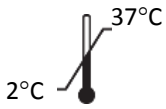
savyonDIAGNOSTICS
Member of the Gamida Diagnostics Division

savvy•gen B. pertussis

REF 613-01H / 613-01L

Test kit for 48 determinations



For Professional Use Only

CE IVD

Savyon® Diagnostics Ltd.
3 Habosem St. Ashdod 7761003
ISRAEL
Tel.: +(972).8.8562920
Fax: +(972).8.8523176
E-mail: support@savyondiagnostics.com

EC REP

European Authorized Representative: Obelis s.a.
Boulevard Général Wahis 53
1030 Brussels, BELGIUM
Tel: +(32) 2. 732.59.54
Fax: +(32) 2.732.60.03
E-Mail : mail@obelis.net

Intended Use

The Savvygen™ B .pertussis test allows the qualitative detection and differentiation of *Bordetella pertussis, Bordetella parapertussis and/or Bordetella holmesii* by real time PCR in respiratory samples from symptomatic patients.. The product is intended for use in the diagnosis of Bordetella alongside clinical data of the patient and other laboratory tests conclusions.

For *in-vitro* professional diagnostic use.

Background

Whooping Cough (Pertussis) is a highly contagious bacterial respiratory tract infection, caused *by Bordetella pertussis* Gram-negative bacilli, an exclusively human pathogen which can affect people of all ages. Transmission of the organism takes place through air droplets produced with cough or sneezes as a result of close direct contact with an infected person. Ninety percent of susceptible household contacts will develop clinically relevant disease following exposure. Despite extensive childhood immunization for several decades, pertussis remains one of the world's leading causes of vaccine preventable deaths. [1,2] The most severe disease occurs in unimmunized infants and young children who are the most vulnerable group with the highest rates of complications and death. The disease is usually milder in adolescents and adults who constitute a reservoir and are a source of spread to young children. [3]

Epidemiology: Pertussis is an endemic disease, but epidemics occur every 3– 5 years. In the USA, 5000–7000 cases are reported each year. It has been reported that 21% of the adults in the United States with prolonged cough (lasting >2 weeks) had pertussis. [4] Estimates from WHO suggest that in 2008 about 16 million cases of pertussis occurred worldwide, 95% of which were in developing countries, and that about 195,000 children died from this disease. [5] Since 2011, increases in the number of cases of pertussis have been repeatedly reported in different regions of the world, even in those with sustained high vaccination coverage. In Europe the situation evolves similarly with many countries observing an increment in cases, mostly in infants, adolescents and adults. [6]

Bordetella parapertussis is closely related to *Bordetella pertussis*. The diseases caused by the two organisms manifests with similar symptoms, but generally parapertussis is milder and of shorter duration than pertussis. Pertussis vaccination does not protect against infection by *B. parapertussis* due to the O-antigen this bacteria holds. This antigen protects *B. parapertussis* against antibodies specific to *B. pertussis*, so the bacteria are free to colonize the host's lungs without being subject to attack by previous antibodies. [7]

Bordetella holmesii**,** an emerging pathogen, which is mistakenly identified as *Bordetella pertussis* by PCR testing. It was reported that up to 29% of the patients diagnosed with pertussis were in fact *B. holmesii* infection. This misdiagnosis undermines the knowledge of pertussis' epidemiology, and may lead to misconceptions on pertussis vaccine's efficacy. [8-10] Several reports from United States and Canada have shown that the organism was detected in nasopharyngeal swab (NPS) specimens of patients with pertussis-like symptoms. [11,12] Although humans may be infected with *B. holmesii*, transmission of *B. holmesii* between humans has not yet been fully elucidated.

Laboratory diagnosis of Pertussis can be performed either directly (polymerase chain reaction or culture), or indirectly by serological tests which measure the specific antibody response. Since the bacteria reside in the upper respiratory tract during the first three weeks of the infection it can be detected during this period only by direct methods. [13-15]

Principles of the Procedure

The Savvygen™ B .pertussis test is designed for detection *of Bordetella pertussis, Bordetella parapertussis and/or Bordetella holmesii* in respiratory specimens and to aid in the assessment of infections caused by these bacteria.

