Two Rapid Diagnostic Procedures for the Identification of *Campylobacter jejuni/coli* in Food Matrix

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Abstract

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Campylobacter species, in particular *C. jejuni* and *C. coli,* cause infections which vary in symptoms, ranging from asymptomatic to severe chronic illness. The only ISO method for the detection of *Campylobacter* spp. until now has been the cultivation by selective enrichment and distinct conditions of growth taking several days to complete. We compared the Singlepath® Campylobacter test which involved 24 h of enrichment in Bolton broth, with PCR-based identification. Chicken meat salad with mayonnaise was spiked with *C. jejuni* and *C. coli* and the detection limit was determined. PCR provided the same detection limit of 10^2 CFU/ml for both strains. The immunotest Singlepath® was positive with *C. jejuni* only, the quantity of cells being 10^3 CFU/ml. *C. coli* was undetectable by Singlepath®, even the concentration of 10^5 did not reveal a positive reaction.

Keywords: thermotolerant campylobacters; PCR; immunotest Singlepath®

Campylobacter jejuni subsp. *jejuni* (*C. jejuni*) is now regarded as the leading cause of bacterial foodborne infection in many developed countries and is responsible for 80–90% of campylobacteriosis (Vandamme 2000). *Campylobacter coli* is responsible for about 7% of human campylobacteriosis cases, but in some areas (e.g. Central African Republic) this number can be as high as 35–40%. The common characteristic of these strains is the ability to multiply at elevated temperature and in microaerophilic (6% O_2, 7% CO_2, 7% H_2, 80% N_2) conditions.

The number of cases in the Czech Republic resembles the situation in the developed countries with incidence growing every year. In the Czech Republic, over 23 000 laboratory-confirmed cases of campylobacter infection were reported according to the data published by SZU in 2003 (National Institute of Public Health 2004). This represents a 90% increase compared to the year 1993 (National Institute of Public Health 2004) in the Czech Republic.

The only available method for the detection of *Campylobacter* spp. until now has been the cultivation by selective enrichment and distinct conditions of growth (ISO/TC 2002). These traditional microbiological isolation and identification methods can often take several days to complete. The aim of this study was the application of a culture-independent method such as simple polymerase chain reaction (PCR) for the detection of *C. jejuni* and *C. coli*, and the available immunoassay. Good sensitivity

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of PCR was proved by several studies (Hoorfar & Cook 2003; Malorny *et al.* 2003) while the main problem with the immunological systems is their low sensitivity (Betts & Blackburn 2003). As the food microbiologists need 25 g of food, to analyse for the presence or absence of a single target organism, an enrichment phase is always necessary. In many foods, clinical and environmental matrices, some components may influence the effectiveness of the PCR assay (Rossen *et al.* 1992; Al Soud & Rådström 2000), and can inhibit the reaction, preventing the signal even when the targets are present.

Here, we present the comparison of *C. jejuni* and *C. coli* PCR assay with the commercial immunochemical test in the spiked chicken meat salad with mayonnaise.

MATERIALS AND METHODS

Bacterial strains. *Campylobacter jejuni* CCM 6212 and *Campylobacter coli* CCM 6211 were grown on Karmali agar plates at 42°C under microaerophilic conditions for 48 h, prior to inoculation into the food sample.

Media. Karmali agar, Bolton broth, Bolton Broth; Bolton Broth Selective Supplement; Campylobacter blood free Selective agar Base (modified CCDA-Preston); CCDA Selective Supplement; Lysed Horse Blood; Stomacher/Stomacher bags with net – lined inserts.

Enumeration of** C. jejuni **and** C. coli. From each spiked food sample (Table 1), a single dilution series in sterile water was made and the cultivation on Karmali agar was carried out in triplicates. The procedure was performed immediately after spiking and after 44 h incubation.

DNA extraction. DNA was extracted from Bolton broth culture using three types of procedure: (*i*) boiling lysis according Englen and Kelley (2000), (*ii*) extraction with 6% Chelex 100 (Josefsen & Hoorfar 2004) and (*iii*) CTAB method in Ausubel *et al.* (1987).

PCR. The *C. jejuni* and *C. coli* PCR was based on the oligonucleotide primers specific for *C. jejuni*, *C. coli* and *C. lari* 16S rRNA sequences gene published by Lübeck *et al.* (2003). The conditions of the reaction and thermocycling were identical with the protocol published by Šabatkova *et al.* (2005). The thermocycler used in this study was a Biometra (Thermocycler T Gradient, Whatman Biometra, Germany).

