JCM
Journals.ASM.org

Detection of non-*jejuni* and -*coli Campylobacter* Species from Stool Specimens with an Immunochromatographic Antigen Detection Assay

Brianne A. Couturier,[a] Marc Roger Couturier,[a,b] Kim J. Kalp,[a] Mark A. Fisher[a,b]

Associated Regional and University Pathologists (ARUP) Laboratories, Institute for Clinical and Experimental Pathology, Salt Lake City, Utah, USA[a]; Department of Pathology, University of Utah School of Medicine, Salt Lake City, Utah, USA[b]

The STAT! Campy immunochromatographic assay for *Campylobacter* antigen was compared to culture for 500 clinical stool specimens. Antigen was detected in six culture-negative, PCR-positive specimens. *C. upsaliensis*, a pathogenic species that is traditionally difficult to recover in routine stool cultures, was detected in two of these culture-negative specimens. This study provides evidence that antigen testing may cross-react with at least one additional non-*jejuni* and -*coli Campylobacter* species that may be missed by routine culture for campylobacteriosis.

Campylobacter spp. are fastidious bacteria that may be difficult to recover in culture due to suboptimal specimen transport and/or storage conditions and lack of universal culture procedures. However, *Campylobacter* antigens may persist in clinical specimens in the absence of viable organisms. The variable clinical specificity reported for several commercially available *Campylobacter* antigen detection assays remains unresolved in the literature (1–4). Several studies have reported higher numbers of antigen-positive than of culture-positive specimens (1, 2, 5), but it is unclear whether these are simply false-positive results or are due to *Campylobacter* spp. in the specimens that are nonviable, viable but nonculturable, or not readily cultivated under commonly employed conditions. This study sought to determine whether culture-negative, antigen-positive specimens, as determined by immunochromatographic assay, actually contained difficult-to-culture *Campylobacter* spp. which could explain the antigen positivity. In addition, we examined the stability and cross-reactivity profile of the immunochromatographic assay to further define the performance characteristics of the test.

A total of 500 fecal specimens submitted over 4 months (July to September 2011 and June 2012) for routine stool culture or *Campylobacter*-specific culture were tested with the Immunocard STAT! Campy antigen assay (Meridian Bioscience, Cincinnati, OH). The routine stool culture for *Campylobacter* was performed by inoculating stool preserved in Cary-Blair transport medium (4°C for ≤72 h) on a Campy CVA plate (Hardy Diagnostics, Santa Maria, CA) and incubated at 42°C in a microaerobic environment (AnaeroPouch-MicroAero, Mitsubishi, Atlanta, GA) for at least 72 h. Colonies resembling *Campylobacter* were Gram stained for morphology and tested for oxidase and hippurate hydrolysis activities. Oxidase- and hippurate hydrolysis-positive isolates with typical colony and Gram stain morphology were identified as *C. jejuni* (6). Hippurate hydrolysis-negative isolates were further identified by matrix-assisted laser desorption–ionization time of flight mass spectrometry (7). Antigen-positive stool specimens (Table 1) were defined by the presence of a red-pink band and bands of any other color were interpreted as negative, according to the package insert. Of the 500 specimens analyzed, 5 (1%) samples were culture positive for *Campylobacter jejuni*, and 15 (3%) were antigen positive, yielding a sensitivity of 80% and specificity of 98% relative to our gold standard of culture (Table 1). Four specimens were both culture and antigen positive, while the remaining culture-positive specimen was antigen negative, and 11 antigen-positive specimens were negative by culture (Table 2). Of the four dual-positive specimens, three were available for stability testing, which was done by repeating culture and antigen detection for 5 consecutive days (the claimed stability period for the antigen assay). All three specimens remained both culture and antigen positive after 5 days of storage at 4°C, indicating that culture and antigen have similar stability characteristics under routine storage conditions. This indicates that the higher observed antigen positivity rate is not likely to be due to instability of culture.

