PCR-BASED TEST FOR THE FORENSIC DETECTION OF FECES; *BACTEROIDES* AS INDICATOR OF FECAL MATTER

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Introduction

In response to the needs of a large urban jail, our laboratory has developed a forensically suitable, PCR-based method for the identification of feces on items of evidence. The test is based on the extensive published research in fecal contamination of water supplies. The method requires that DNA from the questioned stain be recovered (extracted and purified) and analyzed by a combination of PCR and capillary electrophoresis for the presence of 16S rRNA gene of *Bacteroides dorei* (*B. dorei* PCR test) and 16S rRNA gene of other *Bacteroides* species (*Bacteroides* PCR test).

B. dorei is predominantly, but not exclusively, found in human fecal samples. However, *B. dorei* may be absent or below the limit of detection in fecal samples from some individuals. A *Bacteroides* PCR test that detects a wider diversity of *Bacteroides* species and is, thus, less human specific when compared to the *B. dorei* PCR test, detects a *Bacteroides* 16S rRNA gene sequence in human fecal samples that are negative for *B. dorei*.

PCR and Capillary Electrophoresis

Bacteroides dorei gene for 16S rRNA sequence (GenBank: AB242142.1)[1,2]

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   1 agagtttgat cctggctcag gatgaacgct agctacaggc ttaacacatg caagtcgagg
  61 ggcagcatgg tcttagcttg ctaaggctga tggcgaccgg cgcacgggtg agtaacacgt
 121 atccaacctg ccgtctactc ttggccagcc ttctgaaagg aagattaatc caggatggga
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1021 gcaatagcga gtgtgaaggt gctgcatggt tgtcgtcagc tcgtgccgtg aggtgtcggc
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1141 ctgacaagac tgccatcgta agatgtgagg aaggtgggga tgacgtcaaa tcagcacggc
1201 ccttacgtcc ggggctacac acgtgttaca atgggggta cagagggccg ctaccacgcg
1261 agtggatgcc aatccctaaa acccctctca gttcggactg gagtctgcaa cccgactcca
1321 cgaagctgga ttcgctagta atcgcgcatc agccacggcg cggtgaatac gttcccgggc
1381 cttgtacaca ccgcccgtca agccatggga gccgggggta cctgaagtgc gtaaccgcga
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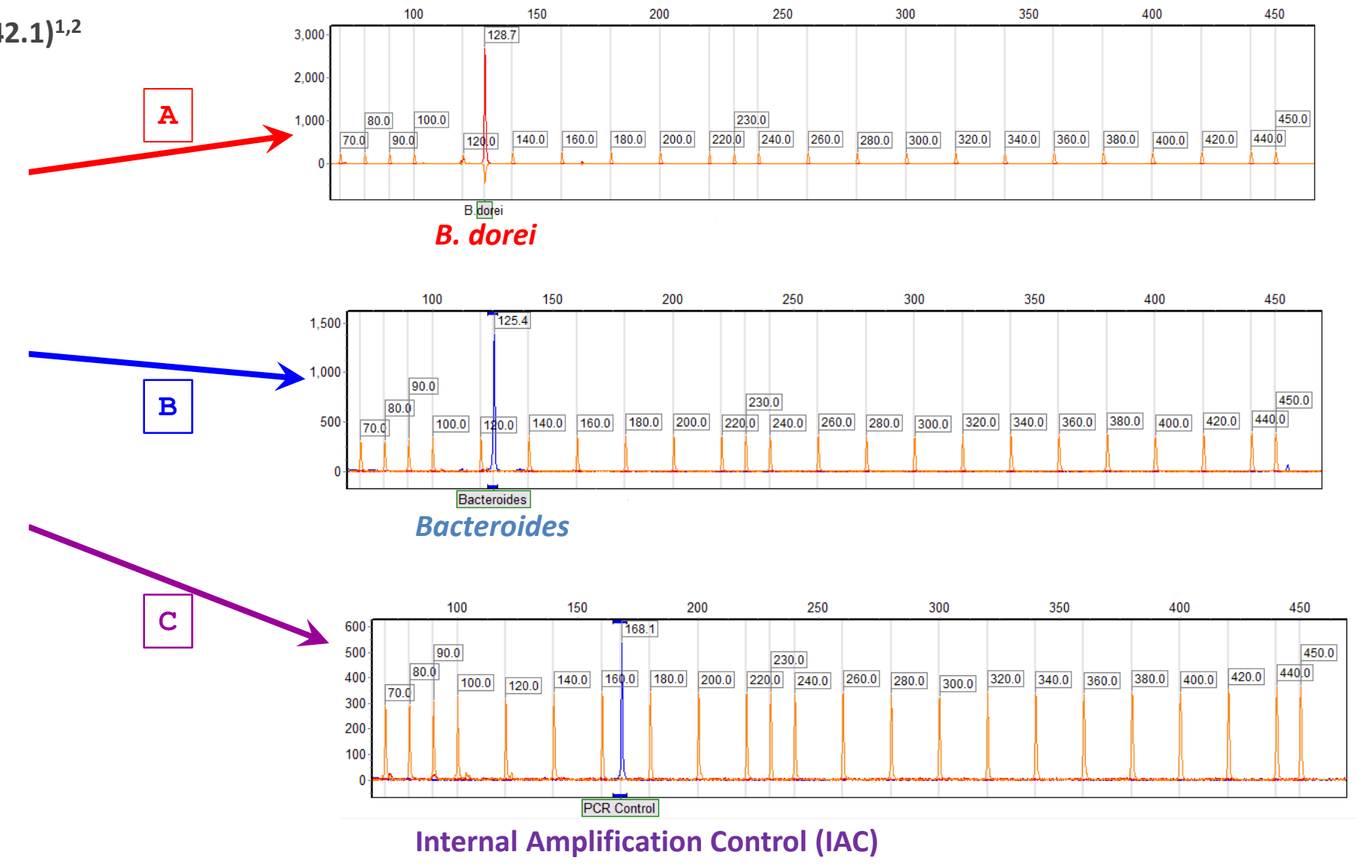


