JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2010, p. 4022–4027 Vol. 48, No. 11
0095-1137/10/$12.00 doi:10.1128/JCM.00486-10
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Comparison of Premier CAMPY Enzyme Immunoassay (EIA), ProSpecT Campylobacter EIA, and ImmunoCard STAT! CAMPY Tests with Culture for Laboratory Diagnosis of *Campylobacter* Enteric Infections▽†

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Received 8 March 2010/Returned for modification 20 May 2010/Accepted 23 August 2010

***Campylobacter* enteritis is a food-borne or waterborne illness caused almost exclusively by *Campylobacter jejuni* and, to a lesser extent, by *Campylobacter coli*. These organisms produce indistinguishable clinical diseases and together represent the second most common cause of bacterial diarrhea in the United States and the leading cause of enteric infection throughout the world. The conventional approach to the laboratory diagnosis of *Campylobacter* enteritis is based on the recovery of the organism from a stool specimen, which requires the use of a specialized medium incubated at 42°C for several days in an artificially created microaerophilic environment. Recently, several commercially available enzyme immunoassays (EIAs) have been developed for the direct detection of *C. jejuni* and *C. coli* in stool specimens. This study compared conventional culture with three EIA methods, the Premier CAMPY EIA (Meridian Bioscience, Cincinnati, OH), the ProSpecT Campylobacter EIA (Remel, Lenexa, KS), and the ImmunoCard STAT! CAMPY test (Meridian Bioscience, Cincinnati, OH), for the detection of *C. jejuni* and *C. coli* in 485 patient stool samples. Discordant results were arbitrated by using an in-house, real-time PCR assay that was developed and validated by a public health reference laboratory. Following analyses of the discrepant specimens by PCR, the sensitivity and specificity of both the Premier CAMPY and ProSpecT Campylobacter EIAs were 99.3% and 98%, respectively, while the ImmunoCard STAT! CAMPY test had a sensitivity of 98.5% and a specificity of 98.2%. By use of the PCR test as the reference standard, culture detected 127 of 135 *Campylobacter*-positive stool specimens, yielding a sensitivity of 94.1%. These results showed that the three EIAs evaluated in this study provide a rapid and reliable alternative for the laboratory diagnosis of enteric infections with *C. jejuni* and *C. coli* and that conventional culture may no longer be recognized as the "gold standard" for diagnosis.**

Campylobacter enteritis is a food-borne and waterborne zoonotic illness that is the leading cause of acute diarrhea and enteritis throughout the world (1). Although 18 species of *Campylobacter* are known, more than 90% of diarrheal infections are caused by *Campylobacter jejuni*, and the remainder are caused primarily by *Campylobacter coli* (3). In the United States, these two species of *Campylobacter* are second only to *Salmonella* as the most common cause of bacterial enteritis, accounting for an estimated 2.4 million symptomatic enteric *Campylobacter* infections per year (2). According to a 2008 study (5) conducted by the Foodborne Diseases Active Surveillance Network (FoodNet) of the Centers for Disease Control and Prevention, which collects data on the incidence of infection caused by food-borne pathogens in the United States, the overall incidence rate of laboratory-confirmed *Campylobacter* infections was 13.0 cases per 100,000 population. Furthermore, FoodNet estimates that as many as 35 times more *Campylobacter* enteric infections may go undiagnosed or unreported each year (5).

Campylobacter jejuni and *C. coli* colonize the gastrointestinal tracts of poultry and a wide variety of animals, including cattle, sheep, swine, and domesticated pets, such as dogs and cats. Most human enteric infections result from the ingestion of undercooked chicken. One study reported that 98% of retail chickens were contaminated with *C. jejuni* and/or *C. coli* (29). Contaminated water or unpasteurized milk may also be sources for sporadic cases of disease or outbreaks of infection (16).

Campylobacter enteritis usually develops within 1 to 7 days after ingestion of a contaminated food or water source, with presenting symptoms of fever, abdominal pain, and mild to severe diarrhea. The disease is self-limited and does not usually require medical or therapeutic intervention except in severe cases. On rare occasions, serious postinfection sequelae, ranging from a transient reactive arthritis to Guillain-Barré syndrome, may develop due to the production of cross-reacting antibodies. Deaths from *Campylobacter* enteric infection are rare and occur primarily in infants, the elderly, or patients with underlying diseases (3).

