PCR-ELISAs for the Detection of *Campylobacter jejuni* and *Campylobacter coli* in Poultry Samples

BioTechniques 30:602-610 (March 2001)

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ABSTRACT

Campylobacter species, primarily *Campylobacter jejuni* and *Campylobacter coli*, are regarded as a major cause of human gastrointestinal disease, commonly acquired by eating undercooked chicken. We describe a PCR-ELISA for the detection of *Campylobacter* species and the discrimination of *C. jejuni* and *C. coli* in poultry samples. The PCR assay targets the 16S/23S ribosomal RNA intergenic spacer region of *Campylobacter* species with DNA oligonucleotide probes designed for the specific detection of *C. jejuni*, *C. coli*, and *Campylobacter* species immobilized on NucleoLink™ wells and hybridized to PCR products modified with a 5' biotin moiety. The limit of detection of the PCR-ELISA was 100–300 fg (40–120 bacterial cells) for *C. jejuni* and *C. coli* with their respective species-specific oligonucleotide probes and 10 fg (4 bacterial cells) with the *Campylobacter* genus-specific probe. Testing of poultry samples, which were presumptive positive for *Campylobacter* following culture on the Malthus V analyzer, with the PCR-ELISA determined *Campylobacter* to be present in 100% of samples (n = 40) with mixed cultures of *C. jejuni*/*C. coli* in 55%. The PCR-ELISA when combined with culture pre-enrichment is able to detect the presence of *Campylobacter* and definitively identify *C. jejuni* and *C. coli* in culture-enriched poultry meat samples.

INTRODUCTION

Campylobacter species, primarily *Campylobacter jejuni* and *Campylobacter coli*, are regarded as a major cause of human gastrointestinal disease, commonly acquired by eating undercooked chicken (6). The percentage of *Campylobacter*-contaminated chicken carcasses varies, often between 50%–90% depending on the time of year and the number of carcasses tested. One study found that as many as 98% of chicken carcasses may be contaminated with *C. jejuni* by the time of sale (18). *Campylobacter*s have fastidious growth requirements, and conventional detection and identification require 4–6 days (4). This has stimulated the development of more sensitive and rapid methods for identifying pathogens present in poultry. These new methods include immunomagnetic separation of pathogens, PCR amplification, pathogen-specific DNA and RNA probes, and identification of pathogen-specific ions by mass spectrometry (7). PCR-based methods (15) are particularly attractive for the detection of pathogenic microorganisms because they can be combined with microtiter, membrane, or the newly developed “real-time technologies” to enable the rapid, sensitive, and specific detection of contaminating bacteria. PCR-based detection methods have previously been described for the detection and discrimination of *Campylobacter* species (2–6,8,9,12,13). These reports describe numerous different targets for the detection and discrimination of *Campylobacter*, including PCR assays based on *Campylobacter* flagellin

(12,13), 16S rRNA (4,8,9), 23S rRNA (3), virulence (6), and, recently, cytolethal distending toxin (CLDT) (2) genes. Some of these reports used microtiter technology for end-point detection, while many relied on standard agarose gel electrophoresis-based detection, which is time consuming, cumbersome, and not amenable to high sample throughput. We describe a PCR-ELISA for the detection of *Campylobacter* species with discrimination of *C. jejuni* and *C. coli* from poultry samples. The assay utilizes NucleoLink™ technology (Nalge Nunc International, Roskilde, Denmark). The NucleoLink surface is a physically modified, thermostable, optically clear resin that allows the covalent binding of 5'-aminated oligonucleotides (10). The PCR assay targets the 16S/23S ribosomal RNA intergenic spacer region (Figure 1) of the *Campylobacter* species using genus-specific primers previously designed in this laboratory (11). DNA oligonucleotide probes designed from the sequences of *C. jejuni*, *C. coli*, and *Campylobacter* species (11), modified with an amine and a 12-T linker between the amine group and the species-specific sequence, were immobilized onto NucleoLink wells. Biotinylated PCR products amplified from *Campylobacter* species were alkali denatured, hybridized to the immobilized probe, and detected using streptavidin-horse radish peroxidase conjugate and the chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). We describe the application of this PCR-ELISA technology for the detection of *Campylobacter* in poultry meat samples.