Singlepath® Campylobacter test. Singlepath® Campylobacter is an immunochromatographic rapid test based on gold-labelled antibodies. The food sample before analysis ought to be treated according to manufacturers instructions in the following way: 25 g solid sample was added to 225 ml Bolton enrichment broth in a 250 ml polystyrene bottle, and the mixture was transferred to the filter unit of a Stomacher bag and homogenised in Stomacher for 1 minute. The homogenate was transferred back to the 250 ml polystyrene bottle, ensuring a headspace of 10–15%. Stomacher bag and the filter unit were discarded. The content of the bottle was incubated for 4 h at 37°C, then transferred to 41.5°C and incubated for a further 44 h. After this time, approx. 1–2 ml of the enriched culture were transferred to an appropriate polypropylene tube and covered with a loose-fitting cap. The tubes were placed in a boiling water bath for 15 min, removed and allowed to cool to room temperature. The last step was the transfer of five free falling drops (about 150–160 µl) into the circular sample port on the test device.

Food matrix and inoculation. Fresh chicken meat salad with mayonnaise purchased in food shop was processed on the same day according to the procedure described above.

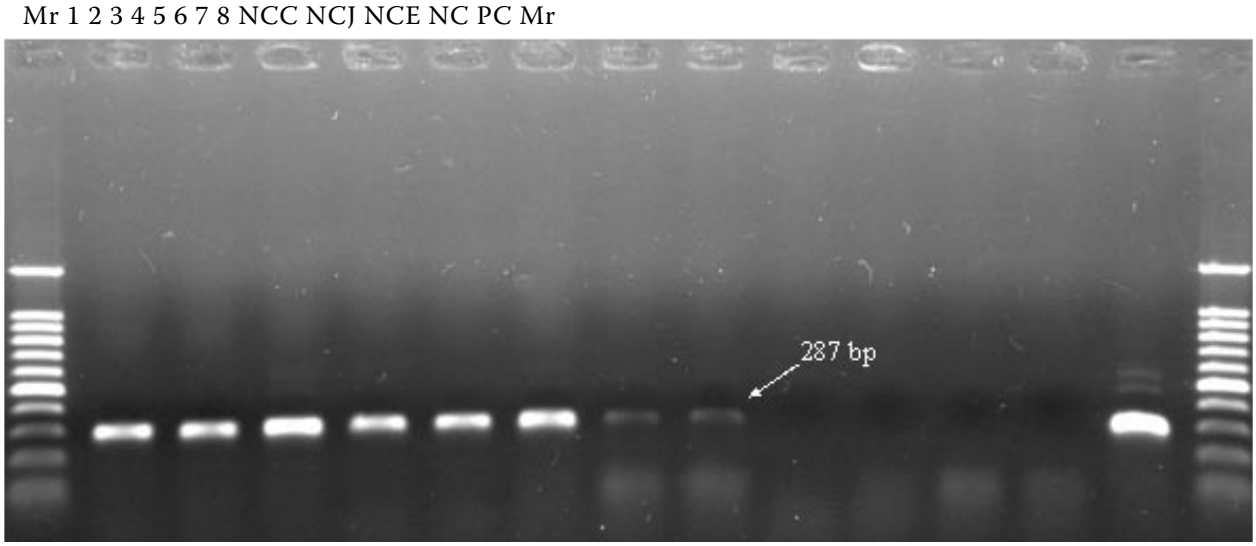
RESULTS AND DISCUSSION

Campylobacter jejuni and *Campylobacter coli* grown on Petri dishes with Karmali agar were harvested and resuspended in physiological saline. Bacterial suspension was diluted prior to the addition to chicken meat salad with mayonnaise. Table 1 shows the concentration at the beginning and after 44 h enrichment in CFU/ml. The cultivation of both strains in Bolton medium was not

Table 1. Concentration of *Campylobacter jejuni* and *Campylobacter coli* at the beginning and after 44 h enrichment (CFU/ml)

Campylobacter jejuni		*Campylobacter coli*	
0 h	44 h	0 h	44 h
10^2	10^2	10^2	10^2
10^3	ND	10^3	ND
10^4	ND	10^4	ND
10^5	10^5	10^5	10^5

ND – not determined



Lines 1–8 contains the PCR products of the samples inoculated with the bacterial strains giving the subsistent CFU/ml before enrichment

