altona DIAGNOSTICS

Instructions for Use

RealStar®
Bordetella PCR Kit 1.0

09/2022 EN

RealStar®

RealStar®

Bordetella PCR Kit 1.0

For use with

ABI Prism® 7500 Fast SDS (Applied Biosystems)

ABI Prism® 7500 SDS (Applied Biosystems)

CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

CFX96™ Real-Time PCR Detection System (Bio-Rad)

LightCycler® 480 Instrument II (Roche)

Mx 3005P™ QPCR System (Stratagene)

Rotor-Gene® 6000 (Corbett Research)

Rotor-Gene® Q5/6 plex Platform (QIAGEN)

VERSANT® kPCR Molecular System AD (Siemens Healthcare)

CE

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1. Intended Use

The RealStar® Bordetella PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection and differentiation of *Bordetella pertussis* and *Bordetella parapertussis* specific DNA.

2. Kit Components

Table 1: Kit components

Lid color	Component	Number of vials	Volume [µl/vial]
Blue	Master A	8	60
Purple	Master B	8	180
Green	Internal Control	1	1000
Red	Positive Control	1	250
White	Water (PCR grade)	1	500

3. Storage

- The RealStar® Bordetella PCR Kit 1.0 is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact altona Diagnostics GmbH for assistance.
- All components should be stored at -25 °C to -15 °C upon arrival.
- Repeated thawing and freezing of Master reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage at +2 °C to +8 °C should not exceed a period of 2 hours.
- Protect Master A and Master B from light.

4. Material and Devices required but not provided

- Appropriate real-time PCR instrument (see chapter 6.1 Real-Time PCR Instruments)
- Appropriate nucleic acid extraction system or kit (see chapter 8.1 Sample Preparation)
- Desktop centrifuge with a rotor for 2 ml reaction tubes
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Vortex mixer
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

NOTE

Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.

NOTE

It is highly recommended to use the 72-well rotor with the appropriate 0.1 ml reaction tubes, if using the Rotor-Gene® 6000 (Corbett Research) or the Rotor-Gene® Q 5/6 plex (QIAGEN).

5. Background Information

Bordetella pertussis and *Bordetella parapertussis* are the causative pathogens of whooping cough, a highly contagious, acute coughing illness in human [1, 2]. Other species of the genus *Bordetella* can also cause respiratory disease in humans. *Bordetella holmesii* was most recently associated with pertussis-like illness [3, 4] and *Bordetella bronchiseptica* infects a broad range of mammals, including humans, occasionally causing cough illnesses. Severe infections can occur in persons who are immunocompromised [5].

All *Bordetella* species that cause respiratory disease in humans carry transposable DNA elements, the so called insertion sequences (IS). These insertion sequences are usually present in multiple copies per genome (see Table 2), allowing the design of PCR systems that display a high sensitivity.

Table 2: *Bordetella* insertion sequences IS481 and IS1001, adapted from Loeffelholz [6]

Presence/no. of copies per genome[1]				
Insertion Sequence	*B. pertussis*	*B. parapertussis*	*B. holmesii*	*B. bronchiseptica*[2]
IS481	+/>50	-/NA	+/8-10	(+)[3]/ND
IS1001	-/NA	+/~20	-/NA	(+)[4]/1-7

[1] Symbols and abbreviations: +, present in all isolates; (+), present in some isolates; -, absent from all isolates; NA, not applicable; ND, not determined.

[2] Human-derived *B. bronchiseptica* isolates only.

[3] One of 73 human-derived isolates was positive.

[4] Four of 73 human-derived isolates were positive.

With more than 50 copies per genome [7], the insertion sequence IS481 is the favourable target for the detection of *Bordetella pertussis*. This target is also present in *Bordetella holmesii*, with copy numbers ranging from 8 to 10 copies per genome [7] and is found infrequently in *Bordetella bronchiseptica* strains [8].

The genome of *Bordetella parapertussis* carries approximately 20 copies of the insertion sequence IS1001, which facilitates a highly sensitive PCR detection, but is also found in some *Bordetella bronchiseptica* strains with copy numbers ranging from 1 to 7 copies per genome [7].

There are differences in the diagnostic needs of clinical versus public health settings. In the clinical setting, the goal is to optimise sensitivity (not to miss cases) while providing rapid results. This ensures appropriate treatment and prevents further transmission. In the public health setting, a high degree of specificity (in most countries a *B. pertussis* infection is reportable, but not an infection with other *Bordetella* species) is needed to avoid unnecessary and ineffective public health interventions [9].

