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Performance of chromID *Clostridium difficile* Agar Compared with BBL *C. difficile* Selective Agar for Detection of *C. difficile* in Stool Specimens

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We evaluated the performance of a new chromogenic medium for detection of *Clostridium difficile*, chromID *C. difficile* agar (CDIF; bioMérieux, France), by comparison with BBL *C. difficile* Selective Agar (CDSA; Becton Dickinson and Company, USA). After heat pretreatment (80°C, 5 min), 185 diarrheal stool samples were inoculated onto the two media types and incubated anaerobically for 24 hr and 48 hr for CDIF and for 48 hr and 72 hr for CDSA. All typical colonies on each medium were examined by Gram staining, and the gram-positive rods confirmed to contain the *tpi* gene by PCR were identified as *C. difficile*. *C. difficile* was recovered from 36 samples by using a combination of the two media. The sensitivity with CDIF 48 hr was highest (100%) and was significantly higher than that with CDIF 24 hr (58.3%; $P<0.001$), because samples with a low burden of *C. difficile* tended to require prolonged incubation up to 48 hr ($P<0.001$). The specificity of CDIF 24 hr and CDIF 48 hr (99.3% and 90.6%, respectively) was significantly higher than that of CDSA 48 hr and CDSA 72 hr (72.5% and 67.1%, respectively; $P<0.001$). CDIF was effective for detecting *C. difficile* in heat-pretreated stool specimens, thus reducing unnecessary testing for toxin production in non-*C. difficile* isolates and turnaround time.

Key Words: *Clostridium difficile*, Chromogenic agar, Performance, ChromID *C. difficile* agar, Toxigenic culture

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Clostridium difficile infection (CDI) is increasingly recognized in both hospital and community settings [1-3]. For culture of *C. difficile*, pre-reduced cycloserine-cefoxitin-fructose agar with a high cycloserine concentration (0.5 g/L) is the most widely used medium, but it requires an incubation of at least 48 hr. In addition, intense growth of stool flora is frequently observed, thereby making detection and isolation of *C. difficile* difficult. To limit the growth of contaminating flora, various treatments such as heat-shock and alcohol-shock may be applied to specimens for culture [4-8]. Recently, a new chromogenic medium for *C. difficile*, chromID *C. difficile* agar (CDIF; bioMérieux, Marcy l'Etoile, France), which can detect *C. difficile* within 24 hr, was introduced to the market. This study aimed to evaluate the performance of CDIF by comparing it with BBL *C. difficile* Selective Agar (CDSA; Becton Dickinson and Company, Sparks, MD, USA).

This prospective study was conducted at Seoul St. Mary's hospital, a 1,300-bed teaching hospital in Seoul, Korea. In total, 185 consecutive diarrheal stool samples from patients suspected of having CDI were included from April to May 2012. Culture was performed within 72 hr of collection, and stool specimens were stored at -4°C until processing. Each specimen (1 mL) was mixed with 1 mL of thioglycollate broth and, after vortexing, placed in an 80°C water bath for 5 min [6-8].

A 100-μL aliquot of this suspension was inoculated onto CDIF and CDSA and spread to obtain isolated bacterial colonies. All media were incubated in a Bactron anaerobic/environmental

Table 1. Comparison of four combinations (CDIF 24 hr, CDIF 48 hr, CDSA 48 hr, and CDSA 72 hr)

	Agar plates				*P* value*					
					CDIF 24 hr vs.			CDIF 48 hr vs.		CDSA 48 hr vs.
	CDIF 24 hr	CDIF 48 hr	CDSA 48 hr	CDSA 72 hr	CDIF 48 hr	CDSA 48 hr	CDSA 72 hr	CDSA 48 hr	CDSA 72 hr	CDSA 72 hr
Sensitivity (95% CI)	58.3% (40.8-74.5)	100% (90.3-100)	83.3% (67.2-93.6)	86.1% (70.5-95.3)	<0.001	0.023	0.012	0.188	0.375	1
Specificity (95% CI)	99.3% (96.3-100)	90.6% (84.7-94.8)	72.5% (64.6-79.5)	67.1% (59.0-74.6)	0.001	<0.001	<0.001	<0.001	<0.001	0.047

*Obtained by exact McNemar test using Bonferroni adjustment.
Abbreviations: CDIF 24 hr, growth of *C. difficile* colonies on chromID *C. difficile* agar (CDIF) at 24 hr incubation; CDIF 48 hr, growth of *C. difficile* colonies on CDIF agar at 48 hr incubation; CDSA 48 hr, growth of *C. difficile* colonies on BBL *C. difficile* Selective Agar (CDSA) at 48 hr incubation; CDSA 72 hr, growth of *C. difficile* colonies on CDSA at 72 hr incubation; CI, confidence intervals.

