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Evaluation of a PCR/DNA Probe Colorimetric Membrane Assay for Identification of *Campylobacter* spp. in Human Stool Specimens

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DNA was extracted from 50 human stool specimens using the QIAamp DNA stool minikit. PCR amplification was followed by post-PCR hybridization to DNA probes specific for the *Campylobacter* genus, *Campylobacter jejuni*, and *Campylobacter coli* in a colorimetric membrane assay. Thirty-two of 38 culture-positive specimens were PCR/DNA probe positive for *C. jejuni*. The assay is rapid and simple and can be applied to stool specimens for the detection of *Campylobacter*.

PCR is currently used for the diagnosis of infectious agents, and there are a number of reports of the application of PCR for the direct detection of *Campylobacter*, the most common cause of enteritis, in stool specimens (5, 6, 7, 10, 11). These and other researchers (1, 2, 4, 9) have also evaluated the application of a range of commercially available and/or in-house methods for the extraction of DNA from stool or other clinical specimens. Our study represents a preliminary evaluation of a PCR/DNA probe colorimetric membrane assay previously developed in this laboratory for the identification of *Campylobacter* in DNA extracted from stool specimens using the newly available QIAamp DNA stool minikit (Qiagen, Hilden, Germany). Fifty stool specimens, including 38 culture-positive and 12 culture-negative specimens, were specifically selected and sent from the department of Medical Microbiology, University College Hospital, Galway, Ireland, for this study. The specimens were routine clinical samples submitted from patients with acute diarrheal disease from both the community and hospital. Specimens were cultured on modified charcoal cefoperazone deoxycholate agar (Oxoid, Basingstoke, United Kingdom) in an atmosphere of 5% CO_2 at 42°C for 48 h. *Campylobacter jejuni* NCTC 12241 was included with each lot of plates processed as a control. Plates were examined for colonies with characteristic morphology, and suspected isolates were confirmed as *Campylobacter* spp. based on Gram stain results and a positive test for oxidase. A heavy suspension of the isolate was prepared in 0.5 ml of saline, and a hippurate hydrolysis diagnostic tablet (Rosco, Taastrup, Denmark) was added. Following incubation at 37°C for 4 h, 5 drops of ninhydrin was added. *C. jejuni* NCTC 12241 was used as a control. Isolates which were positive for hippurate hydrolysis were identified as *C. jejuni*, and those which were negative were identifed as *Campylobacter coli*.

The 38 culture-positive specimens included 30 specimens (set A) which were sent to this laboratory as soon as they were determined to be culture positive for *Campylobacter* and a series of 8 specimens (set B) positive on initial culture which were stored at room temperature and recultured at 2-day intervals until it was no longer possible to recover *Campylobacter* spp. from them. Aliquots of the 50 specimens, including those that had become culture negative as a result of aging, that had been sent for testing with the PCR/DNA probe assay for *Campylobacter* were stored on receipt at −20°C prior to DNA extraction.

Using the QIAamp DNA stool minikit, DNA was extracted from eight specimens per batch according to the manufacturer's instructions, selecting the option to incubate the specimens in lysis buffer at 95°C rather than at 70°C. Five microliters of extracted DNA (undiluted or diluted 1/10 in PCR-grade water; Sigma-Aldrich, St. Louis, Mo.) was included in a 100-μl PCR mixture with biotinylated PCR primers CAMP1F-B (5′-GTT AAGAGTCACAAGCAAGT-3′) and B1-B (5′-C[C/T][A/G][C/T]TGCCAAGGCATCCACC-3′) (MWG-Biotech, Milton Keynes, United Kingdom) designed to amplify the 16S/23S rRNA intergenic spacer of members of the genus *Campylobacter* (8). A positive control consisting of DNA extracted from *C. jejuni* NCTC 8116 and a negative PCR control consisting of PCR-grade water were included in each PCR run. PCR setup was performed in a biological cabinet using dedicated pipettes and aerosol barrier tips to minimize the risk of PCR contamination. Fifty microliters of the biotinylated PCR product was reverse hybridized to DNA probes CAMP4 (5′-GGTAAGCTACTAAGAGCG-3′), CJEJ7 (5′-GCTTAGTT GAGACTAAATCA-3′), and CCOL2 (5′-GACTTAGTTTAG ATATTTTTAG-3) immobilized on a membrane (8). The probes allow the specific identification of members of the *Campylobacter* genus, *C. jejuni*, and *C. coli*, respectively. A positive control probe (POS-DET) for the colorimetric detection component of the assay and a negative control probe were also included on each membrane. Briefly, the PCR products were denatured by heating to 95°C for 7 min. The denatured PCR products were mixed with 1 ml of Hyb/Wash solution (5× SSPE, 0.1% sodium dodecyl sulfate [1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA, pH 7.7]) and incubated with the membrane at 55°C for 1 h. The membranes were washed twice for 10 min in Hyb/Wash solution and once for 5 min in TBS (50 mM Tris-Cl [pH 7.5], 0.15 M NaCl). The

