



# Comparison of ChromID Agar and *Clostridium difficile* Selective Agar for Effective Isolation of *C. difficile* from Stool Specimens

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**Background:** ChromID *Clostridium difficile* agar (IDCd; bioMérieux SA, France) is a recently developed chromogenic medium for rapid and specific isolation of *C. difficile*. We compared the performance of IDCd with that of *Clostridium difficile* Selective Agar (CDSA).

**Methods:** A total of 530 fresh stool specimens were collected from patients with clinical signs compatible with *C. difficile* infection, and cultures for *C. difficile* were performed on IDCd and CDSA. *C. difficile* colonies were identified by spore staining, odor, use of an ANI identification test kit (bioMérieux SA), and multiplex PCR for *tcdA*, *tcdB*, and *tpi*.

**Results:** The concordance rate between IDCd and CDSA was 90.6% (480/530). The positivity rates on IDCd on days 1 and 2 (55.6% and 85.0%, respectively) were significantly higher than those on CDSA (19.4% and 75.6%, respectively) ( $P < 0.001$  for day 1 and  $P = 0.02$  for day 2), but the detection rates on IDCd and CDSA on day 3 were not different (89.4% vs. 82.8%,  $P = 0.0914$ ). On day 3, the recovery rates for non-*C. difficile* isolates on IDCd and CDSA were 30.2% (160/530) and 22.1% (117/530), respectively ( $P = 0.0075$ ). *Clostridium* spp. other than *C. difficile* were the most prevalent non-*C. difficile* isolates on both media.

**Conclusions:** The culture positivity rates on IDCd and CDSA were not different on day 3 but IDCd may allow for rapid and sensitive detection of *C. difficile* within 2 days of cultivation.

**Key Words:** *Clostridium difficile*, Culture, Chromogenic agar, *Clostridium difficile* selective agar

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## INTRODUCTION

Traditionally, *Clostridium difficile* culture has been used for epidemiologic studies and diagnoses of *C. difficile* infection (CDI). Increasing morbidity and mortality rates and the increasing frequency of recurrence and community-acquired CDI demand a reliable culture method for toxigenic *C. difficile* [1, 2]. The effective isolation of *C. difficile* from stool samples requires the use of selective media and spore selection procedures [3-8], and toxigenic culture is regarded as the standard method for detection of toxigenic *C. difficile*. Although bacteriologic culture

is a time-consuming process requiring more than 2 days, it is more straightforward and sensitive than the cytotoxicity neutralization assay [10].

A variety of selective culture media, including cycloserine cefoxitin fructose agar (CCFA) and *C. difficile* selective agar (CDSA; Becton Dickinson, Sparks, MD, USA), have been used for the isolation of *C. difficile* [3-9]. CCFA was the first selective culture medium developed for *C. difficile*, but the original formulation has been modified for commercial distribution [3, 6, 8]. ChromID *C. difficile* agar (IDCd; bioMérieux SA, Marcy l'Etoile, France) is a recently developed chromogenic agar medium for

the rapid and specific isolation of *C. difficile*. The aim of this study was to compare the performance of IDCd with that of CDSA for the rapid and sensitive detection of *C. difficile*.

## METHODS

### 1. Specimens

A total of 530 fresh stool specimens were collected from patients who had clinical signs compatible with CDI and were hospitalized at a teaching hospital in Seoul between January and June 2012. The Institutional Review Board of Sanggye Paik Hospital approved the study protocol.

### 2. *C. difficile* culture and identification

Semiquantitative culture for *C. difficile* was performed as described previously [10] and the extent of growth was rated as follows: grade 1, <10 colonies; grade 2, 10-50 colonies; grade 3, 51-100 colonies; and grade 4, >100 colonies. Briefly, a stool specimen (1.0 mL) was mixed with an equal volume of 70% isopropanol and incubated at room temperature for 30 min. One drop (~100  $\mu$ L) was then inoculated onto prerduced IDCd or CDSA, and the plates were incubated at 37°C under anaerobic conditions (GasPak EZ Anaerobe Pouch; Becton Dickinson) for 72 hr. We observed the plates on days 1, 2, and 3 without interrupting the anaerobic incubation. To evaluate the effect of the alcohol shock, we also inoculated 203 of the 530 stool specimens directly onto IDCd without alcohol shock pretreatment (IDCd-direct). If *C. difficile* was detected on either IDCd or CDSA, the specimen was defined as *C. difficile* positive, and any other bacteria growing on the culture media were defined as non-*C. difficile* isolates. *C. difficile* colonies were identified on the basis of typical morphological features (yellow colony with a ground-glass appearance on CDSA, black colony with an irregular margin on IDCd), spore staining, odor, and the use of an ANI identification test kit (bioMérieux SA).