Several methods have been developed for establishing the

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▽ Published ahead of print on 1 September 2010.

† The authors have paid a fee to allow immediate free access to this article.

laboratory diagnosis of *Campylobacter* enteritis. Some of these involve the direct microscopic detection of the microorganism in stool, the recovery of the organism from culture following the use of a filtration method, or the use of a specialized selective medium for the enhanced recovery of *Campylobacter* from stool (9). Most clinical laboratories do not use the direct microscopic or filtration method, because microscopy is insensitive (22, 25, 33), and filtration is cumbersome and may lack sensitivity (11).

The use of a selective medium is recommended for the optimal recovery of *Campylobacter* from stool samples (9). Some of these selective media are Skirrow's medium (26), charcoal cefoperazone deoxycholate agar (CCDA) (14), and Campy-CVA medium (23a). Once inoculated, the medium is placed in a microaerophilic growth environment, incubated at 42°C for 72 h, and observed daily for the appearance of characteristic *Campylobacter* growth. Most individuals recommend the use of a single medium, such as Campy-CVA or CCDA, for the recovery of *C. jejuni* and *C. coli* from stool specimens (27).

For more than 30 years, culture has been the primary method for establishing the laboratory diagnosis of *C. jejuni* and *C. coli* diarrheal infections. Recently, non-culture-based methods that allow for the direct detection of *Campylobacter* antigens in stool specimens have been developed. Three such methods are commercially available enzyme immunoassays (EIAs): the Premier CAMPY EIA (Meridian Bioscience, Cincinnati, OH), the ProSpecT Campylobacter EIA (Remel, Lenexa, KS), and the ImmunoCard STAT! CAMPY test (Meridian Bioscience, Cincinnati, OH).

The purpose of this study was to comparatively evaluate each of these three EIA methods with conventional culture for detecting *C. jejuni* and *C. coli* in stool specimens collected and transported to the laboratory using Cary-Blair medium. Discordant results were arbitrated by performing an in-house, real-time PCR assay that was developed and validated by the Wadsworth Center, New York State Department of Health (NYSDOH).

MATERIALS AND METHODS

Patient samples. Patient stool specimens for *Campylobacter* testing were obtained from the Microbiology sections of the Kaiser Permanente Regional Reference Laboratories in North Hollywood, CA, and the Laboratory Alliance of Central New York, located in Liverpool, NY. Fecal swab specimens were collected and transported to each laboratory in Cary-Blair transport medium (34). Culture was performed immediately upon specimen receipt in the laboratories.

Culture method. Stool specimens were inoculated onto Campy-CVA medium and incubated in a microaerophilic environment (5% oxygen, 10% carbon dioxide, and 85% nitrogen) created by the use of an evacuation-replacement jar (Anoxomat Mark II; MART Microbiology, Netherlands; distributed by Spiral Biotech, Norwood, MA). Plates were incubated at 42°C for 72 h and were observed daily for the appearance of typical growth. Isolates that were oxidase positive and were observed to be curved, Gram-negative rods following Gram staining were identified as *C. jejuni*/*C. coli* (9). Following culture for *Campylobacter*, the stool specimens were stored refrigerated (4 to 6°C) for as long as 2 weeks prior to batch testing by the three EIA methods. Specimens were then stored frozen (−70°C) for as long as 2 months before PCR testing. These storage conditions appeared to have no detrimental effect on specimen integrity and test results.

EIA methods. The Premier CAMPY EIA (Meridian Bioscience, Cincinnati, OH) and the ProSpecT Campylobacter EIA (Remel, Lenexa, KS) are both microplate assays, while the ImmunoCard STAT! CAMPY test (Meridian Bioscience, Cincinnati, OH) is a lateral-flow immunochromatographic assay. All EIAs were performed by medical technologists in the Microbiology section at the Laboratory Alliance of Central New York.

Each of the three EIAs detects a *Campylobacter* surface antigen, called *Campylobacter*-specific antigen (SA), that is shared by *C. jejuni* and *C. coli*. As such, each EIA method can detect both species of *Campylobacter* in stool specimens but cannot differentiate them.

