CHROMagar™
The Chromogenic Media Pioneer

Performance of CHROMagar™ C.perfringens

Chromogenic Culture Medium for the Detection of Clostridium perfringens

Laboratory

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This report contains 13 pages, including 1 page of annexes

Table des matières

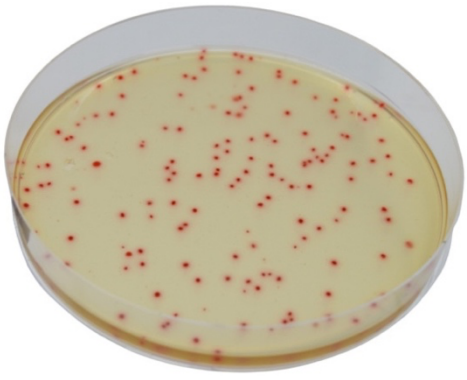
1. Introduction

Clostridium perfringens is a Gram-positive, spore-forming anaerobic bacterium that is widespread in the environment and the intestines of humans and animals. It is one of the most common causes of food poisoning and various other diseases in humans and animals.

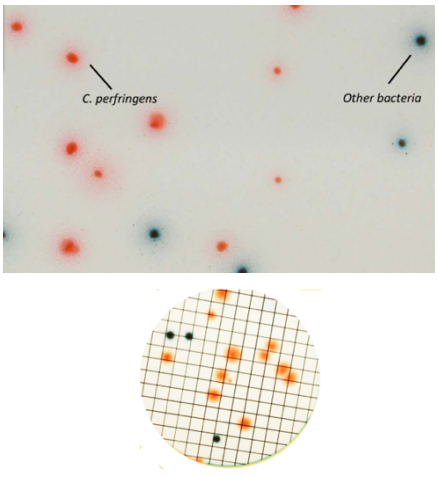
Foodborne illnesses are particularly linked to improperly cooked or stored food. The bacteria produce enterotoxins in the intestines after the ingestion of contaminated food, leading to symptoms. Enteric diseases in livestock (cattle, sheep, pigs, and poultry) is caused by the rapid proliferation of *C. perfringens* in the intestines, leading to severe intestinal damage and sometimes sudden death. The severity depends on the type of toxin produced by different strains.

CHROMagar™ C.perfringens (ref. PF65) has been developed to enable the detection and enumeration of *Clostridium perfringens* in food, water and environmental samples and has been used in veterinary medical diagnostics. On medium plates incubated under anaerobic conditions at 37°C for 24 hours, the colonies of *C. perfringens* grow specifically as orange colonies, being distinguished from other microorganisms growing as blue or metallic blue colonies and selectivity agents inhibit other non-target bacteria.

CHROMagar™ C.perfringens consists of a powder base stored at 15-30°C and two powder supplements (S1 and S2), which are stored at 2-8°C. he medium can be prepared and dispensed into bottles (shelf life is 1 month at 2-8°C) which are melted 1 hour at 100°C before use. Samples can be streaked or spread onto agar plates. In addition, the pour-plate technique with overlay recommended by ISO 15213-2:2023, Microbiology of the food chain — Horizontal method for the detection and enumeration of *Clostridium* spp. — Part 2: Enumeration of *Clostridium perfringens* by colony-count technique (1), is compatible with CHROMagar™ C.perfringens. Water samples can be analysed by the filtration method. Suspected orange colonies can be confirmed by the acid phosphatase test according to ISO 14189:2017 (2). Filtration with cellulose nitrate, cellulose ester or nylon membranes give optimal performances, but cellulose acetate, polyethersulfone or polycarbonate membranes are not recommended.



Clostridium perfringens



Membrane filtration method

This document compiles CHROMagar™ C.perfringens evaluations at two stages:

- In-house evaluations of the chromogenic formula with pure strains.
- Independent laboratory evaluations of the CHROMagar™ C.perfringens formula using food and water samples as well as veterinary specimens.

2. Performance of the CHROMagar™ C.perfringens formula

2.1. Analytical data

Different *Clostridium perfringens* strains, *Clostridium* genus related and other bacterial strains (n=14, n=10 and n=15, respectively) were streaked on CHROMagar™ C.perfringens. Plates were incubated under anaerobic conditions at 37°C for 24 hours. Results are shown below in Tables I and II.

Table I. Bacterial strains tested to evaluate inclusivity of CHROMagar™ C.perfringens.