### 3. Multiplex PCR assay for *tcdA*, *tcdB*, and *tpi*

Multiplex PCR for toxin A (*tcdA*), toxin B (*tcdB*), and triose phos-

phate isomerase (*tpi*) was performed for 180 *C. difficile* isolates, as described previously [11]. The PCR product for *tpi* was 230 bp long if the isolate was *C. difficile*. The PCR product for *tcdA* was 369 bp long if the gene was intact and 110 bp if the isolate contained the variant gene (*tcdA<sup>-</sup>tcdB<sup>+</sup>*). The PCR product for *tcdB* was 160 bp long if the gene was intact.

### 4. Statistical analysis

All statistical analyses were conducted by using SAS Version 9.2 software package (SAS Inc., Cary, NC, USA). Statistical differences in positivity rates among IDCd and CDSA groups at different days were analyzed using the generalized estimating equation (GEE) with logit link method. *P* values <0.05 were considered significant.

## RESULTS

Of the 203 stool specimens cultured with and without alcohol pretreatment, 73 yielded *C. difficile* on at least 1 of the 2 tested media on day 3 (Table 1). The positivity rates for IDCd-direct and IDCd-alcohol on day 3 were 79.5% (58/73) and 87.7% (64/73), respectively, and were not significantly different (*P*=0.5630). Therefore, we further evaluated the performance of IDCd and CDSA with alcohol pretreatment.

Of the 530 alcohol-treated stool specimens used for the comparison of IDCd and CDSA, 180 were identified as *C. difficile* positive by day 3 (Table 2). Of the 180 *C. difficile* isolates, 142

**Table 1.** Comparison of ChromID agar (IDCd) with direct inoculation and IDCd with alcohol pretreatment for *Clostridium difficile*-positive cases

	N (%) of <i>C. difficile</i> isolates among <i>C. difficile</i> -positive cases (total N = 73)		
	IDCd with direct inoculation	IDCd with alcohol pretreatment	<i>P</i>
Day 1	46 (63.0)	48 (65.8)	0.5630
Day 2	56 (76.7)	60 (82.2)	0.1079
Day 3	58 (79.5)	64 (87.7)	0.5630

**Table 2.** Comparison of *Clostridium difficile* and non-*C. difficile* isolates on ChromID agar (IDCd) and *C. difficile* selective agar (CDSA)

	N (%) of <i>C. difficile</i> isolates in <i>C. difficile</i> positive samples (total N = 180)			N (%) of non- <i>C. difficile</i> isolates in <i>C. difficile</i> culture samples (total N = 530)		
	IDCd	CDSA	<i>P</i>	IDCd	CDSA	<i>P</i>
Day 1	100 (55.6)	35 (19.4)	<0.001	70 (13.2)	9 (1.7)	<0.001
Day 2	153 (85.0)	136 (75.6)	0.0245	117 (22.1)	58 (10.9)	<0.001
Day 3	161 (89.4)	149 (82.8)	0.0914	160 (30.2)	117 (22.1)	0.0075

(78.9%) were *tcdA*<sup>+</sup>*tcdB*<sup>+</sup> strains, 11 (6.1%) were *tcdA*<sup>+</sup>*tcdB*<sup>-</sup> strains, and 27 (15.0%) were *tcdA*<sup>-</sup>*tcdB*<sup>-</sup> strains; all of them were *tpi* positive.

On day 3, the final *C. difficile* detection rates on IDCd and CDSA were 89.4% (161/180) and 82.8% (149/180), respectively, and were not significantly different ( $P=0.0914$ ). However, the positivity rates on days 1 and 2 were significantly higher on IDCd than on CDSA (55.6% vs. 19.4% on day 1, 85.0% vs. 75.6% on day 2;  $P<0.001$  for day 1,  $P=0.0245$  for day 2). The recovery rates for non-*C. difficile* isolates on IDCd and CDSA on day 3 were 30.2% (160/530) and 22.1% (117/530), respectively ( $P=0.0075$ ; Table 2). *Clostridium* spp. other than *C. difficile* (COd) were the most prevalent on both media (87 cases on IDCd and 114 cases on CDSA); gram-positive cocci (mostly *Enterococcus* spp.) were the second most prevalent type. Gram-positive bacilli, gram-negative bacilli, and yeasts were also observed on IDCd but were rare on CDSA (Table 3).

