

Multicenter Evaluation of Clinical Diagnostic Methods for Detection and Isolation of *Campylobacter* spp. from Stool

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The use of culture-independent diagnostic tests (CIDTs), such as stool antigen tests, as standalone tests for the detection of *Campylobacter* in stool is increasing. We conducted a prospective, multicenter study to evaluate the performance of stool antigen CIDTs compared to culture and PCR for *Campylobacter* detection. Between July and October 2010, we tested 2,767 stool specimens from patients with gastrointestinal illness with the following methods: four types of *Campylobacter* selective media, four commercial stool antigen assays, and a commercial PCR assay. Illnesses from which specimens were positive by one or more culture media or at least one CIDT and PCR were designated "cases." A total of 95 specimens (3.4%) met the case definition. The stool antigen CIDTs ranged from 79.6% to 87.6% in sensitivity, 95.9 to 99.5% in specificity, and 41.3 to 84.3% in positive predictive value. Culture alone detected 80/89 (89.9% sensitivity) *Campylobacter jejuni/Campylobacter coli*-positive cases. Of the 209 noncases that were positive by at least one CIDT, only one (0.48%) was positive by all four stool antigen tests, and 73% were positive by just one stool antigen test. The questionable relevance of unconfirmed positive stool antigen CIDT results was supported by the finding that noncases were less likely than cases to have gastrointestinal symptoms. Thus, while the tests were convenient to use, the sensitivity, specificity, and positive predictive value of *Campylobacter* stool antigen tests were highly variable. Given the relatively low incidence of *Campylobacter* disease and the generally poor diagnostic test characteristics, this study calls into question the use of commercially available stool antigen CIDTs as standalone tests for direct detection of *Campylobacter* in stool.

Campylobacter infection continues to be a major public health problem. *Campylobacter jejuni* and *Campylobacter coli* are pathogens transmitted commonly through food, causing an estimated 1.3 million cases of illness per year in the United States (1), and yet diagnosis can be challenging because the organism is difficult to isolate, grow, and identify. Recent reports describing clinical laboratory practices for *Campylobacter* diagnostics in Pennsylvania (2) and the Foodborne Diseases Active Surveillance Network (FoodNet) sites (3) highlight the wide range of testing practices in use; currently, no best-practice clinical or public health laboratory guidelines exist for laboratory diagnosis of *Campylobacter* infections. Direct plating onto a *Campylobacter* selective medium, followed by incubation at 42°C under microaerobic conditions for 72 h, has long been considered the "gold standard" for diagnosis (4).

The use of culture-independent diagnostic tests (CIDTs) for *Campylobacter* testing on stool samples is increasing, which may have important implications for both patient management and public health surveillance efforts (5). Stool antigen tests to directly detect *Campylobacter* in fecal samples are fast and generate same-day results, but concerns regarding specificity and positive predictive value (PPV) have been raised (6, 7). There are currently no guidelines on how to interpret and report discordant results between stool antigen tests and culture. In addition, the current

national case definition for a confirmed case of *Campylobacter* requires culture confirmation, whereas persons with positive CIDTs only are classified as probable cases (8). Current reports of *Campylobacter* incidence and trends through FoodNet are also based only on culture-confirmed cases, though CIDT results are

Received 3 August 2015 Returned for modification 30 August 2015

Accepted 24 January 2016

Accepted manuscript posted online 9 March 2016

Citation Fitzgerald C, Patrick M, Gonzalez A, Akin J, Polage CR, Wymore K, Gillim-Ross L, Xavier K, Sadlowski J, Monahan J, Hurd S, Dahlberg S, Jerris R, Watson R, Santovenia M, Mitchell D, Harrison C, Tobin-D'Angelo M, DeMartino M, Pentella M, Razeq J, Leonard C, Jung C, Achong-Bowe R, Evans Y, Jain D, Juni B, Leano F, Robinson T, Smith K, Gittelman RM, Garrigan C, Nachamkin I, *Campylobacter* Diagnostics Study Working Group. 2016. Multicenter evaluation of clinical diagnostic methods for detection and isolation of *Campylobacter* spp. from stool. *J Clin Microbiol* 54:1209–1215. doi:10.1128/JCM.01925-15.