Bacterial Species	Strain #	CHROMagar™ C.perfringens	Caracteristics (toxin gene or source)
Clostridium perfringens	ATCC® 3624	O++, 1.5-2 mm	α -toxin producer
C. perfringens	ATCC® 12916	O+++, 2 mm	α-toxin (*cpa*), enterotoxin (*cpe*) genes
C. perfringens	ATCC® 12920	O+++, 4 mm	Agglutinating type 6. Type A.
C. perfringens	ATCC® 13124	O++, 1-2 mm	α-toxin (*cpa*), Perfringolysin O (*pfoA*) genes
C. perfringens	AR5869	O+++, 0.8-3 mm	ND
C. perfringens	AR5956	O+++, 0.8-3 mm	α-toxin and β-toxin genes (sheep)
C. perfringens	AR5957	O+++, 1-3 mm	α-toxin and β-toxin genes (pig)
C. perfringens	AR5958	O+++, 0.8-1.5 mm	α-toxin and enterotoxin producer
C. perfringens	AR5959	O+++, 2-3 mm	α-toxin and enterotoxin producer
C. perfringens	AR5960	O+++, 1-3 mm	α-toxin gene (bird)
C. perfringens	AR5961	O+++, 0.8-2 mm	α-toxin (*cpa*), enterotoxin (*cpe*) genes (sheep)
C. perfringens	AR5962	O++, 3 mm	α -toxin producer
C. perfringens	AR6027	O+++, 0.8-3 mm	(Stool)
C. perfringens	AR6183	O++, 0.8-2 mm	(Meat)

O, orange; +, colour intensity; size in mm; ND, not determined; AR, CHROMagar™ strain collection.

C. perfringens strains colonies are easily detected in orange on CHROMagar™ C.perfringens under anaerobic conditions at 37°C for 24 hours with sensibility at 100%.

Table II. Microbial strains tested to evaluate exclusivity of CHROMagar™ C.perfringens.

Bacterial species	Strain #	CHROMagar™ C.perfringens
Clostridioides difficile	ATCC© 43255	B++, 1 mm
C. difficile	AR5681	B++, 0.8-1 mm
C. difficile	AR5682	B++, 1 mm
C. difficile	AR5697	unc., 1 mm
C. difficile	AR5698	B+, 0.8-1 mm
C. difficile	AR5737	B+, 0.8-1 m
Clostridium septicum	AR6184	unc., 0.5-0,8 mm
C. sporogenes	AR6185	unc., 0.5-0.8 mm
C. sordellii	ATCC© 9714	O++, 0.8-1 mm
C. clostridioforme	AR6023	B trace

Microbial Species	Strain #	CHROMagar™ C.perfringens
Lactobacillus spp.	AR5215	B DZ
Lactococcus lactis	AR5363	R Trace
Leuconostoc spp.	AR4341	B Trace
Leuconostoc spp.	AR5209	B/R DZ
Leuconostoc spp.	AR6153	-
E. faecalis	AR6061	B trace
E. gallinarum	AR5207	B trace
Serratia marcescens	ATCC® 13880	B DZ
S. marcescens	AR4510	B trace
S. marcescens	AR5569	-
S. marcescens	AR6173	B trace
S. marcescens	AR6234	B trace
S. liquefaciens	AR6146	B trace
Actinomyces viscosus	AR6031	V++, 0.8-1 mm
Candida albicans	ATCC® 10231	unc. DZ

O, orange; B, blue; R, red; V, violet; unc., uncoloured; + colour intensity; size in mm; -, growth absence; DZ, dense zone (some bacterial growth in this agar region, a coloured trace does not present growth); AR, CHROMagar™ strain collection.

Not-target bacteria capable of growing anaerobically on CHROMagar™ C.perfringens are differentiated (blue or uncoloured colonies) or are inhibited (i.e. there is a trace or dense zone or they are flagged as -, in Table II).

C. sordellii can be detected as false positive on CHROMagar™ C.perfringens but it can be distinguished by biochemical tests like indole, proline or acid phosphatase (see points 2.2 and 3.4).

The pour-plate technique with overlay recommended by ISO 15213-2:2023 using pure strains or mixtures of strains (e.g. *C. perfringens* and *C. difficile* or *C. perfringens* and *Actinomyces*) allowed efficient colony enumeration with CHROMagar™ C.perfringens with >70% recovery of *C. perfringens* strains compared to Columbia Nalidixic Acid (CNA) agar.