MATERIALS AND METHODS

Bacterial Strains

The bacterial strains listed in Tables 1 and 2 were obtained from the National Collection of Typed Cultures (NCTC), Public Health Laboratory Service (PHLS) Central Public Health Laboratory, London, UK.

Primers and Probes

All primers and oligonucleotide probes were previously designed in this laboratory (1,11) and were purchased from Genosys Biotechnologies (Cambridgeshire, UK). All primers were biotinylated, and the DNA oligonucleotide probes for *Campylobacter* genus, *C. jejuni*, and *C. coli* species detection were modified with a 5' amine and 12-T linker between the amine group and the sequence-specific oligonucleotide. This linker distanced the probe from the microplate surface as recommended by Nalge Nunc International (Table 3).

Culture Conditions

Campylobacter type strains (Table 1) for DNA isolation were grown on *Campylobacter* Blood-Free Agar (Ox-

oid, Hampshire, UK) at 37°C in a 3.5-L anaerobic jar (Oxoid) with *Campylobacter* gas packs (Oxoid) and palladium catalyst for 48 h. *Campylobacter upsaliensis* strains were grown under the same conditions for 72 h. DNA was extracted using the AGTC Genomic DNA Purification Kit (Edge Biosystems, Gaithersburg, MD, USA) with modifications (11). All non-*Campylobacter* strains used in this study were grown on LB medium (16) overnight (16 h) at 37°C. For the purpose of PCR amplification, a single colony was removed from a plate, emulsified in 100 µL water, and heated to 95°C for 20 min. Five microliters were added to the PCR.

PCR Assay

PCR was performed in a final volume of 100 µL. Each reaction contained 0.25 mM uracil-deoxynucleotidetriphosphate (dU:dNTP) at a ratio of 2:1 dUTP:dTTP (Roche Molecular Biochemicals, East Sussex, UK), 2 mM MgCl₂ (Life Technologies, Paisley, UK), 1× reaction buffer (Life Technologies), 100 ng (12.5 pmol) CAMP1F-B and B1-B primers modified with 5' biotin, 1 U uracil-N-glycosylase (UNG) (Roche Molecular Biochemicals), 0.5 U *Taq* DNA polymerase (Life Technologies), and 5 µL

target material. The target material was purified *Campylobacter* DNA, boiled enriched Malthus media, or boiled emulsified bacterial colony and was added to the PCR and made up to a final volume of 100 µL with Nuclease-free water (Sigma-Aldrich, Dorset, UK). PCR cycling conditions for the CAMP1F-B/B1-B primer set (for the amplification of *Campylobacter* strains) using the Touchdown™ thermocycler (Hybaid, Teddington, UK) were 37°C for 10 min, followed by 35 cycles of 95°C for 30 s, 53°C for 40 s, and 72°C for 40 s. PCR cycling conditions for the A1-B/B1-B (1) primer set (for the amplification of non-*Campylobacter* strains) using the Touchdown thermocycler were 37°C for 10 min, followed by 35 cycles of 95°C for 30 s, 53°C for 40 s, and 72°C for 40 s. A no-template (distilled water) negative control and a positive control of purified *Campylobacter* DNA were included in each PCR run. Ten microliters of PCR product were run on a 1.8% agarose gel in 1× TBE buffer.

Microplate Detection

The solid phase capture oligonucleotides, CJEJ7 specific for *C. jejuni*, CCOL2 specific for *C. coli*, and CAMP4 specific for *Campylobacter*

Figure 1. Schematic representation of the ribosomal RNA operon with the universal PCR primers A1-B/B1-B, *Campylobacter*-specific primer CAMP1FB, the *Campylobacter* species-specific probes CJEJ7, CCOL2, and the *Campylobacter* genus-specific oligonucleotide probe CAMP4.