Editor: C.-A. D. Burnham

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J.M. met authorship criteria but was unreachable for final approval of the byline and article.

Supplemental material for this article may be found at <http://dx.doi.org/10.1128/JCM.01925-15>.

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TABLE 1 Result summary and demographic characteristics of patients for whom specimens were positive, by study site

Site	State	No. of specimens tested	No. of positive cases (% positive)	No. of noncases positive in at least one CIDT	Total no. positive	No. with epidemiological data	Sex: no. of male patients/total no. (%)	Median age, yr (range)	Race: no. of white patients/total no. (%)	Ethnicity: no. of Hispanic patients/total no. (%)
1	CA	340	15 (4.4)	35	50	50	22/50 (44)	49 (1–100)	28/34 (82)	3/36 (8)
2	CO	277	5 (1.8)	26	31	30	9/30 (30)	62 (25–92)	21/24 (88)	7/28 (25)
3	CT	260	7 (2.7)	7	14	14	6/14 (43)	49 (<1–77)	8/11 (73)	2/12 (17)
4	GA	259	6 (2.3)	42	48	45	31/43 (72)	6 (<1–18)	23/43 (54)	6/38 (16)
5	IA	452	20 (4.4)	26	46	45	16/35 (46)	45 (<1–84)	34/36 (94)	0/35 (0)
6	MD	157	3 (1.9)	2	5	4	3/4 (75)	34 (19–58)	1/2 (50)	1/2 (50)
7	MN	504	25 (4.9)	37	62	60	30/59 (51)	44 (1–89)	34/36 (94)	0/47 (0)
8	PA	518	14 (2.7)	34	48	42	20/42 (48)	47 (4–95)	25/38 (66)	2/38 (5)
Total		2,767	95 (3.4)	209	304	290	137/277 (50)	42 (<1–100)	174/224 (78)	21/236 (9)

tracked. There is a clear need to evaluate the performance of culture-independent assays, to better inform microbiologists and clinicians on the use of these tests in patient management, and to ensure the validity of public health surveillance data and also for real-world data for informed considerations on whether and how *Campylobacter* case definitions should be modified. To better understand the performance characteristics of stool antigen CIDTs for *Campylobacter* diagnosis, we conducted a prospective, multi-center study to evaluate the performance of stool antigen tests in comparison to culture and PCR for detection of *Campylobacter* from stool.

MATERIALS AND METHODS

Study sites and stool specimens. From July to October 2010, stool specimens from patients with gastrointestinal illness on whom routine enteric diagnostic laboratory testing, including *Campylobacter* testing, had been ordered were submitted to a participating study site hospital, county, or state public health laboratory in eight states. The testing laboratories and clinical partners were as follows: CA, Sacramento County Public Health Laboratory/University of California Davis Medical Center; CO, Colorado Department of Public Health and Environment/Exempla Saint Joseph Hospital Microbiology Laboratory; CT, Connecticut Emerging Infections Program/Yale-New Haven Hospital; GA, Enteric Diseases Laboratory Branch, CDC/Children's Healthcare of Atlanta; IA, State Hygienic Laboratory; MD, Maryland Department of Health and Mental Hygiene; MN, Public Health Laboratory, Minnesota Department of Health; and PA, Hospital of the University of Pennsylvania/Perelman School of Medicine at the University of Pennsylvania (Table 1). Tests were performed according to the manufacturer's instructions. Hands-on training and webinar instructions on how to perform the stool antigen assays were provided as needed to all study sites before testing began, so all sites were familiar with the immunoassays prior to use. Specimens were cultured immediately upon receipt in accordance with published guidelines (4) or appropriately stored prior to stool antigen testing (0 to 14 days) according to the manufacturer's instructions. This study was approved by institutional review boards at the respective institutions where appropriate.

