chromID™ C. difficile agar (CDIF)

IVD

Chromogenic medium for the detection and identification of *Clostridium difficile*

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SUMMARY AND EXPLANATION

chromID C. difficile agar is a selective chromogenic medium for the detection and identification of *Clostridium difficile* in human specimens *(*stools of symptomatic patients) (1, 2).
The medium contributes to the diagnosis and epidemiological monitoring of *Clostridium difficile* infections (3).
Clostridium difficile is a causative agent of pseudomembranous colitis and more generally of nosocomial or antibiotic-associated diarrhea (4, 5, 6, 7, 8).

PRINCIPLE

chromID C. difficile agar consists of a rich nutritive base combining different peptones and taurocholate which favors the germination of spores (9).
It contains a chromogenic substrate (10) (patent pending) and a mixture of antibiotics which enable:

- the detection and identification of ß-glucosidase-producing *Clostridium difficile* strains based on the typical grey to black color of colonies.
- the inhibition of most Gram-positive and Gram-negative bacteria, yeasts and molds.

CONTENT OF THE KIT

	Ready-to-use medium
REF **43 871**	Pack of 2 x10 plates (90 mm)
	CDIF *

* printed on each plate

COMPOSITION

Theoretical formula.

This medium can be adjusted and/or supplemented according to the performance criteria required:

Meat peptone (porcine)	8.0 g
Taurocholate (bovine)	1 g
Yeast extract	3.5 g
Sodium chloride	6.0 g
Selective mixture	0.27 g
Chromogenic mixture	0.3 g
Agar	13.0 g
Purified water	1 l

pH 7.3

MATERIAL REQUIRED BUT NOT PROVIDED

- Bacteriology incubator.
- GENbox anaer (Ref. 96124) with jar.
- GENbag anaer (Ref. 45534).

or

- Thermoregulated chambers with a controlled atmosphere.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
- All specimens, microbial cultures and inoculated products should be considered infectious and handled appropriately. Aseptic technique and usual precautions for handling the bacterial group studied should be observed throughout this procedure. Refer to "CLSI® M29-A, *Protection of Laboratory Workers from Occupationally Acquired Infections*; *Approved Guideline* – Current Revision". For additional information on handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories – CDC/NIH – Latest edition", or the current regulations in the country of use.
- Culture media should not be used as manufacturing material or components.
- Do not use reagents after the expiry date.
- Do not use reagents if the packaging is damaged.
- The use of this medium may be difficult for people who have problems recognizing colors.
- Do not use contaminated plates or plates that exude moisture.
- Some precipitates and/or light halos may be observed in the agar but these do not affect the performance of the medium.
- The reading and interpretation of the medium should be performed using isolated colonies.
- Interpretation of test results should be made taking into consideration colonial and microscopic morphology and, if necessary, the results of any other tests performed.
- Performance data presented were obtained using the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.

STORAGE CONDITIONS

- **Store the plates at 2-8°C in their box until the expiry date.**
- If not in the box, plates can be stored in the cellophane sachet for 2 weeks at 2-8°C **in the dark.**

SPECIMENS

The medium should be directly inoculated with liquid diarrheal stools or soft stools.
The specimen can be treated or not with ethanol (11).
Good laboratory practices for collection and transport should be respected and adapted to anaerobic bacteria.

INSTRUCTIONS FOR USE

1. **Allow plates to come to room temperature**.
2. Inoculate the specimen immediately after reception in the laboratory.
3. Put the plate in a suitable atmosphere (anaerobiosis) if necessary using a controlled atmosphere generator.
4. Incubate the inverted plates at 37°C.
   The cultures are examined after 24 hours of incubation.

READING AND INTERPRETATION

- After incubation, observe the bacterial growth and the presence of typical colonies: *Clostridium difficile* colonies are grey to black with an irregular or smooth border.
- Appropriate tests should be performed on the specimen to confirm *Clostridium difficile* infection (8)
- Additional biochemical or molecular tests can be performed to confirm identification if the specimen has not been treated with ethanol.

