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Rapid Detection of *Campylobacter jejuni* in Stool Specimens by an Enzyme Immunoassay and Surveillance for *Campylobacter upsaliensis* in the Greater Salt Lake City Area

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The Alexon-Trend, Inc. (Ramsey, Minn.), ProSpecT *Campylobacter* microplate assay was compared with culture on a Campy-CVA plate (Remel, Lenexa, Kans.) and blood-free campylobacter agar with cefoperazone (20 μg/ml), amphotericin B (10 μg/ml), and teicoplanin (4 μg/ml) (CAT medium; Oxoid Limited, Hampshire, England) with 631 patient stool samples. The CAT medium was used to isolate *Campylobacter upsaliensis*. The enzyme immunoassay (EIA) had a sensitivity and a specificity of 89 and 99%, respectively, and the positive and negative predictive values were 80 and 99%, respectively. Even though we extensively looked for *C. upsaliensis* in stool samples from patients from the greater Salt Lake City area, we did not isolate this species during the study period. The overall excellent specificity of the EIA allows rapid detection and treatment of positive patients; however, a negative result should be confirmed by culture when clinical suspicion is high.

Campylobacter is an invasive microorganism that has been associated with both diarrheal and systemic disease in all parts of the world. It is estimated that about 2.4 million cases of human campylobacteriosis occur each year in the United States (24). This infection occurs primarily in infants, elderly people, and patients with underlying disease. Immunocompromised individuals are at higher risk of acquiring campylobacteriosis, and the infection is usually more severe in such individuals (20). Campylobacteriosis is a self-limited disease, and antimicrobial therapy is not generally indicated. However, treatment can decrease the duration and the severity of illness if it is initiated early in the course of infection. Complications are rare; however, it has been reported that infections may be concurrent with reactive arthritis, meningitis, recurrent colitis, acute cholecystitis, pancreatitis, cystitis, and Guillain-Barré syndrome (6, 9, 13, 16, 18, 19).

The genus *Campylobacter* is part of the *Campylobacteriaceae* family (26). This family includes about 18 species and subspecies. Of the *Campylobacter* species that can cause human disease, *Campylobacter jejuni* is the prototype for enteric infections and *Campylobacter fetus* is the prototype for extraintestinal infections. With the refinement of the microbiological techniques for the isolation of *Campylobacter upsaliensis*, this species has been found in association with gastroenteritis in children and human immunodeficiency virus (HIV)-infected individuals (11, 12).

Campylobacter species are non-spore-forming, motile, gram-negative rod organisms that have a comma or S-shape morphology. *Campylobacter* species are microaerophilic organisms that grow best at 37°C, however, *C. jejuni* grows best at 42°C. Visible colonies of *C. jejuni* usually appear on culture medium (e.g., Campy-CVA medium; Remel, Lenexa, Kans.) within 48 to 72 h. Enrichment medium is not required for recovery, since infected humans usually excrete 10^6 to 10^9 CFU of *C. jejuni* per g of stool (5).

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A rapid nonculture assay for detection of *Campylobacter* may be of interest to both the clinician and the microbiology laboratory. Theoretically, same-day results would allow triage of patients for earlier therapy. For those microbiology laboratories without campylobacter culture capability (3.2% of the laboratories participating in the New York Proficiency Survey), a sensitive enzyme immunoassay (EIA) would provide an option for *Campylobacter* detection without lengthy culture procedures. Other rapid tests available either are nonspecific, such as Gram and acridine orange staining, or are available only in the research setting, such as PCR (7).

This study was designed with two goals. The first was to compare a rapid EIA to the "gold standard" traditional culture methods for detection of *Campylobacter* species. The second goal was to determine the prevalence of *C. jejuni* and *C. upsaliensis* gastroenteritis in the greater Salt Lake City area.