Culture. Stool specimens at all study sites were directly inoculated on the following four *Campylobacter* selective media: Campy cefoperazone, vancomycin, amphotericin B (CVA) agar (Remel Inc., Lenexa, KS); Campy Cefex agar (Hardy Diagnostics, Santa Maria, CA); modified cefoperazone charcoal deoxycholate agar (mCCDA) (Remel Inc., Lenexa, KS); and charcoal selective medium (CSM) (Remel Inc., Lenexa, KS). Media were incubated microaerobically for 72 h at either 37°C (mCCDA) or 42°C (CVA agar, Campy Cefex agar, and CSM).

Stool antigen tests. The following four stool antigen tests were performed on all stool specimens at each study site according to the manu-

facturer's instructions. Two are formatted as microplate assays, ProSpecT *Campylobacter* (Remel Inc., Lenexa, KS) and Premier Campy (Meridian Bioscience Inc., Cincinnati, OH), and two are formatted as lateral flow devices, ImmunoCard Stat! Campy (ICS; Meridian Bioscience Inc., Cincinnati, OH) and Xpect Campy (Remel Inc., Lenexa, KS).

PCR. For molecular diagnosis, genomic DNA was isolated using either the QIAamp DNA stool minikit or the automated QIAcube system (Qiagen, Valencia, CA) according to the manufacturer's instructions. Study sites tested all stool specimens using the Seeplex diarrhea-bacterial panel 1ACE detection PCR kit (Seegene Inc., Seoul, South Korea) according to the manufacturer's instructions. Four study sites (CO, GA, MN, and PA) performed PCR testing for their own respective specimens, and study site 4 (GA) performed PCR testing for the other four sites (CA, CT, IA, and MD). Stool specimens were shipped frozen on dry ice, to study site 4 for this PCR testing, once all other testing was complete. This multiplex PCR kit is based on dual priming oligonucleotide technology (DPO) (9), which detects *Campylobacter jejuni* and *Campylobacter coli* (but does not differentiate between these two *Campylobacter* species), *Salmonella* spp. (*Salmonella bongori* and *Salmonella enterica*), *Shigella* spp. (*Shigella flexneri*, *Shigella boydii*, *Shigella sonnei*, and *Shigella dysenteriae*), *Vibrio* spp. (*Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*), and *Clostridium difficile* toxin B. This PCR assay has been previously validated and is reported to be more sensitive than culture for detection of *Campylobacter* from stool (10, 11).

Associated data collection. Demographic and epidemiologic information (e.g., clinical symptoms, illness severity, use of antibiotics during their illness, and use of antibiotics and antacids before illness) were collected from all persons who tested positive for *Campylobacter* by any of the *Campylobacter*-specific culture or CIDT methods evaluated in this study. The majority of these data were already being collected on state-specific interview forms, and interviews were conducted as part of ongoing, routine public health follow-up. In Colorado, Connecticut, Iowa, Maryland, and Minnesota, questions that were not already on a state's form were added and asked by state public health personnel during the study period. California, Georgia, and Pennsylvania obtained information through a structured questionnaire administered by state public health personnel in addition to routine interviews. Pennsylvania also used a prenotification letter.

Data analysis. Illnesses from which specimens were positive by one or more culture media or, if culture negative, positive by at least one stool antigen test and PCR were designated "cases." "Noncases" were defined as illnesses in which specimens were positive only by stool antigen tests or by PCR but not both. Test performance statistics were calculated using standard methods using either the definition of "cases" or *C. jejuni/C. coli*-positive culture using both CVA agar and mCCDA as the gold standard (12). Frequencies and percentages were calculated. Characteristics of patients whose specimens were designated cases or noncases were com-