In favour of highest sensitivity while dispensing highest specificity, the RealStar® Bordetella PCR Kit 1.0 targets the IS481 for the detection of *Bordetella pertussis* and the IS1001 for the detection of *Bordetella parapertussis*.

[1] Zhang X, Weyrich LS, Lavine JS, Karanikas AT, Harvill ET. Lack of cross-protection against *Bordetella holmesii* after pertussis vaccination. Emerg Infect Dis. 2012 Nov;18(11):1771-9.

[2] He Q, Viljanen MK, Arvilommi H, Aittanen B, Mertsola J. Whooping cough caused by *Bordetella pertussis* and *Bordetella parapertussis* in an immunized population. JAMA. 1998 Aug 19;280(7):635-7.

[3] Rodgers L, Martin SW, Cohn A, Budd J, Marcon M, Terranella A, Mandal S, Salamon D, Leber A, Tondella M-L, Tatti K, Spicer K, Emanuel A, Koch E, McGlone L, Pawloski L, LeMaile-Williams M, Tucker N, Iyer R, Clark TA, DiOrio M. Epidemiologic and laboratory features of a large outbreak of pertussis-like illnesses associated with cocirculating *Bordetella holmesii* and *Bordetella pertussis*—Ohio, 2010-2011. Clin. Infect. Dis. 2013 Feb; 56:322–331.

[4] Njamkepo E, Bonacorsi S, Debruyne M, Gibaud SA, Guillot S, Guiso N. Significant finding of *Bordetella holmesii* DNA in nasopharyngeal samples from French patients with suspected pertussis. J Clin Microbiol. 2011 Dec;49(12):4347-8.

[5] Mattoo S, Cherry JD. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to *Bordetella pertussis* and other *Bordetella subspecies*. Clin Microbiol Rev. 2005 Apr;18(2):326-82.

[6] Loeffelholz M. Towards Improved Accuracy of *Bordetella pertussis* Nucleic Acid Amplification Tests. J Clin Microbiol. 2012 Jul, 50(7):2186-2190.

[7] Reischl U, Lehn N, Sanden GN, Loeffelholz MJ. Real-time PCR assay targeting IS481 of *Bordetella pertussis* and molecular basis for detecting *Bordetella holmesii*. J Clin Microbiol. 2001 May;39(5):1963-6.

[8] Tatti KM, Sparks KN, Boney KO, Tondella ML. Novel multitarget real-time PCR assay for rapid detection of *Bordetella* species in clinical specimens. J Clin Microbiol. 2011 Dec;49(12).

[9] http://www.cdc.gov/pertussis/clinical/diagnostic-testing/index.html

6. Product Description

The RealStar® Bordetella PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection and differentiation of *Bordetella pertussis* and *Bordetella parapertussis* specific DNA.

The assay includes a heterologous amplification system (Internal Control) to identify possible PCR inhibition and to confirm the integrity of the reagents of the kit.

Real-time PCR technology utilizes polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labeled with fluorescent reporter and quencher dyes.

Probes specific for *Bordetella pertussis* (Target IS481) DNA are labeled with the fluorophore FAM™ whereas the probes specific for *Bordetella parapertussis* (Target IS1001) DNA are labeled with the fluorophore Cy5. The probe specific for Internal Control (IC) is labeled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of *Bordetella pertussis* (Target IS481) specific DNA and *Bordetella parapertussis* (Target IS1001) specific DNA as well as the detection of the Internal Control in corresponding detector channels of the real-time PCR instrument.

The test consists of two processes in a single tube assay:

- PCR amplification of target DNA and Internal Control
- Simultaneous detection of PCR amplicons by fluorescent dye labeled probes

The RealStar® Bordetella PCR Kit 1.0 consists of:

- Master A
- Master B
- Internal Control
- Positive Control
- Water (PCR grade)

Master A and Master B contain all components (PCR buffer, DNA polymerase, magnesium salt, primers and probes) to allow PCR mediated amplification and detection of *Bordetella pertussis* (Target IS481) specific DNA, *Bordetella parapertussis* (Target IS1001) specific DNA and the Internal Control in one reaction setup.

6.1 Real-Time PCR Instruments

The RealStar® Bordetella PCR Kit 1.0 was developed and validated to be used with the following real-time PCR instruments:

- ABI Prism® 7500 Fast SDS (Applied Biosystems)
- ABI Prism® 7500 SDS (Applied Biosystems)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- LightCycler® 480 Instrument II (Roche)
- Mx 3005P™ QPCR System (Stratagene)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- VERSANT® kPCR Molecular System AD (Siemens Healthcare)

7. Warnings and Precautions

Read the Instructions for Use carefully before using the product.

