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Comparison of CHROMagar C. difficile and taurocholate-CCFA media for isolation of toxigenic *Clostridium difficile* from stools

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BACKGROUND

Clostridium difficile (CD) causes antibiotic-related diarrhea, a major health issue among hospitalized patients. Although PCR-based detection of toxin-related genes from stools is rapid and sensitive, culture of toxigenic isolates is the reference for the diagnosis and epidemiological studies of CD infections. Previously available selective culture media such as taurocholate-cefoxitin-cycloserine-fructose agar (tCCFA) require a 48 h incubation, and there is a need for highly sensitive media allowing overnight detection of CD.

CHROMagar C. difficile (CCd) is a new chromogenic medium for isolation and presumptive identification of CD as UV-fluorescent blue colonies after 24 h (FIG. 1).

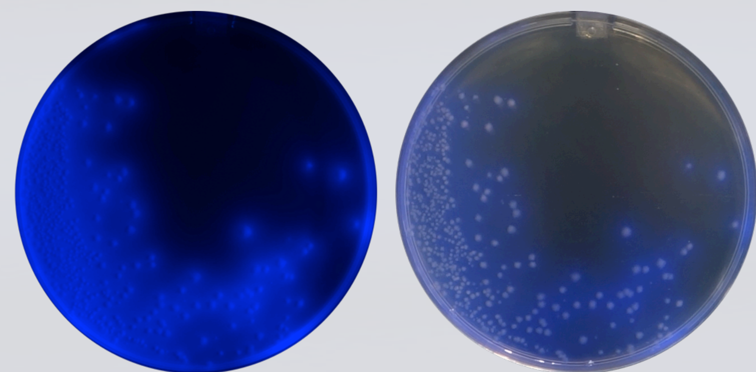


FIG. 1. *C. difficile*, 24 h incubation on CCd agar. UV 365 nm exposure, darkroom (left) and daylight (right).

METHODS

- We first evaluated the growth and colonial aspect on CCd of 40 CD stock strains belonging to 22 PCR-ribotypes, and of various time and geographic origin (TABLE 1).

TABLE 1. PCR-ribotypes of the 40 *Clostridium difficile* stock isolates.

PCR-ribotype	001	002	005	015	018	017	023	014/020	027	029	039/140	056	070	078/126	087	106
No. of isolates	1	1	1	2	1	1	2	7	8	3	1	2	2	5	1	2

- CCd and tCCFA were then prospectively compared for the detection and presumptive identification of toxigenic CD on 594 consecutive diarrheic stool specimens from 572 hospitalized patients.

  Alll stools were also tested for toxigenic CD genes using the Xpert CD assay (Cepheid). Bacterial growth was evaluated after 24 h on CCd and 48 h on tCCFA (35°C, anaerobic atmosphere) Colonial identifications were obtained on a Microflex mass spectrometer (Brüker), and toxigenicity of CD isolates was assessed by an in-house *tcdB-tpi* colony PCR.

  Positive specimens were defined by:
  - growth of toxigenic CD on tCCFA and/or CCd
  - and/or positive Xpert CD assay.

RESULTS

Growth of *Clostridium difficile* stock isolates on CHROMagar C. difficile.

- All isolates tested grew within 24 h as 0.5-3 mm colorless colonies under visible light (Fig. 2, left)
- instant blue fluorescent was present on exposure to 365 nm UV light (Fig. 2, right)
- no difference in size or fluorescence intensity was observed between the various PCR-ribotypes

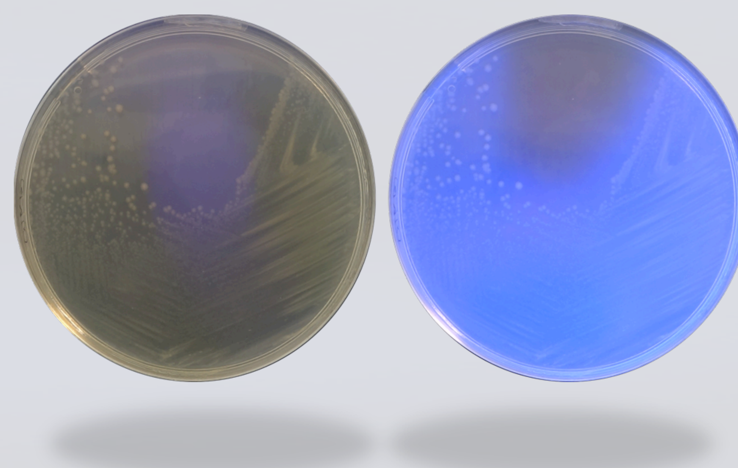


FIG. 2. *C. difficile* PCR-ribotype 027, 24 h anaerobic incubation on CCd agar. Left, visible light. Right, UV light exposure (365 nm).

Prospective analysis of 594 stools from hospitalized patients with diarrhea.

