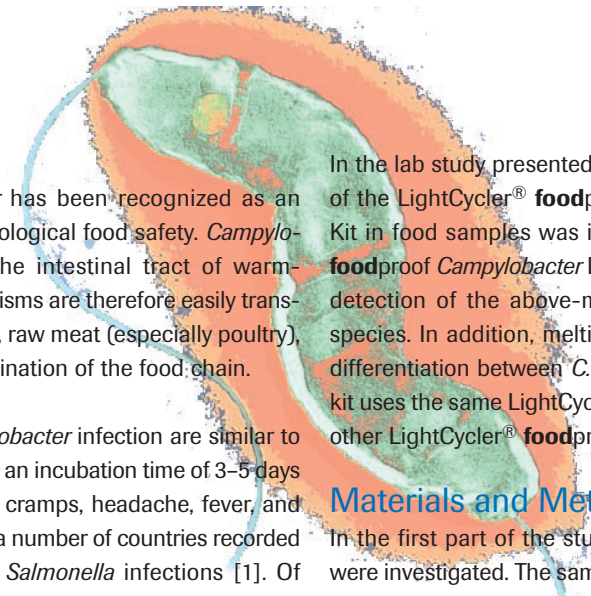
Detection of *Campylobacter* spp. in Food Samples Using the New LightCycler® **food**proof *Campylobacter* Detection Kit

Cordt Grönewald, Matthias Kiehne, Carola Lieb, and Astrid Schneider*
BIOTECON Diagnostics GmbH, Potsdam, Germany
**Corresponding author: cgroenewald@bc-diagnostics.com*

Cordt Grönewald



Introduction

The genus *Campylobacter* has been recognized as an important factor in microbiological food safety. *Campylobacter* can be found in the intestinal tract of warm-blooded animals. The organisms are therefore easily transferred to raw milk products, raw meat (especially poultry), and water, causing contamination of the food chain.

The symptoms of a *Campylobacter* infection are similar to those of Salmonellosis: after an incubation time of 3–5 days it causes diarrhea, nausea, cramps, headache, fever, and insomnia. In the late 1990s, a number of countries recorded more *Campylobacter* than *Salmonella* infections [1]. Of highest relevance are the six species *C. jejuni*, *C. coli*, *C. lari*, *C. fetus*, *C. hyointestinalis* and *C. upsaliensis* [2].

Campylobacter detection in food samples using the currently available microbiological methods as described in ISO 10272 [3] is very difficult, and routine labs in the food industry are usually not equipped for these applications. Moreover, a negative result is available only after three days, and two more days are necessary for biochemical confirmation. Therefore, a great demand for an easy-to-use and rapid method for the safe detection of *Campylobacter* species in food exists.

In the lab study presented in this article, the applicability of the LightCycler® **food**proof *Campylobacter* Detection Kit in food samples was investigated. The LightCycler® **food**proof *Campylobacter* Detection Kit allows the specific detection of the above-mentioned six most important species. In addition, melting-curve analysis enables the differentiation between *C. fetus*, *C. coli* and *C. jejuni*. The kit uses the same LightCycler® Instrument settings as any other LightCycler® **food**proof pathogen detection kit.

Materials and Methods

In the first part of the study, 50 different food matrices were investigated. The samples belong to the food groups egg products (four different samples), milk products (16), poultry (6), fish (9), meat (10) and salad (5). Five grams of each sample were transferred into 45 ml Bolton media and incubated at 42°C for 24 hours at microaerophilic conditions. After being pre-enriched, the food samples were processed using the ShortPrep **food**proof II Kit and the High Pure **food**proof II Kit in parallel. PCR analysis was carried out using the LightCycler® **food**proof *Campylobacter* Detection Kit with 5 µl of each DNA extract. To verify a successful PCR, the internal control of the kit was evaluated for each sample.

In the second part of the analysis, the three species *C. fetus*, *C. coli*, and *C. jejuni* were differentiated. DNA from all species was prepared using the High Pure **food**proof II Kit. Then, DNA was analyzed with the LightCycler® **food**proof *Campylobacter* Detection Kit by melting-curve analysis using the LightCycler® 2.0 Instrument.