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TABLE 1. PCR/DNA probe assay and culture analysis of 50 human stool specimens for *Campylobacter* spp.

Set (*n*)[a]	No. of specimens[b]		
	Culture positive	Culture negative	PCR/DNA probe positive
A (30)	30	0	27
B (8)	0	8	5
C (12)	0	12	0

[a] Set A, culture positive; set B, originally culture positive but currently culture negative; set C, originally culture negative.
[b] Total probe-positive specimens/total culture-positive specimens, 32/38 (84%); probe-positive specimens/currently culture-positive specimens, 27/30 (90%); rescue, 5 of 8 (63%).

membranes were incubated for 20 min in 10 ml of TBS–1% Tween 20 (Sigma-Aldrich) with a 1/2,000 dilution of streptavidin-alkaline phosphatase conjugate (Calbiochem-Novobiochem, La Jolla, Calif.). The membranes were washed twice for 10 min in TBS and once for 5 min in color development solution (0.1 M Tris-Cl [pH 9.5], 50 mM $MgCl_2$, 0.1 M NaCl). The substrates nitroblue tetrazolium (50 μl of a 100-mg/ml solution) and 5-bromo-4-chloro-3-indolylphosphate (37.5 μl of a 50-mg/ml solution) were added to 10 ml of color development solution, and the membranes were incubated in this solution until clearly visible positive signals were obtained from the POS-DET positive control probe. Soaking the membranes in distilled water stopped color development. The specificity of the CAMP4, CJEJ7, and CCOL2 probes had been previously confirmed against a range of *Campylobacter* strains (3) and against a panel of non-*Campylobacter* isolates (8). The detection limits of these probes determined by Southern blot hybridization analysis with digoxigenin-labeled probes were 100 fg for *C. jejuni* DNA with CAMP4 and CJEJ7 and 100 fg to 1 pg for *C. coli* DNA with CAMP4 and CCOL2 DNA probes.

Of the 30 set A (Table 1) specimens, 23 were PCR/DNA probe positive for *Campylobacter* when 5 μl of the undiluted resuspended DNA was included in PCR and an additional 4 were positive when a 1/10 dilution of this DNA was included in the PCR. PCR products from these specimens hybridized to the CAMP4 and CJEJ7 probes (determining the presence of *C. jejuni* in these specimens), yielding a sensitivity of 90% for the PCR/DNA probe-based detection of *Campylobacter* in culture-positive specimens. Five of the eight specimens in set B (Table 1) were DNA probe positive for the presence of *C. jejuni* in the colorimetric membrane assay, which represented a recovery of 63% of previously culture-positive, currently culture-negative specimens with this assay. The PCR products did not hybridize with any other probes present on the membrane. Combining the results of set A and set B, the overall sensitivity of the PCR/DNA probe colorimetric assay compared to culture was 84%. Other researchers have reported sensitivities of 100% (6) and 83% (11) for PCR assays for *Campylobacter* applied directly to DNA extracted from stool specimens, while a sensitivity of 91% was achieved by another research group (10) following overnight enrichment of the stool specimen prior to the application of PCR. An aliquot of DNA from the six culture-positive specimens which were PCR/DNA probe negative for *Campylobacter* was PCR amplified with the universal 16S/23S rRNA primers (A1/B1) as previously described (3) to investigate if the DNA extracted from these specimens was amenable to PCR. The six specimens yielded PCR products with the universal primers.

