ACCURATE DETECTION OF *CAMPYLOBACTER* SPP. ANTIGENS BY IMMUNOCHROMATOGRAPHY AND ENZYME IMMUNOASSAY IN ROUTINE MICROBIOLOGICAL LABORATORY

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Campylobacter spp. are fastidious microorganisms, and their detection by culture depends on the freshness of the stool sample and the skills of the laboratory staff. To improve laboratory diagnosis, assays for the detection of specific antigens have been developed. Here, we evaluated two assays for the detection of *Campylobacter* spp.-specific antigens, i.e., one immunochromatographic test and one enzyme-linked immunosorbent assay (EIA), in 38 frozen *Campylobacter* spp.-positive specimens and prospectively in 533 fresh stool samples with a conventional enzyme immunoassay (EIA) and culture.

Both assays were positive for 36 samples with *Campylobacter jejuni* and one with *Campylobacter coli* among 38 *Campylobacter* spp.-positive frozen samples. One *Campylobacter lari*-positive sample was identified by the immunochromatographic assay (ICA) only. In a prospective study performed within the course of routine microbiology, both assays were positive for 24/25 *C. jejuni* culture-positive samples (positive percent agreement, 96.0% [95% CI: 78.9–100%]). ICA and EIA also were positive for 14 and 10 culture-negative samples, respectively (negative percent agreement: ICA, 97.2% [95% CI: 95.4–98.4%]; EIA, 98.0% [95% CI: 96.4–99.0%]). In conclusion, the high agreement between both antigen-detection assays and culture indicates that both assays may be initially performed followed by culture only upon a positive test result.

Keywords: *Campylobacter*, antigen detection, immunochromatographic assay, enzyme immunoassay, culture

Introduction

Campylobacter jejuni belongs to the most frequent bacterial pathogens that cause gastrointestinal infection in man [1]. The infection with this microorganism is one of the leading causes of hospitalization of adults [2], while *Campylobacter coli*, which causes similar clinical symptoms, is less frequently detected than *C. jejuni*. *Campylobacter* spp. can be isolated from stool specimens on selective media containing antibiotics, which, in combination with a high temperature of incubation (42–43 °C), inhibits the growth of the physiological bacterial flora [3]. Cultural isolation, however, may fail since *Campylobacter* spp. are rather fastidious microorganisms, and the infection can be verified retrospectively in such cases by serology [2]. Therefore, novel assays based on the detection of specific antigens have been developed, which can be performed rapidly, are independent from the bacterial viability and thus also suited for the analysis of frozen samples, and have consequently gained increasing attention in routine

microbiology [4]. The critical impact of the implementation of nonculture diagnostic methods on disease surveillance, however, must be observed [5]. Here, we evaluated prospectively a commercially available immunochromatographic assay (ICA) together with a modified enzyme immunoassay (EIA) that previously has shown moderate sensitivity compared with culture [6], and with the cultural isolation of *Campylobacter* spp. under routine laboratory conditions.

Materials and methods

For the prospective study, we included all fecal specimens submitted from registered practitioners to our laboratory between June and August 2011 for the detection of bacterial pathogens ($n = 533$). When more than one sample was submitted for a patient, only the first specimen was considered. Additionally, 38 stool samples that had previously yielded growth of *Campylobacter* spp.

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(36 × *C. jejuni*, 1 × *C. coli*, and 1 × *Campylobacter lari*) and had been stored at −80 °C were tested with both assays. Fresh samples were immediately homogenized in saline and spread onto two blood-free *Campylobacter* selection agar plates (Oxoid, Wesel, Germany) per sample. Plates were incubated under microaerobic conditions at 37 and 42 °C and screened after 24 and 48 h for bacterial growth. Growth of *Campylobacter* spp. was confirmed by testing of suspicious colonies regarding motility, Gram-staining, and oxidase through applying microbiological routine methods. Species were determined by testing for hippurate and susceptibility to nalidixic acid. One isolate, which was not detected by either ICA or EIA, was confirmed as *C. jejuni* by mass spectrometry. Alongside with culture, all fresh samples were subjected to two commercially available ICA and EIA (RidaQuick *Campylobacter* and RidaScreen *Campylobacter*; both, R-Biopharm, Darmstadt, Germany) for the detection of *C. jejuni* or *C. coli* antigens. Frozen *Campylobacter*-positive samples were thawed and immediately tested in the two antigen-detection assays. All assays were performed and interpreted according to the manufacturer's instructions. Since cultural isolation of *Campylobacter* spp. may yield a considerable rate of false-negative results [2, 7] and PCR could not be performed in the present study, we calculated the positive and negative percent agreement (PPA and NPA, respectively) with 95% confidence interval (95% CI) between the assays and culture.

