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 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 directed by the manufacturer, the plates were washed and incubated with 50x1 washer; Biotec, 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...
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 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 origin 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 sonnei, 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, five Enterococcus faecium, five Aeromonas hydrophila, five Staphylococcus aureus, five Serratia marcescens, six Salmonella group D, five Salmonella group C, five Klebsiella pneumoniae, one Vibrio cholerae, one Vibrio parahaemolyticus, and four Yersinia enterocolitica strains. Moreover, Alexon-Trend reported no cross-reactivity with Campylobacter boyleri, Campylobacter concisus, Campylobacter curvus, Campylobacter fetus, Campylobacter lari, Campylobacter rectus, or Campylobacter spatum; 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 \( \times 10^5 \) CFU/ml. In our hands, the sensitivity of the assay for the detection of C. jejuni was 3 \( \times 10^5 \) CFU/ml, and the sensitivity of the EIA for the detection of C. upsaliensis was 3 \( \times 10^7 \) CFU/ml. The sensitivity of culture for C. jejuni detection in our hands was 3 \( \times 10^5 \) CFU/ml, and the sensitivity of culture for C. upsaliensis detection was 3 \( \times 10^5 \) 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\(^2\) 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\(^9\) to 10\(^10\) 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 in South Australia, Canada, South Africa, and the United States (2, 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 stools. 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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REFERENCES


