

## Evaluation of the Alexon-Trend ProSpecT *Campylobacter* Microplate Assay

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**We evaluated stool specimens known to contain or be free of *Campylobacter* by traditional culture, using the ProSpecT *Campylobacter* microplate assay (Alexon-Trend, Ramsey, Minn.). This rapid enzyme immunoassay for the detection of *Campylobacter*-specific antigens demonstrated 96% sensitivity and 99% specificity and is an acceptable alternative method of *Campylobacter* detection.**

Food- and water-borne bacteria cause gastroenteritis that affects millions of people each year in the United States, which costs billions of U.S. dollars and results in thousands of deaths (2, 15, 24). *Campylobacter jejuni* is the most common cause of bacterial gastroenteritis in the United States, surpassing disease caused by *Salmonella* and *Shigella* spp. combined (1, 8–13, 20, 21, 25, 33, 36, 42). The appropriate identification of the etiologic agent of infectious gastroenteritis is important, since there are differences in treatment; the possibilities of refractory disease and postinfectious sequelae also make identification of the etiologic agent important (3, 20, 32, 35, 38, 40, 43).

*Campylobacter* species are microaerophilic gram-negative, curved bacilli that may be detected in stool by direct microscopy, but more commonly are cultured using selective medium or stool filtration (5–7, 14, 17, 18, 22, 24, 28, 30, 33, 37, 39, 41). More recently, nucleic acid amplification methods and enzyme-linked immunoassays (EIA) have been used to detect these bacteria (16, 19, 26, 27, 31, 37, 44, 45; A. B. John and Y. A. Lue, Program Abstr. 98th Gen. Meet. Am. Soc. Microbiol. 1998, abstr. C-263).

We evaluated the ability of the ProSpecT *Campylobacter* microplate assay (Alexon-Trend, Ramsey, Minn.) to detect *Campylobacter* spp. in clinical stool specimens that were known to contain or be free of *Campylobacter* spp. by traditional culture. Clinical stool specimens were collected and frozen from three institutions; 50 *Campylobacter* culture-positive and 114 *Campylobacter* culture-negative stools were collected simultaneously.

*Campylobacter* species were detected and identified by standard methods (34). Fifteen of the 114 *Campylobacter* culture-negative stool specimens contained the following other bacterial enteric pathogens: six *Salmonella* spp., three *Shigella* spp., three *Yersinia enterocolitica*, and three *Escherichia coli* O157:H7 strains. All of the stool specimens in this study represented samples from individual patients; no duplicate specimens were tested. Seventy-seven of the stool specimens were received in transport medium. The remaining 87 stool samples were received fresh and frozen immediately after culture.

The 164 stool specimens were evaluated for the presence of *Campylobacter* using the the ProSpecT *Campylobacter* microplate assay (Alexon-Trend) according to the manufacturer's

instructions. Stool specimens received in transport medium were not diluted. For the fresh-frozen stool specimens, 0.5 ml of stool was mixed with the diluent provided to obtain the four drops necessary for testing. A positive and negative control well were also prepared using the positive and negative control reagents, respectively. The reactions were read both visually and spectrophotometrically in a single-wavelength spectrophotometer at 450 nm. The validity of each test run was based on appropriate reactions in the positive and negative control wells. These interpretations were performed in accordance with the manufacturer's guidelines. Each stool specimen was tested in duplicate by different medical technologists, who were blinded to the culture results. Each medical technologist recorded a visual interpretation prior to recording the spectrophotometric interpretation. The agreement between the two independent visual interpretations and the two independent spectrophotometric interpretations was determined. The agreement was also determined between the manual and spectrophotometric interpretations. All indeterminate results were repeated. Repetitively indeterminate specimens were recorded as such and considered negatives in calculations, since the EIA was unable to generate a positive result. Upon completion of the study, specimens with discordant culture and EIA results were retested by EIA, and a review of the patient's medical record was performed.

The ProSpecT *Campylobacter* microplate assay correctly characterized 48 of 50 *Campylobacter* culture-positive stool specimens. Two culture-positive stools (one received in transport medium and one fresh-frozen) were characterized as negative by EIA. Repeat EIA testing of these two specimens demonstrated one negative result and one positive result. These specimens were both considered false-negatives, since repeat testing would not have been routinely performed. One hundred and twelve of the 114 *Campylobacter* culture-negative stool specimens were characterized by the ProSpecT *Campylobacter* microplate assay as negative on initial testing. Of the remaining two *Campylobacter* culture-negative stool specimens, one was repeatedly positive and one was initially indeterminate but negative upon repeat testing; these were characterized as false-positive and true-negative, respectively. In this analysis, the ProSpecT *Campylobacter* microplate assay demonstrated 96% sensitivity and 99% specificity. Review of the medical record, however, revealed that the one "false-positive" EIA was from a patient diagnosed with infectious enteritis that may have been campylobacteriosis. It is possible that this could represent a true-positive EIA and a false-neg-

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ative stool culture, since viable organisms are not necessary for detection with the EIA. In this case, the sensitivity and specificity of the ProSpecT *Campylobacter* microplate assay would be increased to 96.1 and 100%, respectively. There was excellent interobserver agreement in both the visual and spectrophotometric test interpretations. Similarly, there was excellent agreement between the visual and spectrophotometric measurements.

The ProSpecT *Campylobacter* microplate assay is an EIA that recognizes a *Campylobacter* surface antigen which is shared by *C. jejuni* and *Campylobacter coli* (10, 42). This EIA demonstrated at least 96% sensitivity and 99% specificity using *Campylobacter* culture-positive and culture-negative specimens as the reference standard. It was rapid and easy to use, with excellent agreement between duplicate manual and duplicate spectrophotometric interpretations. There was no apparent difference between stool samples received in transport media and those that were received fresh. Excellent agreement was also found between the manual and spectrophotometric interpretations. Because one stool specimen was negative on initial testing but positive on repeat testing, we recommend that stool specimens be thoroughly mixed prior to testing. We also suggest that specimens that generate an indeterminate result be retested. Repetitively indeterminate results were never encountered in this assessment. The ProSpecT *Campylobacter* microplate assay appears to be a reliable method for the detection of *C. jejuni* and *C. coli*.

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