LABSYSTEMS DIAGNOSTICS
speaking your language

REF 8100200, 8101200

B. pertussis +

B. parapertussis

Duplex Real-Time PCR

Σ 25 / 100 tests

Instructions for use

IVD

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Instructions for use

For *in vitro* diagnostic use only

A Real-Time PCR assay for the rapid detection and separation of *Bordetella pertussis* and *Bordetella parapertussis* in human respiratory samples.

Products:
8100 200; 25 tests
8101 200; 100 tests

CONTENTS

INTENDED USE

Labsystems Diagnostics' *B. pertussis* + *B. parapertussis* Real-Time PCR kit is developed for the rapid and simultaneous detection of *Bordetella pertussis* and *Bordetella parapertussis* in human respiratory samples. Kit includes an cellular control detecting human genomic DNA to insure the presence of cells in the samples.

The positive result is an aid for the diagnosis of acute *Bordetella pertussis* or *Bordetella parapertussis* infection.

INTRODUCTION

Pertussis, or whooping cough, is an acute, highly communicable infection of the respiratory tract caused by *Bordetella pertussis* bacteria. The disease is transmitted by respiratory droplets from infected individuals. The incubation period is 6-20 days. The infectivity is greatest early in the disease and decreases until the pathogen disappears from the nasopharynx. (1-3)

Bordetella pertussis interferes with ciliary activity and this is responsible for obstruction of airways, because the transport and clearance of mucus is diminished. In addition to directly injuring ciliated epithelial cells *Bordetella pertussis* produces different kind of aggressins and toxins that are important for the colonization, its survival and propagation involved in the pathogenesis of whooping cough (4-5). One of those is pertussis toxin, the main and unique toxin of *Bordetella pertussis* (6).

The characteristic features of pertussis are paroxymal coughing with an inspiratory whoop and/or lymphocytosis (4). In young infants pertussis is a serious disease that can be complicated by pneumonia, convulsions, brain damage, atelectasis, interstitial and subcutaneous emphysema and pneumotorax. In older children and adults the disease is usually mild characterized by a cough lasting 1-3 months. The patient with a typical pertussis may transmit the disease to infants (7).

The definite method of diagnosing whooping cough is by isolating *Bordetella pertussis* in clinical samples. There are, however, many difficulties in culturing, and most studies report recovery rates < 60 % (8). Direct immunofluorescent staining of nasopharyngeal secretions has been used for diagnosis, but this test lacks specificity. PCR method has been a significant improvement in diagnostics of *Bordetella pertussis.* Real-time PCR has been shown to be effective to diagnose pertussis in young children for up to 3 weeks after treatment initiation (9).

To date, most investigations have relied on serologic immunoassays or culture tests. Those technical problems are avoided with the Real-Time PCR assay developed by Labsystems Diagnostics, providing easy, fast and sensitive results.

PRINCIPLE OF THE TEST

The principle of the Labsystems Diagnostics' *B. pertussis* + *B. parapertussis* Real-Time PCR kit is based on the 5' nuclease technique. The kit is composed of ready to use optimized reaction mixture for the amplification and detection. This kit is intended to use on respiratory samples after DNA extraction (not included in the kit).

The kit is suitable for all Real-Time PCR instruments with minimum of three measurement channels (FAM, VIC and ROX). Kit is suitable for both fast instruments (such as ABI 7500FAST Real-Time PCR system from Life Technologies; PikoReal24 from Thermo Scientific; Rotor-Gene Q from Qiagen) as well as standard instruments (such as ABI Prism 7500, 7700 and 7900 from Life Technologies). Kit does not contain passive reference dye.

The target sequences indentified by the kit have been selected from the conserved regions of the pathogen of interest.

Target	***Gene***
Bordetella pertussis	Pertussis Toxin
Bordetella parapertussis	IS1001

The amplification of *Bordetella pertussis* is measured with the FAM fluorescence channel whereas the amplification of *Bordetella parapertussis* is measured with the VIC fluorescence channel. The amplification of the internal control is measured with the ROX channel. The assay can be used in any Real-Time PCR instrument provided with the detection channels for the mentioned fluorescent labels.

KIT CONTENTS

Note:

– Reagents are stored at between +2°C and +8°C, in a separate area.
– The expiration date is printed on each component label and on the package. Do not use reagents after the expiration date.
– Protect from light. The light sensitive amplification mix is packaged in brown vials for protection.
– To avoid contamination, store the positive control before and after first opening in the same area as the extracted samples.