TABLE 2 *Campylobacter* species identified by selective plating media tested

Culture identification	No. of isolates identified in <i>Campylobacter</i> selective medium tested				Total no. of isolates identified in study by species
	CVA agar	Campy- Cefex	CSM	mCCDA	
<i>C. jejuni</i>	63	64	67	66	71
<i>C. coli</i>	4	4	4	4	4
<i>C. jejuni/C. coli</i>	5	5	4	5	5
Subtotal					<i>n</i> = 80
<i>C. upsaliensis</i>	1	0	0	0	1
<i>C. showae</i>	0	1	0	0	1
<i>Campylobacter</i> sp., not <i>C. jejuni/C. coli</i>	3	2	1	2	4
Total	76	76	76	77	86

pared. The chi-square test was used to determine differences between categories. A *P* value of <0.05 was considered statistically significant (13). Variables that were significant in univariate analysis were included in a multivariate model.

RESULTS

A total of 2,767 stool specimens met the study inclusion criteria and were tested by the four *Campylobacter* selective media, four stool antigen tests, and *Campylobacter* species-specific PCR. Results summarized by site are shown in Table 1. The percentage of specimens from cases varied by site and ranged from 1.8% in CO to 4.9% in MN. A total of 95 specimens (3.4%) met the case definition; 86 were positive by selective medium culture, and 9 were culture negative but positive in at least one stool antigen CIDT and PCR. An additional 209 culture-negative specimens were positive in one or more CIDTs but were classified as noncases, since they were positive only by stool antigen tests or by PCR but not both. A breakdown of the *Campylobacter* species identified by selective plating media is shown in Table 2. The majority of *Campylobacter* isolated by selective media were *C. jejuni* (83%) or *C. coli* (5%) with one strain each of *Campylobacter upsaliensis* and *Campylobacter showae*. Nine additional *Campylobacter* isolates were identified to genus level only and were not available for subsequent species-level identification. Five of these were PCR positive in stool and considered *C. jejuni/C. coli* for the analysis. The other four strains were hippurate negative and PCR negative in stool, classifying them as *Campylobacter* sp. other than *C. jejuni/C. coli*. No single culture medium detected all 86 *Campylobacter*-positive specimens, and there was no statistical difference in recovery between the four media. Three media were positive for 76 of 86 culture-positive specimens, and the other, mCCDA, was positive for one additional specimen. The combination of CVA agar and mCCDA resulted in recovery of all 80 *C. jejuni/C. coli* strains and was significantly better than using CVA (*P* = 0.01) or Cefex (*P* = 0.01) agar alone.

Table 3 shows the performance characteristics of the four stool antigen tests using *C. jejuni/C. coli*-positive culture as the reference method. Compared to culture using CVA agar and mCCDA, the two microplate stool antigen assays, PREMIER and ProSpecT, had similar sensitivities (86.3% versus 87.5%), specificities (97.3%

Test method	Reference method				Case definition			
	Culture		Culture		Case definition		Case definition	
	% sensitivity (95% CI) ^b	% specificity (95% CI)	% predictive value (95% CI)	% sensitivity (95% CI)	% specificity (95% CI)	% predictive value (95% CI)	% sensitivity (95% CI)	% specificity (95% CI)
Premier Campy	86.3 (76.7–92.9)	97.3 (96.6–97.9)	48.6 (40.1–57.1)	99.6 (99.2–99.8)	85.4 (77.4–92.8)	97.4 (96.7–97.9)	53.5 (44.9–61.9)	99.5 (99.2–99.8)
ProSpecT <i>Campylobacter</i>	87.5 (78.2–93.8)	97.5 (96.8–98.0)	51.1 (42.4–59.7)	99.6 (99.3–99.8)	87.6 (80.1–94.4)	97.6 (96.9–98.2)	56.9 (48.2–65.3)	99.6 (99.2–99.8)
ImmunoCard Stat!	78.8 (68.2–87.1)	95.9 (95.1–96.6)	36.6 (29.4–44.3)	99.3 (98.9–99.6)	79.8 (69.9–87.6)	95.9 (95.1–96.7)	41.3 (33.8–49.0)	99.3 (98.8–99.6)
Xpect Campy	78.8 (68.2–87.1)	99.2 (98.8–99.5)	75.9 (65.3–84.6)	99.4 (98.9–99.6)	79.6 (69.6–87.4)	99.5 (99.1–99.7)	84.3 (74.7–91.3)	99.3 (98.9–99.6)

^a See Tables S1 and S2 in the supplemental material for detailed results.