The Savvygen™ B .pertussis test is based on amplification of highly specific conserved fragments in the IS481 (*Bordetella pertussis/Bordetella holmesii*), in the hIS1001 gene (*Bordetella holmesii*) and in the pIS1001 gene (*Bordetella parapertussis*). Following extraction of *Bordetella* bacteria DNA the conserved fragments are amplified by Taq DNA in Polymerase Chain Reaction (PCR). The assay is based on the 5'→3' exonuclease activity of Taq DNA Polymerase (figure 1). A fluorophore/quencher dual-labeled probe is annealing to an internal specific sequence. Upon primer elongation, Taq DNA Polymerase displaces and hydrolyzes the probe, thus releasing and activating the fluorophore. The presence of *Bordetella* bacteria is detected by an increase in observed fluorescence during the reaction. The resulting increase in fluorescence signal is proportional to the amount of amplified product in the sample and detected by the real-time PCR instrument.

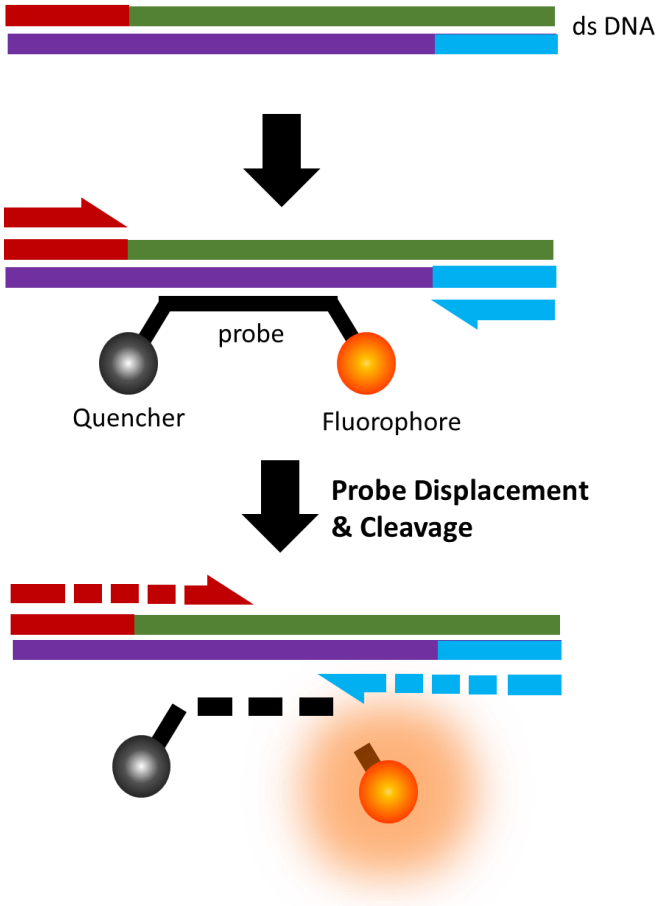


Figure 1. Principle of the Savvygen assay

The Savvygen™ B .pertussis test is a ready-to-use assay containing in each well all the necessary reagents for the reaction in a stabilized format. An internal control allows the identification of a possible inhibition of the reaction. The optical channels used for multiplexed detection of the amplified fragments are outlined in table 1 below:

Table 1. Savvygen™ B .pertussis targets and optical channel detection

Target	Optical channel
Bordetella pertussis / Bordetella holmesii	FAM
Bordetella holmesii	ROX
Bordetella parapertussis	Cy5
Internal Control	*HEX, VIC or JOE

(*) Depending on the equipment used, the proper detection channel should be selected (see table 4)

Materials/ Reagents Provided

Product Description	Contents
Savvygen™ B. pertussis *48 reactions*. Cat.# 613-01H /613-01L	6 x Savvygen™ B. pertussis strips
	1x Savvygen™B. pertussis Positive Control
	1x Water RNAse/DNAse free 1mL
	1x Rehydration Buffer 1.8 mL
	1x Negative Control 1 mL
	Optical caps

Additional Equipment and Material Required

- DNA extraction kit.
- Centrifuge for 1.5 mL tube.
- Vortex.
- Micropipettes (0.5-20 µL, 20-200 µL).
- Powder-free disposal gloves
- Real Time PCR instrument (see table 2A+2B for compatible RT-PCRs).