Direct stool PCR was performed on antigen- or culture-positive stools. DNA was extracted from stool specimens as previously described (8). Primers targeted a *Campylobacter*-specific region of the 16S rRNA gene, and PCR products were sequenced for confirmation (9). Of the 15 antigen-positive specimens, 10 were PCR positive for *Campylobacter* spp. (Table 2). Sequencing these amplicons and analyzing by BLAST using the NCBI refseq_rna database identified the presence of *C. jejuni* ($n = 4$), *C. gracilis* ($n = 1$), and *C. concisus* ($n = 5$). Several of the specimens had potentially mixed sequences, suggesting the presence of more than one species of *Campylobacter* (Table 2). One specimen was culture positive but antigen and PCR negative. The original CVA culture for this specimen yielded fewer than five colonies and may have been below the limit of detection for the antigen and direct stool PCR, but a commercial *Campylobacter* real-time PCR confirmed the presence of *C. jejuni* in this specimen (BioGX, Birmingham, AL, data not shown). Filtration culture was attempted on all antigen-positive specimens (10); however, this method yielded no additional positives beyond the *C. jejuni* obtained by routine culture. Four of the five false-positive specimens that were negative by PCR and culture but positive by STAT! Campy were noted as having very faint pink bands, which should be interpreted as pos-

Received 14 December 2012 **Returned for modification** 18 January 2013
Accepted 24 March 2013
Published ahead of print 3 April 2013
Address correspondence to Mark A. Fisher, mark.fisher@aruplab.com.
B.A.C. and M.R.C. are co-first authors.

doi:10.1128/JCM.03208-12

TABLE 1 Results for culture versus antigen detection assay

Culture result	No. of samples with STAT! Campy result		
	Positive	Negative	Total
No. positive	4	1	5
No. negative	11	484	495
Total no.	15	485	500

itive according to the package insert. It was occasionally difficult to clearly define the color of weakly reactive assays, which may lead to some variability in the interpretation of this test (data not shown). The commercial *Campylobacter jejuni/coli* real-time PCR was performed on these four specimens, and all were negative. The remaining antigen-positive, PCR- and culture-negative specimen showed rapid and strong reactivity immediately after the addition of the specimen. It was tested daily for 10 days with no apparent decrease in reactivity; however, positivity was lost after extended storage at −20°C and multiple freeze-thaw cycles. The source of the strong reactivity in this specimen remains unclear.

The ProSpecT *Campylobacter* enzyme immunoassay (Remel, Lenexa, KS) was previously shown to detect *Campylobacter upsaliensis* (3, 4). To assess whether the false positives observed in our study were due to the presence of uncultured *C. upsaliensis*, species-specific primers were used for direct stool PCR (11). Of the 15 antigen-positive specimens, three were positive for *C. upsaliensis*. Surprisingly, all three appeared to be coinfected with other *Campylobacter* spp., including *C. jejuni*, *C. gracilis*, and *C. concisus* (Table 2). Coinfection with multiple species of *Campylobacter* (particularly *C. upsaliensis*) has been described previously and is thought to be underreported due to culture procedures that are suboptimal for some *Campylobacter* spp. (12). To determine the extent of antigen reactivity with non-*jejuni* and -*coli Campylobacter* spp., 21 clinical or reference isolates of *Campylobacter* spp. were suspended in phosphate-buffered saline (3.0 McFarland, approximately 4.3×10^{10} CFU/ml) and directly tested with the STAT! Campy assay. Of the 10 non-*jejuni* and -*coli Campylobacter* species tested, positive results were observed only for *C. upsaliensis*, and all seven clinical isolates of this species were positive (Table 3). In an attempt to discern whether the reactivity seen in the assay could be clinically relevant, dilutions of *C. jejuni* and *C. upsaliensis* cultures were tested. The assay had approximately 10-fold lower sensitivity for *C. upsaliensis* than for *C. jejuni* (Table 4). To put this in perspective, the assay is 2.5-fold less sensitive in detecting *C. coli*, for which it holds FDA clearance, than *C. jejuni*, according to the manufacturer's package insert. Despite the lower sensitivity, this reactivity for the gastrointestinal pathogen *C. upsaliensis* may explain a portion of the false positives described in this study. *C. upsaliensis* may be inhibited by some *Campylobacter*-selective media, and therefore, it is possible that infections that would otherwise go undiagnosed may be identified using antigen testing. Further prospective studies using culture conditions optimized for the recovery of *C. upsaliensis* will be required to determine the extent to which this species influences STAT! Campy positivity rates. By PCR and sequencing, we determined that five specimens contained *C. concisus*; however, our *C. concisus* isolate failed to cross-react in the antigen detection assay (Table 3). This may indicate that *C. concisus* does not express the antigen(s) detected by the assay and that its presence in the clinical samples may not be the cause of the positive antigen tests. However, as many as four genomospecies have been proposed among isolates identified as *C. concisus* (13), and it is possible that other genomospecies could

