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Evaluation of the Campyslide Agglutination Test for Confirmatory Identification of Selected *Campylobacter* Species

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The utility of a rapid latex slide agglutination test (Campyslide; BBL Microbiology Systems, Cockeysville, Md.) in detecting selected *Campylobacter* spp. was evaluated and compared with that of conventional identification methods. Isolated colonies suggestive of *Campylobacter* spp. were tested directly from primary selective media after incubation at 42°C under microaerophilic conditions. Stock cultures of *Campylobacter jejuni* ($n = 27$) and *C. coli* ($n = 3$) were correctly confirmed to the genus level by latex agglutination when tested in pure cultures or isolated from seeded human feces. A total of 50 fresh clinical isolates of *Campylobacter* spp. (45 *C. jejuni* and 5 *C. coli*) were examined, with complete agreement observed between the latex test and conventional methods. Of 173 non-*Campylobacter* isolates tested from primary plates, only 1 rough strain of *Pseudomonas aeruginosa* produced a false-positive result. Although the manufacturer recommends a 30-min antigen extraction, 1 or 5 min was found to be sufficient. Also, confirmation could be achieved within 24 h of inoculation of clinical specimens, 2 days earlier than with conventional methods.

In recent years, *Campylobacter* spp., particularly *Campylobacter jejuni*, have been recognized as important human pathogens which cause both acute bacterial gastroenteritis and extraintestinal infections (1–5, 7, 8, 10, 13). The incidence of *Campylobacter* infections in the United States has been estimated at 1 to 2 million cases per year (3). *C. jejuni* has been isolated from 3 to 14% of patients with diarrheal illness in Europe and North America (2, 3, 5) and has been found in every country in which surveillance has been reported (2–4, 6, 8). Isolation rates for this organism have approached and often surpassed those of *Salmonella* spp. or *Shigella* spp. in patients with enteric illness (4, 6, 12, 14). Individuals of all ages have been infected with *C. jejuni*, although infections are most frequent in young children and young adults (2, 3, 5, 9). *Campylobacter* infections are especially prevalent among college students, with the fraction of stool cultures yielding *Campylobacter* spp. on U.S. college campuses ranging from 13 to 53% (14).

Early resolution of the clinical symptoms of acute gastroenteritis and clearance of the organism from stools are dependent upon promptly beginning appropriate antibiotic therapy (1). To do this, rapid determination of the presence of *Campylobacter* spp. in feces is required. Currently, the definitive identification of *Campylobacter* spp. by conventional isolation and biochemical techniques requires a minimum of 48 to 72 h. Presumptive identification of these organisms based on Gram staining and oxidase testing requires only 24 to 48 h to accomplish, but the accuracy of this identification is dependent on the proficiency of the technologist. In many laboratories in which the isolation of *Campylobacter* spp. is uncommon, the accuracy of this presumptive identification may not be acceptable. We have evaluated the utility of the Campyslide (BBL Microbiology Systems, Cockeysville, Md.) latex agglutination (LA) test in detecting cell wall antigens of selected *Campylobacter* spp. This test provides a rapid and simple genus-level confirmation of these human pathogens from cultures.

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MATERIALS AND METHODS

Bacterial isolates. All bacterial strains studied were clinical isolates recovered at North Carolina Memorial Hospital (NCMH) or the Student Health Service (SHS) of the University of North Carolina, Chapel Hill. The organisms were either fresh clinical isolates or isolates which were recovered from stool specimens from patients and stored frozen in horse blood at −70°C until used. A total of 253 fresh clinical isolates and stock cultures, including 80 *Campylobacter* spp. and 173 non-*Campylobacter* isolates, were tested by LA. All *Campylobacter* isolates were positively identified to the species level by the methods described by Morris and Patton (11). Non-*Campylobacter* isolates were identified to the species level by Gram staining, catalase and oxidase reactions, and conventional biochemical and physiological methods.

Culture conditions. Fresh stool specimens from patients and stock culture isolates of *Campylobacter* spp. were inoculated onto campylobacter agar or campylobacter CVA agar (BBL). Incubation was for 24 or 48 h at 42°C under a microaerophilic atmosphere provided by the CampyPak Envelope (BBL) and jar system or the Campy Pouch (BBL) system. All *Campylobacter* and non-*Campylobacter* organisms were isolated from the same media and under the same incubation conditions. For experiments involving seeded stools, six human diarrheal stool specimens, confirmed to be negative for *Campylobacter* spp., were pooled, and 0.9-ml aliquots were inoculated with 0.1 ml of a turbid suspension (McFarland no. 2 standard) of each stock culture isolate of *Campylobacter* spp. The seeded stools were inoculated onto campylobacter CVA agar by using sterile Dacron-tipped swabs and were incubated for 24 or 48 h as described above.

