REACTIVITY OF THE CD D-1 LATEX TEST WITH *CLOSTRIDIUM DIFFICILE* AND OTHER BACTERIA

Siripan Wongwanich, Mayura Kusum and Ratanasuda Phan-Urai

National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand

Abstract. The reactivity of a commercial latex test with thirty-three species of bacteria was tested. Toxigenic and nontoxigenic strains of *Clostridium difficile* gave a positive result in the CD D-1 latex test. Cross-reactions were also given by *C. putrificum, C. sporogenes* and proteolytic *C. botulinum.*

INTRODUCTION

A latex agglutination, CD D-1 has been developed by Mitsubishi Chemical Industries, Tokyo. It has been used as a screening test for the presence of *Clostridium difficile* in stool specimens in Japan and Thailand. It was shown that the latex agglutination test could detect low numbers of *C. difficile* present in the selective broth but not cultivatable. Since laboratory services for *C. difficile* detection are not available in rural areas in developing countries and the cause of diarrheal disease is often unknown, a simple, sensitive and rapid latex test for the screening of *C. difficile* antigen in stool specimen would be useful. However, antigen produced by *C. sporogenes,* proteolytic *C. botulinum, Peptostreptococcus anaerobius* and *Bacteroides asaccharolyticus* have been reported to give positive reactions in the latex agglutination test produced by Marion Laboratories (Miles *et al,* 1988; Borriello *et al,* 1987). In previous study, we have detected stool specimens from 9 diarrheal patients (4.4%) that were positive by the latex agglutination test for *C. difficile* but negative by the cytotoxin assay and culture for enteric pathogens (Wongwanich *et al,* 1990). Such discrepant results stimulated us to investigate further the reactivity of the CD D-1 latex kit with toxigenic and nontoxigenic *C. difficile* and other bacteria. Here we report the cross-reactivity of the CD C-1 latex test with other species of clostridia.

MATERIALS AND METHODS

Bacterial strains: The 15 strains of *C. difficile* consisted of 11 toxigenic and 3 non-toxigenic strains which were isolated from diarrheal patients and a reference toxigenic strain of *C. difficile* CDC 4897. The following reference strains were also used: *C. perfringens* CDC 14367, *C. putrificum* ATCC 25784, *C. ramosum* ATCC 25582, *C. septicum* ATCC 1264, *C. sordellii* ATCC 1941, *C. sporogenes* ATCC 19404, *Bacteroides fragilis* ATCC 25285, *Eubacterium lentum* ATCC 25559, *Fusobacterium necleatum* ATCC 25286, *F. varium* ATCC 3601, *Peptostreptococcus anaerobius* ATCC 27337, *P. asaccharolyticus* WAL 3218, *Propionibacterium acnes* ATCC 11828, *P. acnes* CDC 14369, *Prevotella asaccharolytica* ATCC 25260, *Veillonella parvula* ATCC 10790, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *Salmonella typhimurium* ATCC 14028, *Shigella flexneri* ATCC 12022 and *Sh. sonnei* ATCC 25931. All of the isolates other than those were isolated from clinical specimens. The anaerobic bacteria were identified as described previously (Holdeman *et al,* 1977) and facultatively anaerobic species were also identified by established criteria (Zen-yoji *et al,* 1976; Balows *et al,* 1991).

Detection of toxigenic and nontoxigenic strains of *C. difficile:* The *C. difficile* isolates were identified as a toxigenic and a nontoxigenic strain by using the polymerase chain reaction method as described in detailed elsewhere (Kato *et al,* 1991; Kato, personal communication). Toxigenic strain of *C. difficile* contains toxin A and B genes, whereas nontoxigenic strain contains none of those toxigenic genes. Cytotoxicity testing for an identification of toxigenic strain of *C. difficile* was also determined using baby hamster kidney (BHK-21) cells grown in 96-well microtiter plates (Wongwanich *et al,* 1990).

Latex agglutination test: The latex agglutination test for *C. difficile* antigen was performed by using CD D-1 kit (Mitsubishi Chemical Industries, Tokyo). All isolates were grown anaerobically in

brain heart infusion broth for 48 hours at 35°C prior to testing with CD D-1 latex test. Brain heart infusion broth cultures were centrifuged at 3,000 rpm, 4°C for 15 minutes to pellet the cells. Supernatants were examined directly for the presence of reacting antigen as recommended by the manufacturer.