Research Report

genus detection were 5' aminated with a linker of 12 Ts between the 5' end of the oligonucleotide and the amine group. A freshly made coating mixture consisting of 100 nM oligonucleotide, 10 mM EDAC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide), and 10 mM 1-methyl-imidazole (pH 7.0) was prepared. To each NucleoLink well, 100 µL of this solution was added, giving a final concentration of 10 pmol 5'-aminated oligonucleotide in each well. Some wells were coated with Posdet (Table 3) at a concentration of 10 pmol. The Posdet oligonucleotide, consisting of a random sequence with a 5' amine and a 3' biotin, was immobilized on a separate set of wells to serve as a positive control for the detection stage of the assay. The NucleoLink wells were sealed and incubated overnight at 50°C. Following coating with the various oligonucleotide probes overnight, the wells were washed three times, soaked for 5 min, and washed again three times in 100 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.1% Tween® 20 at room temperature. To remove salt residue, the NucleoLink wells were washed three times, soaked for 5 min, and washed three times with deionized sterile water. The coated NucleoLink wells were stored at 4°C in a dry, sealed plastic bag until required but were not stored for longer than 10 months. Following PCR amplification, 10 µL PCR product was added to the NucleoLink wells containing genus-specific or appropriate species-specific immobilized capture oligonucleotide probe, denatured by the addition of 10 µL 1 M NaOH with 0.5 mg/mL thymol blue, and incubated at room temperature for 10 min. Eighty microliters of 6.25× standard saline citrate (SSC), 0.625% blocking reagent (Roche Molecular Biochemicals), 0.125% Tween 20, and 0.5 M NaH₂PO₄ adjusted to pH 6.5 with NaOH were added to each well and incubated at 50°C for 2 h. The wells were washed three times at room temperature with 0.5× SSC and 0.1% Tween 20, soaked for 15 min at 50°C, and washed again three times at room temperature. To detect the immobilized DNA probe-PCR hybrid, 100 µL of a 1:5000 dilution of the streptavidin-horseradish peroxidase conjugate (Life Technologies) in 100 mM Tris-HCl

Table 1. Campylobacter Species Analyzed in This Study

Campylobacter Species	Source
<i>C. jejuni</i>	NCTC 8116
<i>C. jejuni</i>	NCTC 11351 Biotype I
<i>C. jejuni</i>	NCTC 11168 Biotype I
<i>C. jejuni</i>	NCTC 12106 Biotype II
<i>C. jejuni</i>	NCTC 12275 Biotype III
<i>C. jejuni</i>	NCTC 12109 Biotype IV
<i>C. jejuni</i>	Penner 0:19
<i>C. jejuni</i>	Penner 0:4
<i>C. jejuni</i>	Penner 0:6
<i>C. jejuni</i>	Penner 0:3
<i>C. coli</i>	NCTC 11366
<i>C. coli</i>	NCTC 11353
<i>C. coli</i>	NCTC 12110 Biotype I
<i>C. coli</i>	NCTC 12143 Biotype II
<i>C. fetus</i>	NCTC 10842
<i>C. lari</i>	NCTC 11352
<i>C. lari</i>	NCTC 11458
<i>C. lari</i>	NCTC 12144
<i>C. upsaliensis</i>	NCTC 11840