1 AMPLIFICATION MIX, 1 vial (700 µl) / 4 vials
Contains dNTPs, $MgCl_2$, PCR buffer, specific primers and probes, polymerase enzyme

2 NEGATIVE CONTROL, 1.0 ml
Molecular biology grade sterile water. The control must be amplified at the same time and using the same protocol as the patient samples. The negative control verifies the absence of contamination during amplification.

3 POSITIVE CONTROL, 20 µl / 2x 20 µl
Containes two plasmids which are amplified with the primers and probes targeted for *B. pertussis* and *B. parapertussis* in the amplification mix. It verifies the proper performance of the primers and probes. Signal is detected with the same parameters as for the targets.

REAGENT PREPARATION

Reagent	Preparation	Stability of opened reagents (+2°C to +8°C)
1 Amplification mix	Ready for use	1 month *)
2 Negative control	Ready for use	1 month *)
3 Positive control	Ready for use; for quantitation dilute the control (vial 3) with Negative control water (vial 2) for the ten-fold dilution series standard curve	1 month *)

*) The stability of the opened reagents is the maximum only if they are stored properly at +2°C to +8°C. High environmental temperature and contamination may decrease the stability.

MATERIALS REQUIRED BUT NOT PROVIDED

– DNA extraction kit
– Real-Time PCR instrument with at least three detection channels suitable for FAM, HEX/VIC and ROX measurement
– Disposable gloves
– Adjustable pipettes
– Sterile pipette tips with filter
– Vortex mixer
– Centrifuge with a rotor for 2.0 ml tubes
– Sterile nuclease-free microtubes for positive control dilution series
– Sterile nuclease-free PCR tubes, strips or plates recommended by the Real-Time PCR instrument manufacturer

PRECAUTIONS

For *in vitro* diagnostic use only.

Extreme precautions should be taken to prevent contamination of the reactions. Amplification procedures require highly skilled professionals.

Use separate working places for amplification reactions and sample preparations. To avoid contamination, three separate working areas are recommended. Clean area I to store the kit, area II for sample preparation and positive control and designated area III to perform the amplification. Allocate a set of lab coats and pipettes to each area. Never introduce an amplified product in reagent and/or sample preparation areas.

Samples must be prepared under a biological safety hood.

Discard all materials and samples as if capable of transmitting infection. The preferred method of disposal is autoclaving for a minimum of one hour at 121°C. Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 50-500 mg/l free available chlorine. Allow 30 minutes for decontamination to be completed. Spills should be wiped off thoroughly using either an iodophor disinfectant or sodium hypochlorite solution. Materials used to wipe off spills should be added to biohazardous waste matter for proper disposal.

Tubes from specimens and amplification mix must never be opened at the same time.

Wear disposable gloves while handling samples and kit reagents. Afterwards wash hands carefully. Never pipette by mouth.

Do not use same pipettes to handle samples and amplification mix. All pipettes used must be equipped with sterile filter tips.

Do not reuse a strip or plate even if some wells were not used.

Accurate and precise pipetting, as well as following the exact time and temperature requirements, is essential.

Do not eat, drink or smoke in dedicated work areas.

SAMPLE COLLECTION AND HANDLING

Clinical samples should be transported to the laboratory as soon as possible, aliquoted and stored at -70°C or -20°C until use. **Samples should not be repeatedly frozen and thawed.**

Microbially contaminated samples may give erroneous results.

The following methods can be used for DNA extraction of clinical specimens. For manual extractions, use the QIAamp DNA Mini Kit (Qiagen) or QIAamp MinElute Virus Spin Kit (Qiagen) according to the manufacturer's instructions. Briefly, extract 200 µl of sample material and elute the nucleic acid in 50 – 100 µl. Automated DNA extraction can be performed for example with the KingFisher nucleic acid purification system (Thermo Scientific) or MagNA Pure 96 instrument (Roche).

Samples should be processed within one week after the DNA isolation.

Sample types evaluated with this kit: sample transfer medium, nasal aspiration biopsy, respiratory biopsy, nasal swabs and bronchoalveolar lavage.