RESULTS

Table 1 shows the results for reaction of all bacterial strains with the CD D-1 latex test. All 12 toxigenic and 3 nontoxigenic strains of *C. difficile* gave a positive reaction in the latex agglutination test. Two of the three nontoxigenic strains of *C. difficile* showed weak positive reactions. Of the other species of clostridia examined, *C. putrificum* ATCC 25784, two strains of *C. sporogenes* and the three strains of proteolytic *C. botulinum* were positive. We tested only four strains of *C. botulinum* and another strain that was negative was nonproteolytic. None of the other bacterial strains tested reacted in the CD D-1 latex test.

DISCUSSION

This investigation clearly shows that the CD D-1 latex test agglutinated with bacterial products from toxigenic as well as nontoxigenic strains of *C. difficile.* The weak positive reactions observed in two of the three nontoxigenic strains to *C. difficile* may indicate the presence of small amount of materials produced by nontoxigenic strains.

Evaluation of the CDT latex test produced by Marion Laboratories has revealed that *C. sporogenes,* proteolytic *C. botulinum, Peptostreptococcus anaerobius* and *Bacteroides asaccharolyticus* react in the test (Miles *et al,* 1988; Borriello *et al,* 1987). Results from our study have shown, however, that bacterial products from *C. putrificum,* in addition to *C. sporogenes* and proteolytic *C. botulinum,* reacted with the CD D-1 latex test. Neither *P. anaerobius* nor *P. asaccharolyticus (B. asaccharolyticus)* showed positive reactions. Although an uninterpretable CDT latex test result with some strains of *Staphylococcus aureus* has been reported (Miles *et al,* 1988), this result did not occur with any of the strain tested in the current study.

The laboratory diagnosis of *C. difficile* infection usually relies on either direct detection of toxins or bacterial cell antigen in stool or isolation and characterization of the organism. Detection of cytotoxin in stool sample by tissue culture techniques, albeit still the "gold standard", is not widely available outside of major research institutions. Culture methods, although very sensitive, require fairly lengthly processing and additional testing of the isolate to determine toxigenicity, which tends to limit their use for diagnostic purposes. The CD D-1 latex agglutination test which detects a nontoxic bacterial antigen could be available in this region. The easy procedure provides results in less than 20 minutes. In most of health centers in this region where no facilities exist for anaerobic culture and tissue culture, a rapid answer for a severe case may be obtained by the CD D-1 latex test. Positive latex results must be combined with clinical findings and endoscopy to diagnose *C. difficile*-associated diseases.

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Table 1

Reaction of thirty-three species of bacterial strains with the latex agglutination test.

Organism	No. positive/ No of strains tested	Organism	No. positive/ No. of strains tested
Anaerobic bacteria			
C. botulinum (nonproteolytic)	0/1	*Prevotella asaccharolytica* ATCC 25260	0/1
C. botulinum (proteolytic)	3/3	*Propionibacterium acnes* ATCC 11828	0/1
C. difficile (toxigenic strains)	11/11	*P. acnes* CDC 14369	0/1
C. difficile (nontoxigenic strains)	3/3	*Veillonella parvula* ATCC 10790	0/1
C. bifermentans	0/4	*Campylobacter jejuni*	0/9
C. litus-eburense	0/1	**Nonanaerobic bacteria**	
C. perfringens CDC 14367	0/1	*Enterobacter* sp	0/4
C. perfringens type A	0/8	*Escherichia coli* ATCC 25922	0/1
C. putrificum ATCC 25784	1/1	*E. coli*	0/10
C. ramosum ATCC 25582	0/1	*Klebsiella pneumoniae*	0/4
C. septicum ATCC 1264	0/1	*K. oxytoca*	0/12
C. sordellii ATCC 1941	0/1	*Proteus* sp	0/3
C. sporogenes ATCC 19404	1/1	*Pseudomonas aeruginosa*	0/2
C. sporogenes	1/1	*Salmonella typhimurium* ATCC 14028	0/1
Bacteroides fragilis ATCC 25285	0/1	*Shigella dysenteriae*	0/1
B. fragilis	0/3	*Sh. flexneri* ATCC 12022	0/1
Eubacterium lentum ATCC 25559	0/1	*Sh. sonnei* ATCC 25931	0/1
Fusobacterium nucleatum ATCC 25286	0/1	*Staphylococcus aureus* ATCC 25923	0/1
F. varium ATCC 3601	0/1	*S. aureus*	0/19
Peptostreptococcus anaerobius ATCC 27337	0/1	*S. epidermidis* ATCC 12228	0/1
P. asaccharolyticus WAL 3218	0/1	*Vibrio cholerae* O-1	0/5