- Before first use check the product and its components for:
  - Integrity
  - Completeness with respect to number, type and filling (see chapter 2. Kit Components)
  - Correct labelling
  - Frozenness upon arrival
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimens and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.

- Use separated and segregated working areas for (i) sample preparation, (ii) reaction setup and (iii) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

8. Procedure

8.1 Sample Preparation

Extracted DNA is the starting material for the RealStar® Bordetella PCR Kit 1.0.

The quality of the extracted DNA has a profound impact on the performance of the entire test system. It is recommended to ensure that the system used for nucleic acid extraction is compatible with real-time PCR technology. The following kits and systems are suitable for nucleic acid extraction:

- QIAamp® DNA Mini Kit (QIAGEN)
- QIAsymphony® (QIAGEN)
- NucliSENS® easyMAG® (bioMérieux)

- MagNA Pure 96 System (Roche)
- m2000sp (Abbott)
- Maxwell® 16 IVD Instrument (Promega)
- VERSANT® kPCR Molecular System SP (Siemens Healthcare)

Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the nucleic acid extraction procedure for use with RealStar® Bordetella PCR Kit 1.0 has to be validated by the user.

If using a spin column-based sample preparation procedure including washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 1 min at approximately 17,000 x g (~ 13,000 rpm), using a new collection tube, prior to the elution of the nucleic acid.

CAUTION	
	***If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.***

CAUTION	
	***The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.***

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support (see chapter 14. Technical Assistance).

8.2 Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The RealStar® Bordetella PCR Kit 1.0 contains a heterologous Internal Control (IC), which can either be used as a PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a PCR inhibition control.

- If the IC is used as a PCR inhibition control, but not as a control for the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Internal Control	1 µl	12 µl
Volume Master Mix	**21 µl**	**252 µl**

- If the IC is used as a control for the sample preparation procedure and as a PCR inhibition control, add the IC during the nucleic acid extraction procedure.
- No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the specimen. The IC should always be added to the specimen/lysis buffer mixture. The volume of the IC which has to be added, always and only depends on the elution volume. It represents 10 % of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC per sample must be added into the specimen/lysis buffer mixture.
- If the IC was added during the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Volume Master Mix	**20 µl**	**240 µl**

CAUTION

If the IC (Internal Control) was added during the sample preparation procedure, at least the negative control must include the IC.

CAUTION

No matter which method/system is used for nucleic acid extraction, never add the IC directly to the specimen.

8.3 Reaction Setup

- ► Pipette 20 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- ► Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (Positive or Negative Control).

Reaction Setup	
Master Mix	20 µl
Sample or Control	10 µl
Total Volume	**30 µl**

- ► Make sure that each Positive Control and at least one Negative Control is used per Master Mix and run.
- ► Thoroughly mix the samples and controls with the Master Mix by pipetting up and down.
- ► Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.
- ► Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1,000 x g (~ 3,000 rpm).

9. Programming the Real-Time PCR Instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed programming instructions regarding the use of the RealStar® Bordetella PCR Kit 1.0 on specific real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

9.1 Settings

► Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default
Passive Reference	ROX™

9.2 Fluorescence Detectors (Dyes)

► Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
Bordetella pertussis specific DNA	Target IS481	FAM™	(None)
Bordetella parapertussis specific DNA	Target IS1001	Cy5	(None)
Internal Control	Internal Control	JOE™	(None)

9.3 Temperature Profile and Dye Acquisition

► Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature [°C]	Time [min:s]
Denaturation	Hold	1	-	95	02:00
Amplification	Cycling	45	-	95	00:15
			Yes	58	00:45
			-	72	00:15

10. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the RealStar® Bordetella PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

10.1 Validity of Diagnostic Test Runs

10.1.1 Valid Diagnostic Test Run (qualitative)

A **qualitative** diagnostic test run is **valid**, if the following control conditions are met:

Control ID	Detection Channel		
	FAM™	Cy5	JOE™
Positive Control [*Bordetella pertussis* and *Bordetella parapertussis*]	+	+	+/-*
Negative Control	-	-	+

* The presence or absence of a signal in the JOE™ channel is not relevant for the validity of the test run.