Campyslide LA test. The Campyslide test is an LA assay for the confirmatory genus-level identification of the four major *Campylobacter* pathogens of human illness (*C. jejuni*, *C. coli*, *C. laridis*, and *C. fetus* subsp. *fetus*) from cultures. The test is based upon the detection of cell wall antigens found in these species by use of an anti-*Campylobacter* antibody-coated latex bead suspension. Initially, the

Campyslide test was assessed with stock strains of *Campylobacter* spp. in pure cultures or isolated from seeded human feces. The performance of the test was subsequently evaluated with fresh clinical specimens. Both *Campylobacter* spp. and non-*Campylobacter* spp. were tested directly from primary plates of *Campylobacter* selective media. A limited amount of the organism, either as isolated colonies or as confluent growth from these plates, was required to perform the LA test. The LA test was performed in accordance with the instructions of the manufacture with minor modifications. Briefly, bacterial isolates were suspended in 0.2 ml of extraction reagent to achieve a slightly turbid suspension (McFarland no. 0.5 to 1 standard) and were extracted for 1, 5, or 30 min at room temperature. This step was followed by the addition of 0.2 ml of neutralization reagent. The suspension was vortexed thoroughly, and a change in color from yellow to a mid- to deep purple was indicative of neutralization. If the suspension remained yellow, at least 1 additional drop (5 to 20 μl) of neutralization reagent was added to neutralize the mixture. The neutralized antigen extracts (25 μl) were placed onto two circles of a test slide, and 1 drop (25 μl) each of reactive latex and control latex was dispensed for each organism tested. The extract and latex were mixed with plastic stirrers, and the test slide was rotated at 100 rpm on a mechanical rotator for 3 to 4 min. The test slide was then read for distinct visible agglutination. If a sample produced weak agglutination, the test slide was rotated for an additional 6 min before being read again. A positive antigen control and a negative reagent control were tested with each set of specimens. Agglutination was scored as positive, equivocal, or negative. Equivocal results were considered negative.

RESULTS AND DISCUSSION

Initial studies with stock cultures of 27 strains of *C. jejuni* and 3 strains of *C. coli* were positive by LA when tested in pure cultures or after isolation from seeded human feces (Table 1). Studies on fresh clinical specimens were then performed. The prevalence of *Campylobacter* spp. between 28 October 1986 and 30 April 1987 was 17.9% (37 of 207) for SHS, and that for NCMH was 1.6% (14 of 881). A total of 34 (92%) *C. jejuni* and 3 (8%) *C. coli* strains were isolated from SHS, while 12 (86%) *C. jejuni* and 2 (14%) *C. coli* strains were found at NCMH. Of 51 clinical isolates, 50 were correctly confirmed to the genus level by the LA test (Table 1). One *Campylobacter* strain isolated from NCMH was not tested.

Of 173 non-*Campylobacter* isolates tested from primary plates, only 1 rough strain of *Pseudomonas aeruginosa* repeatedly produced a positive agglutination result. However, atypical agglutination reactions with either the reactive or control latex or both were observed for eight other isolates of this organism. Reactions of this type were rapid, were atypical in appearance, and occurred while the antigen extracts were being stirred with the antibody-coated latex particles. These reactions could not be consistently reproduced upon repeat testing of the isolated organisms and were considered equivocal results. Table 2 summarizes the results for the non-*Campylobacter* isolates tested by LA.

Although the manufacturer recommends a 30-min antigen extraction for the LA test, we found 1 or 5 min to be sufficient. Antigens extracted for different times from 44 fresh clinical isolates of *Campylobacter* spp. (24 isolates for 5- and 30-min extraction comparisons; 20 isolates for 1- and 5-min extraction comparisons) performed equally well when tested by LA (Table 3). A total of 83 non-*Campylobacter* isolates were extracted for 5 and 30 min; 79 isolates showed negative results at both extraction times, and 4 isolates of *P. aeruginosa* showed atypical agglutination. Three separate test kits were used for this comparison, and 11 of the 83 non-*Campylobacter* isolates were *P. aeruginosa*. A comparison of 1- and 5-min extraction times revealed negative results for 49 non-*Campylobacter* isolates and atypical agglutination for 4 isolates of *P. aeruginosa*. Four separate kits were used for this comparison, and 10 of the 53 organisms examined were *P. aeruginosa*.

