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IDAHO DEPARTMENT OF  
HEALTH & WELFARE

## Introduction of the Bp\_pCB1\_prn Three Target *Bordetella pertussis* Real Time PCR Assay

Amanda J. Bruesch, MS

The Idaho Bureau of Laboratories has been using a real time PCR assay for detection of *Bordetella pertussis* in nasopharyngeal samples using the IS481 target and an internal control. Performance of the test was verified against samples that were deemed true positives or negatives using the Cepheid *Bordetella pertussis* ASR as a gold standard. While the assay has been in use for over a year at the lab, there has been great interest in adding an additional target to the assay to increase specificity. Previous research has shown that detection of the IS481 insertion sequence, while easily amplified, can lead to false positive results as the insertion sequence is found in other *Bordetella* species. In order to alleviate the potential for false positive results, the pertactin (*prn*) gene was selected as a third target in our assay. The pertactin gene is present as a single

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copy per cell in only two species of *Bordetella*, *pertussis* and *bronchiseptica*. However, the combination of both the pertactin gene and IS481 is only found in *B. pertussis*. Addition of the *prn* gene as a target also satisfies all of the requirements recommended by CDC for a specific diagnostic real time PCR assay for the detection of *B. pertussis*. As shown in Table 1, a sample must meet a specific amplification profile to be considered positive for the presence of *B. pertussis* DNA. All three targets must be amplified to positively identify *B. pertussis* DNA. Amplification of the insertion sequence or the pertactin gene, but not both, indicates the presence of another *Bordetella* species, and not *B. pertussis*.



## Season's Greetings from the IBL Microbiology Team

**Front, left to right:** Rachel Ketterling, Sadika Kobic, Amanda Bruesch  
**Back, left to right:** Walt Delong, Kari Getz, Vivian Lockary, Rachel Beukelman,  
Dr. Chris Ball, Raemi Nolevanko, Joanna Lewis, Steve Gregoire, Colleen Greenwalt  
**Not pictured:** Vonnita Barton

Table 1. Amplification profiles for known *Bordetella* species.

	IS481	Internal Control (IC)	Pertactin gene (prn)
<i>B. pertussis</i>	+	+	+
<i>B. parapertussis</i>	-	+	-
<i>B. holmesii</i>	+	+	-
<i>B. avium</i>	-	+	-
<i>B. bronchiseptica</i>	-	+	+

To verify the Bp\_pCB1\_prn *Bordetella pertussis* real time PCR assay for use in testing clinical samples, we evaluated its performance using genomic DNA extracted from 30 samples with known Bp\_pCB1 values. Of the 30 samples tested: 10 were extracted from cultures of *B. pertussis*, *B. avium*, *B. bronchiseptica*, *B. holmesii*, and *B. parapertussis*, and 20 were clinical samples. Performance of the assay was measured in a variety of ways (Table 2). Accuracy, or the ability to correctly identify a positive or negative sample, was calculated at 100%. Positive predictive value, or the ability to correctly identify a positive sample, was 100% with this assay, and negative predictive value or the ability to correctly identify a negative sample, was also 100%.

Table 2. Performance Characteristics of the Bp\_pCB1\_prn *B. pertussis* Assay

Bp_pCB1_prn Performance	Accuracy	100%
	Positive Predictive Value	100%
	Negative Predictive Value	100%

The results of this verification study show that the addition of the pertactin gene as a third target in our assay will result in more specific and sensitive results. Our ability to differentiate *B. pertussis* from other *Bordetella* species is very important in both the diagnosis and treatment of whooping cough in residents of the state of Idaho.

## Forum Findings

(words found in this issue and Fall issue – solution on last page)