Table 2A. Compatible Real Time PCR instruments (Low-Profile)

Bio-Rad	***Applied Biosystems***
CFX96 Touch™ Real-Time PCR Detection System	7500 Fast Real-Time PCR System
Roche	7500 Fast Dx Real-Time PCR System
LightCycler ®480 Real-Time PCR System	QuantStudio™ 12K Flex 96-well Fast
LightCycler ®96 Real-Time PCR System	QuantStudio™ 6 Flex 96-well Fast
Agilent Technologies	QuantStudio™ 7 Flex 96-well Fast
AriaMx Real-Time PCR System	QuantStudio™ 5 Real-Time PCR System
DNA-Technology	ViiA™ 7 Fast Real-Time PCR System
DTlite Real-Time PCR System	***Cepheid***
DT prime Real-Time Detection Thermal Cycler	SmartCycler®*
	Qiagen
	*Rotor-Gen® Q**

Table 2B. Compatible Real Time PCR instrument (High-Profile)

Bio-Rad	***Applied Biosystems***
CFX96 Touch Deep Well Real-Time PCR Detection System	*7500 Real-Time PCR System*
iCycler iQ Real-Time PCR Detection System	*QuantStudio™ 12K Flex 96-well*
iCycler iQ 5 Real-Time PCR Detection System	*QuantStudio™ 6 Flex 96-well*
DNA-Technology	*QuantStudio™ 7 Flex 96-well*
DTlite Real-Time PCR System	*QuantStudio™ 5 Real-Time PCR System*
DT prime Real-Time Detection Thermal Cycler	*ViiA™ 7 Real-Time PCR System*
Stratagene /Agilent Technologies	***Qiagen***
Mx3000P™ Real Time PCR System	*Rotor-Gen® Q**
Mx3005P™ Real Time PCR System	***Cepheid***
Analytik Jena Biometra	*SmartCycler®**
TOptical	***Abbot***
qTOWER 2.0	*Abbot m2000 RealTime System*

* Once the product has been reconstituted following the appropriate procedure, transfer it in the specific Rotor-Gene® Q or SmartCycler® tubes.

Precautions

Amplification technologies can amplify target nucleic acid sequences over a billion-fold and provide a means of detecting very low concentrations of target. Care must be taken to avoid contamination of samples with target molecules from other samples, or amplicons from previous amplifications. Follow these recommendations to help control contamination.

1. Separate pre-amplification steps from post-amplification steps. Use separate locations for pre- and post-amplification. Use dedicated lab equipment for each stage. Prepare samples in a laminar flow hood using dedicated equipment to minimize contamination. Set up the post-amplification area in a low-traffic area with dedicated equipment.
2. The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.
3. Use disposable containers, disposable barrier pipette tips, disposable bench pads, and disposable gloves. Avoid washable lab wear.
4. Use a diluted bleach solution (0.2% sodium hypochlorite) to treat waste from the post-amplification and detection areas, as the waste contains amplicon. Use the bleach solution to wipe down equipment and bench areas, as well as to treat drains used to dispose of liquid waste.
5. Use negative controls to monitor for possible contamination during reaction setup. If reagent contamination is detected, dispose of the suspect reagents.
6. Do not use after the expiration date stated on the box.
7. Specimens must be treated as potentially infectious as well as all reagents and materials that have been exposed to the samples and handled in the same manner as an infectious agent. Take necessary precautions during the collection, storage, treatment and disposal of samples.

Transport and Kit Storage

The Savvygen kits can be shipped and stored at 2-37ºC until expiration date stated in the label.
After resuspension of the Positive Control, store at -20ºC. Avoid repeated freeze/thaw cycles.
It is recommended to make aliquots of the positive control and stored at -20ºC once resuspended in order to avoid freeze & thaw cycles.

Test Procedure

Specimen Collection, Processing and DNA Extraction

In order to obtain an adequate sample, the procedure for sample collection must be followed closely and according to the manufacturer's instructions. The specimens should be transported as fast as possible and to be stored at the indicated temperatures conditions.

Nucleic Acid (NA) Extraction: for pre-treatment and NA isolation, it is recommended to use an appropriate DNA extraction kit according to manufacturer´s protocol. NA Extraction may be carried out manually or automatically using commercially available extraction kits. Several extraction systems were validated for this kit including:

- Savvygen Extractor (Savyon Diagnostics) using the Savvygen Respiratory extraction kit
- Maxwell®16 Viral Total Nucleic Acid Purification Kit, using the Maxwell® 16 instrument (Promega).
- Invisorb® Spin Universal Kit (Stratec).
- EZ1 Virus Mini Kit, using EZ1 instrument (Qiagen).
- RIDA® Xtract (r-Biopharm).