The concordance rate between IDCd and CDSA on day 3 was 90.6% (480/530). Of the 50 discordant cases, 31 were IDCd+/

CDSA- and 19 were IDCd-/CDSA+. Of the 31 IDCd+/CDSA- cases, 12 were COd and 19 were not isolated on CDSA. Of the 19 IDCd-/CDSA+ cases, 10 were COd, 4 were gram-positive cocci, and 5 were not isolated on IDCd.

Following semiquantitative culture of the *C. difficile* positive cases ( $n=161$  on IDCd and  $n=149$  on CDSA), the proportions of *C. difficile* colonies exhibiting higher degrees of growth (grades 3 and 4) on IDCd and CDSA were 29.8% and 15.4% on day 1 ( $P=0.0002$ ), 59.0% and 60.4% on day 2 ( $P=0.4919$ ), and 69.6% and 72.5% on day 3 ( $P=0.5635$ ), respectively (Table 4).

## DISCUSSION

A variety of culture media have been described for the selective isolation of *C. difficile*. CCFA, which contains cefoxitin, cycloserine, and fructose, has long been the first choice among *C. difficile* media, but the original formulation has been slightly modified for commercial distribution [2, 6, 8]. CDSA is a bloodless agar that contains cefoxitin, cycloserine, and mannitol instead of fructose [9, 12]. Spore selection techniques are also important for effective isolation of *C. difficile*, and it has been reported that alcohol pretreatment of stool specimens leads to better recovery of *C. difficile* than heat shock techniques [7, 8].

Selective culture media for *C. difficile* have been evaluated by several investigators, who have shown variable recovery rates ranging from 42.6% to 99.6% depending on the type of medium, alcohol pretreatment, and/or incubation time [3-5, 8, 9, 12-15]. In our study, the positivity rates on IDCd-direct, IDCd-alcohol, and CDSA on day 3 (79.5%, 89.4%, and 82.8%, respectively) suggested reliable performance across different types of media and treatments.

A chromogenic culture medium such as IDCd enables rapid detection of *C. difficile* colonies. Although the final *C. difficile*

**Table 3.** Non-*Clostridium difficile* isolates from 530 stool specimens recovered on ChromID agar (IDCd) and *C. difficile* selective agar (CDSA) on day 3

Microorganism	N (%) of isolates recovered	
	IDCd	CDSA
<i>Clostridium</i> spp. other than <i>C. difficile</i>	87 (54.4)	114 (97.4)
Gram-positive cocci	40 (25.0)	1 (0.9)
Gram-positive rods	25 (15.6)	2 (1.7)
Gram-negative rods	3 (1.9)	0
Yeasts	3 (1.9)	0
Peptostreptococci	2 (1.3)	0
Total	160 (100*)	117 (100)

\*Because of rounding, percentage may not total 100.

**Table 4.** Comparison of the extent of growth by semiquantitative culture for *Clostridium difficile* with ChromID agar (IDCd) and *C. difficile* selective agar (CDSA)

Grade*	N (%) of cases on IDCd (total N = 161)			N (%) of cases on CDSA (total N = 149)		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
0	61 (37.9)	10 (6.2)	0 (0)	114 (76.5)	13 (8.7)	0 (0)
1	32 (19.9)	35 (21.7)	33 (20.5)	1 (0.7)	19 (12.8)	15 (10.1)
2	20 (12.4)	21 (13.1)	16 (9.9)	11 (7.4)	27 (18.1)	26 (17.4)
3	14 (8.7)	27 (16.8)	27 (16.8)	10 (6.7)	36 (24.2)	26 (17.4)
4	34 (21.1)	68 (42.2)	85 (52.8)	13 (8.7)	54 (36.2)	82 (55.1)
Total	161 (100)	161 (100)	161 (100)	149 (100)	149 (100)	149 (100)

*P* value between IDCd and CDSA for grade 3 plus grade 4:  $P=0.0002$  on day 1,  $P=0.4919$  on day 2, and  $P=0.5635$  on day 3.