The first goal was to evaluate the performance of the ProSpecT *Campylobacter* microplate assay (EIA; Alexon-Trend, Inc., Ramsey, Minn.) with that of *Campylobacter* isolation agar medium with 631 stool specimens submitted to ARUP Laboratories during the 1999 summer season. Samples were collected at four different facilities in Utah (University of Utah Health Sciences Center, Primary Children's Medical Center, Utah Public Health Laboratory, and Cottonwood Hospital Medical Center) and were sent to ARUP Laboratories. All samples were obtained from patients with suspected bacterial diarrhea. Only liquid or nonformed stools (i.e., those that took the shape of the container) collected from ambulatory patients or those hospitalized for less than 3 days were tested. The ProSpecT *Campylobacter* microplate assay was performed as directed by the manufacturer. Briefly, after the stool samples were mixed thoroughly in bacterial specimen diluent and added to the appropriate wells, the samples were incubated at room temperature for 60 min. Stool samples in Cary-Blair or enteric transport medium were added directly to the microplate well for testing. After the wells were thoroughly washed (50x1 washer; Biotech, Windoski, Vt.) and the enzyme conjugate was added, the samples were incubated at room temperature for 30 min. The plates were washed and incubated with

TABLE 1. Comparison of *Campylobacter* isolation by culture to detection by EIA

Medium and result	No. of samples with the following result by EIA:		EIA			
	Positive	Negative	% Sensitivity	% Specificity	% PPV[a]	% NPV[b]
Campy-CVA and CAT media			89	99	80	99
Positive	16	2				
Negative	4	609				
Campy-CVA medium			93	99	70	99
Positive	14	1				
Negative	6	610				
CAT medium			93	99	65	99
Positive	13	1				
Negative	7	610				

[a] PPV, positive predictive value.
[b] NPV, negative predictive value.

color substrate at room temperature for 10 min. The reaction was stopped with the stop solution, and the results were read spectrophotometrically at 450 nm.

Stool samples submitted to the laboratory in sterile containers or in transport medium were inoculated on Hektoen enteric agar, MacConkey agar, Campy-CVA medium (with cefoperazone at 20 μg/ml, vancomycin at 10 μg/ml, and amphotericin B at 2 μg/ml), and Columbia sheep blood agar plates. With the exception of the plates with Campy-CVA medium, all the plates were incubated at 37°C. The plates with Campy-CVA medium were incubated under microaerophilic conditions in a 42°C incubator. The plates with samples from the patients were evaluated daily for 3 days. In addition, blood-free campylobacter agar with cefoperazone at 8 mg/ml, amphotericin B at 10 mg/ml, and teicoplanin at 4 mg/ml (CAT medium; Oxoid Limited, Hampshire, England) was included for isolation of *C. upsaliensis* (3). The plates with CAT medium were incubated under microaerophilic conditions in a 37°C incubator for 4 days. Suspicious catalase-positive and/or oxidase-positive colonies were Gram stained to look for the "comma" or "gull wing" morphology, and hippurate hydrolysis was used as a confirmatory test for the identification of *C. jejuni*. Hippurate-negative isolates were identified on the basis of susceptibility testing with nalidixic acid disks (30 mg) and cephalothin disks (30 mg). A positive sample was reported upon *Campylobacter* species isolation from either Campy-CVA or CAT medium.

Sensitivity, specificity, and positive and negative predictive values were calculated for determination of the performances of the EIA, CAT medium, and Campy-CVA medium for isolation of *Campylobacter* spp. In addition, statistical analysis was performed by the McNemar test.

Of the 631 stool samples evaluated, 18 samples from different patients were positive for *C. jejuni* for an overall positivity rate of 2.8%. Sixteen samples were positive by both culture and the EIA, and two samples were positive by culture but negative by EIA, thus giving the assay an 89% sensitivity. Of the 613 samples negative by culture, four were positive by EIA, thus giving the assay a 99% specificity (Table 1). The positive and negative predictive values for the EIA compared to the results obtained with both culture media were 80 and 99%, respectively. Upon reanalysis of the four samples with discrepant results that were positive by EIA and negative by culture, all four samples were transported to ARUP Laboratories under optimal transport conditions and were positive upon repeat EIA and negative by culture at ARUP Laboratories. Furthermore, the culture results for these four samples were negative when the samples were tested at the site of origination of the specimen. None of the four patients who submitted the four samples were receiving any type of antibiotics at the time that the stool samples were collected. Thus, the negative culture result was not due to nonviable organisms. A positive EIA result and a negative culture result could be a result of the assay's cross-reactivity with other *Campylobacter* species that are not detected by culture.

