

Detection of *Bordetella pertussis* by Homogeneous Strand Displacement Amplification for use on the BDProbeTec™ ET System

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ABSTRACT

■ Development of a pertussis vaccine in the 1950s has made whooping cough an uncommon disease in developed countries although reported cases have increased since 1980. We have developed an assay for *Bordetella pertussis* targeting a sequence within the pertussis toxin promoter (ptx) gene. The assay uses strand displacement amplification (SDA) and real-time detection that employs a universal fluorescence energy transfer detector probe. The *B. pertussis* assay is part of a respiratory panel currently under development (along with assays for *Legionella pneumophila* and *Mycoplasma pneumoniae*) for use on the BDProbeTec™ ET System. This assay features an internal control that may be used to control for sample inhibition. The approximate analytical sensitivity of the BDProbeTec™ ET *B. pertussis* assay is less than 100 copies of target DNA per reaction. The assay is specific for *B. pertussis* when tested with other bacteria, including other members of the genus *Bordetella*. The assay successfully detected ten ATCC™ strains of *B. pertussis*.

INTRODUCTION

■ *Bordetella pertussis* causes whooping cough (pertussis), pneumonia, and bronchitis in humans. Pertussis occurs in epidemic cycles and reported cases of pertussis have been on the rise since 1980. Common methods for the detection of *Bordetella pertussis*, such as direct-fluorescent antibody (DFA) staining, have variable sensitivities and may be technique dependent. The organism may lose viability in transport and is fairly-slow growing on culture media.

A method has been developed for determining the presence or absence of *Bordetella pertussis* DNA in respiratory or other samples. The method employs nucleic acid primers to amplify a target within the pertussis toxin promoter (ptx) region, using strand displacement amplification (SDA) with real-time detection on the BDProbeTec™ ET System. The assay uses a universal detection system and incorporates an optional internal amplification control to indicate assay inhibition.

As part of a respiratory panel, along with *Legionella pneumophila*, *Mycoplasma pneumoniae*, and *Chlamydiaceae* family assays, the *Bordetella pertussis* assay* has the potential to expand the capabilities of the BDProbeTec™ ET System.

ACKNOWLEDGEMENTS

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METHODS

ASSAY DEVELOPMENT. The assay has been statistically optimized for potassium phosphate, dimethyl sulfoxide, glycerol, potassium hydroxide, bicine, enzyme levels and primer concentrations. The target region from ATCC™ strains 53894 and 9340 were cloned into separate pUC19 plasmids to use as target sources in optimization experiments. We sequenced the SDA target region of ten ATCC™ strains and found that most were similar to either strain 53894 or 9340. The SDA primer binding sequences within the pertussis toxin promoter region vary slightly between the two strains, however, the assay detects both sequence variants.

INTERNAL CONTROL. Internal amplification control (IAC) target was prepared by mutating the pertussis toxin promoter region of a clone of ATCC strain 53894. Duplex reactions co-amplify IAC at 80 copies/ reaction with native target. Monoplex reactions amplify native target alone. The dual-dye capability of the BDProbeTec™ ET System allows detection of both native target detector probe and IAC detector probe to occur in 60 minutes. Figure 2.

DATA ANALYSIS. All experiments were performed using the BDProbeTec™ ET System. Data were analyzed using the Time to Threshold (T3) algorithm developed for this instrument. Negative samples never achieve the threshold value and are assigned a T3 value of 60. Positive samples have T3<60.

ANALYTICAL SENSITIVITY / LIMIT OF DETECTION. To determine the analytical sensitivity of the *Bordetella pertussis* assay in both monoplex and duplex formats, SDA was performed on dilutions of each cloned target nucleic acid sequence. Sixteen replicates were tested at each target level with and without IAC. Figure 3.

SPECIFICITY. Dilutions of a McFarland #1 standard were made with 10 different ATCC™ strains of *Bordetella pertussis*. Each strain was tested in triplicate using the duplex assay format. Figure 4.

CROSS-REACTIVITY. Cross-reactivity was tested by challenging the assay with a variety of microorganisms, including bacteria, fungi, and viruses. Organism suspensions containing approximately 1×10^9 organisms/mL were resuspended in PBS/BSA buffer and assayed in the duplex BDProbeTec™ ET *Bordetella pertussis* assay. Three replicates of each organism were examined in the presence of the IAC to validate negative results. Figure 5.