C J O A O H Y E M A J D K B N L W H Q I P F B T J K R C N S  
 X I E H T A P T L I E J E B O T C P N N W E P S K E I C D D  
 I P M H B Y T L I T K T B R F Z P S J V Z W R E P E J M W J  
 X M N E T X E D E L A K T F N I K U I E U S I T R W S U L G  
 C S V B D R H C C L I N M O N I T O R S C A A E U Z N P N P  
 J A U N E N T K A B O B N K W K O M N T E O N G H S Y B D I  
 Z S R T A I A C Y C D B I R W C G O Z I Q X T D T X S J M P  
 I Q T B O H T P N U M M G T O T C D W G E C I O L E M I A O  
 V U A N A A I O B O O U P L P G T U G A O B V H A M S C S T  
 S F D H M P I P L A S M I D M E D I A T E D I D E I T G I Y  
 L N Q A I T E W F N Y H I E K C C A A I W T R E H P R A Y U  
 H B S T C E C N A L L I E V R U S S C O N R A I C E A C P J  
 G E S E E E U A E D V C I V D J H C U N G Q L F I F I Y U M  
 M B F E R D O Y A M M Q O X A U Y E Z S X Z N I L E N D F Z  
 T N S M D N W B U Y A Y H Z T J K N T T G H Z D B C G Z V D  
 I X V E R V S R Y Z S S R F P V T A D L G U V O U J F U V P  
 I N F L U E N Z A E Z L E G I B V Q A T N L R M P H E V A D  
 N O I T C A E R N I A H C E S A R E M Y L O P D C P M A E T  
 U L Q N W O T E P A C E L C A F V S D B Q F D Y R H S F E F  
 T V N B Z C Z W B R C H U Q U L S C H Y T R S G H T M G O K

### WORD LIST

AMPC  
 ANTIVIRAL  
 BETA LACTAMASE  
 CAPETOWN  
 CARBAPENEMASE  
 CEFEPIME  
 COLI  
 DETECTION  
 DRUG SUSCEPTIBILITY  
 ESBL  
 INFECTION CONTROL  
 INFLUENZA  
 INVESTIGATION  
 JEJUNI  
 KPC  
 MODIFIED HODGE TEST  
 MONITOR  
 PANDEMIC  
 PERTUSSIS  
 PLASMID MEDIATED  
 POLYMERASE CHAIN  
 REACTION  
 PUBLIC HEALTH  
 STRAIN  
 SUBTYPE  
 SURVEILLANCE  
 SUTTERELLA

## Idaho Bureau of Laboratories Campylobacter Surveillance Project

Vivian Lockary, MPH, MT(ASCP) and Christopher L. Ball, PhD

Campylobacteria are the most commonly reported enteric bacterial pathogens in Idaho (Idaho Reportable Disease Summaries). From 2004 through 2008, *Campylobacter* was reported to cause approximately 1.5 times more illness than *Salmonella* species (Table 1). While standard laboratory procedures suggest that only *C. jejuni*, *C. coli*, and *C. fetus* are clinically important, the use of the Capetown Protocol for *Campylobacter* and *Helicobacter* has shown that up to 17 different species or subspecies of Campylobacteriaceae can be isolated from clinical specimens.

Table 1. Idaho Reportable Disease Statistics

Year	<i>Campylobacter</i>	<i>Salmonella</i>
2004	238	159
2005	226	151
2006	245	179
2007	214	155
2008	277	199

In 2007, the Idaho Bureau of Laboratories (IBL) designed a preliminary study to determine the prevalence of Campylobacteriaceae other than *C. jejuni* and *C. coli* from a sub-sampling of local stool cultures. The intention of our study was to estimate the prevalence and diversity of *Campylobacter* species in the Treasure Valley and determine if a larger study on the epidemiology and prevalence of *Campylobacter* in Idaho was warranted.

Blinded stool samples were obtained from St. Alphonsus Regional Medical Center Microbiology lab for primary isolation of Campylobacteriaceae using the Capetown Protocol. Identification of *Campylobacter* species (other than *C. jejuni* and *C. coli*) and *Campylobacter*-related species was determined by partial 16S ribosomal RNA gene sequence analysis.

The Capetown Protocol utilizes passive filtration. A watery emulsion of stool in saline is placed onto a membrane filter (0.6 µm) on non-selective media (blood agar) for 15 minutes. Gravity causes the water to pass through the filter, carrying the slender organisms with it. The filter is removed and the plate is incubated with an increased hydrogen-microaerophilic atmosphere for six days. Eight culture plates containing various selective

agents, under varying incubation conditions, would be required to isolate all the *Campylobacter* recovered by the Capetown Protocol.