^b CI, confidence interval.

TABLE 4 Summary of results for culture-negative specimens that were positive in at least one CIDT ($n = 218$)^b

No. of culture-negative specimens	No. of EIAs ($n = 4$) positive	Result by test:				
		Premier Campy	ProSpecT	ImmunoCard Stat! Campy	Xpect Campy	Seegene multiplex PCR, <i>Campylobacter</i> result
7 ^a		Pos	Pos	Pos	Pos	Pos
1 ^a		Neg	Pos	Neg	Neg	Pos
1 ^a		Neg	Neg	wk Pos	Inv	Pos
1		Pos	Pos	Pos	wk Pos	Neg
11		Neg	Neg	Neg	Neg	Pos
2		NT	NT	NT	NT	Pos
153	1					Neg
38	2					Neg
4	3					Neg

^a Cases per case definition.

^b Abbreviations: Pos, positive; Neg, negative; wk Pos, weakly positive; Inv, invalid; NT, not tested; EIA, enzyme immunoassay.

versus 97.5%), and positive predictive values (PPVs) (48.6% versus 51.1%). The lateral flow assays, ICS and Xpect, were less sensitive than the microplate assays (78.8% each). Correspondingly, the Xpect assay had the highest specificity (99.2%) and PPV (75.9%) of the four stool antigen assays tested. The ICS test had the lowest specificity (95.9%) and PPV (36.6%) of the four stool antigen assays tested. A detailed summary of the stool antigen tests and PCR results for the 80 specimens with *C. jejuni/C. coli* isolated by culture is provided in Table S1 in the supplemental material. Of the 21 *C. jejuni/C. coli* culture-positive specimens that were negative by one or more of the immunoassays ($n = 11$, Premier; $n = 10$, ProSpecT; $n = 17$, both ICS and Xpect), 17/21 were positive by PCR, indicating that the PCR assay generated four false-negative results (see Table S1 in the supplemental material).

Among the 2,681 samples that were culture negative for *Campylobacter*, the stool antigen tests were positive in 2.8% ($n = 74$, Premier), 2.6% ($n = 69$, ProSpecT), 4.1% ($n = 109$, ICS), and 0.8% ($n = 23$, Xpect) of specimens, respectively, for a total of 218 specimens. Nine of these were confirmed as cases by PCR, indicating nine false-negative culture results (Table 4). Another 196 culture-negative stool antigen CIDT-positive samples (67 [Premier], 61 [ProSpecT], 101 [ICS], and 16 [Xpect]) were negative by PCR and deemed to be false positives. An additional 13 specimens were positive by PCR only and likely represented true positives, although they did not meet the study case definition. The performance characteristics of the stool antigen tests using the consensus culture or stool antigen CIDT plus PCR case definition are also shown in Table 3. Using this case definition, stool antigen CIDT sensitivities were relatively similar to those observed using *C. jejuni/C. coli*-positive culture as the reference method, with the lateral flow assays being less sensitive (79.8%, ICS; 79.6%, Xpect) than the microplate assays (85.4%, Premier; 87.6%, ProSpecT). Specificity was highest for the Xpect assay (99.5%), comparable to the microplate assays (Premier [97.4%] versus ProSpecT [97.6%]), and lowest for the ICS assay (95.9%). The PPV improved slightly for all four assays, with the lowest value seen for the ICS test (41.3%) and the highest seen for the Xpect assay (84.3%), respectively. After resolution by PCR testing, culture using a combination of CVA agar and mCCDA had a sensitivity of 89.9% (80/89) for *C. jejuni/C. coli*-positive stool specimens.