Positive Control Preparation

Note: The Positive Control vial contains high-copy number template of the assay targets with a contamination risk. Therefore, it is recommend re-suspend the vial in a separate laboratory area or a special cabinet.

Open the Positive control pouch to re-suspend the lyophilized B. pertussis Positive Control (red cap vial) with 100 µl of Water RNAse/DNAse free (transparent cap vial) supplied. To ensure a complete re-suspension, vortex the vial thoroughly. After first use, dispense into aliquots in order to avoid multiple freeze-thaw cycles, and store them at -20ºC.

***PCR Protocol Program*.**

Set your thermocycler to the following conditions below:

Table 3. Real time RT-PCR profile

Step	Temperature	Time	Cycles
Initial denaturation	95ºC	2 min	1
Denaturation	95ºC	10 sec.	45
Annealing/Extension	60ºC	50 sec.	

Note: *Set the fluorescence data collection during the extension step (*) through the FAM (Bordetella pertussis / Bordetella holmesii), ROX (Bordetella holmesii), Cy5 (Bordetella parapertussis) and HEX, JOE or VIC channels (Internal Control (IC)).*

Depending on the equipment used select the proper detection channel (*table 4*). For the following systems: Applied Biosystems 7500 Fast Real-Time PCR or the Stratagene Mx3005P™ Real Time PCR check that passive reference option ROX is not marked.

A. Reconstitute the required reaction wells.

Calculate the number of required reactions including samples and controls. It is highly recommended to run at least one positive and one negative control per run.

1. Peel off protective aluminum seal from the strips/plate
2. Pipette 15 µL of Rehydration Buffer (Blue cap vial) into each well.

B. Add samples and controls according to real-time PCR experimental plate set up.

1. Pipette 5 µL of DNA sample into each sample well.
2. Pipette 5 µL of resuspended *B. pertussis* Positive Control (red cap vial) into each positive control well.
3. Pipette 5 µL of Negative Control (orange cap vial) into each negative control well.
4. Cover the wells with the caps provided. Spin down briefly if needed.

C. Performing PCR.

1. Place the strips in the Real Time PCR instrument.
2. Start the run.

The fluorescence detection channels of common Real Time PCR Thermocyclers are specified in Table 4.

Table 4: Detection fluorescence channels of different Real Time PCR systems

RT- PCR THERMOCYCLER	***System Detection channels***	***Savvygen probes channels***	***Remarks***
Roche LightCycler® 96 or Roche LightCycler®480II	465/510	FAM	Color Compensation is required only for LC480 system
	533/580	HEX	
	533/610	ROX	
	618/660	Cy5	
Applied Biosystems ABI 7500 fast	FAM	FAM	Passive reference option ROX is not mark
	VIC	HEX	
	ROX	ROX	
	Cy5	Cy5	
Bio-Rad CFX96 ™	FAM	FAM	
	HEX	HEX	
	ROX	ROX	
	Cy5	Cy5	
Abbott® m2000rt	FAM	FAM	
	VIC	HEX	
	ROX	ROX	
	Cy5	Cy5	
Stratagene Mx3000P™ / Mx3005PTM	FAM	FAM	Passive reference option ROX is not mark
	HEX	HEX	
	ROX	ROX	
	Cy5	Cy5	
Agilent AriaMx	FAM	FAM	
	HEX	HEX	
	ROX	ROX	
	Cy5	Cy5	

Interpretation of results

Interpretation of results can be automatically performed if programed by the user using the RT-PCR instrument software following manufacturer´s instructions. It is required to run assay controls (positive and negative controls) in each run to validate the reaction.

Note: *The positive control well should demonstrate positive signals for all assay targets (B. pertussis, B. holmesii and B. parapertussis) while the negative control well should demonstrate an absence of signal (except internal control target).*

The result interpretation is summarized and done according to Table 5.

Positive sample- A sample is considered a positive, if the obtained amplification curve signal is below threshold cycle 40

Negative sample- A sample is considered as negative for the target if there is no evidence of amplification signal in the detection system but the internal control is positive.

Internal control- The Internal Controls must show an amplification curve, which verifies the correct functioning of the amplification mix. Sometimes, the detection of the internal control is not necessary because a high copy number of the pathogen DNA template can cause preferential amplification of target sequence.

Positive control- The positive controls used in each run, must show 3 amplification curves for *B. pertussis, B. holmesii and B. parapertussis* which validates the reaction.