Table 2. Non-Campylobacter Strains Analyzed in This Study

Non-Campylobacter Strains	Source
<i>Staphylococcus epidermidis</i>	NCTC 11047
<i>Proteus rettgeri</i>	NCTC 7475
<i>Proteus mirabilis</i>	NCTC 10975
<i>Lactococcus lactis</i>	NCTC 0662
<i>Bacillus cereus</i>	NCTC 7476
<i>Bacillus subtilis</i>	NCTC 10400
<i>Klebsiella aerogenes</i>	NCTC 9528
<i>Aeromonas hydrophila</i>	NCTC 8049
<i>Enterococcus faecalis</i>	NCTC 775
<i>Escherichia coli</i>	NCTC 10418
<i>Listeria monocytogenes</i>	NCTC 11994
<i>Salmonella poona</i>	NCTC 4840
<i>Pantonea agglomerans</i>	NCTC 9381
<i>Pseudomonas aeruginosa</i>	NCTC 10332
<i>Salmonella enteritidis</i>	NCTC 4444
<i>Salmonella typhimurium</i>	NCTC 0074

(pH 7.5), 150 mM NaCl, 0.1% Tween 20, and 0.5% blocking reagent were added to each well and incubated for 1 h at room temperature. The strips were washed three times, soaked for 5 min, and washed three times with 100 mM Tris-HCl (pH 7.5), 150 mM NaCl, and

0.1% Tween 20 at room temperature. One hundred microliters of TMB were added to each well, sealed, and incubated for approximately 30 min at room temperature. To stop the reaction, 100 µL 1 M H₂SO₄ were added, and the absorbance was measured at 450 nm in an

Research Report

EAR 400AT-SLT (SLT-Lab-Instruments, Salzburg, Austria) plate reader.

Analysis of Broiler Samples

Samples were collected from a total of 40 broiler chickens from four different points along the processing line in a chicken processing plant. The presumptive presence of *Campylobacter* was determined by using the Malthus V^M analyzer (Malthus Instruments, West Sussex, UK) and by conventional plate count method on 25 g chicken neck skin flap samples. Aliquots (100 μ L) of liquid media from samples identified as presumptive/suspect positive for *Campylobacter* on the Malthus V analyzer were spread-plated on *Campylobacter*-selective agar. Three colonies were selected at random from each plate for

biotyping with the API-Campy strips (BioMerieux, Marcy-l'Étoile, France). PCR was performed directly on the presumptive positive Malthus *Campylobacter*-selective liquid media by heating 100 μ L liquid medium to 95°C for 20 min. The bacterial cell lysate (5 μ L) was added to the PCR mixture and analyzed by PCR-ELISA.

RESULTS

To determine the detection limit of the *C. jejuni*- and *C. coli*-specific oligonucleotide probes and *Campylobacter* genus oligonucleotide probes in PCR-ELISA format, serial dilutions of purified *C. jejuni* and *C. coli* DNA ranging from 1 ng to 10 fg were PCR-amplified with CAMP1F-B/B1-B primer set (344

bp for *C. jejuni*, 503 bp for *C. coli*). Ten microliters of each PCR product were hybridized to NucleoLink wells with immobilized CJEJ7, CCOL2, or CAMP4 probes as described. The cut-off value for the detection level was set at the mean of the background signal plus three standard deviations ($\bar{x} \pm 3$ SD). The mean absorbance value on NucleoLink strips for background signals, as determined by hybridizing 10 μ L PCR to which no template DNA was added, to NucleoLink wells containing CJEJ7, CCOL2, or CAMP4 oligonucleotide probes, were 0.104 ± 0.02 for CCOL2, 0.2 ± 0.01 for CJEJ7, and 0.029 ± 0.005 for CAMP4. From these cut-off values, a positive value for each oligonucleotide probe was determined as A values greater than 0.165, 0.23, and 0.045 positive for CCOL2, CJEJ7, and

Figure 2. Sensitivity of detection of biotinylated amplified PCR products of 1 ng to 10 fg purified *Campylobacter* DNA with the probes CJEJ7 (A), CCOL2 (B), and CAMP4 (C) immobilized on NucleoLink wells. The detection limits of A, B, and C are indicated by the horizontal lines. (A) Detection of *C. jejuni* with CJEJ7 species-specific oligonucleotide probe. (B) Detection of *C. coli* with CCOL2 species-specific oligonucleotide probe. (C) Detection of *C. jejuni* and *C. coli* DNA with the *Campylobacter* genus-specific CAMP4 oligonucleotide probe.