Twelve culture-negative specimens (set C) (Table 1) were included in the study, and DNA extracted from these specimens was PCR amplified with the *Campylobacter* genus-specific primers and with universal PCR primers. No PCR products were obtained from these 12 samples with the *Campylobacter* genus-specific primers. Ten of the twelve samples yielded PCR products with the universal primers, indicating that PCR-quality DNA was extracted from these specimens. The remaining two samples failed to yield PCR products with this primer set, most likely because of copurification of PCR inhibitors in these specimens. No DNA probe signals were obtained from these specimens when the A1/B1 PCR products were hybridized to the membrane-bound DNA probes for *Campylobacter* spp.

The aim of this study was to investigate the application of a PCR/DNA probe colorimetric membrane assay originally developed for the identification of *Campylobacter* spp. in poultry meat to the identification of *Campylobacter* spp. in human stool specimens. We decided to evaluate the QIAamp DNA stool minikit for the extraction of PCR-quality DNA from stools, as it was newly available and at the outset of this study the performance of this kit for the extraction of PCR-quality DNA from stool specimens had not previously been reported on in the literature. Ninety-six percent of the specimens yielded DNA that was amenable to PCR following extraction with the QIAamp DNA stool minikit. Therefore, the QIAamp DNA stool minikit was capable of extracting PCR-quality DNA efficiently from stool specimens. A recent report (4) evaluated the sensitivity of the QIAamp DNA minikit for the extraction of PCR-quality DNA from liquid stool specimens and reported a sensitivity of 86% based on positive PCR signals for the target under investigation.

This PCR/DNA probe colorimetric assay is simple to perform; the membranes containing the immobilized DNA probes can be easily prepared in large batches using specialized equipment (X-Y 3000 dispensing platform [BioDot Inc., Irvine, Calif.]) as previously described (8) or manually using a hand-held pipette and spotting the required probes onto the membrane. The membrane strips with immobilized probes can be stored for 6 months at 4°C in an airtight container. As several probes can be immobilized on a single membrane strip, it is possible to simultaneously screen a sample for the presence of more than one species. We have previously reported (8) finding mixed cultures of *C. jejuni* and *C. coli* in 55% of Irish poultry meat samples using this technology. In this application of the technology we found 100% of the PCR/DNA probe-positive specimens to be positive for *C. jejuni*, which correlates with the phenotypic results obtained at University College Hospital, Galway, for these specimens. Currently conventional culture performed at University College Hospital, Galway, requires 48 h to obtain results, which must be followed by biochemical tests to discriminate *C. jejuni* from *C. coli.* The extraction of DNA directly from fecal specimens followed by the application of the PCR/DNA probe colorimetric membrane assay can be performed in a single working day, with definitive identification of *C. jejuni* and *C. coli* and with the capability of identifying the presence of mixed cultures of these organisms. This rapid turnaround time to results may be advantageous for

testing of certain specimens, for example, pediatric specimens or specimens from elderly patients. The extraction of DNA from the fecal specimens that is amenable to PCR may also provide for molecular epidemiology to be performed on the DNA should additional information regarding the source of the infection be required. It is of interest that five of eight aged specimens from which it was no longer possible to isolate *Campylobacter* species by culture were positive on testing with the PCR/DNA probe assay. This finding suggests that the process described may have a particular application in circumstances where transport of specimens to the laboratory is delayed or where samples have been improperly stored during transport to the laboratory. We have now designed species-specific probes for *Campylobacter lari* and *Campylobacter upsaliensis* which will be included on future membrane strips. This small preliminary study demonstrates that the newly available QIAamp DNA stool minikit extracts high-quality DNA from stool specimens for the identification of *Campylobacter* spp. using a PCR/DNA probe colorimetric membrane assay.

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