An initial comparison in qualitative detection of 12 Bordetella pertussis QCMD panel samples (QAB094132 QCMD 2016 Bordetella pertussis DNA Programme) by RealStar® Bordetella PCR Kit 1.0 and RIDA® GENE Bordetella real-time PCR Kit was used to show general comparability in sensitivity and specificity of both assays.

After this 50 human clinical nasopharyngeal swabs were processed by NucliSENS® easyMAG® system using generic protocol for DNA and RNA extraction (BioMeriéux). For this purpose retrospective positive and negative tested Bordetella routine samples were included to this study. All sample eluates were analyzed in parallel with RealStar® Bordetella PCR Kit 1.0 and RIDA® GENE Bordetella PCR real-time PCR Kit on LightCycler 480 instrument II (Roche) according to the manufacturer's instructions. The RIDA® GENE Bordetella PCR real-time PCR Kit detects and differentiates B. pertussis, B. parapertussis and B. holmesii, whereas the RealStar® Bordetella PCR Kit 1.0 detects and differentiates B. pertussis and B. parapertussis.

Results and Discussion

Comparison of the 2 commercial real-time PCR Kits by running QCMD proficiency panel sample material (2016 Bodetella pertussis DNA EQA Programme)

This test will give information of general comparability of assays. All samples not indicated as educational have to show same results to ensure suitability of assays for detection of Bordetella from human sample material.

Table 1: Results of QAB094132 QCMD 2016 Bordetella pertussis DNA EQA Programme

QCMD BPDNA16 samples	Bordetella Type	RIDA®GENE Bordetella real-time PCR Kit			RealStar® Bordetella PCR Kit 1.0	
		B.pert./ B.holm.	*B. holm.*	*B. parapert.*	*B. pert./ B.holm*	*B. parapert.*
sample 01 *core*	*B. pertussis*	ct 32	-	-	ct 31	-
sample 02 *	*B. bronchoseptica (IS481+)*	-	-	-	ct 40	-
sample 03 *core*	*B. pertussis*	ct 30	-	-	ct 29	-
sample 04 *core*	*B. parapertussis*	-	-	ct 33	-	ct 38
sample 05 *	*B. pertussis*	ct 33	-	-	ct 33	-
sample 06 *	*B. holmesii (IS481+)*	ct 32	ct 28	-	ct 31	-
sample 07 *core*	-	-	-	-	-	-
sample 08 *core*	*H. influenzae*	-	-	-	-	-
sample 09 *	*B. pertussis*	ct 32	-	-	ct 30	-
sample 10 *core*	*B. pertussis*	ct 29	-	-	ct 27	-
sample 11 *core*	*B. pertussis*	ct 27	-	-	ct 27	-
sample 12 *core*	*B. pertussis*	ct 25	-	-	ct 24	-

Samples are marked by * are defined as "educational" by QCMD. Bacterial load is low.

Core proficiency samples are reviewed by the QCMD Scientific Expert(s) and are expected to be reported correctly within the EQA challenge / distribution.

All core samples are detected correctly.

Only one sample (sample 02, marked as educational) showed discordant results between the 2 assays.

One sample (sample 06, marked as educational) demonstrated the correct differentiation between B. pertussis and B. holmesii by RIDA®GENE Bordetella PCR Kit.

In general the results show that a study based on comparison of results of Bordetella in respiratory samples done with RIDA®GENE Bordetella PCR Kit and RealStar® Bordetella PCR Kit 1.0 is reasonable since both assays are showing correct results for all "core" samples and a comparable sensitivity in the "educational" samples of external proficiency panel sample material.

Retrospective analysis of respiratory samples previously tested positive or negative for Bordetella

50 respiratory samples were tested with RIDA®GENE Bordetella real-time PCR Kit and RealStar® Bordetella PCR Kit 1.0

Table 2: Results shown in fourfold table. Total number of tested samples: 50

total number of tested samples: 50	RealStar® Bordetella PCR Kit 1.0 **positive result**	RealStar® Bordetella PCR Kit **negative result**
RIDA®GENE Bordetella real-time PCR Kit **positive result**	**17**	**3**
RIDA®GENE Bordetella real-time PCR Kit **negative result**	**3**	**27**

17 out of 50 samples were detected positive by both assays, 27 out of 50 samples were detected negative by both assays.

3 samples were detected positive by RealStar® Bordetella PCR Kit 1.0 only, whereas also 3 samples were detected positive by RIDA®GENE Bordetella real-time PCR Kit only.

In total 6 samples out of 50 samples stayed discordant. 5 out of these 6 samples do show ct values higher than 36, which indicates very low pathogen load.

Table 3: Discordant Samples

		RIDA®GENE Bordetella real-time PCR Kit				RealStar® Bordetella PCR Kit 1.0		
		B.pert./holm.	B. holm.	B. parapert.	IC	B.pert./holm.	B. parapert.	IC
22	82-1	-	-	ct 36	+	-	-	+
35	86-1	-	-	-	+	ct 36	-	+
41	89-1	-	-	ct 36	+	-	-	+
42	89-4	-	-	ct 31	+	-	-	+
43	89-5	-	-	-	+	ct 36	ct 36	+
45	91-3	-	-	-	+	ct 38	-	+

Conclusion

The QCMD 2016 Bordetella pertussis DNA EQA Programme results of both assays indicate a comparable sensitivity of RIDA®GENE Bordetella real-time PCR Kit and RealStar® Bordetella PCR Kit 1.0. Under this consideration, all 6 discordant routine samples can be discussed as weak positive samples which are close or below the sensitivity of both assays. Sequencing for confirmation will not be possible due to low DNA content.

The overall performance of the RIDA®GENE Bordetella real-time PCR Kit in comparison to RealStar® Bordetella PCR Kit 1.0 can be considered as to be equal.

References

[1] Bordetella pertussis. Nieves DJ, Heininger U.: Microbiol Spectr. 2016 Jun; 4(3). doi: 10.1128/microbiolspec.EI10-0008-2015.

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