TABLE 2 Direct *Campylobacter* spp. PCR and sequencing results for specimens generating discrepant results between antigen detection and culture

Specimen	Result using:			
	STAT! Campy	Culture	Stool PCR and sequencing[a]	*C. upsaliensis* PCR
CM-508	Positive	*C. jejuni*	*C. jejuni*	Negative
CM-105	Positive	*C. jejuni*	*C. jejuni*	Negative
CM-168	Positive	*C. jejuni*	*C. jejuni*	Negative
CM-44	Positive	*C. jejuni*	*C. jejuni*[b]	Positive
CM-216	Positive	Negative	*C. gracilis*[b]	Positive
CM-372	Positive	Negative	*C. concisus*[b]	Positive
CM-23	Positive	Negative	*C. concisus*	Negative
CM-136	Positive	Negative	*C. concisus*	Negative
CM-351	Positive	Negative	*C. concisus*[b]	Negative
CM-422	Positive	Negative	*C. concisus*	Negative
CM-411	Positive	Negative	Negative	Negative
CM-45	Positive	Negative	Negative	Negative
CM-474	Positive	Negative	Negative	Negative
CM-423	Positive	Negative	Negative	Negative
CM-65	Positive	Negative	Negative	Negative
CM-485	Negative	*C. jejuni*	Negative	Negative

[a] Identifications were based on CLSI guidelines of >99% identity for species-level identification.
[b] These sequences had occasional mixed bases.

TABLE 3 Analysis of antigenic cross-reactivity for multiple clinical or reference *Campylobacter* isolates

Campylobacter spp. (no. of isolates)	STAT! Campy result
C. coli (1)	Positive
C. curvus (2)	Negative
C. concisus[a]	Negative
C. fetus (1)	Negative
C. gracillis (1)	Negative
C. jejuni (1)	Positive
C. lari (2)	Negative
C. rectus (1)	Negative
C. sputorum (1)	Negative
C. upsaliensis (7)	Positive
C. ureolyticus (2)	Negative
C. volurcis (1)	Negative

[a] ATCC 51562.

TABLE 4 Relative sensitivities of antigen detection from *C. jejuni* and *C. upsaliensis* cultures

McFarland standard	Result for indicated culture	
	C. jejuni	*C. upsaliensis*
2.0	Positive	Positive
1.0	Positive	Positive
0.5	Positive	Positive
1:10 dilution[a]	Positive	Negative
1:100 dilution[a]	Negative	ND[b]

[a] 0.5 McFarland (1.0×10^8 CFU/ml) was diluted 10-fold and 100-fold, as indicated.
[b] ND, not determined.

cross-react *in vitro*. Focused studies involving multiple well-characterized representatives of each genomospecies of *C. concisus* will be required to better define the presence or extent of antigenic reactivity in this assay. One limitation of this study is that antigen-negative specimens were discarded and thus unavailable for retrospective testing by *C. upsaliensis*- or *C. concisus*-specific PCRs. Therefore, we were unable to determine whether any other specimens containing *C. upsaliensis* or *C. concisus* were not detected by the STAT! Campy assay.

Reactivity with *C. upsaliensis* was clearly established in this study, but that alone does not account for all false-positive specimens seen. False-positive results may also be explained by variability in test interpretation, as positives may be scored subjectively in terms of whether faint reactivity is truly pink or brown. One published report also suggested that blood in the stool may cause false-positive results (5). Of the 500 specimens tested in our study, 31 (6%) were reported as grossly bloody specimens; however, none of those were positive for *Campylobacter* antigen, suggesting that bloody stools may not be a significant problem with this assay. Limited ancillary testing was ordered on the majority of these specimens: eight were evaluated by ovum and parasite examination, six by *Clostridium difficile* PCR, five for *Giardia*, and two for *Cryptosporidium* by fecal antigen enzyme-linked immunosorbent assay. Testing for gastrointestinal pathogenic viruses was not performed on any of the specimens. Overall, no causative agent of gastroenteritis was identified by testing at our laboratory for any of the discrepant specimens.