The Premier CAMPY and ProSpecT Campylobacter tests are both microplate EIAs and are performed similarly. Basically, the patient stool specimen is emulsified and suspended in a diluent, and a small volume of the stool diluent is transferred to a well in a microtiter plate that is coated with rabbit polyclonal anti-*Campylobacter* SA. An incubation period follows, which allows for capture by the polyclonal antibody of any *Campylobacter* SA that might be present in the sample. The well is washed to remove unbound material, followed by the addition of an enzyme-conjugated polyclonal anti-*Campylobacter* SA antibody. The well is incubated to allow for any enzyme-conjugated antibody binding, followed by another washing step. Finally, a volume of colorless substrate is added to the well. In a positive reaction, the enzyme-antibody conjugate bound to the well by the *Campylobacter* SA converts the substrate to a colored reaction product that is detected spectrophotometrically. In a negative reaction, no colored reaction product is produced, because no *Campylobacter* SA is present in the well for the enzyme-antibody conjugate to bind. Appropriate positive and negative specimens are included in each test run, as required by the manufacturer. Results may be read visually or with the use of a spectrophotometer. In this study, all microwell EIA results were read with a spectrophotometer. Both EIAs can be completed in less than 2 h.

The ImmunoCARD STAT! CAMPY test is a lateral-flow-based immunoassay that uses a monoclonal antibody specific for *C. jejuni* and *C. coli* SA. The test is performed using a disposable, self-contained rectangular test cartridge. A volume of stool specimen that has been suspended in a diluent is transferred to the specimen port of the test cartridge. If present in the specimen, *Campylobacter* SA binds to the monoclonal antibody-colloid conjugate in the membrane filter as the specimen migrates through the device. The *Campylobacter* capture monoclonal antibody bound to the assay membrane at the Test position of the device's central window binds the antigen–anti-*Campylobacter* antibody–colloidal-gold complex and produces a visible pink-red line. If no *Campylobacter* SA is present in the specimen, no complex is formed, and no pink-red line develops at the Test position of the device's central window. The Control Line serves as the assay control by showing adequate flow of the diluted specimen through the test device, improper assay execution, and/or deterioration of test reagents. The Control Line is a goat anti-mouse antibody bound at the Control position of the reading window. A visible pink-red line at the Control position of the device's central window should be present each time a specimen or control is tested. If no pink-red Control Line is seen, adequate specimen flow has not occurred, and the test is considered invalid. The total assay time is less than 30 min.

A total of 485 stool specimens (127 culture positive and 358 culture negative) were tested by both the Premier CAMPY and the ProSpecT Campylobacter microplate EIA, while a subset of 300 stool specimens (127 culture positive and 173 culture negative) were tested using the ImmunoCard STAT! CAMPY test.

Real-time PCR assay. Stool specimens that gave discordant results by culture and any of the three EIAs were sent to the NYSDOH for arbitration by performing an in-house multiplex real-time PCR assay that was developed and validated for detecting both *C. jejuni* and *C. coli* in a single reaction directly from stool specimens (17, 21). One set of oligonucleotide primers and the corresponding probe amplify a gene target, the hippuricase gene (*hipO*), unique to *C. jejuni*. The second set of oligonucleotide primers and the corresponding probe amplify a gene target, the serine hydroxymethyltransferase gene (*glyA*), unique to *C. coli*. The PCR primers and probes used in the PCR assay are shown in Table 1. Additionally, a plasmid inhibition control is utilized which includes heterologous fruit fly DNA flanked by the primer binding sites for *C. jejuni* DNA cloned into a plasmid. This control, included at a low quantity in each real-time PCR assay mixture, assesses whether inhibitory factors that could lead to a false-negative result may be present. All stool specimens (0.2 g) underwent DNA extraction using the easyMag automated extraction system (bioMerieux Inc., Durham, NC). The multiplex PCR was performed on the ABI 7500 FAST instrument (Applied Biosystems Inc., Foster City, CA) using the LightCycler-FastStart DNA Master Hybridization Probes master mix (Roche Applied Science, Indianapolis, IN).