Four medical technologists in the bacteriology laboratory at NCMH were asked to independently evaluate the Campyslide test and comment on its performance. In a blind study, they were given five strains of *C. jejuni* and five non-*Campylobacter* isolates (*Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Citrobacter freundii*, and *P. aeruginosa*) to test by LA. Extraction times of 1 and 5 min (two technologists for each) were used. All individuals successfully and correctly completed their evaluations. Weakly positive agglutination reactions were observed by one technologist for the *C. jejuni* isolates after the initial 3- to 4-min rotation of the test slide. In accordance with the instructions of the manufacturer, the test slide was rotated for an additional 6 min before being read again. Strong positive reactions were observed at this time. The *P. aeruginosa* isolate showed various degrees of atypical agglutina-

TABLE 1. Campyslide test results for stock culture strains and fresh clinical isolates of *Campylobacter* spp.

Organism(s)	No. of positive organisms[a]	
	C. jejuni	*C. coli*
Stock strains (pure cultures)	27	3
Stock strain in seeded feces[b] (24-h incubation)	16	3
Stock strain in seeded feces (48-h incubation)	27	3
Fresh clinical isolates	45	5

[a] All tested organisms were positive.
[b] Some stock strains showed no visible growth in seeded feces after incubation for 24 h. Growth subsequently occurred after an additional 24 h of incubation.

TABLE 2. Campyslide test results for 173 non-*Campylobacter* isolates

Organism	No. positive/ no. tested
Acinetobacter calcoaceticus subsp. *anitratus*	0/4
Bacteroides fragilis	0/1
Citrobacter freundii	0/4
Enterobacter cloacae	0/21
Enterobacter aerogenes	0/7
Enterobacter agglomerans	0/1
Escherichia coli	0/71
Klebsiella pneumoniae	0/10
Klebsiella oxytoca	0/5
Lactobacillus spp.	0/5
Proteus mirabilis	0/5
Pseudomonas aeruginosa	1/27[a]
Staphylococcus spp. (coagulase negative)	0/4
Yeasts	0/7
Yersinia enterocolitica	0/1

[a] One rough strain of *P. aeruginosa* produced a positive agglutination result. Atypical agglutination reactions were observed for eight other isolates of this organism.

TABLE 3. Detection of *Campylobacter* isolates by LA following different antigen extraction times

Extraction time (min)	No. of positive organisms[a]	
	C. jejuni	*C. coli*
30	32[b]	5[c]
5	21	3
1	19	1

[a] All tested organisms were positive.
[b] Eleven isolates of *C. jejuni* were tested by LA following 30 min of antigen extraction only.
[c] Two isolates of *C. coli* were tested by LA following 30 min of antigen extraction only.

tion with the latex test for all of the technologists. The procedure was well received with the exception of the neutralization step. More often than not, the technologists had to add more than an equal volume of neutralization reagent to neutralize the extraction mixture.

All *Campylobacter* isolates from SHS were confirmed within 24 h of inoculation of clinical specimens onto selective media, 2 days earlier than with conventional methods routinely used in the bacteriology laboratory at NCMH. Stools from SHS were processed in batches on a given day, immediately placed under strict microaerophilic conditions (CampyPak Envelope or Campy Pouch), and incubated undisturbed for 18 to 24 h at 42°C. This procedure was not feasible for the bacteriology laboratory at NCMH, since processing of all specimens is offered on a 24-h basis. Therefore, *Campylobacter* plates were routinely placed in a candle jar as the stools were processed until they could be transferred to jars with CampyPak Envelopes by day-shift personnel. This procedure may have resulted in a delay of growth of the *Campylobacter* isolates by an additional 24 h and, therefore, a subsequent delay in identification. Under these conditions, the Campyslide test would save only 1 day in confirming a positive result for *Campylobacter* spp. in a clinical specimen.

In conclusion, the Campyslide LA test provides a sensitive and specific test for the confirmatory serological identification of *Campylobacter* spp. from primary selective media. The test is rapid and simple to perform and, with the exception of an atypical agglutination reaction with some isolates of *P. aeruginosa*, the results are distinct and easily interpreted. The antigen extraction time can be decreased to 1 or 5 min, making the test more suitable for a busy clinical microbiology laboratory. Isolated colonies or confluent growth suggestive of *Campylobacter* spp. can be tested as early as 18 to 24 h after incubation of the primary plates, making early detection and prompt administration of antibiotics feasible.

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