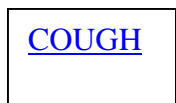


Bordetella parapertussis
PCR reagents
Detection with real time PCR reagents

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Overview:



Whooping cough presents in infants with a series of coughs followed by apnea or vomiting. Astute clinicians recognize these repetitive spells of cough, cough, cough, apnea or vomiting as whooping cough. These apneas may result in episodes of deep cyanosis. The vomiting may make weight gain very difficult. The spells of cough-apnea-vomiting may last for many weeks. Maternal immunity provides little protection for the infant.

B. pertussis was originally isolated in 1906 by Bordet and Gengou (Bordet, J and Gengu, O. 1906. Le microbe de la Coqueluche. Ann. Inst. Pasteur 20:731-741.) The most sensitive diagnosis is with PCR.

Bordetella helensi has been a source of illness in immune suppressed patients. This organism may be isolated from blood cultures. Bordetella bronchoseptica is a common infection in animals, but not often in humans. B. parapertussis may cause a milder form of whooping cough.

B. parapertussis was found by Grace Eldeering and Pearl Kendrick, Michigan Department of Health, in 1938 as 10 of 1498 pertussis cough plate cultures. These 10 organisms were found on subculture to be unusually large and grew on plain infusion agar and in broth. Although they agglutinated with B bronchosepticus antiserum, they were non-motile. Of the six patients infected with the new B para-pertussis, one had severe, two had moderate, two had light and one had very light disease.(1)

Primers and probe are available for amplification and detection of the B. parapertussis. The B. parapertussis primers are specific for an 88 bp portion of the repeated IS1001. The B. parapertussis detection probe is a molecular beacon. (2)

Products

Products	Catalog No.	Quantity
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B. parapertussis FAM-BHQ1 Primer-probe PP400		0.055ml
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Store at -20C. 25-20 µl reactions

Typical use: Add 2X master mix and DNA, then thermal cycle.

Attostar reagent contains primers and probe. Detection at 510nm.

AttoMaster 2X Mix for qPCR	AM10	1.25 ml
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Store at -20°C. 125-20 ul reactions

Contains Taq polymerase (requires heat activation), dNTPs (0.4 mM) with optimal dUTP to dTTP ratio, heat labile UDG, Mg(6 mM), and buffer.

Typical use: Add Attostar Primer-probe, DNA, and then thermal cycle.

B. parapertussis Plasmid	PLAS400	0.25ml
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Store at -20C)

Typical use: make serial 10 fold dilutions in TE for standard curve, diluting 5 ul into 45 ul TE.

Detection of *Bordetella parapertussis* DNA:

Thermal cycle conditions for PCR reactions on RotorGene*

*Similar cycle conditions and reaction volumes may be used on many other thermal cyclers.

95C 120 sec (activation for AttoMaster polymerase)

40 cycles

95C 15 seconds

60C 30 seconds RotorGene Channel Setup FAM/Sybr, Cy5; Gain 7

72C 30 seconds

FAM/Sybr has a source of 470nm and Detector 510nm (LightCycler use F1)

Cy5 has a source of 625nm and Detector 660hp nm

Quasar 670 has the same fluorescent absorption and emission as Cy5.

Thermal cycle conditions for PCR reactions on LightCycler

95C 120 sec (activation for AttoMaster Gold polymerase)

40 cycles

95C 15 seconds

60C 30 seconds acquire fluorescent signal on F1 gain =1

72C 30 seconds

40C 30 seconds cool

Use of the *Bordetella parapertussis* plasmids:

Dilute the plasmid in TE to prepare a standard curve. Common dilutions would be 10-fold from 200 to 0.02pg/ml. The 0.02 pg/ml plasmid dilution contains 10 copies of plasmid in 2 μ l

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Attostar Primers/probe MM400 in RotorGene-PCR 20µl reactions

RotorGene Reagents needed for 20 ul PCR final reaction tube volumes										
Reaction tube number	1	2	3	4	5	6	7	8	9	10
Attostar Primer-Probe FAM labeled (10X)	2	4	6	8	10	12	14	16	18	20 µl
Master mix (2X)	10	20	30	40	50	60	70	80	90	100 µl
Dispense 12 ul / reaction tube										
Add 8 ul DNA / reaction tube										

Attostar Primers/probe MM400 in LightCycler-PCR 20 µl reactions

LightCycler Reagents needed for 20 ul PCR final reaction tube volumes										
Reaction tube number	1	2	3	4	5	6	7	8	9	10
Attostar Primer-Probe (10X)	2	4	6	8	10	12	14	16	18	20 µl
Master mix (2X)	10	20	30	40	50	60	70	80	90	100 µl
BSA 1 mg/ml	1	2	3	4	5	6	7	8	9	10 µl
Dispense 13 ul / reaction tube										
Add 7 ul DNA / reaction tube										

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Extraction / amplification control with T4 bacteriophage

Adding T4 bacteriophage (BAC130) to the sample provides DNA for extraction, amplification, and reaction condition PCR controls.

When added to a sample, T4 adds a known amount of DNA. The T4 DNA can then be extracted, amplified, and detected as a control. T4 controls for the efficiency of DNA extraction, the presence of PCR amplification inhibitors, intact amplification reagents (DNA polymerase, buffer, dNTPs), and instrument function (thermal cycling and fluorescent detection system).

The T4 DNA may be detected in a separate PCR reaction (using FAM labeled T4 probe in PP100). Or the T4 DNA and test organism DNA may be detected using a multiplex reaction (using Quasar 670 labeled T4 probe in PP160 and FAM labeled test organism probe).

Brief procedure for use of T4 as extraction and amplification control:

- Add 5µl T4 bacteriophage to the sample. Proceed with DNA extraction. Dilutions of the bacteriophage may be made to give a final PCR Ct value that is about 35. At this dilution, the phage is more sensitive, i.e. more likely, to detect a poor extraction or the presence of PCR inhibitors in the reaction.

Please refer to the BAC130, PP100, and PP160 product literature.

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References:

1. Eldering G, Kendrick P. BACILLUS PARA-PERTUSSIS: A SPECIES RESEMBLING BOTH BACILLUS PERTUSSIS AND BACILLUS BRONCHISEPTICUS BUT IDENTICAL WITH NEITHER. J Bacteriol. 1938 June; 35(6): 561-72
2. <http://www.molecular-beacons.org/Introduction.html>

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For technical support, contact Attostar@Attostar.com

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