INTERNAL AMPLIFICATION CONTROL EFFECTIVENESS. The ability of the BDProbeTec™ ET *Bordetella pertussis* assay's IAC to identify an inhibited sample was evaluated by examining the effect of increasing levels of type III Histones. This testing employed the duplex assay format with 100 copies target plasmid (strain 53894). Eight replicates were run at each inhibitor level. Figure 6.

NON-SPECIFIC DNA TOLERANCE. The tolerance of the BDProbeTec™ ET *Bordetella pertussis* duplex assay to non-specific DNA was evaluated. Eight replicates at each of eight non-specific DNA levels were tested in the duplex assay format in the presence of 100 copies target plasmid (strain 53894). Figure 7.

COMPATIBILITY WITH SAMPLE EXTRACTION KITS. Compatibility with the Qiagen and Genra DNA extraction kits was demonstrated by extracting 100 copies/rxn of cloned native target plasmid (*Bordetella pertussis* ATCC 53894) along with 80 copies/reaction of IAC plasmid (Figure 7). Two hundred microlites samples were extracted with the QIAamp® DNA Blood Mini Kit (blood and body fluid spin protocol), and 50 µL samples were extracted using the Genra® PureGene DNA Isolation Kit (body fluids protocol) according to the instructions of each manufacturer. Eight replicates were assayed in the BDProbeTec™ ET *Bordetella pertussis* system and compared to an unprocessed control (N=8). Figure 8.

RESULTS

Figure 1. BDProbeTec™ ET Liquid SDA Workflow

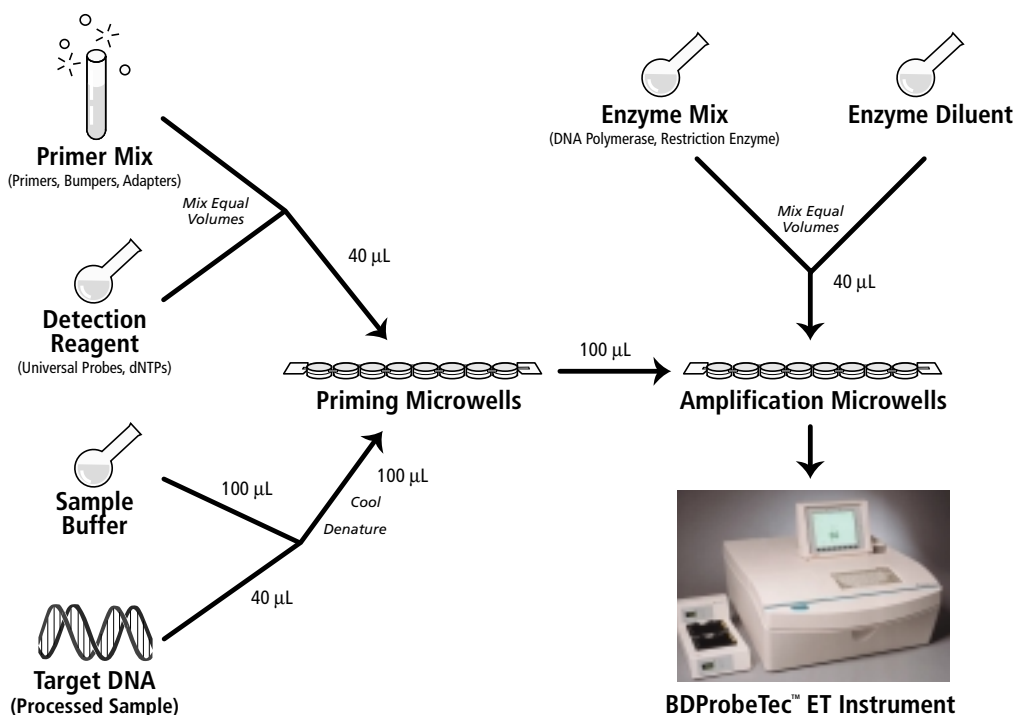
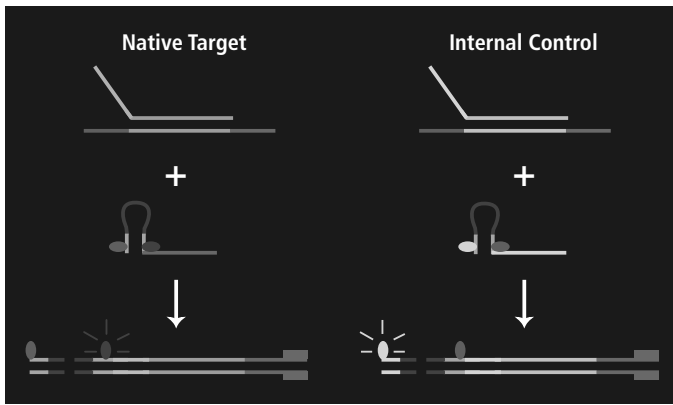


