RIDA®GENE Bordetella

REF PG2505

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1. Intended use

For *in vitro* diagnostic use. RIDA®GENE Bordetella is a real-time multiplex PCR for the direct, qualitative detection and differentiation of the *Bordetella pertussis, Bordetella parapertussis* and *Bordetella holmesii* from human nasopharyngeal swabs and washes. The RIDA®GENE Bordetella real-time multiplex PCR is intended for use as an aid in diagnosis of whooping cough caused by *Bordetella pertussis*, *Bordetella parapertussis* and *Bordetella holmesii*, respectively.

2. Summary and Explanation of the test

Bordetella pertussis is a gram-negative bacteria that cause an acute respiratory infection called pertussis or whooping cough. *Bordetella holmesii*, *Bordetella parapertussis* and *Bordetella bronchiseptica* cause less common a whooping cough–like illness that is in general milder. Pertussis can cause a serious illness in people of all age groups, which can be life-threatening particular in infants. The WHO (World Health Organization) estimates about 16 million cases of pertussis worldwide in 2008, resulting in about 195,000 children deaths from the disease.[1] In 2012, 41,800 cases of pertussis were reported in the U.S.[2] It is estimated that 3-35 % of the *Bordetella* infections are caused by *B. parapertussis*.[3,4] *B. parapertussis* was identified during a 3 year study in 14 % of the samples as cause of pertussis illness in the US.[5] In a French study *B. holmesii* was detected in about 20 % of the *Bordetella* infections in adolescents and adults.[6]
Transmission of *Bordetella* infection occurs by the respiratory route with airborne droplets. After incubation period of 7–10 days the clinical course of the illness proceeds through three stages: catarrhal stage (1-2 weeks) with common cold symptoms, paroxysmal stage (1-2 weeks) characterized by paroxysms of numerous, rapid coughs followed by vomiting and convalescent stage (6-10 weeks) characterized by disappear of the paroxysmal coughs.[7,8]
In populations with high vaccination coverage of infants and children the transmission of pertussis still occurs, because protection from vaccination lasts for 5-10 years and protection after natural infection wanes after 10 to 15 years.
Thus, in highly vaccinated populations transmission of the disease occurs from adolescent and adults to infants or among people with no more vaccination protection.[8,9] A recent study suggests lack of cross-protection against *B. holmesii* after pertussis vaccination.[10]
Several laboratory methods, including culture, serology and PCR, are available for diagnosis of pertussis. Culture is almost 100 % specific and can be as high as 50 % in the first 2 weeks after cough onset, which further declines with time. Culture requires special media and takes at least one week. Pertussis serology diagnosis is inappropriate in the early stage of the disease.
Antibodies can be detected at earliest two weeks after infection or vaccination. ELISA assay with either pertussis toxin (PT) or filamentous hemagglutinin (FHA) as a test antigen can be used. FHA is formed by all *Bordetella* species, while PT is formed

only by *B. pertussis*. PT is contained in all vaccines, while FHA is component of many acellular pertussis vaccines. With a serological assay the immune responses against infection and vaccination cannot be distinguished. Real-time PCR allows a rapid, sensitive and specific detection in the first 4 weeks after cough onset.
In addition real-time PCR allows the differentiation of the pathogenic *Bordetella* species.[8,9]

3. Test principle

RIDA®GENE Bordetella is a real-time multiplex PCR for the direct, qualitative detection and differentiation of *Bordetella pertussis*, *Bordetella parapertussis* and *Bordetella holmesii* (*IS481, IS1001*) from nasopharyngeal swabs and washes. Following DNA isolation, the specific gene fragments for *Bordetella pertussis* (IS481), *Bordetella parapertussis* (pIS1001) and *Bordetella holmesii* (IS481, hIS1001) are amplified (if present). The amplified targets are detected with hydrolysis probes, which are labeled at one end with a quencher and at the other end with a fluorescent reporter dye (fluorophore). In the presence of a target the probes hybridize to the amplicons. During the extension step the Taq-polymerase breaks the reporter-quencher proximity. The reporter emits a fluorescent signal, which is detected by the optical unit of a real-time PCR instrument. The fluorescence signal increases with the amount of formed amplicons. The RIDA®GENE Bordetella assay contains an Internal Control DNA (ICD) as an internal control of sample preparation procedure and/or to determine possible PCR-inhibition.

4. Reagents provided

Tab. 1: Reagents provided (Reagents provided in the kit are sufficient for 100 determinations)

Kit Code	Reagent	Amount		Lid Color
1	Reaction Mix	2x	1050 µl	yellow
2	Taq-Polymerase	1x	80 µl	red
D	Internal Control DNA	2x	1700 µl	orange
N	No Template Control	1x	450 µl	white
P	Positive Contro l	1x	200 µl	blue

5. Storage instructions

- Protect all reagents from light and store at -20 °C. All reagents can be used until the expiration date. After expiry the quality guarantee is no longer valid.
- Carefully thaw reagents before using (e.g. in a refrigerator at 2 – 8 °C).
- Reagents can sustain up to 20 freeze/thaw cycles without influencing the assay performance (e.g. after the first thawing separate it in aliquots and freeze immediately).
- During PCR preparation all the reagents should be stored cold in an appropriate way (2 – 8 °C).