The chromogenic differentiating (orange and blue colonies), and selecting (inhibition of annex flora) performances of CHROMagar™ C.perfringens are observed using the filtration method with cellulose nitrate, cellulose ester or nylon membranes. The intensity of the orange colour on the anaerobically incubated membrane filter may intensify when exposed to ambient air.

2.2. Confirmatory test of *C. perfringens* detection in water

C. perfringens is present in the intestinal tract of animals and humans in the form of vegetative cells and spores. This bacterium is widely recognised as an indicator of faecal pollution, as its spores survive in water for months, indicating distant or intermittent faecal pollution. Monitoring for *C. perfringens* has proved useful for assessing the quality of water resources and for checking water treatment steps.

ISO 14189:2017, which specifies a method for the enumeration of vegetative cells and spores of *C. perfringens* by membrane filtration method in samples of water, recommends an acid phosphatase test for confirmation after subculturing of characteristic colonies on a rich medium such as blood agar. The acid phosphatase test requires the use of Fast Blue B salt (CAS n° 14263-94-6) in the acid phosphatase reagent and develops a purplish colour within 3 to 4 min for colonies spread on filter paper when the reaction is positive (2).

A method to detect and confirm *C. perfringens* without subculture but directly from CHROMagar™ C.perfringens plates incubated anaerobically for 24 h at 37°C was developed. Typical orange colonies can be examined by using in this case Fast Bleu RR salt (CAS n° 14726-29-5) in the test reagent without modifying its standard preparation and use protocols with a gain of 1 day compared to ISO 14189. A positive acid phosphatase reaction for colonies spread on filter paper gives a purplish colour in 3 to 4 minutes (Table III).

Table III. Acid phosphatase testing to confirm *C. perfringens*.

Species	Strain #	CNA agar		CHROMagar™ C.perfringens	
		Fast Blue B salt	Fast Bleu RR salt	Fast Blue B salt	Fast Bleu RR salt
Clostridium perfringens	AR5959	-	+ weak	-	+ 
C. perfringens	ATCC® 13124	+ weak	++	-	+ 
C. perfringens	AR6323	+ weak	++	-	+ weak 
C. sordellii	ATCC© 9714	-	-	-	- 
C. difficile	AR5681	-	-	-	- 

+, positive reaction; -, negative reaction; AR, CHROMagar™ strain collection.

3. Independent laboratory evaluation of the product

CHROMagar™ C.perfringens tests using pure strains, food products and veterinary specimens were carried out by third-party laboratories.

3.1. Performance of CHROMagar™ C.perfringens with pure strains

Several bacterial species from strain collections were used to assess the inclusivity and exclusivity of CHROMagar™ C.perfringens.

Table IV. Bacterial strains used to evaluate CHROMagar™ C.perfringens.

Laboratory, Country (citation)	Bacterial strains	Sensitivity	Specificity	Comments
Actalia, France (3)	**Inclusivity:** *C. perfringens* ATCC® 13124 *C. perfringens* ATCC® 12916 *C. perfringens* AD 246/*C. perfringens* 1221 (poultry) *C. perfringens* 214 (environment, n=5) **Exclusivity:** *C. sporogenes* Act74-001 *C. pasteurianum* Act74-019 *C. bifermentans* Act74-065 / Act74-198 *C. tyrobutyricum* Act74-014 (milk product) (n=5) *Escherichia coli* LMG 8063 *Enterococcus faecalis* CNRZ 134 *Citrobacter freundii* ATCC® 8454 *Bacillus cereus* ADQP 407 *Staphylococcus aureus* LMG 8195 *Lactobacillus plantarum* ATCC® 8014 (n=6)	100%	100%	Comparison of CHROMagar™ C.perfringens and TSC plates, anaerobic incubation at 37 °C for 20±2 hours Streaking and pour-plate methods
Du Pont, USA	**Inclusivity:** *C. perfringens* (veterinary, n=18) **Exclusivity:** *C. paraputrificum* (n=20) *Pediococcus pentosaceus* (n=1) *L. plantarum* (n=1) *E. faecalis* (n=1)	100%	100%	Comparison of CHROMagar™ C.perfringens and TSC plates, anaerobic incubation at 37 °C for 24 hours
Faculty of Veterinary Medicine, Ghent University, Belgium (4)	**Inclusivity:** *C. perfringens* from different animals (n=25) **Exclusivity:** *C. difficile* (n=4) *B. galinarum* (n=1) *B. amyloliquefaciens* (n=1) *B. subtilis* (n=1) *E. faecalis* (n=1)	100%	100%	Non-*C. Perfringens* grew blue colonies or were inhibited
Microsept, France (5)	**Inclusivity:** *C. perfringens* (food and water, (n=15) **Exclusivity:** *Clostridium* spp. (n=6) *Paeniclostidrium sordellii* (n=1) *Leuconostoc mesenteroides (n=2)* Lactic acid bacteria (n=8) Enterococci (n=3) *Weissella viridescens* (n=1) *P. pentosaceus* (n=1) *Enterobacter cloacae* (n=1) *S. marcescens* (n=1) *C. freundii* (n=1)	100%	70% ↓ 95% with acid phosphatase test	Comparison of CHROMagar™ C.perfringens and TSC plates, anaerobic incubation at 37 °C for 24 hours