Figure 2. Diplex Universal Detection



Internal Amplification Control

- Verifies negative results and identifies inhibitory samples
- Same priming sequences as native target but with mutated internal region
- Native target and IAC detected using probes labeled with different dyes

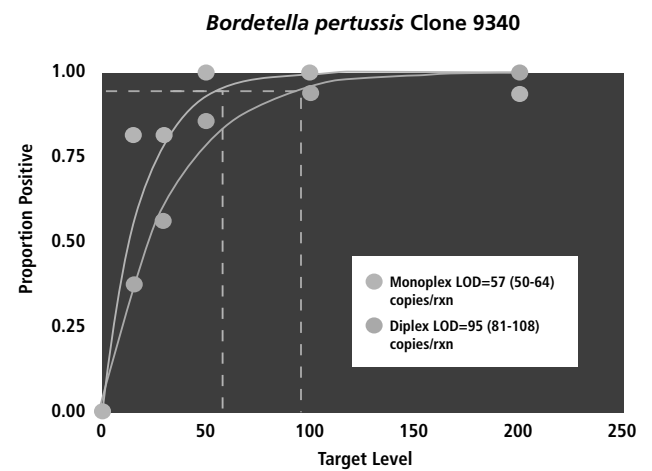
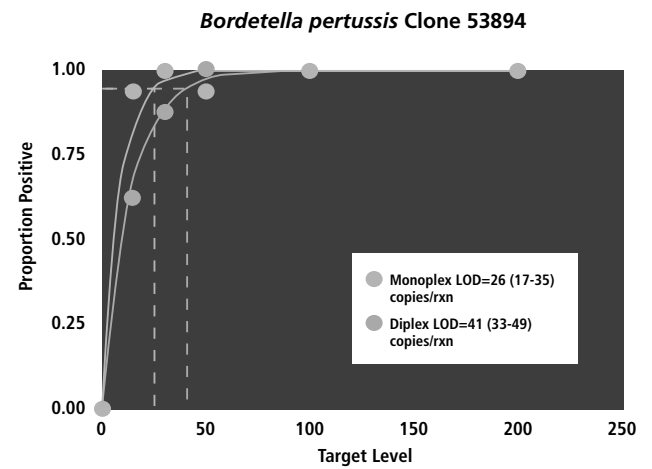
Figure 4. Specificity

	ATCC Strain #
<i>Bordetella pertussis</i>	9797
<i>Bordetella pertussis</i>	8478
<i>Bordetella pertussis</i>	1038
<i>Bordetella pertussis</i>	11615
<i>Bordetella pertussis</i>	12743
<i>Bordetella pertussis</i>	53894
<i>Bordetella pertussis</i>	51445
<i>Bordetella pertussis</i>	9832 *
<i>Bordetella pertussis</i>	12742
<i>Bordetella pertussis</i>	9340

All *Bordetella pertussis* strains were detected at < 1000 organisms per reaction

*Two of three replicates were positive.

Figure 3. Analytical Sensitivity/Limit of Detection



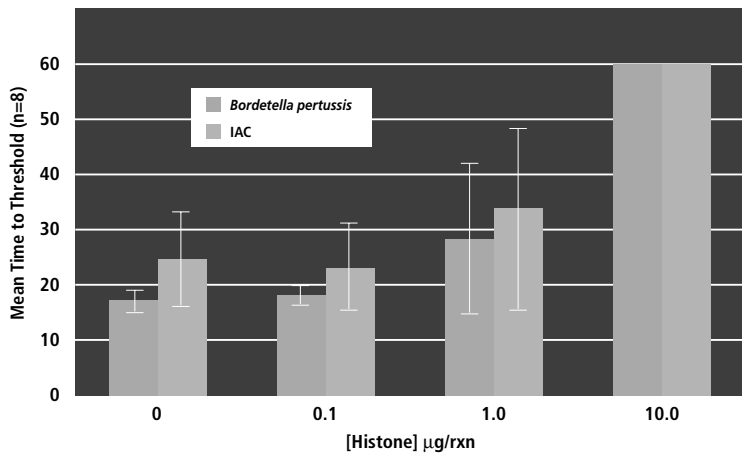
Addition of the IAC does not significantly affect the LOD. Data in parentheses represent upper and lower 95% bounds of confidence.