A). *B. dorei* signal is observed when the sequence in RED (bases 180-305) is amplified by ROX labeled forward primer (HF-183[3]) and unlabeled reverse primer (Bac-287[3]).

B). *Bacteroides* signal is observed when the sequence shown in BLUE (bases 561-683) is amplified by 6-FAM labeled forward primer (HuBac566F[4]) and unlabeled reverse primer (HuBac692R[4]).

C). The Internal Amplification Control (IAC) template is the sequence shown in PURPLE (bases 848-1017) amplified by 6-FAM labeled forward primer and unlabeled reverse primer.

Table 1. PCR Reactions and Conditions

PCR component	5 µL PCR	50 µL PCR
2x PCR Buffer	2.5 µL	25 µL
B. dorei/IAC or *Bacteroides/IAC* Primer Mix	1.0 µL	10 µL
AmpliTaq Gold DNA Polymerase (ABI)	1.25 U	12.5U
DNA	1.25 µL	12.5 µL

10 min. 95°C; (15 sec. 95°C; 30 sec. 61°C; 30 sec. 72°C); 10 min. 72°C; hold at 10°C

26 cycles

Table 2. Repeatability and Reproducibility: *Bacteroides, B. dorei* and IAC

Operator 1	***Bacteroides***	***B. dorei***	**IAC**
Number of Experiments	26	26	26
Signal Position (Average)	125.3 bp	128.9 bp	167.8 bp
Standard Deviation	0.15 bp	0.14 bp	0.12 bp
Operator 2			
Number of Experiments	6	14	48
Signal Position (Average)	125.4 bp	128.9 bp	167.8 bp
Standard Deviation	0.04 bp	0.13 bp	0.15 bp

Limit of Detection of *B. dorei* and *Bacteroides* PCR Tests

Serial dilutions of *B. dorei* DNA were made in the presence of various amounts of human DNA (K562). Peak heights for *B. dorei* and IAC, and *Bacteroides* and IAC PCR products are shown.