Twenty two of the isolates recovered from 393 cultures (5.6%) were identified as *Campylobacter* or *Campylobacter*-related species (e.g. *Arcobacter*, *Helicobacter*, and *Sutterella wadsworthensis*). Interestingly, *C. jejuni* represented only 13.6% of these isolates (3 of 22). The significance of our findings is highlighted when one considers the limitations of routine detection methods for *Campylobacter* species in clinical laboratories. For instance, use of selective media that includes cephalothin, colistin, and polymixin B inhibits some strains of *C. jejuni*, and *C. coli*, as well as *C. fetus* subsp. *fetus*, *C. jejuni* subsp. *doylei*, *C. upsaliensis*, and *A. butzleri*. Increased hydrogen is a requirement for the primary isolation and growth of some *Campylobacter* species, including two recovered in this study, *C. consisus* and *C. curvus*. Although Campy-CVA is highly recommended if only a single medium can be used, *C. consisus*, *C. curvus*, and *C. showae* strains recovered in this study demonstrably did not grow on CVA. Moreover, *C. showae* is considered a strict anaerobe so alternative incubation strategies may need to be considered.

*C. curvus/consisus* and *S. wadsworthensis* have been reportedly associated with bloody gastroenteritis (Abbott, 2005; Engberg, 2000). Additionally, *C. curvus/consisus* has possibly been linked to Brainerd's Diarrhea, a chronic diarrheal illness typically affecting older adults (Vugia, 2006).

Our findings suggest that the highest yield of *Campylobacter* from stool samples requires the use of non-conventional methods, a combination of media, and varied incubation conditions. We concluded that routine use of the Capetown Protocol and combination media are impractical for reasons of expense and analyst time. In cases of persistent diarrhea or when Brainerd-like symptoms are present, cultures for non-*C. jejuni* and non-*C. coli* species may be appropriate when primary culture is unrevealing.

Our sincere thanks go to John Burch and the microbiology staff at St. Alphonsus Regional Medical Center in Boise for their collaborative efforts in this study.

## References

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## Influenza Season 2009/10

September 1, 2009 through May 31, 2010

September 1 through November 30, 2009, the Idaho Bureau of Laboratories tested 1494 respiratory specimens for Idaho and U.S. Influenza Surveillance.

- 2009 Pandemic Influenza AH1N1 detected in 677 specimens (45% positivity)
- No seasonal influenza subtypes detected (seasonal AH1, AH3, or B)
- CDC reports that so far all 2009 H1N1 influenza A viruses remain similar to the virus chosen for the 2009 H1N1 vaccine, and remain susceptible to the antiviral drugs oseltamivir and zanamivir with rare exception.

For national update go to the CDC FluView "Weekly Influenza Surveillance Report" at <http://www.cdc.gov/flu/weekly>

## Upcoming Teleconferences

**January 6, 2010**  
**10:00**

Recent Updates to CLSI Antimicrobial Susceptibility Testing Documents

**January 19, 2010**  
**11:30**

Cases in Clinical Microbiology

To receive email notification of upcoming teleconferences, contact Dave Eisentrager at

[Eisentra@dhw.idaho.gov](mailto:Eisentra@dhw.idaho.gov)

## Forum Findings Solution (Over,Down,Direction)

AMPC(28,18,W)	ANTIVIRAL(23,5,S)	BETALACTAMASE(14,1,SW)
CAPETOWN(11,19,W)	CARBAPENEMASE(1,5,SE)	CEFEPIME(26,15,N)
COLI(16,7,SW)	DETECTION(12,1,SW)	DRUG SUSCEPTIBILITY(24,18,NW)
ESBL(4,14,NW)	INFECTION CONTROL(1,16,NE)	INFLUENZA(1,17,E)
INVESTIGATION(20,1,S)	JEJUNI(25,1,SE)	KPC(17,4,N)
MODIFIED HODGE TEST(24,17,N)	MONITOR(13,5,E)	PANDEMIC(8,8,NW)
PERTUSSIS(21,1,SE)	PLASMID MEDIATED(8,10,E)	PUBLICHEALTH(25,17,N)
POLYMERASE CHAIN REACTION(23,18,W)		STRAIN(27,9,S)
SUBTYPE(2,7,NE)	SURVEILLANCE(17,12,W)	SUTTERELLA(1,10,NE)