Table 2. The number of agar plates presenting growth according to the medium, time, and colony-count-grades

Colony-count-grade (colony number)	No. of agar plates presenting growth			
	CDIF 24 hr	CDIF 48 hr	CDSA 48 hr	CDSA 72 hr
1+ (1-10)	-	10	4	1
2+ (11-50)	2	3	4	-
3+ (51-100)	-	1	2	-
4+ (≥101)	19	1	20	-

Abbreviations: CDIF 24 hr, growth of *C. difficile* colonies on chromID *C. difficile* agar (CDIF) at 24 hr incubation; CDIF 48 hr, growth of *C. difficile* colonies on CDIF agar at 48 hr incubation; CDSA 48 hr, growth of *C. difficile* colonies on BBL *C. difficile* Selective Agar (CDSA) at 48 hr incubation; CDSA 72 hr, growth of *C. difficile* colonies on CDSA at 72 hr incubation; CI, confidence intervals.

chamber (Shel Lab, Cornelius, OR, USA) at 37°C, either for 24 hr (CDIF) or for 48-72 hr (CDSA) as recommended by the manufacturer and then removed into air for a maximum of 15 min for counting colonies. CDIF plates were reincubated anaerobically for a further 24 hr and further counts were performed.

The colonies were read by two investigators and the presence of *C. difficile* was evaluated semiquantitatively as follows: 1+ for 1-10 colonies, 2+ for 11-50 colonies, 3+ for 51-100 colonies, and 4+ for more than 100 colonies. Growth of typical colonies of *C. difficile* (gray to black color with an irregular or smooth border on CDIF and flat, yellow colonies with a ground glass-like appearance and a slightly filamentous edge on CDSA) were regarded as positive on each medium. All the colonies were examined by Gram staining, and the gram-positive rods were subjected to PCR to detect the *tpi* gene, a housekeeping gene of *C. difficile* [9]. If a colony harbored the *tpi* gene, it was determined to be *C. difficile*.

By using this criterion, growth of *C. difficile* confirmed to contain the *tpi* gene in any medium was defined as true positive. False positive was defined as growth of an isolate showing typical colony morphology of *C. difficile* but not identified as *C. difficile*. The four combinations (CDIF 24 hr, CDIF 48 hr, CDSA 48 hr, and CDSA 72 hr) were compared statistically by using the exact McNemar test with Bonferroni adjustment for proportions and the Mantel-Haenszel chi-squared test for trend analysis. $P<0.05$ was considered statistically significant. The Institutional Review Board of Seoul St. Mary's Hospital approved this study (Protocol No. KC11SISI0655).

C. difficile was recovered from 36 stool samples (19.5%) by using a combination of two media. The number of stool samples positive for *C. difficile* was 21 (58.3%) and 36 (100%) on CDIF at 24 hr and 48 hr, respectively, and 30 (83.3%) and 31 (86.1%) on CDSA at 48 hr and 72 hr, respectively. The sensitivity of CDIF at 48 hr was significantly higher than that of CDIF 24 hr ($P<0.001$, exact McNemar test using Bonferroni adjustment), but it was not significantly higher than that of CDSA 48 hr or CDSA 72 hr (Table 1). Of the 36 CDIF-positive samples, the distribution of colony-count-grades was as follows; 1+ in 10 samples, 2+ in 5 samples, 3+ in one sample, and 4+ in 20 samples. The proportion of samples that presented growth at 24 hr according to the colony-count-grade was 0% in grade 1+, 40% in grade 2+, 0% in grade 3+, and 95% in grade 4+ (P for trend <0.001, Mantel-Haenszel chi-squared test; Table 2).

With CDIF, non-*C. difficile* isolates presenting gray to black colonies were recovered from approximately 9% of samples (16/185; 10 gram-positive bacillus [GPB], 1 gram-negative bacillus [GNB], 1 GPB/GNB, and 2 GPB/gram-positive coccus [GPC]/GNB), and they were much less common at 24 hr of incubation (two isolates). With CDSA, non-*C. difficile* isolates presenting typical, yellow colonies were recovered from approximately 30% (55/185; 53 GPB and 2 GPC) of samples, and 47 of them were recovered at 48 hr. Among them, the number of agar plates showing false-positive isolates from the 149 *C. difficile*-negative stool samples was one on CDIF at 24 hr, 14 on

Table 3. Non-*C. difficile* isolates presenting typical colonies recovered from 185 stool specimens

Gram stain morphology	No. of agar plates							
	CDIF				CDSA			
	C. difficile true positive 36 samples		*C. difficile* true negative 149 samples		*C. difficile* true positive 36 samples		*C. difficile* true negative 149 samples	
	24 hr	48 hr	24 hr	48 hr	48 hr	72 hr	48 hr	72 hr
GPB	-	-	1	10	5	5	40	48
GPC	1	2	-	-	1	1	1	1
GNB	-	-	-	1	-	-	-	-
GPB/GNB	-	-	-	1	-	-	-	-
GPB/GPC/GNB	-	-	-	2	-	-	-	-
Total	1	2	1	14	6	6	41	49