6. Additional necessary reagents and necessary equipment

The RIDA®GENE Bordetella real-time PCR Assay is suitable for use with following extraction platforms and real-time PCR instruments:

Tab. 2: Necessary equipment

Extraction platform	
R-Biopharm	RIDA® Xtract
Promega	Maxwell® RSC
bioMérieux	NucliSENS easy®MAG™
Real-time PCR instrument:	
Roche	LightCycler® 480II, LightCycler® 480 z
Agilent Technologies	Mx3005P
Applied Biosystems	ABI 7500
Bio-Rad	CFX96™
QIAGEN	Rotor-Gene Q

Note: Only use 0.1 ml tubes on the Rotor-Gene Q (QIAGEN).

If you want to use other extraction platforms or real-time PCR instruments please contact R-Biopharm at mdx@r-biopharm.de.

- Sterile, media-free Nylon flocked swabs (e.g. Copan Diagnostic Inc., catalogue no. 552C)
- RIDA®GENE Color Compensation Kit IV (PG0004) for run the LightCycler® 480II and LightCycler® 480 z
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with a rotor for the reaction vials
- Vortexer
- Pipettes (0.5 – 20 µl, 20 – 200 µl, 100 – 1000 µl)
- Filter tips
- Powder-free disposal gloves
- PCR water (nuclease-free water)

7. Precautions for users

For *in-vitro* diagnostic use.
This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories have to be followed. The instruction manual for the test procedure has to be followed. Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes. During handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure. Do not smoke, eat or drink in areas where samples or reagents are being used.

- Extraction, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- Samples must be treated as potentially infectious as well as all reagents and materials being exposed to the samples and have to be handled according to the national safety regulations.
- Do not use the kit after the expiration date.

All reagents and materials used have to be disposed properly after use. Please refer to the relevant national regulations for disposal.

For more details see Safety Data Sheets (SDS) at www.r-biopharm.com.

8. Collection and storage of samples

8.1 Sample preparation from Nasopharyngeal Swabs

For DNA isolation from dry swabs, use a commercially available DNA isolation kit (e.g. RIDA® Xtract (R-Biopharm)) or DNA extraction system (e.g. Maxwell® RSC (Promega)). Extract DNA according to the manufacturer's instructions.

For DNA isolation from nasopharyngeal swabs the following procedure is recommended: Add 400 µl PCR water into a preparation tube. Insert the swab into the water and cut or break the swab stem. Cap the preparation tube tightly, vortex shortly and continue according to manufacturer's instruction of the DNA-extraction kit or DNA extraction system.

The RIDA®GENE Bordetella assay contains an Internal Control DNA that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient. The Internal Control DNA can either be used as PCR inhibition control or as extraction control for the sample preparation procedure and as PCR inhibition control.

If the Internal Control DNA is used only as a PCR inhibition control, 1 µl of the Internal Control DNA should be added to the Master- Mix (see Tab.4).

If the Internal Control DNA is used as an extraction control for the sample preparation procedure and as PCR inhibition control, 20 µl of Internal Control DNA has to be added during extraction procedure. The Internal Control

DNA should always be added to the specimen-lysis buffer mixture and must **not** be added directly to the specimen. We also recommend to add 1 µl of the Internal Control DNA to the negative control and positive control PCR Mix.

8.2 Sample preparation from Nasopharyngeal Washes

For DNA isolation from nasopharyngeal washes add the appropriate volume according to manufacturer's instruction of the DNA-extraction kit or DNA-extraction system.

For DNA isolation from dry swabs, use a commercially available DNA isolation kit (e.g. RIDA® Xtract (R-Biopharm)) or DNA extraction system (e.g. Maxwell® RSC (Promega)). Extract DNA according to the manufacturer's instructions.

The RIDA®GENE Bordetella assay contains an Internal Control DNA that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient. The Internal Control DNA can either be used as PCR inhibition control or as extraction control for the sample preparation procedure and as PCR inhibition control.

If the Internal Control DNA is used only as a PCR inhibition control, 1 µl of the Internal Control DNA should be added to the Master- Mix (see Tab.4).

If the Internal Control DNA is used as an extraction control for the sample preparation procedure and as PCR inhibition control, 20 µl of the Internal Control DNA has to be added during extraction procedure. The Internal Control DNA should always be added to the specimen-lysis buffer mixture and must **not** be added directly to the specimen. We also recommend to add 1 µl of the Internal Control DNA to the negative control and positive control PCR Mix.