As part of the real-time PCR validation testing, 50 blinded stool specimens from this study were submitted to the NYSDOH for analyses. The specimens included 25 that were culture positive for *Campylobacter* and yielded positive results by all three EIA methods and another 25 stool specimens that were negative for *Campylobacter* by culture as well as the three EIA methods. The results of the real-time PCR assay correlated perfectly with the culture and EIA results for the 50 blinded stool specimens tested, validating the reliability of the real-time PCR assay for arbitrating discordant results.

TABLE 1. PCR primers and probes used in the real-time PCR assay

Primer or probe[a]	Nucleotide sequence (5′ → 3′)	T_m (°C)	Location within target[b]	Gene detected[c]
Cj-*FI* (forward)	TGCTAGTGAGGTTGCAAAAGAATT	58.2	918–941	*hipO* (100 bp)
Cj-*RI* (reverse)	TCATTTCGCAAAAAAATCCAAA	60.9	1018–997	
Cj-*FAM* probe	ACGATGATTAAATTCACAATTTTTTTCGCCAAA	68.1	975–943	
Cc-*FI* (forward)	CATATTGTAAAACCAAAGCTTATCGG	58.3	331–357	*glyA* (133 bp)
Cc-*RI* (reverse)	AGTCCAGCAATGTGTGCAATG	58.2	464–444	
Cc-*VIC* probe	TAAGCTCCAACTTCATCCGCAATCTCTCTCTAAATTT	68.8	431–397	

[a] Primers and probes were designed by using the Primer Express program, version 2.0 (Applied Biosystems, Foster City, CA). The melting temperatures (T_m) of the primers ranged from 58 to 60°C, and those of the probes ranged from 68 to 70°C. The TaqMan probes were conjugated with fluorescent reporter dyes FAM 495 (*C. jejuni*-specific probe; Cj-*FAM*) or VIC 538 (*C. coli*-specific probe; Cc-*VIC*) at the 5′ ends and with the quencher dye TAMRA 555 at the 3′ ends (Applied Biosystems).

[b] The positions of the oligonucleotides are listed relative to the initiation codons (+1 methionine) of the respective genes.

[c] The nucleotide sequences were retrieved from the GenBank sequence database (http://www.ncbi.nlm.nih.gov) under accession numbers Z36940 (*hipO*) and AFI 36494 (*glyA*).

Statistical analyses. Statistical analyses of the data to determine sensitivity, specificity, and positive and negative predictive values were performed using standard methods as described by Ilstrup (15).

RESULTS

A total of 485 stool specimens (127 culture positive and 358 culture negative) were tested by the Premier CAMPY and ProSpecT Campylobacter microwell plate EIAs. The Premier and ProSpecT assays detected 126 of the 127 culture-positive specimens, with a single discordant result obtained for each EIA, involving 2 different stool specimens. Of the 358 stool specimens that were culture negative for *Campylobacter*, the Premier CAMPY and ProSpecT Campylobacter EIAs detected 14 and 15 positive specimens, respectively. Using culture as the reference method, Table 2 shows the sensitivity, specificity, and positive and negative predictive values for each test following statistical analyses. Compared to culture, both microwell assays had identical sensitivities of 99.2% and comparable specificities (96.1% versus 95.8%). Also, each assay had a 90% positive predictive value and a 99.7% negative predictive value.

The discordant culture and EIA microwell specimens were tested by real-time PCR to arbitrate the discrepant results. Of the 2 discordant culture-positive, EIA-negative specimens, both gave positive PCR results, indicating that these specimens were truly culture positive for *Campylobacter*, with each EIA microwell test producing a single false-negative test result. However, for the 14 and 15 culture-negative specimens that tested positive by the Premier and ProSpecT assays, respectively, arbitration of the discordant results by real-time PCR showed that 8 specimens positive by EIA but negative by culture were confirmed as truly positive by real-time PCR, indicating that culture produced 8 false-negative results. Using PCR as the test for arbitration, the Premier and ProSpecT EIAs had 6 and 7 false-positive results, respectively.