Abbreviations: CDIF 24 hr, growth of *C. difficile* colonies on chromID *C. difficile* agar (CDIF) at 24 hr incubation; CDIF 48 hr, growth of *C. difficile* colonies on CDIF agar at 48 hr incubation; CDSA 48 hr, growth of *C. difficile* colonies on BBL *C. difficile* Selective Agar (CDSA) at 48 hr incubation; CDSA 72 hr, growth of *C. difficile* colonies on CDSA at 72 hr incubation; CI, confidence intervals; GPB, gram-positive bacillus; GPC, gram-positive coccus; GNB, gram-negative bacillus.

CDIF at 48 hr, 41 on CDSA at 48 hr, and 49 on CDSA at 72 hr, resulting in a specificity 99.3%, 90.6%, 72.5%, and 67.1%, respectively (Table 3). The specificity of CDIF 24 hr and CDIF 48 hr was significantly higher than that of CDSA 48 hr and 72 hr ($P<0.001$, exact McNemar test using Bonferroni adjustment; Table 1), and this low specificity of CDSA resulted from the frequent growth of non-*C. difficile* GPB (Table 3).

Toxigenic culture is a new reference method for diagnosis of CDI, as it is more sensitive than the fecal cytotoxin assay [10-12]. However, it is a two-step method that consists of isolating *C. difficile* strains on a selective medium and then testing the colonies for toxin production by PCR. To reduce unnecessary PCR tests and turnaround time, the occurrence of endogenous flora showing colonies of typical color should be low.

CDIF is a new chromogenic medium containing taurocholate and a chromogen mix that allows isolation of *C. difficile* in 24 hr. Although direct plating or alcohol pre-treatment is recommended by the manufacturer, we pre-treated stool samples to facilitate the plate reading and used heat-shock rather than alcohol-shock to shorten the turnaround time. The heat pre-treatment seemed to inhibit the growth of GPC and GNB effectively, as growth of GPC or GNB was only observed on rare occasions (one sample [0.5%] on CDIF at 24 hr, six samples [3.2%] on CDIF at 48 hr, two samples [1.1%] on CDSA at 48 hr, and two samples [1.1%] on CDSA at 72 hr among 185 stool samples). This finding is similar to that of Yim *et al*. [13], where GPC or GNB isolates were recovered from 3.1% (23/738) of samples on CDIF at 48 hr and superior to that of Perry *et al*. [14], where GPC or GNB isolates were recovered from 3.0% (11/368) and 9.0% (33/368) of samples on CDIF at 24 hr and 48 hr, respectively. In both studies [13, 14], alcohol pre-treatment was used.

Based on our results, the sensitivity for recovering CDIF at 24 hr was low (58.3%), but after 48 hr incubation, it increased to 100%. This finding agrees with that of Eckert *et al*. [15], who showed that prolonging incubation enhanced the recovery of *C. difficile* from 74.1% to 87%, but appears to contrast with the findings of Perry *et al*. [14], who showed that the sensitivity of CDIF at 24 hr incubation was 96.3%. However, looking into the study more closely, the recovery rate of *C. difficile* with CDIF at 24 hr incubation was different between the samples which were positive and negative for Vidas immunoassay; it was high (94.5%) in Vidas-positive samples, but low (68%) in Vidas-negative samples. In both studies, the recovery rate on CDIF at 24 hr was somewhat higher than that in our study. Further evaluation is needed to investigate whether this difference results from a difference in pre-treatment, because Eckert *et al*. [15] used the direct plating method and Perry *et al*. [14] used the alcohol-shock method.

The colony-count-grades in stool samples from which *C. difficile* was recovered on CDIF at 24 hr and 48 hr (3.8±0.6 and 1.5±0.9, respectively) indicate that a 24 hr incubation is sufficient for stool samples with a high burden (more than 50 colonies per agar plate), but the samples with a low burden (less than 50 colonies per agar plate) of *C. difficile* require prolonged incubation of up to 48 hr. This finding is similar to that of Shin *et al*. [16], where the no-growth-plates were 37.9% and 6.2% on day 1 and 2, respectively. For CDSA, of the 31 stool samples from which *C. difficile* was recovered, all but one were recov-

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ered after 48 hr of incubation, indicating that prolonged incubation of up to 72 hr was not necessary.

In conclusion, CDIF was highly effective for detecting *C. difficile* in heat-pretreated stool specimens, thus reducing unnecessary testing for toxin production on non-*C. difficile* isolates and turnaround time, and this medium exhibited the best sensitivity at 48 hr, especially with stool samples with low burden of *C. difficile*.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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