Research Report

CAMP4, respectively (Figure 2). The background for the species-specific probes, CJJE7 and CCOL2, was tenfold higher than the genus-specific probe, CAMP4. These cut values gave a detection limit for CJJE7 and CCOL2 probes in the PCR-ELISA of 100–300 fg (40–120 bacterial cells) for *C. jejuni* and *C. coli* DNA, respectively, while the CAMP4 genus-specific probe had a detection limit of 10 fg (4 bacterial cells) for both *C. jejuni* and *C. coli* DNA (Figure 2). The detection limits of *C. jejuni*- and *C. coli*-purified DNA, following PCR amplification, on a 1.8% agarose gel were 1 pg for *C. jejuni* DNA and 10 pg for *C. coli* DNA. The sensitivities of detection of the CJJE7, CCOL2, and CAMP4 probes were evaluated using Southern blot hybridization (17) assays. CAMP4 and CJJE7 had a detection limit of 100 fg for *C. jejuni* DNA, while CAMP4 and CCOL2 had a detection limit of 100 fg to 1 pg with *C. coli* DNA (data not shown).

The specificity of CAMP4 for the detection of members of the Campylobacter genus and the species-specific detection of CJJE7 and CCOL2 DNA probes was evaluated against a panel of Campylobacter strains, two *C. jejuni* (NCTC 8116 and 11351), one *C. coli* (NCTC 11366), one *Campylobacter fetus* (NCTC 10842), three *Campylobacter lari* (NCTC 11352, 11458, and 12144) and one *Campylobacter upsaliensis* (NCTC 11840) (Table 1) in the microplate format and previously by Southern blot and DNA probe colorimetric membrane hybridization (11). In this study, the CJJE7, CCOL2, and CAMP4 probes were also evaluated against additional *C. jejuni* (NCTC 11168, 12106, 12109, 12275, and Penner serotypes 0:19, 0:4, 0:3, 0:6) and *C. coli* (NCTC 11353, 12110, and 12143) strains, representing the various Campylobacter biotypes, by Southern blot hybridization analysis (data not shown). The CAMP4 binds to all members of the Campylobacter genus, while CJJE7 binds specifically to *C. jejuni* and CCOL2 to *C. coli*. The non-Campylobacter strains used to test the specificity of the CJJE7, CCOL2, and CAMP4 probes are listed in Table 2. PCR amplification of 5 µL emulsified colony heated to 95°C for 20 min from each of these species was performed

Table 3. Sequences and Descriptions of the Oligonucleotides Used in This Study

Oligo-nucleotide	Sequence (5'→3')	T _m (°C)	Modification
aA1-B	BIO-AGTCGTAACAAGGTAGCCG-	58.0	Biotinylated
aB1-B	BIO-CYRYTGCCAAGGCATCCACC-	68.6	Biotinylated
aCAMP1F-B	BIO-GTTAAGAGTCACAAGCAAGT-	53.4	Biotinylated
aCAMP4	AMI-GGTAAGCTACTAAGAGCG-	51.7	Aminated/12 Thymidine
aCJJE7	AMI-GCTTAGTTGAGACTAAATCA-	51.1	Aminated/12 Thymidine
aCCOL2	AMI-GACTTAGTTTAGATATTTTATG-	45.9	Aminated/12 Thymidine
POSDT	AMI-NNNNNNNNNNNNNN-BIO	32.0	Aminated/Biotinylated

BIO, biotinylation; AMI, amination; Y=C+T; R=A+G
 aThe orientation of the oligonucleotide primers and probes are shown in Figure 1.

with A1-B/B1-B primer set, and PCR products of the appropriate size were generated for all species (300–700 bp). PCR amplification with the CAMP1F-B/B1-B primer set failed to yield PCR products for any of the species, while hybridization of 10 µL A1-B/B1-B PCR products from these organisms to CJJE7, CCOL2, and CAMP4 probes in the PCR-ELISA did not yield positive A readings. This was consistent with the results we obtained following Southern blot analysis and the DNA probe colorimetric membrane hybridization analysis (11) of these PCR products with each of the oligonucleotide probes.