QUALITY CONTROL

Protocol:

The medium can be tested using the following strains :

- *Clostridium difficile* ATCC® BAA2155 (equivalent to the strain CCUG 60276)

Range of expected results:

Strain	Results at 37°C
Clostridium difficile ATCC® BAA2155 incubation in anaerobic atmosphere	Growth and grey-black color after 24 hours

Note:

It is the responsibility of the user to perform Quality Control taking into consideration the intended use of the medium, and in accordance with any local applicable regulations (frequency, number of strains, incubation temperature, etc.).

LIMITATIONS OF THE METHOD

- Some micro-organisms other than *C. difficile* may develop weakly on the medium producing colonies with a typical color: *C. tertium, C. clostridioforme*, *Bacteroïdes, Lactobacillus*.
- Colonies produced on chromID™ C. difficile agar must not be used with the VITEK®2 ANC identification card and the ATB™ antimicrobial susceptibility test strips dedicated to anaerobes (tendency to globally overestimate resistance). It is recommended to subculture on a non-selective medium.
- Growth depends on the requirements of each individual microorganism. It is therefore possible that certain *Clostridium difficile* strains which have specific requirements may not develop.

PERFORMANCE

A study was performed at 2 sites (Spain and Germany) using clinical specimens.
737 specimens (liquid diarrheal stools or soft stools) were inoculated on chromID™ C. difficile agar and on a conventional medium for which reading is recommended after 48 hours.

The fecal specimens were inoculated either directly (n=474), or after treatment with ethanol (n=263).

Specimens inoculated directly (without ethanol treatment):

Out of the 474 specimens tested, 78 were confirmed to be positive for *C. difficile* by PCR.

	Recovery % (*)	**Sensitivity (%)**
chromID C. difficile 24h	**99** (72/73*)	**92** (72/78)
Other culture medium 48h	55 (40/73*)	51 (40/78)

* Number of specimens found to be positive / Number of specimens found to be positive at 24 hours on chromID C. difficile and/or at 48 hours on the other medium.

The specificities obtained for chromID C. difficile agar (24h) and the other medium (48h) are 88% and 90% respectively.

Specimens inoculated after treatment with ethanol

Out of the 263 specimens tested, 41 were confirmed to be positive for *C. difficile* by PCR.

	Recovery % (*)	**Sensitivity (%)**
chromID™ C. difficile 24h	**89** (33/37*)	**80** (33/41)
Other culture medium 48h	81 (30/37*)	73 (30/41)

* Number of specimens found to be positive / Number of specimens found to be positive at 24 hours on chromID C. difficile and/or at 48 hours on the other medium.

The specificities obtained for chromID C. difficile agar (24h) and the other medium (48h) are 98% et and 93% respectively.

WASTE DISPOSAL

Unused reagents may be considered as non hazardous waste and disposed of accordingly.
Dispose of used reagents as well as any other contaminated disposable material following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

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2. DELMEE M. et al. - Laboratory diagnosis of *Clostridium difficile*-associated diarrhea: a plea for culture. - *J Med Microbiology* - 2005; vol. 54, p.187-191 Microbiology unit, Louvain University, Brussels, Belgium.
3. STUART H. et al. – Clinical Practice Guidelines for *Clostridium difficile* Infection in Adults: 2010 Update by the Society fo Health care Epidemiology of America (SHEA) and the Infection Diseases Society of America (IDSA). – Infect Control Hosp Epidemiol – 2010, vol. 31, p. 431-455.
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8. RILEY T.V., BOWMAN A., CARROLL S.M. - Diarrhoea associated with *Clostridium difficile* in a hospital population - *Med. J. Aust.,* 1983*,* vol. 1, n° 4, p. 166-169.
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10. PERRY D. J. et al. – Evaluation of a Chromogenic Culture Medium for isolation of *Clostridium difficile* within 24 hours. – Journal of clinical microbiology - 2010, p. 3852-3858.
11. RILEY T. V. et al. – Comparison of alcohol shock enrichment and selective enrichment for the isolation of *Clostridium difficile*. – Epidemiol. Infect. – 1987, vol. 99, p. 355-359.

INDEX OF SYMBOLS

Symbol	Meaning
	Catalogue number
	*In vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limitation
	Use by
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Protect from light

BIOMÉRIEUX
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