Anaerobic incubation, 24h at 37°C. TSC, tryptose sulphite cycloserine agar

In the study conducted by Microsept (5), five out of seven strains related to *Clostridium* spp., namely *C. butyricum*, *C. glycolicum*, *C. sordellii*, *C. biofermentants*, and *Paeniclostridium sordellii*, as well as *Enterococcus faecalis* and *E. faecium* of water origin and bacteria of food/probiotic origin such as Lactobacilli, *Leuconostoc mesenteroides* and *Streptococcus thermophilus* were detected as false positives on CHROMagar™ C.perfringens (70% specificity). All but one out of two strains of *Lactobacillus sakei* were negative in the acid phosphatase test (see table V), bringing the specificity to 95%).

3.2. Detection and enumeration of *C. perfringens* in food products

Actalia, France (3), conducted a study to evaluate the sensitivity of CHROMagar™ C.perfringens with food products in anaerobic incubation at 37 °C for 20±2 hours. Naturally contaminated meat, beef heart, poultry sausage and raspberry pastry samples (n=7) were used to compare CHROMagar™ C.perfringens, tryptose sulphite cycloserine (TSC) agar and the colony count technique at 46 °C (6; NF V08-061:2009) with streaking and pour-plate methods. For all food samples, detection of *C. perfringens* as orange colonies was possible using CHROMagar™ C.perfringens with both streak and pour-plate methods, whereas TSC agar failed to detect C. *perfringens* with the streak method and substantial annex flora grew in one pour-plate sample. The colony count technique at 46 °C detected *C. perfringens* in only 3 out of 7 food products.

Artificially contaminated (at $1x10^3$ *C. perfringens* CFU/g) meat and poultry sausage samples (n=3), were used to compare CHROMagar™ C.perfringens with streaking and pour-plate methods and TSC agar with the pour-plate method. The recovery of *C. perfringens* at >90% was equivalent for both media. Colony enumeration with CHROMagar™ C.perfringens was facilitated as the orange colour is evident and does not fade once the plates are in aerobic conditions, whereas on TSC agar colonies tend to lose their black coloration once exposed to air.

3.3. Detection and enumeration of *C. perfringens* in veterinary specimens

3.3.1. Analyses of poultry faeces

In healthy animals or in subclinical enteric disease, the number of *C. perfringens* in the intestine is often low. Isolation and fast detection of the organism's proliferation in the intestinal tract is essential in veterinary medical diagnosis and veterinary research. Husta *et al.*, 2020 (4) evaluated four selective culture media allowing to detect and enumerate *C. perfringens* in poultry faeces spiked with different *C. perfringens* strains (CP10, $NetB^-$; CP20, $NetB^-$; CP56, $NetB^+$; JIR4869, NetB+). On Columbia blood agar, a typical haemolytic zone appears around *C. perfringens* colonies, while on Shahidi-Ferguson perfringens agar, an opaque halo can be observed and on TSC agar colonies are black. On CHROMagar™ C.perfringens colonies were orange, whereas other strains were differentiated in blue. There was no significant difference between the four tested media for recovery of different *C. perfringens* strains from fresh poultry faeces. The limit of quantification was 10^3 CFU/mL for all agars.

CHROMagar™ C.perfringens showed the highest specificity, especially when low *C. perfringens* loads were present in the faeces. The orange colour of *C. perfringens* colonies differentiates the target bacteria from other strains growing as blue colonies. Therefore, CHROMagar™ C.perfringens can be recommended when timely and easy detection and enumeration of *C. perfringens* from complex samples such as faeces is needed.