10.1.2 Invalid Diagnostic Test Run (qualitative)

A **qualitative** diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

10.2 Interpretation of Results

10.2.1 Qualitative Analysis

Detection Channel			Result Interpretation
FAM™	Cy5	JOE™	
+	+	+*	*Bordetella pertussis* and *Bordetella parapertussis* specific DNA detected.[1,2]
+	-	+*	*Bordetella pertussis* specific DNA detected.[1]
-	+	+*	*Bordetella parapertussis* specific DNA detected. [2]
-	-	+	Neither *Bordetella pertussis* nor *Bordetella parapertussis* specific DNA detected. The sample does not contain detectable amounts of *Bordetella pertussis* or *Bordetella parapertussis* specific DNA.
-	-	-	PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the Internal Control in the JOE™ detection channel is not required for positive results either in the FAM™ detection channel or in the Cy5 detection channel. A high *Bordetella pertussis* (Target IS481) and/or *Bordetella parapertussis* (Target IS1001) DNA load in the sample can lead to reduced or absent Internal Control signals.

[1] A positive signal in the FAM™ channel could also be due to the presence of *Bordetella holmesii* or *B. bronchiseptica* DNA in the sample.

[2] A positive signal in the Cy5 channel could also be due to the presence of *Bordetella bronchiseptica* DNA in the sample.

11. Performance Evaluation

11.1 Analytical Sensitivity

The analytical sensitivity of the RealStar® Bordetella PCR Kit 1.0 is defined as the concentration (copies/µl of the eluate) of *Bordetella pertussis* (Target IS481) or *Bordetella parapertussis* (Target IS1001) specific DNA molecules that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified *Bordetella pertussis* (Target IS481) DNA and *Bordetella parapertussis* (Target IS1001) DNA.

Table 3: PCR results used for the calculation of the analytical sensitivity with respect to the detection of *Bordetella pertussis* (Target IS481) specific DNA

Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]
31.600	18	18	100
10.000	18	18	100
3.160	18	18	100
1.000	18	18	100
0.316	18	14	78
0.100	18	8	44
0.032	18	8	44
0.010	18	0	0
0.003	18	0	0

Table 4: PCR results used for the calculation of the analytical sensitivity with respect to the detection of *Bordetella parapertussis* (Target IS1001) specific DNA

Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]
31.600	18	18	100
10.000	18	18	100
3.160	18	18	100
1.000	18	18	100
0.316	18	14	78
0.100	18	9	50
0.032	18	2	11
0.010	18	0	0
0.003	18	0	0

The analytical sensitivity of the RealStar® Bordetella PCR Kit 1.0 was determined by probit analysis:

- For the detection of *Bordetella pertussis* (Target IS481) specific DNA, the analytical sensitivity is 0.74 copies/µl [95% confidence interval (CI): 0.39 - 2.08 copies/µl]
- For the detection of *Bordetella parapertussis* (Target IS1001) specific DNA, the analytical sensitivity is 0.60 copies/µl [95% confidence interval (CI): 0.35 - 1.54 copies/µl]

11.2 Analytical Specificity

The analytical specificity of the RealStar® Bordetella PCR Kit 1.0 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that all relevant *Bordetella* genotypes will be detected.

The analytical specificity of the RealStar® Bordetella PCR Kit 1.0 was evaluated by testing a panel of genomic RNA/DNA extracted from bacteria related to *Bordetella pertussis* and *Bordetella parapertussis* and other pathogens causing similar symptoms as *Bordetella pertussis* and *Bordetella parapertussis*.

The RealStar® Bordetella PCR Kit 1.0 did not cross-react with any of the following pathogens:

- Human adenovirus 1
- Human adenovirus 4
- Enterovirus, Coxsackie A3
- Human metapneumovirus A2
- Human metapneumovirus B2
- Influenza A virus
- Influenza B virus
- Human parainfluenza virus 1
- Human parainfluenza virus 2
- Human parainfluenza virus 3
- Human parainfluenza virus 4a/b
- Human respiratory syncytial virus A
- Human respiratory syncytial virus B
- *Chlamydophila pneumoniae*
- *Chlamydophila psittaci*
- *Corynebacterium diphteriae*
- *Haemophilus influenzae*
- *Legionella pneumophila*
- *Moraxella catarrhalis*
- *Mycobacterium avium*
- *Mycoplasma pneumoniae*
- *Neisseria meningitidis*
- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*
- *Streptococcus pneumoniae*
- *Streptococcus pyogenes*
- *Bordetella petrii*
- *Bordetella trematum*
- *Bordetella hinzii*
- *Bordetella avium*
- *Bordetella bronchiseptica* IS481-

11.3 Precision

Precision of the RealStar® Bordetella PCR Kit 1.0 was determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots). Total variability was calculated by combining the 3 analyses.