One of the four samples with discrepant results, which was positive by EIA and negative for *Campylobacter* species by culture, was from a patient with an overwhelming *Vibrio parahaemolyticus* infection. *V. parahaemolyticus* could have overgrown the *Campylobacter* organisms present in the patient's stool sample. The other three patients had either persistent or recurrent diarrhea, but no intestinal pathogen was isolated.

The specificity of the assay was challenged by reacting the EIA with the following organisms isolated from patient's samples: five *Shigella soneii*, five *Shigella flexneri*, four *Escherichia coli*, four *Proteus mirabilis*, two *Proteus vulgaris*, five *Pseudomonas aeruginosa*, one *Clostridium difficile*, five *Enterobacter cloacae*, five *Citrobacter koseri*, four *Enterococcus faecalis*, four *Enterococcus faecium*, five *Aeromonas hydrophila*, five *Staphylococcus aureus*, five *Serratia marcescens*, six *Salmonella* group D, five *Salmonella* group C, four *Klebsiella pneumoniae*, one *Vibrio cholera*, one *Vibrio parahaemolyticus*, and four *Yersinia enterocolitica* strains. Moreover, Alexon-Trend reported no cross-reactivity with *Campylobacter buyzleri*, *Campylobacter concisus*, *Campylobacter curvus*, *Campylobacter fetus*, *Campylobacter lari*, *Campylobacter rectus*, or *Campylobacter sputorum*; however, *Campylobacter coli*, which is associated with mild disease, did cross-react (Alexon-Trend package insert). In our study, *C. upsaliensis* (ATCC 49815) also cross-reacted with the EIA. The *Campylobacter*-specific antigen that is detected by the ProSpecT *Campylobacter* microplate assay may be shared by *C. upsaliensis*, thus allowing it to cross-react. This cross-reactivity will be of clinical use since this species has been associated with human gastroenteritis (14).

Compared to the results obtained with Campy-CVA medium alone, the sensitivity and specificity of the EIA were 93 and 99%, respectively (Table 1). The EIA positive and negative predictive values were 70 and 99%, respectively. In comparison to the results obtained with CAT medium, the sensitivity, specificity, and positive and negative predictive values of the EIA were 93, 99, 65, and 99%, respectively (Table 1). Of interest is the number of *C. jejuni* isolates that grew on one

medium but not the other. Three isolates grew on CAT medium but did not grow on Campy-CVA medium. This could be due to the sensitivities of these isolates to the antibiotics in the Campy-CVA medium or, less likely, to sampling error upon inoculation of the culture medium. Four isolates were not detected on the plates with CAT medium but were isolated on the plates with Campy-CVA medium. We believe that overgrowth with normal enteric flora obscured detection of *C. jejuni* on the plates with CAT medium.

The observed difference between the results of EIA compared to the results obtained with both culture media (Campy-CVA and CAT media) and the results obtained with Campy-CVA medium alone were not statistically significant (by the McNemar test, $P > 0.25$ and $P > 0.05$, respectively). Thus, a laboratory that does not perform *Campylobacter* culture can reliably substitute the ProSpecT *Campylobacter* microplate assay. However, there was a statistically significant difference between the results of the EIA and the results obtained with CAT medium alone ($P < 0.05$). The use of the ProSpecT *Campylobacter* microplate assay is superior to the use of CAT medium alone.