9. Test procedure

9.1 Master-Mix preparation

Calculate the total number of PCR reactions (sample and control reactions) needed. One positive control and negative control must be included in each assay run.

We recommend calculating an additional volume of 10% to compensate imprecise pipetting (see Tab. 3, Tab. 4). Thaw, mix gently and briefly centrifuge the Reaction Mix, the Taq-Polymerase, the Positive Control, the No Template Control and the Internal Control DNA before using. Keep reagents appropriately cold during working step (2 – 8 °C).

Tab. 3: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD as extraction and PCR inhibition control)

Kit code	Master-Mix components	Volume per reaction	10 reactions (10 % extra)
1	Reaction Mix	19.3 µl	212.3 µl
2	Taq-Polymerase	0.7 µl	7.7 µl
	Total	**20 µl**	**220 µl**

Mix the components of the Master-Mix gently and briefly spin down.

Tab. 4: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD only as PCR inhibition control)

Kit code	Master-Mix components	Volume per reaction	10 reactions (10 % extra)
1	Reaction Mix	19.3 µl	212.3 µl
2	Taq-Polymerase	0.7 µl	7.7 µl
D	Internal Control DNA	1.0 µl	11 µl
	Total	**21.0 µl**	**231.0 µl**

Mix the components of the Master-Mix gently and briefly spin down.

9.2 Preparation of the PCR-Mix

Pipette 20 µl of the Master-Mix in each reaction vial (tube or plate).

Negative control: Add 5 µl No Template Control to the pre-pipetted Master-Mix.

Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control DNA to the negative control PCR-Mix.

Sample: Add 5 µl DNA-Extract to the pre-pipetted Master-Mix.

Positive control: Add 5 µl Positive Control to the pre-pipetted Master-Mix.

Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control DNA to the positive control PCR-Mix.

Cover tubes or plate. Spin down and place in the real-time PCR instrument. The PCR reaction should be started according to the PCR instrument Set-up (see Tab. 5, Tab. 6, Tab. 7, Tab. 8).

9.3 PCR Instrument Set-up

9.3.1 DNA real-time PCR profile

Tab. 5: DNA real-time PCR profile for LightCycler® series and Rotor-Gene Q

Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	10 sec, 95 °C 15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

Tab. 6: DNA real-time PCR profile for Mx3005P, ABI7500, CFX96™

Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	15 sec, 95 °C 30 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

9.3.2 Universal real-time PCR profile

Note: the universal real-time PCR profile should only be used for DNA assays when combining RIDA®GENE DNA and RNA real-time PCR assays in one run.

Tab. 7: Universal real-time PCR profile for LightCycler® series

Reverse Transcription	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	10 sec, 95 °C 15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

Tab. 8: Universal real-time PCR profile for Mx3005P, ABI7500, Rotor-Gene Q and CFX96™

Reverse Transcription	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	15 sec, 95 °C 30 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

9.4 Detection channel set-up

Tab. 9: Selection of appropriate detection channels

Real-time PCR instrument	Detection	Detection Channel	Note
Roche LightCycler® 480II	*B. pertussis/B. holmesii*	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) Is required**
	ICD	533/580	
	B. holmesii	533/610	
	B. parapertussis	618/660	
Roche LightCycler® 480 z	*B. pertussis/B. holmesii*	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) Is required**
	ICD	540/580	
	B. holmesii	540/610	
	B. parapertussis	610/670	
ABI 7500	*B. pertussis/B. holmesii*	FAM	**Check that passive reference option ROX is none**
	ICD	VIC	
	B. holmesii	ROX	
	B. parapertussis	Cy5	
Agilent Techn. Mx3005P	*B. pertussis/B. holmesii*	FAM	**Check that reference dye is none**
	ICD	HEX	
	B. holmesii	ROX	
	B. parapertussis	Cy5	
Bio-Rad CFX96™	*B. pertussis/B. holmesii*	FAM	**-**
	ICD	VIC	
	B. holmesii	ROX	
	B. parapertussis	Cy5	
Qiagen Rotor-Gene Q	*B. pertussis/B. holmesii*	Green	**The gain settings have to be set to 5, according to the default settings**
	ICD	Yellow	
	B. holmesii	Orange	
	B. parapertussis	Red	

10. Quality control

The analysis of the samples is done by the software of the used real-time PCR instrument according to the manufacturer` s instruction. Positive and negative controls have to show correct results (see Tab. 10, Fig. 1, Fig. 2, Fig. 3) in order to determine a valid run.

The Positive Control for *B. pertussis, B. holmesii* and *B. parapertussis* has a concentration of 10^3 copies/µl. In each PCR run it is used in a total amount of 5×10^3 copies.

