Rapid Detection of Bordetella pertussis and Bordetella parapertussis DNA in Clinical Samples by Duplex Real Time PCR Assay Using Taqman Probes
Sanjib Bhattacharyya*, Manjeet Khubbar, Susan Koehler, Ajaib Singh and Steve Gradus; City of Milwaukee Health Department Laboratory (MHDL), Milwaukee, Wisconsin

Abstract
Detection of Bordetella pertussis, the causative agent for whooping cough in Wisconsin has been based in the development of rapid nucleic acid tests, since treatment of disease at an early stage is crucial to control outbreaks. In this study, we report a rapid and sensitive assay for simultaneous detection of B. pertussis and B. parapertussis in clinical samples by duplex real time PCR assay. The assays were used in the clinical laboratory to identify B. pertussis and B. parapertussis in clinical samples. The assay was designed to detect B. pertussis and B. parapertussis in single or duplex, 24 cycle PCR using Syber Green and Taqman probes. The PCR was performed in 25 µl volume with 2 µl template DNA and 2 µl of the internal control (IC) in separate duplex assay. The Taqman PCR Cycling conditions were as follows:

Methods
1. DNA extraction from patient samples: DNA was extracted from NP swabs using QIAamp DNA Mini kit (Qiagen, Valencia, CA) with minor modifications for various samples. The swabs were washed in PBS and suspended in 200µl PBS buffer with 20 µl Qagen Protease K, followed by manufacturer’s protocols and eluted with 50 µl of AE buffer.

2. PCR controls: Laboratory controls of B. pertussis and B. parapertussis were used as positive extraction controls. Human β-ACT controls were used as inhibition controls.

3. Duplex PCR Assay in SmartCycler II: Duplex Taqman PCR assays were performed with Circoviridae IC bears, 400 pmol primers, 200 pmol IC, 200 pmol of labeled probes and 2 µl template DNA in 25 µl volume. Amplification using Omnimix IC bears was performed in 25 µl volume.

4. Real Time PCR: A Comparison between Cepheid B. pertussis ASR and MHDL Duplex Assay

Results
1. The MHDL Taqman PCR demonstrated very high sensitivity and specificity for detecting B. pertussis and B. parapertussis in single reaction.

2. The turnaround time for MHDL Duplex PCR assay is 29 min.

3. Cepheid B. pertussis ASR bead assay is very specific for B. pertussis and equally sensitive when template concentration is increased.

Acknowledgement
Funding from Wisconsin State Laboratory of Hygiene Sub-Grant BTFY04-1 for the Laboratory Response to Bioterrorism Preparedness in Wisconsin.

Conclusions
1. The MHDL Taqman PCR demonstrated very high sensitivity and specificity for detecting B. pertussis and B. parapertussis in single reaction

2. The turnaround time for MHDL Duplex PCR assay is 29 min.

3. Cepheid B. pertussis ASR bead assay is very specific for B. pertussis and equally sensitive when template concentration is increased.

4. Amplification using Omnimix IC beads provide high performance, reproducibility and ease during the assays.