PCR amplification with the primer set CAMP1F-B/B1-B was performed on 5 µL Malthus liquid from 40 chicken neck skin flap samples that were cultured for 30 h and yielded presumptive positive results on the Malthus analyzer. All of the samples analyzed were positive with the Campylobacter genus-specific probe (CAMP4), 40 were *C. jejuni* positive with the CJJE7 probe, and 22 gave positive hybridization signals with CCOL2, which is specific for *C. coli*. The presence of mixed PCR products for *C. jejuni* and *C. coli* corresponding to a mixed culture of the organisms was also confirmed by gel electrophoresis and Southern blot analysis with digoxigenin (DIG)-labeled (Roche Molecular Biochemicals) CJJE7 and CCOL2 probes (data not shown). Aliquots (100

µL) of liquid media from samples identified as presumptive/suspect positive for Campylobacter on the Malthus V analyzer were also spread-plated on Campylobacter-selective agar. Three colonies were selected at random from each plate for biotyping with the API-Campy strips. The API-Campy strips clearly identified most colonies as *C. jejuni*, some as *C. coli*, and in 14% of the colonies randomly selected, the API-Campy strips yielded unacceptable profiles for designation as Campylobacter species.

DISCUSSION

We report on the development of PCR-ELISAs for Campylobacter detection with discrimination of *C. jejuni* and *C. coli* and describe the application of the technology to the detection of Campylobacter in poultry meat samples.

The PCR assay targets amplification of 16S/23S rRNA intergenic spacer region in Campylobacter using a CAMP1F-B/B1-B primer set specific for amplification of members of the Campylobacter genus. This PCR amplification assay was combined with genus- and species-specific probes for *C. jejuni* and *C. coli* formulated as an ELISA using NucleoLink technology. The sensitivity study showed a detection limit for both *C. jejuni*- and *C. coli*-purified DNA of 100–300 fg with the species-specific probes CJJE7 and

CCOL2 and 10 fg with the genus-specific probe CAMP4. For other detection formats, Southern blot hybridization analysis and DNA probe membrane-based colorimetric detection, CAMP4 and CJEJ7 DNA probes had the same detection limits for *C. jejuni* DNA, while CAMP4 and CCOL2 had the same detection limits for *C. coli* DNA. In the microplate detection format, we consistently achieved a tenfold lower detection limit with CAMP4 for *C. coli* and *C. jejuni* DNA. The tenfold lower sensitivity that was achieved with CAMP4 in the microplate ELISA is a direct reflection of the tenfold lower background signal seen with this probe by comparison with background signals for CJEJ7 and CCOL2 in this format. The NucleoLink surface has been previously used to detect human papilloma virus, *Salmonella typhimurium* DNA prepared from boiled bacterial cells, RNA from PYV potato virus following RT-PCR, DNA from λ -bacteriophage, and DNA from the fungus *Phytophthora fragariae* (10). Compared with gel electrophoresis, the detection limit for all the systems was 10–100-fold lower and comparable with the detection limits described in our assay.

Specificity studies showed that the primer set (CAMP1F-B/B1-B) amplified only members of the *Campylobacter* genus (11). The specificities of the *Campylobacter* probes CJEJ7 specific for *C. jejuni*, CCOL2 specific for *C. coli*, and CAMP4 specific for members of the *Campylobacter* genus were evaluated on the NucleoLink strips and previously by Southern blot analysis and DNA probe colorimetric membrane assay (11). The oligonucleotide probes did not cross-react with 16S/23S rRNA PCR products from other non-*Campylobacter* species.