Negative control- The negative controls included in each run must show the absence of signal for *B. pertussis, B. holmesii and B. parapertussis* which validates the reaction.

Invalid run- The assay should be considered as invalid and a new run should be performed if there is signal of amplification for one of the pathogens in the negative control well or absence of signal in the positive control well.

Note: *If an amplification curve for the internal control is not shown, the sample should be retested by dilution of the original sample 1:10. Alternatively it is recommended to repeat the nucleic acid extraction due to possible problems caused by PCR inhibitors.*

Table 5. Results interpretation

B. pertussis / B. holmesii (FAM)	***B. holmesii (ROX)***	***B. parapertussis (Cy5)***	**Internal control**	**Negative Control**	**Positive Control**	**Interpretation**
POS	NEG	NEG	POS / NEG	NEG	POS	*B. pertussis* **Positive**, B. holmesii and B. parapertussis **Negatives**
POS	POS	NEG	POS / NEG	NEG	POS	*B. holmesii* ***Positive****, B. pertussis and B. parapertussis* ***Negatives***
NEG	NEG	POS	POS / NEG	NEG	POS	*B. parapertussis* ***Positive****, B. holmesii and B. pertussis* ***Negatives***
NEG	NEG	NEG	POS	NEG	POS	*B. pertussis, B. holmesii and B. parapertussis* ***Negatives***
POS	POS	POS	POS	NEG	POS	Invalid Run
NEG	NEG	NEG	NEG	NEG	NEG	Invalid Run

POS: Amplification of curve signal
NEG: No amplification curve signal

Limitations of the test

- All results should be used and interpreted in the context of a full clinical evaluation as an aid in the diagnosis of respiratory infection.
- This test was only validated for nasal, nasopharyngeal and throat swabs.
- Inappropriate specimen collection, transport, storage and processing or a viral load in the specimen below the analytical sensitivity can result in false negative results.
- Error results may occur from improper sample collection, handling, storage, technical error, sample mix-up, or because the number of organisms in the sample is below the analytical sensitivity of the test.
- The presence of PCR inhibitors may cause invalid results.
- A false positive result with other targets is possible due to contamination with PCR products from previous testing.
- As with all PCR-based in vitro diagnostic tests, extremely low levels of target below the analytical sensitivity of the assay may be detected, but results may not be reproducible.
- If a certain sample result is Invalid then the sample should be repeated from DNA extraction.

Quality Control

In order to confirm the appropriate performance of the molecular diagnostic technique, an Internal Control (IC) is included in each reaction. Besides, a positive and a negative control must be included in each assay to interpret the results correctly.

Performance Characteristics

Clinical Sensitivity and Specificity

Clinical performance characteristics of the Savvygen™ B. pertussis test were assessed in a clinical study. The study specimens consisted of 35 respiratory specimens (nasopharyngeal aspirates and prenasal swabs) from symptomatic patients which were tested by the Savvygen™ B .pertussis test and compared to RIDA®GENE Bordetella kit(r-Biopharm).

Bordetella pertussis were detected in 14 samples, but only 1 specimen could be confirmed as Bordetella parapertussis positive. 20 specimens resulted negative for *Bordetella pertussis, Bordetella parapertussis* or *Bordetella holmesii*. The results show a high sensitivity and specificity to detect Bordetella using the Savvygen™ B .pertussis Real Time PCR Detection Kit.

Analytical Sensitivity

A serial dilution test was conducted to evaluate the analytical sensitivity of the Savvygen™ B. pertussis test for *B. pertussis, B. holmesii* and *B. parapertussis* pathogens. This assay has a detection limit of ≥10 DNA copies per reaction (Figure 2, 3 and 4).

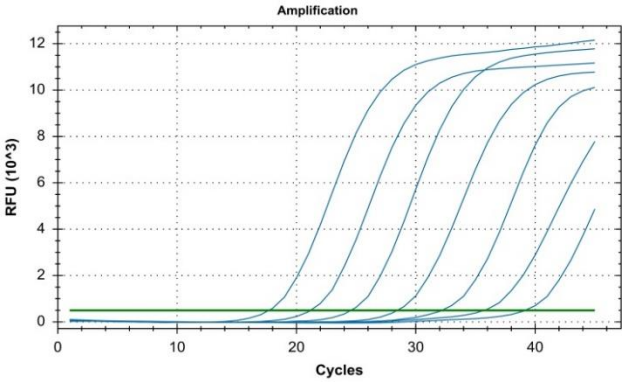


Figure 2. Amplification plot for 10-fold dilution series of *B. pertussis / B. holmesii* template ranging from 10^7 to 10^1 copies/reaction (FAM channel).