Results

To evaluate the general ability of both immunoassays to detect *Campylobacter* species, we analyzed 38 frozen samples known to be positive for *Campylobacter* sp. (36 × *C. jejuni*, 1 × *C. coli*, and 1 × *C. lari*) with both assays. Both assays yielded positive results with all 36 *C. jejuni*-positive samples and one *C. coli*-positive sample (Table 1). The ICA also detected *C. lari* in one sample (although the assay had not been designed to detect this species).

Table 1. Detection of *Campylobacter* spp. antigens by ICA or EIA in 38 frozen stool samples from which *Campylobacter* spp. had been isolated previously

<i>Campylobacter</i> sp.	ICA	EIA
<i>C. jejuni</i> (n = 36)	36	36
<i>C. coli</i> (n = 1)	1	1
<i>C. lari</i> (n = 1)	1	0

When both assays were tested prospectively under routine laboratory conditions and alongside with bacterial culture, the positivity rates of the ICA and the EIA were 7.1% (38/533 samples; 95% CI, 5.2–9.7%) and 6.4% (34/533 samples; 95% CI, 4.6–8.8%), respectively, compared with 4.7% (25/533 samples; 95% CI, 3.2–6.9%) for culture. This is a higher agreement between the two assays

and culture than that recently observed by Giltner et al. [8] who have reported positivity rates of 11.3% for a different EIA and 1.7% for previously performed culture as well as 5.88% for this EIA batch-tested following 2 weeks of culture, which had yielded 2.52% positive results, respectively. PCR revealed false-positive and EIA-positive samples in that study.

Both immunoassays missed one (both the same specimen) of 25 culture-positive samples (this isolate was confirmed as *C. jejuni* by mass spectrometry); ICA and EIA, however, yielded positive results with 14 and 10 culture-negative stool samples, respectively (Table 2). Notably, there was a considerable agreement between ICA and EIA regarding the culture-negative samples, i.e., six samples yielded positive results with both assays and might therefore represent false-negative culture results. Eight samples were ICA-positive only and negative by EIA while four samples were EIA-positive only. In comparison to culture, the PPA for both assays was 96.0% (95% CI, 78.9–100%) while the NPA was 97.2% (95% CI, 95.4–98.4%) for the ICA and 98.0% (95% CI, 96.4–99.0%) for the EIA. *C. coli* or other species were not isolated. Considering all 571 (38 frozen and 533 fresh) stool samples, PPA and NPA for the ICA were 98.4% (95% CI, 90.7–100%) and 97.2% (95% CI, 95.4–98.4%) versus 96.8% (95% CI, 88.5–99.8%) and 98.0% (95% CI, 96.4–99.0%) for the EIA as compared with culture.

Table 2. Comparison of ICA and EIA for the detection of *Campylobacter* spp.-specific antigens with cultural isolation of *C. jejuni* from 533 fresh stool samples

		Culture	
		Positive	Negative
ICA	Positive	24	14
	Negative	1	494
EIA	Positive	24	10
	Negative	1	498

Discussion

Both assays yielded accurate results when used under routine laboratory conditions. An only slightly lower PPA (87.1%; 95% CI, 70.5–95.5%) of the same ICA with culture/PCR than reported by us can be calculated for data recently published from Spain [9]. This difference may be due to distinct patient populations or different transport conditions and/or times. The NPA (97.0%; 95% CI, 94.1–98.6%), however, is similar to what we observed. Furthermore, our data for the ICA also are comparable to those published for a different ICA, the immunoCard STAT! Assay [7, 10, 11]. Like the ICA evaluated by us, the immunoCard STAT! assay also detects antigens derived from non-*jejuni* and non-*coli* *Campylobacter* spp. [12]. Although confirmation by PCR would have been desirable but unfortunately not feasible, some ICA-positive

but culture-negative samples in our study might have been true positives as previously shown for both the immuno-Card STAT! and the RidaQuick assay [7, 9], although also false-positive results have been reported for both assays [9, 13, 14].

The new generation of the EIA tested has been improved compared with the previous type, which only had a sensitivity of 69% and a specificity of 87% compared with culture [6]. Considering the present results for all fresh and frozen samples and culture “gold standard”, sensitivity and specificity of the current product are 96.8% (95% CI, 88.5–99.8%) and 97.2% (95% CI, 95.4–98.4%), respectively, which is in the range of data reported for other, similar assays [11, 15–17].

In conclusion, the high PPA and NPA of both assays with bacterial culture suggest a step-wise diagnostic procedure in the laboratory diagnosis of campylobacteriosis, i.e., the initial performance of one of these assays and – upon a positive immunoassay result – bacterial culture that enables *Campylobacter* species differentiation (which, however, could also be achieved by PCR [18]). Due to its short incubation time (ICA, 15 min vs. EIA, 105 min), the ICA may be particularly interesting for the analysis of low sample numbers.

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Conflict of interest

The authors declare that they have no conflict of interest.

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