The variability data are expressed in terms of standard deviation and coefficient of variation based on threshold cycle (C_t) values. At least 6 replicates per sample were analyzed for intra-assay variability, inter-assay and inter-lot variability.

Table 5: Precision data for the detection of *Bordetella pertussis* (Target IS481) and *Bordetella parapertussis* (Target IS1001) specific DNA

Bordetella pertussis **(Target IS481) and** ***Bordetella parapertussis*** **(Target IS1001)**		**Average Treshhold Cycle (C_t)**	**Standard Deviation**	**Coefficient of Variation [%]**
Intra-Assay Variability	Target IS481	30.84	0.12	0.40
	Target IS1001	30.44	0.14	0.46
Inter-Assay Variability	Target IS481	30.83	0.12	0.37
	Target IS1001	30.63	0.20	0.65
Inter-Lot Variability	Target IS481	30.76	0.12	0.38
	Target IS1001	30.45	0.10	0.34
Total Variability	Target IS481	30.79	0.12	0.40
	Target IS1001	30.56	0.20	0.65

Table 6: Precision data for the detection of the Internal Control

Internal Control	**Average Threshold Cycle (C_t)**	**Standard Deviation**	**Coefficient of Variation [%]**
Intra-Assay Variability	27.05	0.15	0.55
Inter-Assay Variability	26.71	0.16	0.61
Inter-Lot Variability	26.94	0.17	0.63
Total Variability	26.82	0.23	0.84

12. Limitations

- Strict compliance with the Instructions for Use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors (e.g. heparin) may cause false negative or invalid results.
- Potential mutations within the target regions of the *Bordetella pertussis* (Target IS481) and *Bordetella parapertussis* (Target IS1001) genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogens.
- As with any diagnostic test, results of the RealStar® Bordetella PCR Kit 1.0 need to be interpreted in consideration of all clinical and laboratory findings.
- Many *Bordetella* species carry transposable DNA elements, the so called insertion sequences (IS). Notably IS481 is present with high copy numbers in the genome of *Bordetella pertussis* and IS1001 occurs in the genome of *Bordetella parapertussis*. The transposable element IS481 is also found with medium copy numbers in the genome of *Bordetella holmesii* and with a very low incidence in the genome of some strains of *Bordetella bronchiseptica*. The transposable element IS1001 can also be present in low copy numbers in the genome of *Bordetella bronchiseptica*.

13. Quality Control

In accordance with the altona Diagnostics GmbH EN ISO 13485-certified Quality Management System, each lot of RealStar® Bordetella PCR Kit 1.0 is tested against predetermined specifications to ensure consistent product quality.

14. Technical Assistance

For customer support, please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**
phone: **+49-(0)40-5480676-0**

15. Literature

Versalovic, James, Carroll, Karen C., Funke, Guido, Jorgensen, James H., Landry, Marie Louise and David W. Warnock (ed). Manual of Clinical Microbiology. 10th Edition. ASM Press, 2011.

Cohen, Jonathan, Powderly, William G, and Steven M Opal. Infectious Diseases, Third Edition. Mosby, 2010.

16. Trademarks and Disclaimers

RealStar® (altona Diagnostics); ABI Prism® (Applied Biosystems); NucliSENS®, easyMAG® (bioMérieux); CFX96™ (Bio-Rad); FAM™, JOE™, ROX™ (Life Technologies); Maxwell® (Promega); Rotor-Gene®, QIAamp®, QIAsymphony® (QIAGEN); LightCycler® (Roche); VERSANT® (Siemens Healthcare); Mx 3005P™ (Stratagene).

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

The RealStar® Bordetella PCR Kit 1.0 is a CE-marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/EC.

Product not FDA cleared or approved.

Not available in all countries.

17. Explanation of Symbols

Symbol	Explanation
IVD	*In vitro* diagnostic medical device
LOT	Batch code
CAP	Cap color
REF	Catalogue number
CONT	Content
NUM	Number
COMP	Component
GTIN	Global trade item number
	Consult instructions for use
	Contains sufficient for “n” tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
	Caution: Highlights operating instructions or procedures which, of not followed correctly, may result in personal injury or impact product performance. Contact altona Diagnostics Technical Support for assistance.

Symbol	Explanation
i	Note: Information is given to the user that is useful but not essential to the task at hand.
	Version

Notes:

always a drop ahead.

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