Tab. 10: For a valid run, the following conditions must be met:

Sample	Assay result	ICD Ct	Target gene Ct
Positive control	Positive	NA *[1]	See Quality Assurance Certificate
Negative control	Negative	Ct > 20	0

*[1] *No Ct value is required for the ICD to make a positive call for the positive control.*

If the positive control is not positive within the specified Ct range but the negative control is valid, prepare all new reactions including the controls.

If the negative control is not negative but the positive control is valid prepare all new reactions including the controls.

If the required criteria are not met, following items have to be checked before repeating the test:

- Expiry of the used reagents
- Functionality of the used instrumentation
- Correct performance of the test procedure

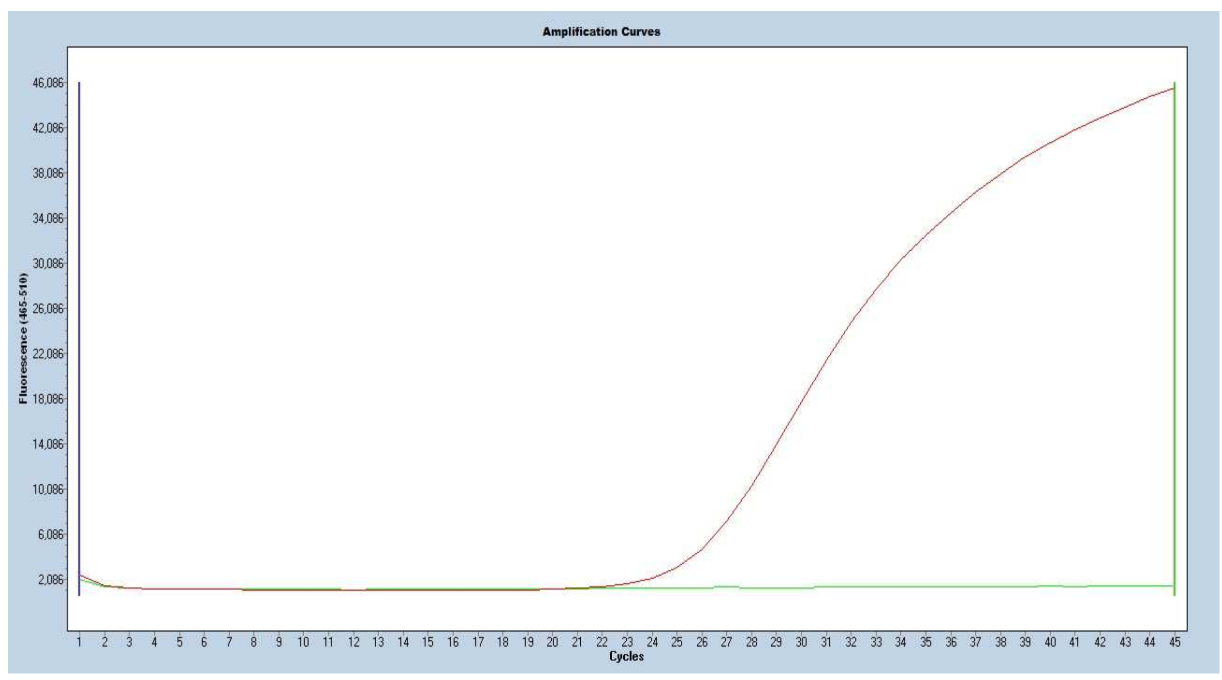

Fig. 1: Correct run of the positive and negative control (*Bordetella pertussis*) on the LightCycler® 480II