\*Extent of growth by semiquantitative culture for *C. difficile*: grade 0, no growth; grade 1, <10 colonies; grade 2, 10-50 colonies; grade 3, 51-100 colonies; grade 4, >100 colonies.

detection rates were not significantly different between IDCd and CDSA on day 3, the positivity rate was significantly higher on IDCd than on CDSA on day 1 (55.6% vs. 19.4%,  $P < 0.001$ ) and on day 2 (85.0% vs. 75.6%,  $P = 0.0245$ ). Recent development in synthetic enzymatic substrates has allowed improved detection and identification of microorganisms in various specimens. Chromogenic culture media contain multiple substrates that allow bacteria to form colored colonies depending on their enzymatic activity. This feature facilitates the differentiation of target pathogens within polymicrobial cultures with high specificity [16, 17]. In our study, the proportion of *C. difficile* colonies showing a high extent of growth (grades 3 and 4) was greater on IDCd culture plates than on CDSA plates on day 1 (29.8% vs. 15.4%,  $P = 0.0002$ ), although the proportions were not significantly different on days 2 and 3 (Table 4). The chromogenic enzymatic substrates and suitable germinants in IDCd may allow better production of *C. difficile* colonies on this medium than on CDSA [4, 5, 17].

The effects of alcohol pretreatment on both media could be excluded in our study because we simultaneously applied the alcohol-pretreated stool specimens on both media. Perry et al. [15] reported that the culture positivity rate on IDCd at 24 hr was 96.3%. One plausible explanation for this difference may be a sample selection bias in the study by Perry et al. (i.e., a predominance of enzyme immunoassay [EIA]-positive samples were selected for evaluation) [15]. In our study, the culture positivity rate on CDSA after 3 days was not significantly different from that on IDCd but was much higher than the rate reported for CDSA in previous studies [9, 12]. The higher culture positivity rate in our study was probably due to alcohol pretreatment and/or the longer duration of culture [7-9]. The positivity rate was slightly higher for IDCd-alcohol than for IDCd-direct in our study, but there was no significant difference between the two results on day 3 (Table 1; 87.7% vs. 79.5%;  $P = 0.5630$ ).

Previous studies using IDCd or CDSA with or without alcohol pretreatment have reported that the recovery rates for non-*C. difficile* isolates were from 53% to 95.7%, and COd are the most prevalent non-*C. difficile* isolates [9, 14, 15]. In our study, the culture positivity rates for non-*C. difficile* isolates correlated with the duration of culture, and COd were the most prevalent non-*C. difficile* isolates on both media. Gram-positive cocci and gram-positive bacilli were also frequently observed on IDCd but were rare on CDSA. Nerandzic and Donskey [9] reported that *Staphylococcus epidermidis* and *Candida* spp. were also commonly recovered on CCFA and CDSA. These results indicate that IDCd and CDSA are selective but not specific for *C. difficile*. Thus, dif-

ferentiation of *C. difficile* from COd is important for accurate and rapid diagnosis of *C. difficile* infection. On CDSA, *C. difficile* colonies are usually yellow with a ground-glass appearance, but colony morphological features cannot definitively differentiate *C. difficile* from COd, because COd also forms yellow ground-glass colonies on CDSA that are nearly indistinguishable from those of *C. difficile* [9]. Therefore, when suspicious colonies are found on CDSA, additional diagnostic procedures, such as spore staining, ANI tests, and/or PCR assays for *tcdA* and *tcdB*, must be performed [10, 11, 18]. However, on IDCd, *C. difficile* colonies exhibit remarkably clear and distinctive morphological features (black with irregular margins) [14, 15]. In our study, all the suspected *C. difficile* colonies on IDCd were *tpi* positive, although 15% of them were non-toxin-producing strains. The *C. difficile* colonies became larger and the black coloration of the colonies and the irregularity of the margins increased with increase in the duration of culture.

In conclusion, the advantage of IDCd is that although the culture positivity rates were not significantly different between IDCd and CDSA on day 3, IDCd may allow for rapid and sensitive detection of *C. difficile* within 2 days of cultivation (with or without alcohol pretreatment).

## Authors' Disclosure of Potential Conflicts of Interest

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

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