Table 3 shows the statistical analyses for the test results resolved by using the PCR assay to arbitrate discordant results. The sensitivities of each of the EIAs were still identical at 99.3%, while the specificities compared closely (99.3% versus 98%), and the positive and negative predictive values for each EIA improved to 95% and 99.7%, respectively. Importantly, by using real-time PCR for the arbitration of discordant results, the sensitivity of culture resolved from 100% (127/127 specimens, as shown in Table 2) to 94.1% (127/135 specimens, as shown in Table 3).

A subset of 300 (127 culture-positive and 173 culture-negative) stool specimens was tested using the ImmunoCard STAT! CAMPY assay. As shown in Table 2, the ImmunoCard STAT! CAMPY assay detected 125 of the 127 culture-positive specimens but gave positive test results for 10 of the 173 culture-negative specimens. Using culture as the standard of reference, the ImmunoCard STAT! CAMPY assay had a sensitivity of 98.4% and a specificity of 94.2%, with positive and negative values of 92.6% and 98.8%, respectively.

The real-time PCR assay was used to arbitrate the 12 discordant results obtained with the ImmunoCard STAT! CAMPY test. The two culture-positive stool specimens that produced negative ImmunoCard results gave positive reactions, confirming that these were true false-negative ImmunoCard test results. On the other hand, of the 10 culture-negative stool specimens that produced positive STAT! CAMPY ImmunoCard results, 7 specimens were positive by the real-time PCR test, indicating that these were likely false-negative culture results.

Table 3 shows the resolved statistical analyses for the ImmunoCard STAT! CAMPY test when the real-time PCR assay

TABLE 2. Statistical analyses of the Premier CAMPY, ProSpectT Campylobacter, and STAT! CAMPY EIA methods using culture as the reference method

Assay	Positive specimens		Negative specimens		Predictive value (%)	
	No. positive by test/culture	Sensitivity (%)	No. negative by test/culture	Specificity (%)	Positive	Negative
Meridian EIA	126/127	99.2	344/358	96.1%	90.0	99.7
Remel EIA	126/127	99.2	343/358	95.8%	89.4	99.7
Meridian STAT!	125/127	98.4	163/173	94.2%	92.6	98.8

TABLE 3. Statistical analyses of the Premier CAMPY, ProSpecT Campylobacter, and STAT! CAMPY EIA methods following real-time PCR arbitration of discordant results

Assay	Positive specimens		Negative specimens		Predictive value (%)	
	No. positive by test/culture	Sensitivity (%)	No. negative by test/culture	Specificity (%)	Positive	Negative
Meridian EIA	134/135	99.3	344/350	98.3	95.7	99.7
Remel EIA	134/135	99.3	343/350	98.0	95.0	99.7
Meridian STAT!	132/134	98.5	163/166	98.2	97.8	98.8

was used to arbitrate discordant test results. The sensitivity of the STAT! CAMPY test remained about the same (98.5%), but its specificity increased to 98.2%. The positive and negative predictive values also increased to 97.8% and 98.8%, respectively. By using real-time PCR as the reference test to arbitrate the discordant results for the ImmunoCard test, the corrected sensitivity for culture resolved from 100% (127/127) to 94.8% (127/134).

DISCUSSION

The genus *Campylobacter* consists of motile, non-spore-forming, Gram-negative rods and includes at least 18 different species (28). *Campylobacter* is oxidase positive and grows best in a reduced-oxygen atmosphere containing 5% to 10% oxygen. All campylobacters grow at 37°C, but *C. jejuni* and *C. coli*, the two species that account for almost all cases of *Campylobacter* enteritis, grow best when incubated at 42°C (9). This thermophilic growth requirement, along with a specialized selective growth medium, is used by most clinical laboratories to optimize the recovery of *C. jejuni* and *C. coli* from stool specimens.

Campylobacters isolated from stool are typically identified by Gram stain examination of the bacterial colony along with the performance of an oxidase test. Colonies that are oxidase positive, exhibit a Gram stain appearance of curved to S-shaped Gram-negative rods, and grow on a specialized selective medium incubated at 42°C under microaerophilic conditions can be reliably identified as *Campylobacter* spp. (9). Hydrolysis of sodium hippurate can be used to distinguish *C. jejuni* from *C. coli*, but this differentiation is not usually necessary, because these two *Campylobacter* species produce clinical diseases that are indistinguishable. Also, some strains of *C. jejuni* may be negative for hippurate hydrolysis (9).