Culture and stool antigen test results for the six non-*C. jejuni/C. coli* culture-positive specimens are shown in Table S2 in the supplemental material. Only two strains were positive by one

or more of the stool antigen tests; the *C. upsaliensis* strain was positive by the Premier and ProSpecT assays and one *Campylobacter* (not *C. jejuni* or *C. coli*) strain was positive by the ProSpecT assay only. The other four strains were negative by all four stool antigen tests and PCR.

Of 304 patients with any positive *Campylobacter* laboratory results generated in this study, 290 (95%) had some demographic, clinical, or treatment information available (Tables 1 and 5). Compared to patients whose specimens were designated non-cases, patients whose specimens were cases were significantly more likely to report having gastrointestinal symptoms such as diarrhea and fever, to have a greater number of stools, and to have taken an antibiotic during their illness (Table 5). In addition, cases were less likely to be hospitalized, had a shorter length of hospitalization, and were less likely to report taking an antibiotic in the 4 weeks before illness onset. In a multivariate model that included all of these factors, cases remained significantly more likely to have diarrhea (odds ratio [OR] = 9.3; 95% confidence interval [CI], 1.1 to 74.8) and fever (OR = 4.4; 95% CI, 2.1 to 9.5) and less likely to be hospitalized for their illness (OR = 0.3; 95% CI, 0.13 to 0.64).

DISCUSSION

Initial published evaluations of *Campylobacter* stool antigen CIDTs reported comparable or better sensitivities than conventional culture methods (10, 14, 15). More recent evaluations (6, 16) and clinical case investigations (7, 17) have questioned the specificity of these assays. We undertook a large multicenter study of four *Campylobacter* stool antigen CIDTs and multiple selective media to determine the real-world performance of stool antigen CIDTs and correlate positive results with clinical data.

Compared to culture using CVA agar and mCCDA, the four stool antigen CIDTs varied in sensitivity (78.8% to 87.5%) and specificity (95.9% to 99.2%). However, several of them had more false positives than true positives, and only one had a reasonable positive predictive value (Xpect Campy, 75.9%). The other three had PPVs of 51% or lower. Performance improved slightly with PCR analysis of discrepant samples (Table 3), but three stool antigen tests still had PPVs less than or equal to 57% due to the high number of false positives, making them unacceptable as stand-alone diagnostics for *C. jejuni/C. coli* infection. Only the Xpect Campy assay had a PPV that might be viewed as acceptable for this test population, 84.3%, and this test is not at this time FDA cleared, though it is available in Europe for clinical use. The neg-

TABLE 5 Comparison of epidemiologic characteristics between patients whose specimens were cases and those whose specimens were noncases ($n = 299$)

Characteristic	No. positive/no. total (%), unless otherwise indicated		P value
	Case ($n = 94$)	Noncase ($n = 205$)	
Median age, yr (range)	32 (<1–77)	46 (<1–100)	NS ^a
Sex, female	38/90 (42)	105/196 (54)	NS
Race, white	61/74 (82)	117/155 (75)	NS
Ethnicity, Hispanic	10/77 (13)	12/166 (7)	NS
Diarrhea	70/72 (97)	99/131 (76)	<0.0001
Median no. of stools (range)	10 (3–144)	7 (3–100)	0.0007
Median length of diarrhea, days (range)	6 (3–70)	10 (1–78)	NS
Bloody diarrhea	23/62 (37)	41/124 (33)	NS
Fever	48/67 (72)	44/116 (38)	<0.0001
Nausea	36/61 (59)	55/113 (49)	NS
Vomiting	24/67 (36)	43/125 (34)	NS
Abdominal cramps	57/66 (86)	86/117 (74)	NS
Median days of illness (range)	10 (5–29)	14 (3–60)	NS
Hospitalization	23/80 (29)	81/166 (49)	0.0029
Median length of hospitalization, days (range)	2 (<1–57)	4 (<1–41)	0.0017
Death	0/87 (0)	7/169 (4)	NS
Contact with others with diarrhea	10/60 (17)	7/104 (7)	NS
Drug taking			
Antibiotics for illness	49/63 (78)	59/119 (50)	0.0002
Antibiotics in 4 wk before illness	4/62 (6)	26/111 (23)	0.0057
Antacids in 4 wk before illness	18/60 (30)	46/120 (38)	NS

