Bordetella pertussis
Whooping cough
Detection with real time PCR reagents

Overview:

COUGH

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.

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

Primers and probe are available for amplification and detection of the B. pertussis. The B. pertussis primers are specific for an 83 bp portion of the repeated IS481. This B. pertussis amplification reagent detects B. pertussis, holmesii, and bronchoseptica.

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Products

Products	Catalog No.	Quantity
B. pertussis FAM-BHQ1 Primers-probe	**PP300**	**0.055ml**

Store at –20ºC. 25-20 µl reactions
Typical use: Add 2X master mix and DNA, then thermal cycle.
Attostar reagent contains primers and probe. Detect at 510 nm.

AttoMaster 2X Mix for qPCR	**AM10**	**1.25 ml**

Store at –20ºC. 125-20 µl 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. pertussis Plasmid	**PLAS300**	**0.25ml**

Store at –20C.
Typical use: make serial 10 fold dilutions in TE for standard curve, diluting 5 µl into 45µl TE.

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Detection of Bordetella pertussis DNA:

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Thermal cycle conditions for PCR reactions on RotorGene*

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

25ºC 10 min (UDG treatment time)
95ºC 120 sec (activation for AttoMaster polymerase)
40 cycles
95ºC 15 seconds
60ºC 30 seconds RotorGene Channel Setup FAM/Sybr, Cy5; Gain 7
72ºC 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.

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Thermal cycle conditions for PCR reactions on LightCycler

25ºC 10 min (UDG treatment time)
95ºC 120 sec (activation for AttoMaster polymerase)
40 cycles
95ºC 15 seconds
60ºC 30 seconds acquire fluorescent signal on F1 gain =1
72ºC 30 seconds
40ºC 30 seconds cool

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Use of the Bordetella pertussis plasmid:

Dilute the plasmid in TE to prepare a standard curve. Common dilutions would be 10-fold from 200 to 0.002pg/ml. The 0.02 pg/ml plasmid dilution contains 10 copies of plasmid in 2 μl.

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Attostar Primers/probe PP300 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 PP300 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.

T4 multiplex reaction (PP160)

RotorGene Multiplex PCR 20µl reactions

	Multiplex 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 PP160	2	4	6	8	10	12	14	16	18	20	µl
Primer-Probe mix for TEST organism	2	4	6	8	10	12	14	16	18	20	µl
2 x master mix	10	20	30	40	50	60	70	80	90	100	µl
Dispense 14 ul / reaction tube											
Add 6 ul DNA / reaction tube											

Reaction tube number	11	12	13	14	15	16	17	18	19	20	
Attostar Primer-Probe PP160	22	24	26	28	30	32	34	36	38	40	µl
Primer-Probe mix for TEST organism	22	24	26	28	30	32	34	36	38	40	µl
2 x master mix	110	120	130	140	150	160	170	180	190	200	µl
Dispense 14 ul / reaction tube											
Add 6 ul DNA / reaction tube											

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Reference:

1) http://www.molecular-beacons.org/Introduction.html

For technical support, contact Attostar@Attostar.com

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