Figure 5. Cross Reactivity. (All organisms tested at ~10⁶ organisms/reaction)

<i>Bordetella</i> species	Non- <i>Bordetella</i> species	Non- <i>Bordetella</i> species	Non- <i>Bordetella</i> species
<i>B. avium</i> ATCC 35086	<i>Acinetobacter calcoaceticus</i> ATCC 13339	<i>Moraxella osloensis</i> ATCC 19976	Adenovirus-5 ABI Type 5
<i>B. bronchiseptica</i> ATCC 10580	<i>Actinomyces israelii</i> ATCC10049	<i>Mycobacterium tuberculosis</i> ATCC 27294	<i>Blastomyces dermatitidis</i> ATCC 4292
<i>B. hinzii</i> ATCC 51783	<i>Aeromonas hydrophila</i> ATCC 7966	<i>Neisseria gonorrhoeae</i> ATCC 19424	<i>Chlamydia pneumoniae</i> ABI AR-39
<i>B. holmesii</i> ATCC 51541	<i>Alcaligenes faecalis</i> ATCC 8750	<i>Neisseria meningitidis</i> ATCC 13077	<i>Chlamydia trachomatis</i> ABI LGV2
<i>B. parapertussis</i> ATCC 15311	<i>Branhamella catarrhalis</i> ATCC 25285	<i>Neisseria mucosa</i> ATCC 19696	<i>Coccidioides immitis</i> ATCC 7366
	<i>Candida albicans</i> ATCC 44808	<i>Peptostreptococcus anaerobius</i> ATCC 27337	<i>Cryptococcus neoformans</i> ATCC 36556
	<i>Citrobacter freundii</i> ATCC 8090	<i>Porphyromonas asaccharolytica</i> ATCC 25260	<i>Cytomegalovirus</i> -ABI AD-169
	<i>Corynebacterium diphtheriae</i> ATCC 11913	<i>Pseudomonas pertucinogena</i> ATCC 190	Enterovirus (Echovirus) ABI 11
	<i>Corynebacterium jeikeium</i> ATCC 43734	<i>Pseudomonas aeruginosa</i> ATCC 27853	Herpesvirus-1 ABI MacIntyre
	<i>Cryptococcus neoformans</i> ATCC 36556	<i>Prevotella oralis</i> ATCC 33269	<i>Histoplasma capsulatum</i> ATCC 12700
	<i>Eikenella corrodens</i> ATCC 23834	<i>Salmonella choleraesuis</i> serotype <i>enteritidis</i> ATCC 13076	Influenza virus A- BDAD PR8
	<i>Enterobacter aerogenes</i> ATCC 13048	<i>Salmonella choleraesuis</i> serotype <i>typhi</i> ATCC 19430	Influenza virus B-BDAD HK/5/72
	<i>Enterobacter cloacae</i> ATCC 13047	<i>Serratia marcescens</i> ATCC 8100	<i>Mycoplasma pneumoniae</i> -ABI Haflica
	<i>Enterococcus faecalis</i> ATCC 29212	<i>Staphylococcus aureus</i> , protein A-producing ATCC 12598	Parainfluenza 1 virus-BDAD Sendai
	<i>Enterococcus faecium</i> ATCC 19434	<i>Staphylococcus aureus</i> , non-protein A-producing ATCC 25923	Rhinovirus ABI 80-015
	<i>Escherichia coli</i> ATCC 11775	<i>Staphylococcus epidermidis</i> ATCC E155	Resp. Syncytial virus, Long strain ABI 74-093
	<i>Fusobacterium nucleatum</i> ATCC 25586	<i>Stenotrophomonas maltophilia</i> ATCC 13637	
	<i>Haemophilus influenzae</i> ATCC 33533	<i>Streptococcus</i> group B ATCC 12386	
	<i>Haemophilus parainfluenzae</i> ATCC 7901	<i>Streptococcus pneumoniae</i> ATCC 6303	
	<i>Klebsiella pneumoniae</i> subsp. <i>ozaenae</i> type 4 ATCC 11296	<i>Streptococcus pyogenes</i> ATCC 19615	
	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> ATCC 13883	<i>Streptococcus mutans</i> ATCC 25175	
	<i>Lactobacillus acidophilus</i> ATCC 4356	<i>Veillonella parvula</i> ATCC 10790	
	<i>Legionella micdadei</i> ATCC 33204		
	<i>Legionella pneumophila</i> ATCC 33152		