^a NS, not statistically significant.

ative predictive value of all stool antigen CIDTs was very high, which is not surprising given the low prevalence of *Campylobacter* infections in the tested populations at the eight study sites. Culture was more sensitive than or comparable to the most sensitive stool antigen CIDTs but without the false positives.

A number of previous studies evaluated several of the stool antigen CIDTs reported in our study. Granato et al. (14) found that stool antigen assays (Premier Campy, ProSpecT, and ImmunoCard Stat!) had high sensitivities (~99%), specificities (~98%), and PPVs (95 to 98%). In that study of 485 preselected stool samples, there were 127 culture-positive samples, giving a culture-positive prevalence of 18.5%. Bessede et al. (10) in a prospective analysis of 242 stool samples with 23 culture-positive cases (9.5% prevalence) found the ImmunoCard Stat! to have a sensitivity of ~70% and the Premier Campy a sensitivity of ~80%. In a study of U.S. military patients with a culture-positive prevalence of 36.8% ($n = 182$ cases), Tribble et al. (15) found ProSpecT to have 95% sensitivity, 94% specificity, and a PPV of 96%. A study of infants with diarrhea in developing countries of South America found ProSpecT to have 60.6% sensitivity and 88.8% specificity for *C. jejuni/C. coli* infections (18). Our prospective multicenter study included 2,767 samples with 86 culture-positive patients (3.1% prevalence). Using either culture as the reference method or a case definition that included stool antigen CIDT positivity with a positive PCR result, performance characteristics of stool antigen tests for the detection of *C. jejuni/C. coli* differed substantially from performance characteristics reported by other investigators. In particular, we found lower sensitivity of these assays and, for the most part, poor positive predictive values. There are a number of possible explanations for these differences.

The prevalence of infection in our study, 3.1%, is low compared to many of the previously published studies. However, this prevalence in an unselected population likely represents the true prevalence of *Campylobacter* seen in most U.S. clinical microbiology laboratories. Even in the face of what would be considered high specificity, the PPVs for the three stool antigen tests (Premier Campy, ProSpecT, and ImmunoCard Stat!) were approximately 41 to 57%. Stool antigen CIDT studies showing excellent performance using preselected sampling for *Campylobacter*-positive specimens (6, 14) may show different performance characteristics once testing is performed in real time, as reported by Giltner et al. (6). In that study, where testing with Premier Campy had a PPV of 91% in lab validation studies, the test performed poorly in real-time use, with only 75% sensitivity and 42.9% PPV (6).

With technology for detecting microbial agents moving away from culture to nonculture techniques, such as immunoassays for antigen detection and detection of microbial nucleic acids, we need to consider whether discordant results (e.g., stool antigen test positive, culture negative) are truly false positives or if they represent true infections that were not detected by culture. In the case of stool antigen tests for *Campylobacter* antigen detection, cross-reactivity with species of *Campylobacter* other than *C. jejuni* or *C. coli* has been reported (18–21), so perhaps these other *Campylobacter* species present in the stool samples account for discordant results. In our study, we had an insufficient number of other identified species, such as *C. upsaliensis* ($n = 1$) and *C. showae* ($n = 1$), to make any conclusion about the ability of stool antigen CIDTs to detect these species (see Table S2 in the supplemental material). Additionally, the clinical significance of *C. showae* as a human pathogen is unknown. The fact that non-*C. jejuni/C. coli*

species were detected by selective culture and *Campylobacter concisus* was detected by filtration in one study site (data not shown) but not by all stool antigen tests, however, supports the conclusion that discordant results likely represent truly false-positive antigen tests rather than detection of less common species. This is further supported by the lack of concordance of positive test results between methods for the noncases (Table 4). As such, we and several authors have suggested caution when interpreting positive *Campylobacter* stool antigen results (6, 7, 17), and there may be implications for patient management, especially when *Campylobacter*-positive antigens are seen in combination with positive results for other pathogens such as Shiga toxin-producing *Escherichia coli* (7).