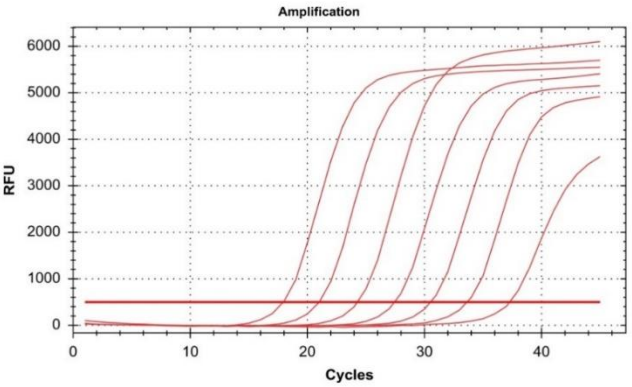


Figure *3*. Amplification plot for 10-fold dilution series of B*.* holmesii template ranging from 10^7 to 10^1 copies/reaction (ROX channel).

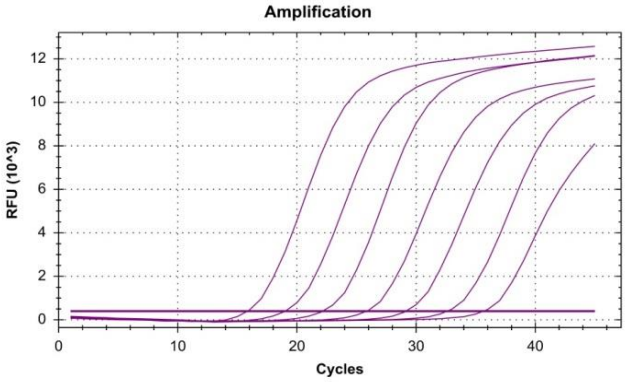


Figure 4. Amplification plot for 10-fold dilution series of B. parapertussis template ranging from 10^7 to 10^1 copies/reaction (Cy5 channel).

Analytical specificity

The analytical specificity of the Savvygen™ B. pertussis was evaluated *for Bordetella pertussis, Bordetella holmesii* and *Bordetella parapertussis* assays was tested within the panel of following microorganisms, where no cross-reactivity was seen between any of the species (table 6).

Table *6*. Cross-reactivity testing**.**

Pathogen	Analytical-Reactivity Test		
	Savvygen™ B. pertussis		
	B. pertussis	*B. holmesii*	*B. parapertussis*
Haemophilus influenzae	-	-	-
Human Adenovirus 5	-	-	-
Human coronavirus 229E	-	-	-
Human metapneumovirus A and B	-	-	-
Human parainfluenza 1, 2, 3 and 4 viruses	-	-	-
Human rhinovirus	-	-	-
Influenza A/California/7/2009(H1N1) virus	-	-	-
Influenza A/Perth/16/2009(H3N2) virus	-	-	-
Influenza A/New Caledonia/20/99(H1N1) virus	-	-	-
Influenza A/Switzerland/9715293/2013	-	-	-
Influenza A/Turkey/Germany R2485+86/2014	-	-	-
Influenza B/Brisbane/60/2008 virus	-	-	-
Influenza B/Florida/04/06 virus	-	-	-
Influenza B/Phuket/3073/2013	-	-	-
Legionella bozemanii	-	-	-
Legionella micdadei	-	-	-
Legionella dumoffii	-	-	-
Legionella longbeachae	-	-	-
Legionella pneumophila	-	-	-
Methicillin-resistant *Staphylococcus aureus*	-	-	-
Mycoplasma pneumoniae	-	-	-
Moraxella catarrhalis	-	-	-
Respiratory syncytial virus (RSV)	-	-	-
Staphylococcus aureus subsp. *aureus*	-	-	-
Streptococcus pneumoniae	-	-	-

Analytical reactivity

The reactivity of the Savvygen™ B. pertussis was confirmed by the real time amplification using *Bordetella pertussis, Bordetella parapertussis* and *Bordetella holmesii* as template.

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Symbols for IVD Components and Reagents

Manufacturer	Use by	For i*n vitro* diagnostic use only
Lot number	Temperature limitation	Consult instructions for use
Catalogue number	Contains sufficient for <n> test	Buffer (sample diluent)