The analytical sensitivity of the EIA for the detection of *C. jejuni* (ATCC 33290) and *C. upsaliensis* (ATCC 49815) was determined. Isolates of both *Campylobacter* species (1.0 McFarland standard) were serially diluted in liquid stool samples. The samples were then divided in half; one part was streaked for Campylobacter detection on Columbia sheep blood agar plates and the other half was evaluated by the EIA. The manufacturer reported the analytical sensitivity of the EIA to be 5×10^5 CFU/ml. In our hands, the sensitivity of the assay for the detection of *C. jejuni* was 3×10^6 CFU/ml, and the sensitivity of the EIA for the detection of *C. upsaliensis* was 3×10^7 CFU/ml. The sensitivity of culture for *C. jejuni* detection in our hands was 3×10^2 CFU/ml, and the sensitivity of culture for *C. upsaliensis* detection was 3×10^5 CFU/ml. This is consistent with the results of Aspinall et al. (2), who reported the sensitivity of culture for detection of *C. upsaliensis* to be in the range of 10^5 CFU/g of stool. Even though a large number of organisms is required for the EIA to be positive, on average, patients infected with *C. jejuni* excrete 10^6 to 10^9 CFU/g of stool. The analytical sensitivity of this EIA might explain why the EIA missed two of the positive patient samples analyzed in this study.

As part of this evaluation, we surveyed patients' stool samples for *C. upsaliensis* using the selective CAT medium. Aspinall et al. (2, 3) have reported that CAT medium is suitable for culture of *C. upsaliensis* from patient stool samples. Most cases of human *C. upsaliensis* infections have been reported from Europe (Denmark, England, France, Ireland, and Sweden); however, *C. upsaliensis* has also been isolated from patients from Australia, Canada, South Africa, and the United States (3, 7, 9, 11, 14, 15, 17, 21, 23, 24). Cats and dogs have been reported to be potential reservoirs of *C. upsaliensis* (4, 10).

C. upsaliensis has been associated with mild infective enteritis that lasts for <7 days, particularly in children and travelers (17, 25). Other extraintestinal manifestations include breast abscess in an elderly woman and possible pneumonia in children (8, 16).

The epidemiology of *C. upsaliensis* in the United States is not well characterized. In 1989, the Centers for Disease Control reported 11 cases of *C. upsaliensis* infection in humans from the United States (21). Of the 11 patients reported to have *C. upsaliensis* infections, 5 had diarrhea and 1 had bloody stool. The majority of these patients had been exposed to dogs, cats, or rats.

In an extensive analysis of 631 stool samples, we did not isolate *C. upsaliensis* from any of the cultures with the stool culture medium used (CAT medium). Because of the low concentration of the antibiotics present in CAT medium, it allowed the growth of a large number of normal flora present in the stool samples. This observation complicated the work of the medical technologist reading the plates. The technologist performed Gram staining and oxidase testing to rule out the presence of *C. upsaliensis*. Our experience with *C. upsaliensis* strain ATCC 49815 has shown that it does not grow as well as *C. jejuni* on any culture medium used. Moreover, it can easily be overgrown by the normal flora present in the stool samples.

Rapid detection of *C. jejuni* in patients with gastroenteritis is clinically relevant if therapy is initiated early in the infection process (22). However, no therapeutic effect was observed if treatment was delayed for several days until *C. jejuni* was isolated (1). The ProSpecT *Campylobacter* microplate assay would allow same-day treatment. This EIA is easy to perform and is amenable to testing in small laboratories. In terms of assay performance, the ProSpecT *Campylobacter* microplate assay is less sensitive than the gold standard, culture (89% sensitivity). However, the high specificity of the ProSpecT *Campylobacter* microplate assay (99%) allows a firm diagnosis to be made with a positive result. Another advantage of the EIA is the detection of *C. upsaliensis*, which causes diarrhea in children and HIV-infected patients, especially in geographic locations where this species is prevalent. The cost-effectiveness of this assay requires evaluation since the direct cost of the EIA is $8 more than that of culture.

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