Evaluation of poultry samples with the PCR-ELISA, which were presumptive positive for *Campylobacter* following culture on the Malthus V analyzer, confirmed the presence of *Campylobacter* in all samples and demonstrated the presence of mixed cultures of *C. jejuni* and *C. coli* in 22 out of 40 samples. In a previous study, 101 broiler chicken samples were tested (11), and 55 out of the 101 samples were found to be PCR/DNA probe positive for *C. jejuni* and *C. coli* (54.4%). In the current

study, the 40 broiler chicken samples analyzed following Malthus culture represent a random subset of the above 101 samples. PCR amplification with the CAMP 1-F/B1-B primer pair was performed on different aliquots (100 μ L) of Malthus-enriched culture media by the principle authors in both studies. Post-PCR detection and identification of the PCR products were performed using the same DNA probes, CAMP4, CJEJ7, and CCOL2, in the two different detection formats. A complete correlation in the relative percentage of mixed cultures and the samples from which they were derived was achieved in both studies. Analytical profile index (API) analysis on some of the colonies selected following spread-plating of an aliquot of the Malthus-enriched media yielded inconclusive results for 14% of the colonies tested. Hydrolysis of hippurate in the API strips is frequently used to distinguish *C. jejuni* from *C. coli*, but the test is not always reliable (14). The PCR-ELISA detected the presence of *Campylobacter* and identified the *Campylobacter* species present in 100% of the samples analyzed. The ability to differentiate *C. jejuni* from *C. coli* is particularly important because erythromycin-based treatments are not adequate if the enteritis is caused by *C. coli*, which may be more likely to be resistant to this antibiotic. Rapid assays capable of discriminating these two species would be particularly useful in outbreak situations in which large numbers of specimens need to be handled quickly so that the appropriate therapy can be instituted (5).

PCR assays based on *Campylobacter* flagellin (12,13), 16S rRNA (4,9), 23S rRNA (3), and, recently, CLDT (2) genes have previously been described for the detection of *Campylobacter*. A number of these assays rely on gel-based analysis (2,3,4) Southern blot with nonradioactive DIG (5) or enhanced chemiluminescence-labeled (12) probes to detect PCR products and increase the sensitivities of the assays. These methods can be cumbersome and restrict the number of samples that may be speedily processed. An earlier study has described a PCR assay combined with microplate hybridization for the detection and discrimination of *C. jejuni* and *C. coli* in chicken fecal samples

Research Report

both before and after selective enrichment (13). The detection limits of four *Campylobacter* cells with the PCR-ELISA in this study using the genus-specific CAMP4 oligonucleotide probe is comparable to 1–20 cells detected in a previous PCR-ELISA (13). It is also comparable to PCR assay sensitivities of 100 cells (6) and to the PCR assay sensitivities of 5–50 cfu achieved in a study (9) that describes PCR/DNA probes based on a 16S ribosomal RNA sequence for the detection of *Campylobacter* in stool specimens. Other research groups report sensitivities of 1–5 cells with a PCR assay targeted to the 23S rRNA gene (3) and a PCR/DNA probe for the *flaA* gene with a detection limit of four cells.

The PCR-ELISA described in this study is very simple and can be performed within one day following culture-based enrichment of poultry samples. This represents significant timesaving over conventional microbiological methods and also offers the ad-

vantage of being able to definitively identify *C. jejuni* and *C. coli* and discriminate between the organisms in mixed cultures.

ACKNOWLEDGMENTS

The authors would like to thank Paul White for collection of the broiler chicken samples used in this study and Henrik Rasmussen, Janne Knudsen, and Tina Kristensen at Nunc A/S for technical support. This work was supported by the Department of Agriculture Non-Commissioned Food Research Programme.

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Received 18 January 2000; accepted 31 August 2000.

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