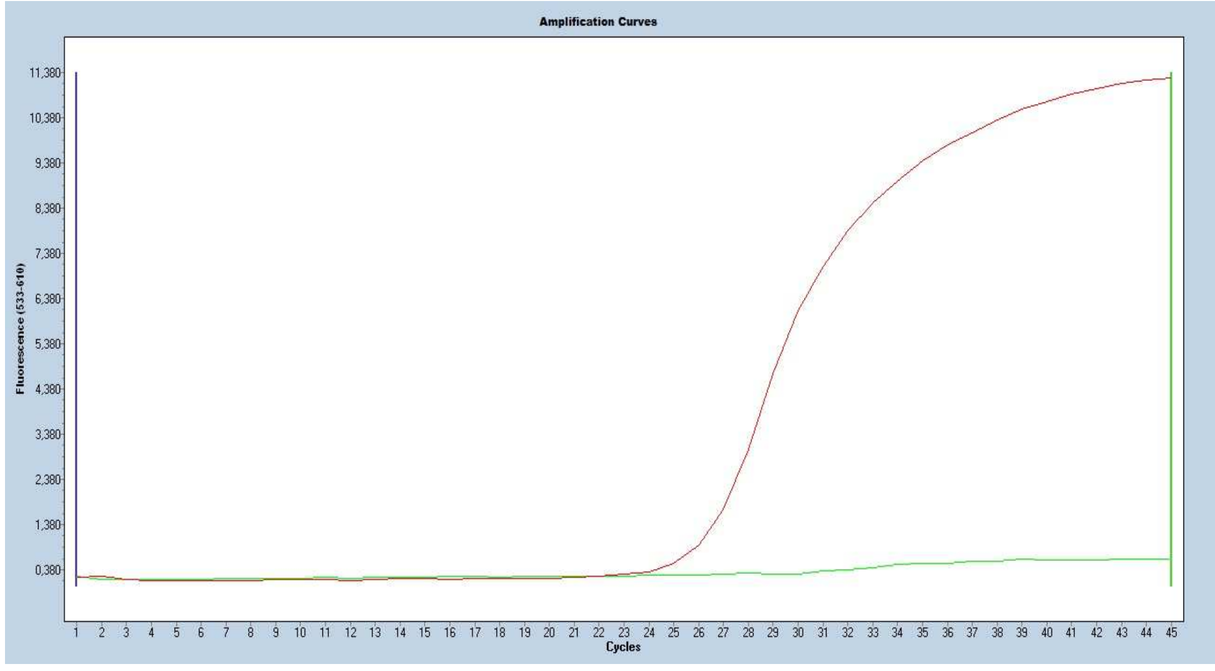

Fig. 2: Correct run of the positive and negative control (*Bordetella holmesii*) on the LightCycler® 480II

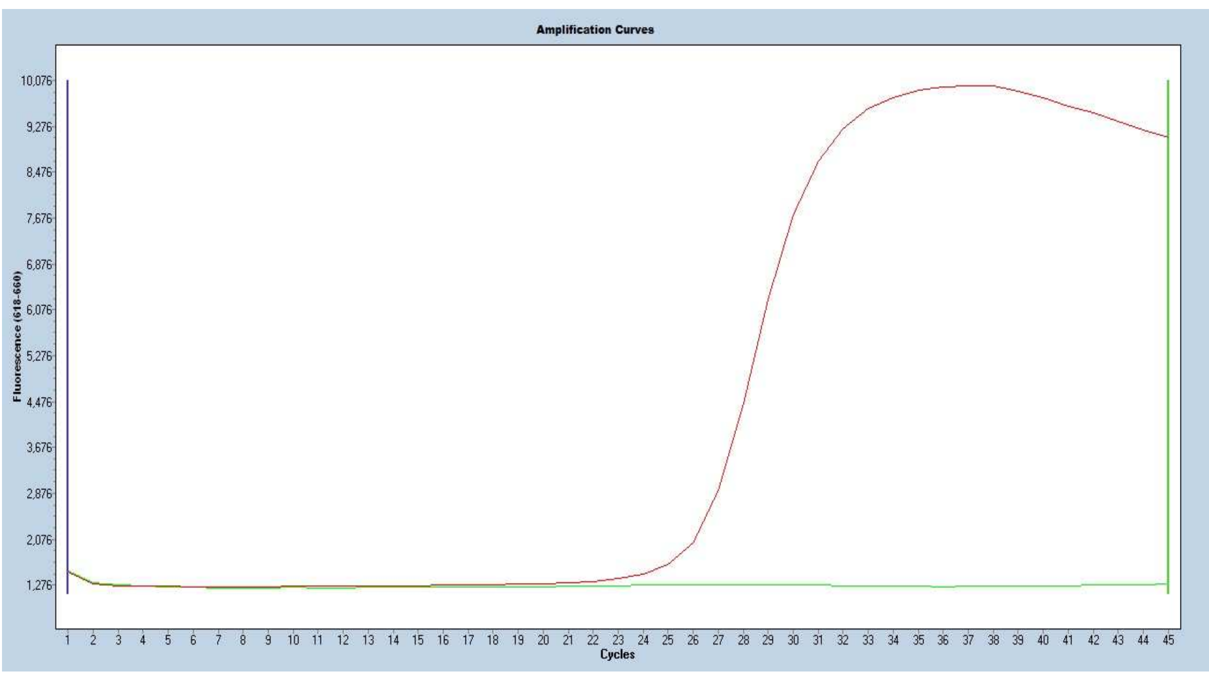

Fig. 3: Correct run of the positive and negative control (*Bordetella parapertussis*) on the LightCycler® 480II

11. Result interpretation

The result interpretation is done according to Table 11.