Overall, the STAT! Campy assay may detect infection with *Campylobacter* spp. other than *C. jejuni*/*coli*; however, this attribute alone would not account for all of the perceived false positives encountered in this study. Additional studies using well-characterized and highly sensitive molecular assays capable of identifying a variety of *Campylobacter* spp. may help clarify the role of antigen testing in clinical diagnostics for campylobacteriosis.

ACKNOWLEDGMENTS

The study was partially funded by Meridian Bioscience.

We thank the Clinical Microbiology laboratories of ARUP for their help with processing specimens.

REFERENCES

1. **Granato PA, Chen L, Holiday I, Rawling RA, Novak-Weekley SM, Quinlan T, Musser KA.** 2010. Comparison of premier CAMPY enzyme immunoassay (EIA), ProSpecT Campylobacter EIA, and ImmunoCard STAT! CAMPY tests with culture for laboratory diagnosis of Campylobacter enteric infections. J. Clin. Microbiol. **48**:4022–4027.
2. **Bessede E, Delcamp A, Sifre E, Buissonniere A, Megraud F.** 2011. New methods for detection of campylobacters in stool samples in comparison to culture. J. Clin. Microbiol. **49**:941–944.
3. **Hindiyeh M, Jense S, Hohmann S, Benett H, Edwards C, Aldeen W, Croft A, Daly J, Mottice S, Carroll KC.** 2000. Rapid detection of Campylobacter jejuni in stool specimens by an enzyme immunoassay and surveillance for Campylobacter upsaliensis in the greater Salt Lake City area. J. Clin. Microbiol. **38**:3076–3079.
4. **Dediste A, Vandenberg O, Vlaes L, Ebraert A, Douat N, Bahwere P, Butzler JP.** 2003. Evaluation of the ProSpecT microplate assay for detection of Campylobacter: a routine laboratory perspective. Clin. Microbiol. Infect. **9**:1085–1090.
5. **Myers AL, Jackson MA, Selvarangan R.** 2011. False-positive results of Campylobacter rapid antigen testing. Pediatr. Infect. Dis. J. **30**:542.
6. **Murray PR, Baron EJ.** 2007. Manual of clinical microbiology, 9th ed. ASM Press, Washington, DC.
7. **Khot PD, Couturier MR, Wilson A, Croft A, Fisher MA.** 2012. Optimization of matrix-assisted laser desorption ionization-time of flight mass spectrometry analysis for bacterial identification. J. Clin. Microbiol. **50**:3845–3852.
8. **Couturier BA, Hale DC, Couturier MR.** 2012. Association of Campylobacter upsaliensis with persistent bloody diarrhea. J. Clin. Microbiol. **50**:3792–3794.
9. **Linton D, Owen RJ, Stanley J.** 1996. Rapid identification by PCR of the genus *Campylobacter* and of five *Campylobacter* species enteropathogenic for man and animals. Res. Microbiol. **147**:707–718.
10. **Le Roux E, Lastovica AJ.** 1998. The Cape Town protocol: how to isolate the most campylobacters for your dollar, pound, Franc, yen, etc., p 30–33. *In* Lastovica AJ, Newell D, Lastovica EE (ed), Proceedings of the 9th International Workshop on *Campylobacter*, *Helicobacter* and Related Organisms. Institute of Child Health, Cape Town, South Africa.
11. **Chaban B, Ngeleka M, Hill JE.** 2010. Detection and quantification of 14 *Campylobacter* species in pet dogs reveals an increase in species richness in feces of diarrheic animals. BMC Microbiol. **10**:73. doi:10.1186/1471-2180-10-73.
12. **Lastovica AJ.** 2006. Emerging *Campylobacter* spp.: the tip of the iceberg. Clin. Microbiol. Newsl. **28**:49–56.
13. **Man SM.** 2011. The clinical importance of emerging *Campylobacter* species. Nat. Rev. **8**:669–685.