We found significant differences in demographics and clinical symptoms between cases and noncases in our study (Table 5). The fact that noncases were less likely to have clinical symptoms consistent with *Campylobacter* infection lends further weight to the conclusion that discordant results truly represent false-positive antigen results and that noncases likely had a different illness. Differences in age and gender seen between cases and noncases may reflect differences in testing practices (e.g., use of stool antigen assays in nursing home populations) or regional variations in use of nonculture testing and should be further explored.

In addition to studying the performance characteristics of stool antigen tests for detecting *Campylobacter* in stool, we had the opportunity to reexamine the performance characteristics of commonly used selective culture media for isolation of *Campylobacter*. Although evaluations of the many commercially available *Campylobacter* selective media have been published over the past 30 years, none have evaluated *Campylobacter* vancomycin-amphotericin (CVA) agar, one of the most commonly used selective media in the United States. We found that the four selective culture media used were equivalent in performance. We did find, however, that a combination of CVA agar and mCCDA isolated all 80 *C. jejuni*/*C. coli* culture-positive samples, increasing the sensitivity over a single medium by 5 to 10%. This confirms the results of a study from the early 1990s that a combination of media had better sensitivity than any single medium (22).

In conclusion, while convenient to use, no stool antigen CIDT offered the necessary combination of high sensitivity, high specificity, and moderate to high positive predictive value needed in a standalone diagnostic test. Two stool antigen tests, Premier Campy and ProSpecT, had sensitivities similar to that of culture but poor PPVs due to a large number of false positives. Only one stool antigen CIDT, Xpect Campy, had an acceptable PPV for this test population (84%), but the sensitivity of this assay was less than 80% and this assay is not yet FDA cleared. The utility of a diagnostic test is dependent upon the prevalence of the disease in the population being tested. While *Campylobacter* causes a high burden of disease, it still has a relatively low incidence in the United States. Given this low incidence and the resulting poor positive predictive values of antigen detection based on the performance data that we present, our study calls into question the validity of using commercially available stool antigen assays for direct detection of *Campylobacter* in stool. Our study also provides real-world data in which informed considerations can be made on whether and how *Campylobacter* case definitions should be modified in the future. Highly sensitive and more specific nucleic acid amplification assays have more recently been approved by the FDA and may

be preferable to stool antigen assays as alternatives to culture for detecting *Campylobacter* in stool (23–26).

ACKNOWLEDGMENTS

We thank the following members of the *Campylobacter* Diagnostics Study Working Group: Jan Pruckler and Patrick Kwan (Enteric Diseases Laboratory Branch, CDC); Sharon Greene and Cassandra Harrison (Enteric Diseases Epidemiology Branch, CDC); Jonelle McKey, Theresa Stanley, Donna Peace, Kathleen Temple, and Charles Ash (Children's Healthcare of Atlanta, GA); Holly Fogarty (Spencer Hospital Laboratory, Spencer, IA); Jill Noble (Mercy Medical Center, Des Moines, IA); and Charlotte Taylor, Scott Fisher, Anupama Ganeshkumar, Victoria Lappi, Jennifer Palm, Jaime Christensen, Brian Nefzger, Dan Hammersley, and Dave Boxrud (Minnesota Department of Health). We thank Remel, Hardy Diagnostics, Meridian Biosciences, Seegene Inc., and Qiagen for providing laboratory supplies.

We thank the Association for Public Health Laboratories for funding this study.

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