Tab.11: Sample interpretation

Target genes				
B. pertussis/ B. holmesii	***B. holmesii***	***B. parapertussis***	**ICD**	**Result**
positive	negative	negative	**positive/negative**	***B. pertussis* detected**
positive	**positive**	negative	**positive/negative**	***B. holmesii* detected***
negative	negative	**positive**	**positive/negative**	***B. parapertussis* detected**
negative	**positive**	negative	**positive/negative**	**Invalid**
negative	**positive**	**positive**	**positive/negative**	**Invalid**
positive	negative	**positive**	**positive/negative**	***B. pertussis* and *B. parapertussis* detected**
positive	**positive**	**positive**	**positive/negative**	***B. holmesii* and *B. parapertussis* detected**
negative	negative	negative	**positive**	**Target genes are not detected**
negative	negative	negative	negative	**Invalid**

Note: *see also point 8 in chapter 12: Limitations of the method

B. pertussis, B. parapertussis or *B. holmesii* is detected, if the sample DNA and the Internal Control DNA show an amplification signal in the detection system.

B. pertussis, B. parapertussis or *B. holmesii* is also detected, if the sample DNA shows an amplification signal but none for the Internal Control DNA in the detection system. The detection of the internal amplification control is not necessary because high concentrations of the amplicon can cause a weak or absent signal of the Internal Control DNA.

B. pertussis, B. parapertussis or *B. holmesii* is not detected, if the sample DNA shows no amplification signal, but an amplification signal for the Internal Control DNA in the detection system. An inhibition of the PCR reaction can be excluded by the detection of the Internal Control DNA.

A sample is invalid, if the sample DNA and Internal Control DNA show no amplification signal in the detection system. The sample contains a PCR inhibitor.

The extracted sample needs to be further diluted with PCR water (1:10) and re-amplified, or the isolation and purification of the sample has to be improved.

12. Limitations of the method

1. The result of molecular analysis should not lead to the diagnosis, but always be considered in the context of medical history and symptoms of the patient.
2. This assay is only validated for human nasopharyngeal swabs and washes.
3. Inappropriate specimen collection, transport, storage and processing or a pathogen load in the specimen below the analytical sensitivity can result in false negative results.
4. The presence of PCR inhibitors may cause invalid results.
5. Mutations or polymorphisms in primer or probe binding regions may affect detection of new variants resulting in a false negative result with the RIDA®GENE Bordetella assay.
6. As with all PCR based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection (LoD) may be detected, but results may not be reproducible.
7. A positive test result does not necessarily indicate the presence of viable organisms. However, a positive result is indicative for the presence of the target gene for *B. pertussis, B. parapertussis* and *B. holmesii,* respectively (IS481, IS1001).
8. In case of a positive signal for *B. pertussis* and *B. holmesii* a coinfection cannot be ruled out due to the detected target genes.

13. Performance characteristics

13.1 Analytical sensitivity

The RIDA®GENE Bordetella real-time multiplex PCR has a detection limit of ≥ 10 DNA copies per reaction for *B. pertussis, B. parapertussis* and *B. holmesii*, respectively.

The following figures 4, 5 and 6 show dilution series of *B. pertussis, B. parapertussis* and *B. holmesii* (each 10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II.

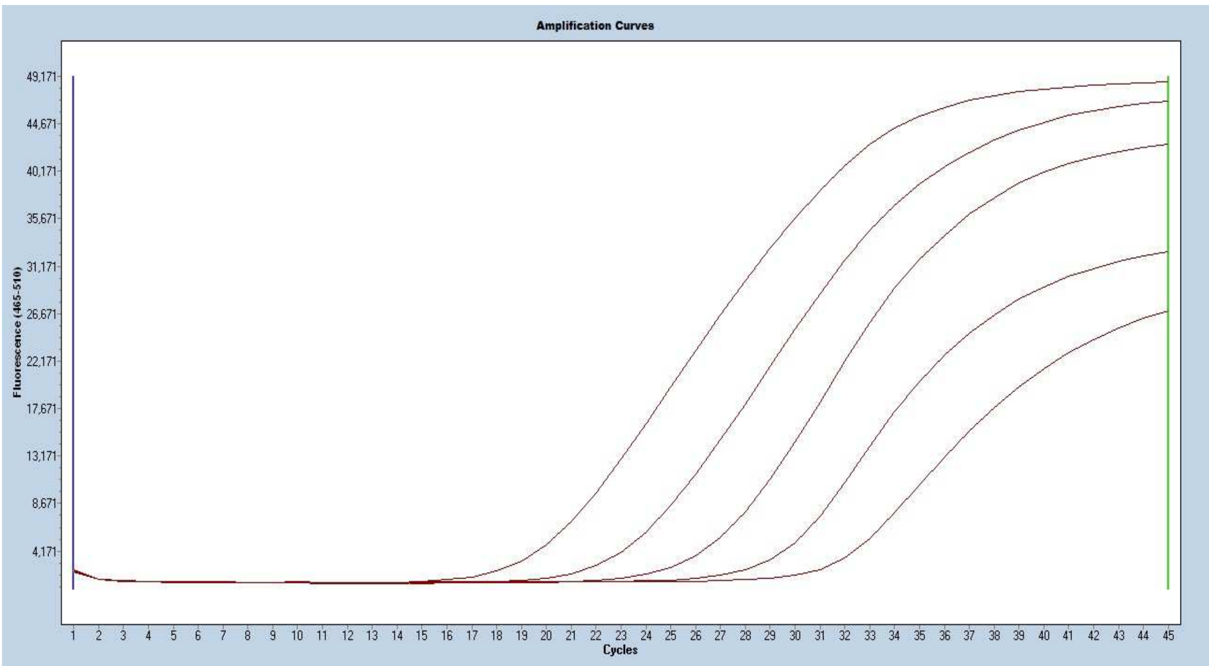

Fig. 4: Dilution series *Bordetella pertussis* (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