- A total of 174 (29.3 %) stools were CD toxins genes-positive and/or grew toxigenic CD.
  - 170/174 were Xpert CD-positive, **8 CCd-negative, 48 tCCFA-negative**
  - 166/174 grew toxigenic CD on CCd, **4 Xpert CD-negative**
  - 122/174 grew toxigenic CD on tCCFA, **all Xpert CD and CCd-positive.**

- False positive on CCd were non toxigenic CD (n = 38), and *Clostridium glycolicum* (n = 10), a mostly environmental species. *C. glycolicum* colonies (Fig. 3, A) were similar to CD colonies (Fig. 3, B).

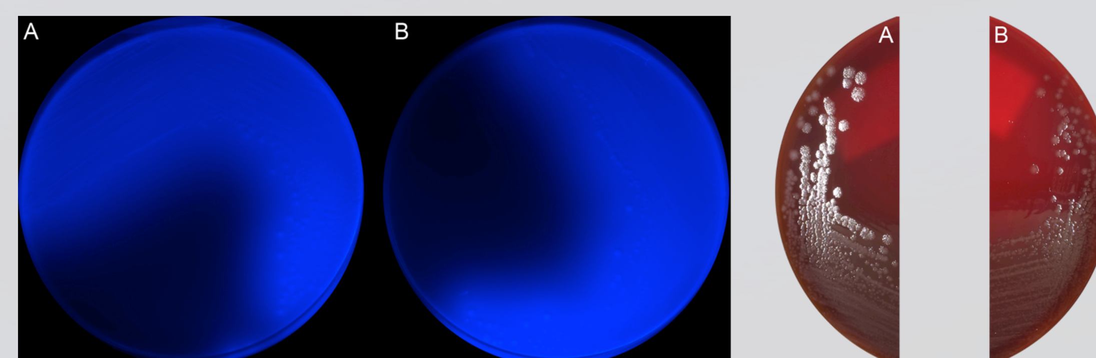


FIG. 3. Colonies of *C. glycolicum* (A) and *C. difficile* (B) on CCd (left) and tCCFA (right). Anaerobic incubation at 35°C, 24 h (CCd) and 48 h (tCCFA).

- False positive on tCCFA were non toxigenic CD (n = 38), based on colonial aspect and specific smell.

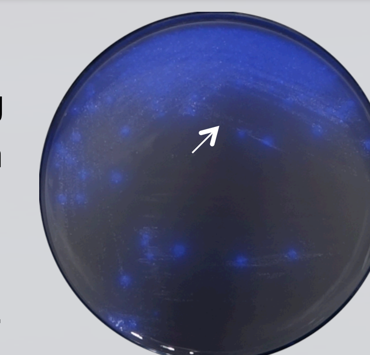
- With 36% more toxigenic isolates recovered, the sensitivity of CCd on primary plating was significantly higher than that of tCCFA ($P < 0.01$) (TABLE 3).

TABLE 3. Sensitivities, specificities, predictive positive and negative values of tCCFA (48 h) and CCd (24 (h) on primary plating of 597 clinical stools specimens

Medium	No. of isolates				Sensitivity	PPV	Specificity	PNV
	True +	False +	True -	False -				
CCd	166	48*	380	8	**95.4%***	77.6%	88.8%	**97.9%***
tCCFA	122	14**	458	52	70%	89.7%	**97%***	89.8%

* $P < 0.01$

- UV-based visualization allowed to detect scant CD colonies among contaminant flora, *e.g.* enterococci (right, white arrow) and non fluorescent *Clostridium innocuum*.



- PCR was not inhibited when bacteria were picked directly from CCd.

SUMMARY

- CHROMagar C. difficile allows specific recognition of CD as UV-fluorescent colonies.
- UV fluorescence is particularly helpful when contaminant flora is present.
- *Clostridium glycolicum* was the only non-CD false positive detected.
- 28% stools tested positive with the Xpert assay did not grow CD on tCCFA
- < 5% stools tested positive with the Xpert assay did not grow CD on CCd
- 4 samples grew toxigenic CD on CCd, although they were Xpert CD-negative

Based on its high sensitivity, CCd can be recommended for routine isolation of CD in diarrheic stools. However, separate detection of toxigenicity is required, as with other culture media.

REFERENCES

Perry, J. D., K. Asir, D. Halimi, S. Orenga, J. Dale, M. Payne, R. Carlton, J. Evans, F. K. Gould. 2010. Evaluation of a chromogenic culture medium for isolation of *Clostridium difficile* within 24 hours. J. Clin. Microbiol. **48:**3852-58.

Bauer, M. P., D. W. Notermans, B. H. B. van Benthem, J. S. Brazier, M. H. Wilcox, M. Rupnik, D. L. Monnet, J. P. van Dissel, E. J. Kuiper. 2011. *Clostridium difficile* infection in Europe: a hospital-based study. *Lancet* **377:**63-73.

Wannet, W. J. B., M. Reessink, H. A. Brunings, and H. M. E. Maas. 2013. *Clostridium difficile* ribotype diversity at six health care institutions in the United States. J. Clin. Microbiol. **51:**1938-40.