Table 3. *B. dorei* PCR test: limit of detection

B. dorei **DNA (pg)**	**Human DNA (pg)**	***B. dorei*** **signal (RFU)**	**IAC signal (RFU)**
2.5	625	2477	6321
2.5	62.5	2547	6559
2.5	0	3459	8128
0.25	625	280	1349
0.25	62.5	298	1359
0.25	0	251	916
0.025	625	18	984
0.025	62.5	26	838
0.025	0	31	681
0 (Negative Control)	625	0	1052
0 (Negative Control)	62.5	0	991
0 (Negative Control)	0	0	887

Table 4. *Bacteroides* PCR test: limit of detection

B. dorei **DNA (pg)**	**Human DNA (pg)**	***Bacteroides*** **signal (RFU)**	**IAC signal (RFU)**
0.625	625	>9000	6680
0.625	62.5	>9000	6481
0.625	0	>8800	5975
0.0625	625	1039	1443
0.0625	62.5	1172	1652
0.0625	0	1454	1929
0.00625	625	114	1276
0.00625	62.5	37	869
0.00625	0	37	1403
0 (Negative Control)	625	0	854
0 (Negative Control)	62.5	0	854
0 (Negative Control)	0	0	889

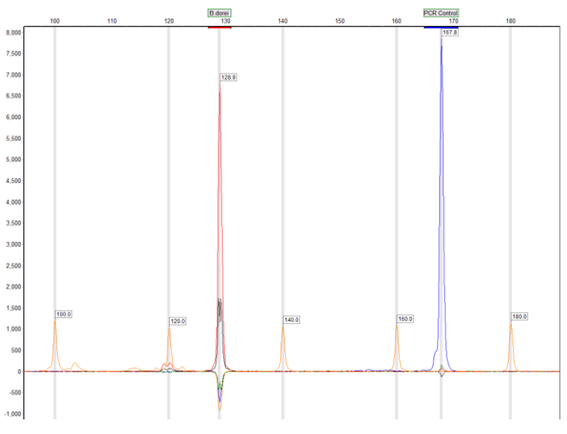
Specificity of *B. dorei* and *Bacteroides* PCR Tests

Human fecal samples (DNA extracted and purified with Qiagen QIAamp Fast DNA Stool Mini kit)
Fecal samples from 11 individuals were tested: all samples were positive for *Bacteroides* and eight were positive for *B.dorei.*

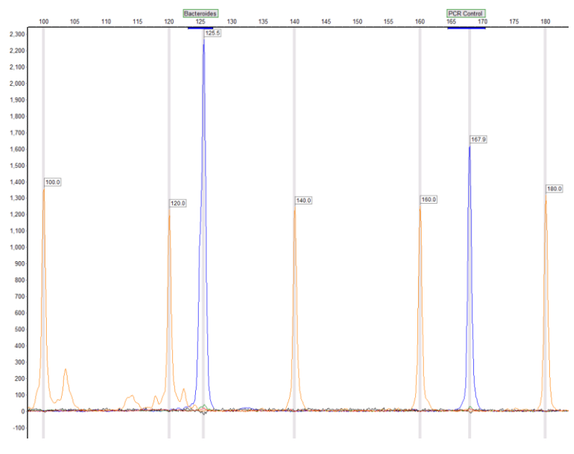
Human body fluid samples from nine individuals.
4 blood samples, 9 saliva samples, 3 semen samples and 6 urine samples were tested: all samples were negative for both, *Bacteroides* and *B.dorei*

Animal fecal samples.
Fecal samples from two dogs, one cat, one geese and one parrot were tested. All samples were negative for *B.dorei,* but positive for *Bacteroides.*

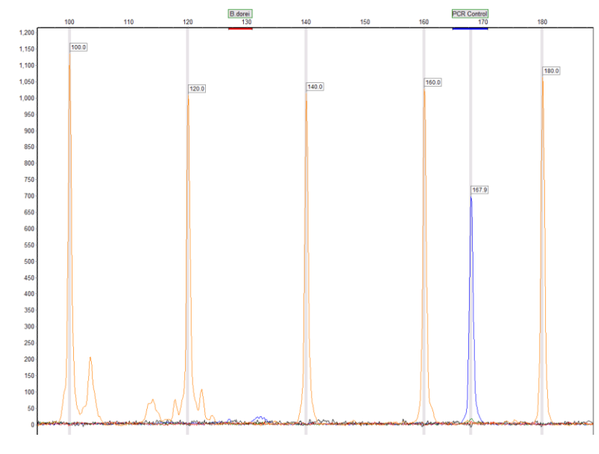
B. dorei positive test
(only 100 – 180 bp range is shown)



Bacteroides positive test
(only 100 – 180 bp range is shown)



Negative Control
(only 100 – 180 bp range is shown)



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