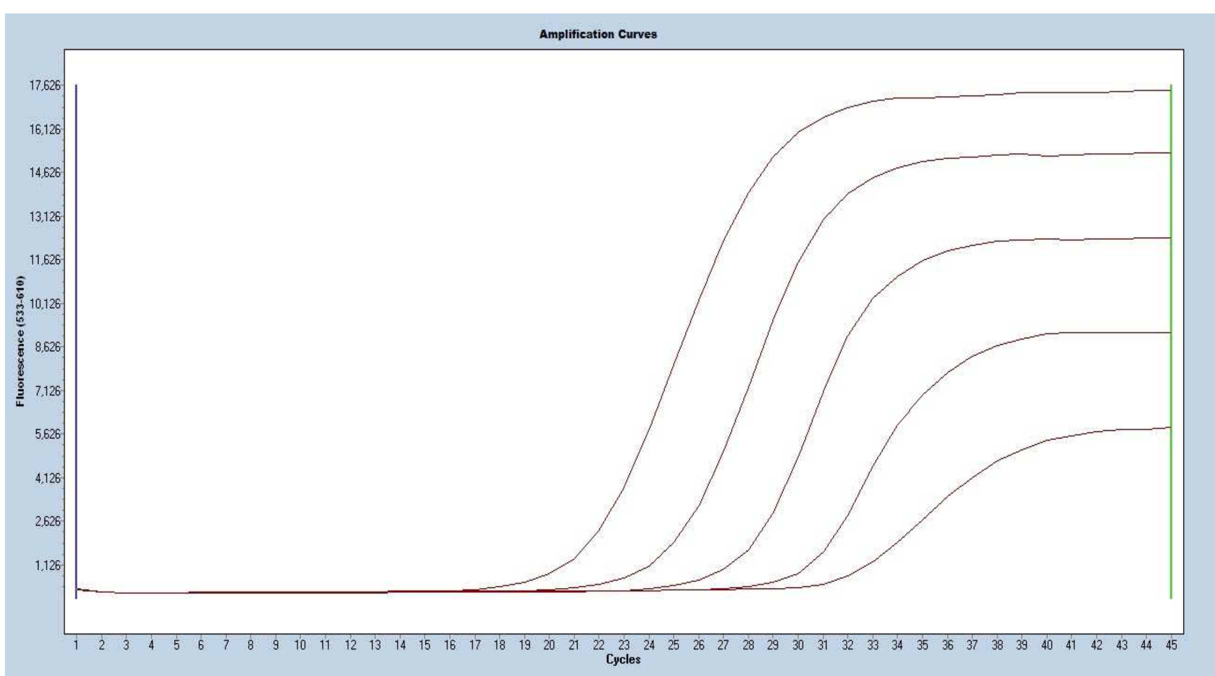

Fig. 5: Dilution series *Bordetella holmesii* (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