Campylobacter jejuni and *C. coli* have been recognized as important causes of gastrointestinal infection since the 1970s. Infected patients may present with mild to severe symptoms that may include fever, abdominal cramps, and diarrhea with or without the presence of fecal leukocytes (9). Symptomatic infection is usually self-limited, lasting from a few days to more than a week. As many as 10% of untreated patients may experience a relapse of infection (3).

Postinfectious complications may occur following *Campylobacter* enteritis, but they are rare. Guillain-Barré syndrome (GBS), an acute paralytic disease of the peripheral nervous system, may develop, with an estimated incidence of 1 per 1,000 infections (20). *Campylobacter*-induced GBS is thought to result from the host's immune response to ganglioside-like epitopes present in the core region of the *Campylobacter* lipopolysaccharide (10), which can mediate damage to peripheral nerves, where ganglioside targets are highly enriched (35).

Reactive arthritis may develop in 2 to 7% of patients following *Campylobacter* enteritis, with pain and joint swelling developing often within 2 weeks of infection. This reactive arthritis syndrome may last for as long as 12 months (27). The immune pathogenesis of the reactive arthritis is unknown, but it is thought to be strongly associated with HLA B27 positivity (6).

With the development of reliable laboratory techniques for the detection or recovery of *Campylobacter* in stool, *C. jejuni* and *C. coli* have emerged as the leading cause of bacterial enteritis worldwide and the second most common cause of diarrheal disease in the United States (1, 2). Conventional methods available for the laboratory diagnosis of *Campylobacter* enteritis include microscopy, filtration, and culture.

Gram stain smear examination of stool specimens can be performed as a rapid and inexpensive method for establishing the presumptive diagnosis of *Campylobacter* enteritis. However, due to their thin morphology, campylobacters cannot be readily visualized with the safranin counterstain that is used in most Gram stain procedures. Instead, carbol fuchsin or 0.1% aqueous basic fuchsin can be substituted as the counterstain to achieve visualization of the organism in direct smears of stool (22, 25, 33). Evaluation of this direct microscopic method in stool specimens collected from patients with acute diarrheal disease shows a sensitivity ranging from 66% to 94% and a specificity of >95% (22, 25, 33).

Filtration techniques (8, 12, 32) involve the selective recovery of *Campylobacter* from a stool specimen based on the organism's motility and thin morphology, which allow it to pass through a membrane filter. In the filtration method, a suspension of stool specimen is passed through a cellulose acetate membrane filter (pore sizes, 0.45 to 0.65 μm). Due to their active motility and slender morphology, campylobacters readily pass through the filter, while the other microorganisms are trapped on the filter. A portion of the filtrate is then removed and inoculated onto a culture plate that is incubated at 37°C under microaerophilic conditions. One study has shown that the filtration method is not as sensitive as culture using selective medium, because at least 10^5 CFU of *Campylobacter* per ml must be present in the sample to allow detection by the filtration method (11).

Culture is used by most laboratories for establishing the diagnosis of *Campylobacter* diarrheal disease. Several selective media have been developed for the enhanced recovery of *Campylobacter* from stool specimens. The most common culture methods involve the use of blood-based, antibiotic-containing media, such as Skirrow's, Butzler's, and Campy-BAP

media (9). Also, a non-blood-based, charcoal-containing selective medium, called charcoal cefoperazone deoxycholate agar (CCDA) (14), has been developed and reported to be more sensitive than the filtration method or the use of Skirrow's medium for the cultural recovery of *Campylobacter* from stool specimens (8).

Several enrichment broths, such as Campy-thio (24), Preston enrichment (4), and *Campylobacter* enrichment (18) broth, have been developed for the enhanced recovery of *Campylobacter* from stool. However, because symptomatic patients usually excrete 10^6 to 10^9 CFU of *Campylobacter* per g of stool, broth enrichment is usually unnecessary and is not considered cost-effective (3). Broth enrichment may be useful when low numbers of the organism may be present in the specimen due to a delay in specimen transport to the laboratory or if the specimen is collected following the acute stage of the disease (19).