TEST PROCEDURE

PCR Reaction set-up

The PCR reaction is performed in a final volume of 30 µl. The composition of the amplification reaction is as follows:

Amplification mix	25 µl
Extracted sample or control	5 µl
Total	30 µl

PCR amplification program

After placing the PCR tubes into the instrument the following protocol is used:

TEMPERATURE	TIME	CYCLES
95°C	1 min	1
95°C	3 sec	45
60°C	35 sec	
20°C	10 sec	1

Note 1: Select "none" in "passive reference" or " reference dye" when programming.

Note 2: Detection in the FAM, HEX/VIC and ROX channels should be activated.

Detailed programming guides for each Real-Time PCR instrument are available upon request.

NOTES:

1. Use of duplicates is preferable. **Mix well.**
2. **Avoid contamination**: When removing aliquots from the reagent vials, use aseptic technique to avoid contamination. Use a new pipette tip for each sample. Do not touch the walls of the wells with pipette tips.
3. Perform at least one negative and one positive control amplification in every PCR run to verify the reaction performance.
4. Do not store and handle Amplification mix in the same area as the samples and positive control to avoid contamination.

RESULTS

Programming of the instruments is carried out according to the instrument's User Manual. Fluorescence data are plotted against the number of cycles. The threshold cycle (C_t or C_q) serves as a tool for calculation of the starting template amount in each sample. The threshold is adjusted to a value above the baseline, but must be located in the log-linear range of the PCR. Before determining the C_t value, ensure that the baseline is positioned correctly and adjust if necessary.

The negative control should not give a detectable signal on any channel.

The positive control should give a detectable signal at both FAM and VIC channels below 35 cycles.

Interpretation of the Results

A positive sample displays a C_t value.

If a C_t value can not be calculated, the sample is considered as negative or inhibited or contains a limited number of target cells.

No $C_{t\ value}$	**Negative**
$35 \leq C_t \leq 44$	**Low positive**
$C_t < 35$	**Positive**

When a low positive sample is detected, sample should be retested.

	Bordetella parapertussis		
		Ct value detected	**No Ct value**
Bordetella pertussis	**Ct value detected**	*B. pertussis* **+** *B. parapertussis* **+**	*B. parapertussis* **–** *B. pertussis* **+**
	No Ct value	*B. parapertussis* **+** *B. pertussis* **–**	*B. parapertussis* **–** *B. pertussis* **–**

The quality of the sample taken and/or inhibition for the amplification reaction is verified using the internal control. If the C_t value is less than 32, the DNA extraction is performed correctly and the number of cells used in the assay is adequate.

LIMITATION OF THE PROCEDURE

Because no single method leads to the definitive diagnosis, the results of the present method should be interpreted in conjunction with the clinical condition, epidemiological situation and other laboratory methods.

It is recommended that the assay is performed by qualified and trained laboratory technician.

PERFORMANCE CHARACTERISTICS

Analytical specificity
The specificity of the primers and probes in the *B. pertussis* + *B. parapertussis* Real-Time PCR kit was determined by sequence analysis (viral, bacterial and human). The kit was experimentally evaluated with real-Time PCR on the following pathogens:
Bordetella pertussis
Bordetella parapertussis
Chlamydophila pneumoniae (strain CM-1 and strain AR-39)
Chlamydia trachomatis (strains UW-36/Cx, UW-43/Cx, BOUR)
Haemophilus influenzae
Influenza A, B
Legionella pneumophila subs. Pneumophila
Mycobacterium tuberculosis
Mycoplasma pneumoniae
Mycoplasma bovis
Mycoplasma genitalium
Mycoplasma fermentas
Mycoplasma arginini
Streptococcus pneumoniae

No cross-reaction between *Bordetella pertussis* and *Bordetella parapertussis* and no amplification with any of the other pathogens were observed.

Tests were also performed on human DNA extracts negative for *Bordetella pertussis* and *Bordetella parapertussis*. No amplification of sequences of human origin were observed.

External evaluation
Clinical performance evaluation of Labsystems Diagnostics' B. pertussis + B. parapertussis Duplex Real-Time PCR was performed in a European Hospital Laboratory. Altogether 80 respiratory patient samples (previously tested positive for *B. pertussis* (N=29) or *B. parapertussis* (N=12) or negative for both (N=39) were selected for the analysis. 23/29 samples originally tested positive for *B. pertussis* were positive in one or both tests for pertussis, 9/11 samples originally tested positive for *B. parapertussis*. All 39 samples that had originally negative for both agents, remained negative in both tests.