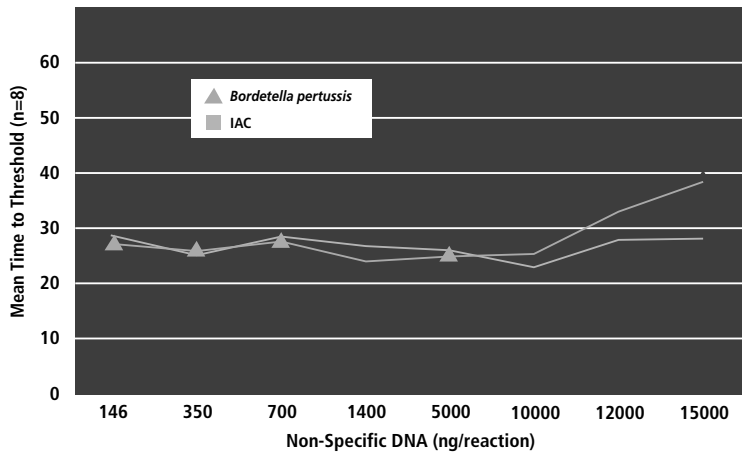
None of the organisms tested cross-reacted in the BDProbeTec™ ET *Bordetella pertussis* Assay.

Figure 6. IAC Effectiveness. SDA Inhibition with Histones (type III)



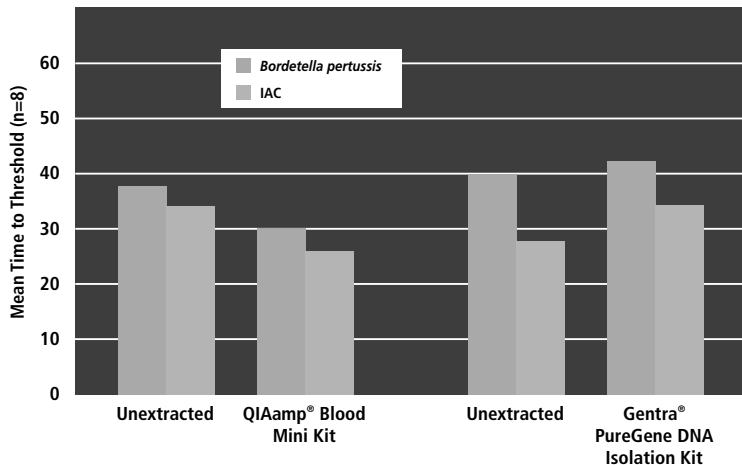
IAC is effective at indicating SDA inhibition. Error bars represent +/- 1 Standard Deviation.

Figure 7. Tolerance to Non-specific DNA



Both *Bordetella pertussis* and internal amplification control targets are tolerant to a wide range of Non-specific DNA levels.

Figure 8. Compatibility with Extraction Methods



The BDProbeTec™ ET *Bordetella pertussis* assay is compatible with the QIAamp® Mini Blood Kit and the Gentra® PureGene DNA Isolation Kit.

CONCLUSIONS

- A sensitive and specific assay has been developed for the detection of *Bordetella pertussis* DNA using the BDProbeTec™ ET System.
- The assay is compatible with both the QIAamp® and Gentra® DNA extraction kits and is tolerant to varying levels of non-specific DNA.
- The assay has been developed with an optional internal control which has been shown to be effective at verifying negative results and identifying inhibitory samples.
- The *Bordetella pertussis* assay does not cross-react with other bacteria, viruses, or fungi likely to be encountered in respiratory samples. As part of a respiratory panel currently under development, along with *Legionella pneumophila*, *Mycoplasma pneumoniae*, and *Chlamydiaceae* family assays, the *Bordetella pertussis* assay has the potential to expand the capabilities of the BDProbeTec™ ET System.