Results

Testing 50 different food samples, we showed that there was no influence of the food matrix on the PCR performance of the LightCycler® **food**proof *Campylobacter* Detection Kit. The internal control for each sample was clearly positive; this was independent of the sample preparation kit used. Table 1 shows the CP values of the internal control for the different food groups and sample preparation kits.

Table 1: CP values of the internal control in six different food groups using the ShortPrep foodproof II Kit and the High Pure foodproof II Kit

Food Group	ShortPrep **food**proof II Kit*			High Pure **food**proof II Kit		
	CP_{min}	CP_{mean}	CP_{max}	CP_{min}	CP_{mean}	CP_{max}
Egg Products	31.34	31.71	32.14	31.18	31.25	31.32
Milk Products	31.02	31.64	31.97	31.10	31.41	31.92
Poultry	31.29	31.65	31.81	31.28	31.40	31.50
Fish	31.30	31.75	32.00	31.13	31.27	31.53
Meat	31.48	31.79	32.09	31.07	31.41	31.69
Salad	31.43	31.52	31.74	31.24	31.39	31.50

**Some samples were diluted 1:10*

The differentiation of the three different species via melting-curve analysis is shown in Figure 1. The melting temperatures of all four targets are different and clearly distinguishable.

Discussion

The new LightCycler® **food**proof *Campylobacter* Detection Kit was tested under routine conditions with different food matrices in combination with the ShortPrep **food**proof II Kit and with the High Pure **food**proof II Kit. In both cases, the kit demonstrated its suitability and applicability for the routine use in a food laboratory. Sample preparation is easy and superior to the more difficult culture methods. The most relevant species can be identified, facilitating better tracing of contaminations during food processing. The LightCycler® **food**proof *Campylobacter* Detection Kit offers a rapid and safe detection method for specific *Campylobacter* species such as *C. fetus*, *C. coli* and *C. jejuni*. ■

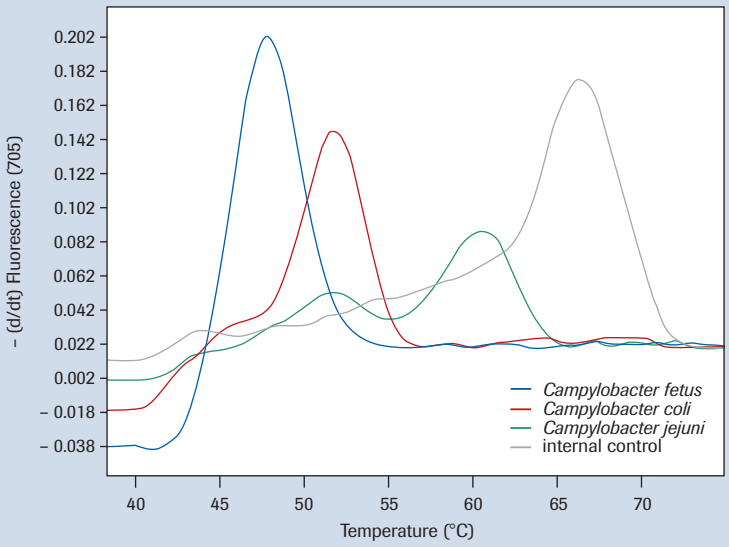


Figure 1: Melting peaks of the species *C. fetus*, *C. coli,* and *C. jejuni*, and the internal control.

Product	Pack Size	Cat. No.
LightCycler® foodproof *Campylobacter* Detection Kit	1 Kit (96 reactions for max. 90 samples)	04 351 797 001
ShortPrep foodproof II	1 Kit (96 isolations)	03 358 062 001
High Pure foodproof II	1 Kit (100 isolations)	03 358 054 001
MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi)	1 Kit (192 isolations)	03 264 785 001

References

1. Food Safety Authorities of Ireland (2002) Control of Campylobacter species in the food chain. Food Safety Authorities of Ireland, Dublin
2. Takkinen J, Ammon A (2003) Eurosurveillance 8 (11):219
3. ISO 10272, Edition 1995-10

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