B. pertussis	in-house +	in-house -	Total
LDx+	19	4	23
LDx-	0	57	57
Total	19	61	80

B. parapertussis	in-house +	in-house -	Total
LDx+	8	1	9
LDx-	1*	70	71
Total	9	71	80

* One sample was detected strongly positive for pertussis and weak positive for parapertussis with in-house assay

Quality control panels
Assay specificity and sensitivity is tested regularly using external quality control panels.

TROUBLESHOOTING

Cause/Error	Solution
Positive control is negative	
1. One of the reaction components was not added	Ensure that all components have been added
2. The positive control was not stored properly	Store all components according to manufacturers instructions
3. Positive control has been degraded	Follow the general manufacturers instructions
4. Wrong PCR profile	Check the programming of the Real-Time PCR instrument

Cause/Error	Solution
Negative control is positive	
1. Carry over contamination	Repeat the entire experiment with fresh reagents. Handle samples, kit components and consumables as prescribed.

Cause/Error	Solution
Internal control is negative	
1. No DNA in the sample	Repeat extraction procedure to ensure the DNA extraction. Choose another DNA isolation method.
2. Inhibitory substances	Dilute DNA sample and repeat the experiment. Repeat the extraction procedure to ensure that no contaminants are present.

Cause/Error	Solution
Very weak fluorescence signal	
1. Incorrect instrument settings	Check channel settings
2. Improper storage of the Amplification mix	Store all components according to manufacturers instruction. Protect the Amplification mix from light exposure.

NOTICE TO THE PURCHASER

LIMITED LICENSE.
The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use solely in the human in vitro diagnostic field (in accordance with applicable FDA and other regulatory requirements) and may not be used for any other commercial use, including without limitation repacking or resale in any form. No general patent or other license of any kind other than this specific right of use from purchase is granted hereby by Labsystems Diagnostics Oy.

REFERENCES:

1. Edwards, K.M., M.D. Decker, and E.A. Mortimer (1999) Pertussis vaccine. In *Vaccines*. Orenstein, S.A.P. (ed). Philadelphia, USA:W.B. Saunders company, 293-344.
2. Hoppe, J.E. (1999) *Bordetella*. In *Manual of clinical microbiology*. Murray, P.R., Barron, E.J., Pfaller, M.A., Tenover, F.C., and Yolken, R.H. (eds). Washington, USA: American Society for Microbiology. 7th edition, 614-624.
3. Lapin, J.H. (1943). Whooping cough. Charles C. Thomas, Springfield, IL.
4. Stevenson, A. and Roberts, M. (2003) Use of *Bordetella bronchiseptica* and *Bordetella pertussis* as live vaccines and vectors for heterologous antigens. *FEMS Imm. Med. Microb*. 37: 121-128.
5. Weiss, A. A., E. L. Hewlett, G. A. Myers, and S. Falkow. (1984). Pertussis toxin and extracytoplasmic adenylate cyclase as virulence factors in Bordetella pertussis . *J. Infect. Dis*. 150: 219–222
6. Weiss, A.A.,, Hewlett, E.L,, Myers, G.A. et al. (1984) Pertussis Toxin and Extracytoplasmic Adenylate Cyclase as Virulence Factors of Bordetella pertussis *J Infect Dis*. 150(2): 219-222
7. Mils, K.H.G. (2001) Immunity to *Bordetella pertussis. Mic. Infect*. 3: 655-677.
8. Lind-Brandberg, L., Welinder-Olsson, C., Lagergard, T., Taranger, J., Trollfors, B., and Zackrisson, G. (1998) Evaluation of PCR diagnosis of *Bordetella pertussis* and *Bordetella parapertussis* infections. *J. Clin. Microbiol*. 36: 679-683.
9. Bidet, P., Liquori, S., De Lauzanne A., Caro V., Lorrot M., Carol A, *et al*. (2008) Real-Time PCR Measurement of Persistence of Bordetella pertussis DNA in Nasopharyngeal Secretions during Antibiotic Treatment of Young Children with Pertussis. *J Clin Microbiol*. 46(11):3636-38.

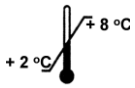
CE-mark

REF
Catalog number

Contains sufficient for < n > tests

Use by YYYY-MM

LOT
Batch code



Temperature limitation

IVD
In vitro diagnostic medical device

Manufacturer

Consult instructions for use

CONTROL +
Positive control

CONTROL -
Negative control

Potential biohazardous material