1 – *C. coli*, 4.10^5 CFU/ml; **2** – *C. jejuni*, 4.10^5 CFU/ml; 3 – *C. coli,* 4.10^4 CFU/ml; 4 – *C. jejuni***,** 4.10^4 CFU/ml; **5** – *C. coli*, 4.10^3 CFU/ml; 6 – *C. jejuni***,** 4.10^3 CFU/ml; **7** – *C. coli*, 4.10^2 CFU/ml; 8 – *C. jejuni***,** 4.10^2 CFU/ml; NCC – negative control of *C. coli*, NCJ – negative control of *C. jejuni;* NCE – negative control of extraction; NC – negative control of the PCR; PC – positive control of PCR; Mr – 100bp DNA Ladder; Lines NCC and NCJ contain the PCR products of the samples not inoculated with the bacterial strains

Figure 1. Qualitative detection of *Campylobacter jejuni* and *Campylobacter coli* in food samples before enrichment. DNA was isolated using Chelex 100

effected by the presence of food matrix, because the numbers of living cells stayed on the same level after 44 h. This enrichment procedure was required for Singlepath® Campylobacter test. The main task of the study was a verification of PCR sensitivity by immunochemical test and an assessment of possible inhibitory factors.

Figure 1 shows the signals obtained by PCR before the enrichment with *Campylobacter jejuni* and *Campylobacter coli,* when DNA was isolated using Chelex 100. A positive PCR signal was obtained with all inoculated samples of both tested species. It was found that food matrix had not displayed any suppression effect on PCR products. Selectivity and detection limit are the critical parameters that define the accuracy of a PCR assay (Hoorfar & Cook 2003). The usage of primers derived from DNA coding 16S rRNA, presumed that both *C. jejuni* and *C. coli* would be confirmed with the equal precision.

At first sight the data summarised in Table 2 clearly showed that PCR sensitivity for both tested strains was equal. The next information from the Table 2 is finding that PCR sensitivity is much higher than immunochemical test. Regardless on producer assurance, that Singlepath® Campylobacter test display the same sensitivity for *C. jejuni* and *C. coli*, data in Table 2 showed opposite result. The immunotest Singlepath® was positive with *C. jejuni* only and the quantity of cells was 10^3 CFU/ml. *C. coli* was undetectable by Singlepath®, even the concentration of 105 was not giving positive reaction. Figure 2 illustrates the positive and negative results of Singlepath® Campylobacter test.

Chicken meat salad with mayonnaise was spiked with different concentrations of *C. jejuni* and *C. coli* cells and the detection limit was determined. A simple ISO-compatible PCR-based method has been adopted for analysis of complex food matrix for the presence of *C. jejuni* and *C. coli*. The procedure was giving the same detection limit 10^2 CFU/ml for both strains, while Singlepath® Campylobacter test was sensitive for *Campylobacter jejuni* only. Presented results reflect the proficiency of our laboratory acquired during

Table 2. Comparison of PCR and Singlepath® Campylobacter

CFU/ml after inoculation	Results of Singlepath®		PCR results					
			CTAB		CHELEX		thermal lyse	
	C. coli	*C. jejuni*	*C. coli*	*C. jejuni*	*C. coli*	*C. jejuni*	*C. coli*	*C. jejuni*
4×10^5	N	P	P	P	P	P	P	P
4×10^4	N	P	P	P	P	P	P	P
4×10^3	N	p	P	P	P	P	p	p
4×10^2	N	N	p	p	p	p	N	N
0	N	N	N	N	N	N	N	N

N – negative signal, P – positive signal, p – limit of detection

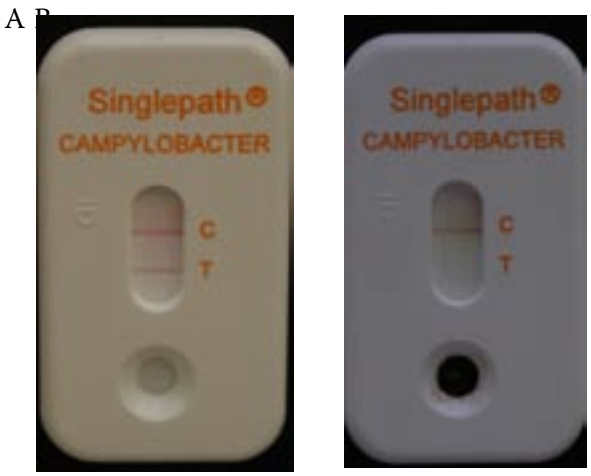


Figure 2. Examples of positive and negative results by Singlepath® Campylobacter

A – positive reaction, B – negative reaction

validation of PCR/based method for detection of thermotolerant campylobacters (Josefsen *et al*. 2004).

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