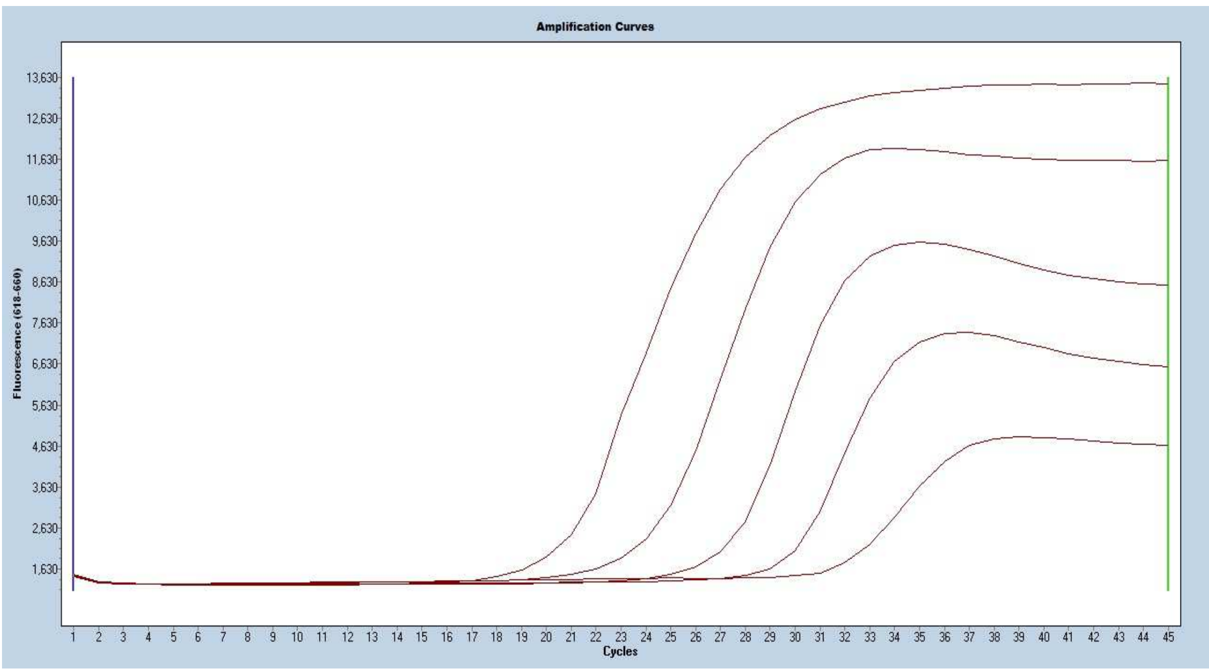

Fig. 6: Dilution series *Bordetella parapertussis* (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

The detection limit of the whole procedure depends on the sample matrix, DNA extraction and DNA concentration.

13.2 Analytical specificity

The RIDA®GENE Bordetella real-time multiplex PCR is specific for *Bordetella pertussis, Bordetella holmesii* and *Bordetella parapertussis* from nasopharyngeal swabs and washes. No cross-reaction could be detected for the following species (see Tab.12):

Tab. 12: Cross-reactivity testing

Acinetobacter baumannii	-	Coxsackie virus B4, human	-	Parainfluenza virus, serotype 3	-
Arcobacter butzleri	-	Cytomegalovirus, human	-	Parainfluenza virus 4b strain CH19503, human	-
Adenovirus 1, human, strain Adenoid 71	-	*E. coli* (O26:H-)	-	*Proteus vulgaris*	-
Adenovirus 7, human, strain Gomen	-	*E. coli* (O6)	-	*Pseudomonas aeruginosa*	-
Adenovirus 40, human, srain Dugan	-	*E. coli* (O157:H7)	-	Respiratory syncitial virus, human, strain Long	-
Adenovirus 41,human, strain Tak	-	*Enterobacter cloacae*	-	Respiratory syncitial virus, human, strain 9320	-
Aeromonas hydrophila	-	*Enterococcus faecalis*	-	Rhinovirus, human, Genogruppe A	-
Bacillus cereus	-	Epstein-Barr-Virus B95-8 strain	-	*Salmonella enteritidis*	-
Bacteroides fragilis	-	*Haemophilus influenzae* Rd	-	*Salmonella typhimurium*	-
Campylobacter coli	-	Herpes simplex virus 1 strain McIntyre	-	*Serratia liquefaciens*	-
Campylobacter fetus	-	Herpes simplex virus 2 strain MS	-	*Shigella flexneri*	-
Campylobacter lari	-	Influenza virus A/PR/8/34	-	*Staphylococcus aureus*	-
Campylobacter jejuni	-	*Klebsiella pneumoniae* strain MGH78578	-	*Staphylococcus epidermidis*	-
Campylobacter upsaliensis	-	*Klebsiella oxytoca*	-	*Staphylococcus haemolyticus SM131*	-
Candida albicans	-	*Legionella pneumophila* subsp. *pneumophila*	-	*Staphylococcus hominis* subsp. *novobiosepticus R22*	-
Citrobacter freundii	-	*Mycoplasma pneumoniae* Strain FH of Eaton Agent	-	Streptococcus pneumoniae strain NCTC 7465	-
Clostridium difficile	-	*Metapneumovirus, human*	-	Varicella Zoster Virus (Type B)	-
Clostridium perfringens	-	*Neisseria meningitides, strain* FAM18	-	*Vibrio parahaemolyticus*	-
Clostridium sordellii	-	Parainfluenza virus 1 strain C35, human	-	*Yersinia enterocolitica*	-
Coronavirus 229E, human	-	Parainfluenza virus 2 strain Greer, human	-		

14. Version history

Version number	Chapter and designation
2019-05-17	3. Test principle 6. Additional necessary reagents and necessary equipment 9.4 Detection channel set-up 11. Result interpretation 13.2 Analytical specificity

15. Explanation of symbols

General symbols

IVD	For *in vitro* diagnostic use
	Consult instructions for use
LOT	Lot number
	Expiry
	Store at
REF	Article number
Σ	Number of tests
	Date of manufacture
	Manufacturer

Testspecific symbols

Not applicable

16. Literature

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