Molecular detection techniques, such as nucleic acid amplification and EIA, have also been used for the diagnosis of *Campylobacter* enteric infection. Nucleic acid amplification techniques, such as that used in this study (15, 21) and another reported elsewhere (23), are in-house-developed amplification assays that are not commercially available for routine laboratory use. On the other hand, antigen detection methods for the direct detection of *C. jejuni* and *C. coli* in stool specimens have been commercially available for several years. One of the first commercially available EIAs was the ProSpecT Campylobacter kit. Evaluation of this kit by several investigators (7, 13, 30) showed that it had a sensitivity ranging from 80 to 96% and a specificity of >97%. Other EIA kits are now also commercially available for diagnostic use.

This study comparatively evaluated the performance of three commercially available EIAs, the Premier CAMPY EIA, the ProSpecT Campylobacter EIA, and the ImmunoCard STAT! CAMPY test, with an established culture method for detecting *C. jejuni* and *C. coli* in stool specimens. Specimens with discordant test results were arbitrated by performing an in-house real-time PCR assay that was developed and validated by a public health reference laboratory. After arbitration of discordant test results by real-time PCR, statistical analyses of the data showed that each of the three EIA methods performed better than culture in detecting *C. jejuni* and *C. coli* in stool specimens, but false-negative and false-positive results were observed with each of the EIA methods.

Using the PCR assay as the reference method, the number of false-negative EIA results was extremely low compared to culture: the two EIA microwell tests had 1 false-negative result each, while the ImmunoCard method gave false-negative results for 2 specimens. By comparison, 8 false-negative culture results were observed in this study. The higher sensitivity of the EIAs than of culture offers obvious advantages for improved patient care and increased public health awareness of the true prevalence of *Campylobacter* enteric infections in the community (9). In addition, the use of the more-sensitive EIAs allows for improved diagnosis and therapeutic management of patients who might develop the postinfectious complications of reactive arthritis or GBS following *Campylobacter* infection (20, 27).

False-positive results were also observed for each of the three EIAs. The Premier and ProSpecT microplate assays had 6 and 7 false-positive results, respectively, while the ImmunoCard test had 3 specimens with false-positive results. The false-positive EIA results could, in fact, be due to false-negative PCRs caused by hippurate-negative isolates of *C. jejuni* that lacked the hippurase gene, *hipO*. This possibility was not investigated as part of our study. However, this explanation may be unlikely, because one report (31) has suggested that the phenotypic lack of expression of hippurase is due not to a lack of the hippurase gene but rather to the inability of the hippurate assays to detect strains of *C. jejuni* that are low-level producers of hippurase.

A more likely explanation for the false-positive EIA results may be sensitivity or specificity differences between the assays. Since different inoculum amounts are used in the PCR and EIAs, discordant results may occur if low levels of *Campylobacter* are in the specimen. For instance, in the PCR assay, 0.2 g of stool is extracted and eluted in 200 μl of buffer, with only 5 μl used for analysis, whereas a much larger volume, up to 0.2 ml, is used for EIAs. Another explanation for the possible false-positive EIA results may be a rare occurrence of cross-reactivity to various components found in stool specimens.

The results of this study showed that the Premier CAMPY and ProSpecT Campylobacter microplate EIAs had identical sensitivities of 99.3%, while the lateral-flow ImmunoCard STAT! CAMPY test had a very acceptable performance sensitivity of 98%. Given its convenience in use and the short turnaround times for final test results, the ImmunoCard test may have applications as a STAT method and/or for routine use in small-volume laboratories. In addition, the use of any of these EIAs offers the potential for providing same-day results and eliminates the need and expense associated with using a 42°C incubator and special devices for creating a microaerophilic environment. Finally, the resolved sensitivity of culture following real-time PCR arbitration of discordant test results was 94.1%, suggesting that the three EIAs evaluated in this study provide rapid and reliable alternatives for the laboratory diagnosis of *Campylobacter* enteric infections and that conventional culture may no longer be the recommended method for diagnosis.

ACKNOWLEDGMENT

This study was partially funded by a grant provided by Meridian Bioscience, Cincinnati, OH.

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