3.3.2. Analyses of animal intestinal tracts

Du Pont, USA, carried out a study to assess the sensitivity of CHROMagar™ C.perfringens and TSC agar with gastrointestinal mucus samples from various animal types (n=15). Detection of *C. perfringens* as orange colonies was possible with a sensibility of 100% after anaerobic incubation at 37°C for 24 hours (2017, unpublished data).

A study completed at the Faculty of Veterinary Medicine, Ghent University, Belgium, with cloacal swabs (n=10) incubated anaerobically for 24 h at 37°C on Columbia blood agar (CBA supplemented with 12 mg/L kanamycin and 30,000 IU/L of polymyxin B) and on plates of CHROMagar™ C.perfringens. On both media, colonies of C. perfringens were isolated, CHROMagar™ C.perfringens, showed orange colonies which were identified as *C. perfringens* (sensitivity 100%, specificity 100%), whereas the supplemented CBA also allowed the growth of annex flora (2017, unpublished data).

3.4. Acid phosphatase test to confirm *C. perfringens*

Microsept (5) tested the method described in point 2.2. to detect and confirm *C. perfringens* directly from CHROMagar™ C.perfringens plates using *C. perfringens* strains from different sources (n=15), *Clostridium* spp. strains (n=7) and other bacterial strains (n=18). TSC and Columbia with 5% sheep blood agar or TSA plates were included in the study.

All *C. perfringens* strains from CHROMagar™ C.perfringens and Columbia blood plates (the latter were subcultures of TSC plates) tested positive for acid phosphatase in 3 to 4 minutes with the Fast Bleu RR salt reagent.

The results of *Clostridium* spp. strains (*C. butyricum*, *C. glycolicum*, *C. sordellii*, *C. biofermentants*, *Paeniclostridium sordellii* and *C. septicum*) were negative. Strains not belonging to the *Clostridium* genus developed blue colonies or were inhibited on CHROMagar™ C.perfringens after incubation at 37°C for 24 hours. Some such strains developed orange colonies, in particular *Enterococcus faecalis* and *E. faecium* of water origin, and bacteria of food/probiotic origin, such as Lactobacilli, *Leuconostoc mesenteroides* and *Streptococcus thermophilus*, were negative for acid phosphatase. One out of two *Lactobacillus sakei* (strain B, smoked salmon source) gave a positive result in the acid phosphatase test (specificity 98%, Table V). Definitive bacterial identification may require further tests directly on colonies in dairy products.

The acid phosphatase reaction is a rapid confirmation test that can be performed directly on colonies growing on CHROMagar™ C.perfringens medium, compatible with the filtration method.

Table V. Results of acid phosphatase tests on bacterial strains.

Strain (number of strains or internal reference)	Source	CHROMagar™ C.perfringens			TSC	TSA
		Colony aspect	Acid phosphatase	Conclusion	Colony aspect	Acid phosphatase
C. perfringens (n=15)	various	orange	positive	*C. perfringens*	black	positive
C. butyricum (2)	river water	orange	negative	not *C. perfringens*	black	negative
Clostridium sp. (4)	oulet water	orange	negative	not *C. perfringens*	black	negative
C. glycolicum (5)	pond water	orange	negative	not *C. perfringens*	black	negative
C. sordellii (11)	Thyme	orange	negative	not *C. perfringens*	black	negative
C. bifermentans (12)	composite Food	orange	negative	not *C. perfringens*	black	negative
Paeniclostridium sordellii (N)	DSMZ strain	orange	negative	not *C. perfringens*	black	negative
C. septicum (P)	DSMZ strain	no growth	/	/	white	positive
Leuconostoc mesenteroides (A)	food	blue	negative	not *C. perfringens*	no growth	negative
Lactobacillus sakei (B)	smoked salmon	orange	positive	possible *C. perfringens*	no growth	positive
L. gasseri (C)	probiotic	blue	negative	not *C. perfringens*	no growth	negative
L. reuteri (D)	probiotic	orange	negative	not *C. perfringens*	no growth	negative
Bifidobacterium lactis (E)	probiotic	blue	negative	not *C. perfringens*	no growth	negative
L. paracasei (F)	dairy	no growth	/	/	no growth	positive (weak)
L. mesenteroides (G)	meat product	orange	negative	not *C. perfringens*	no growth	negative
Streptococcus thermophilus (H)	probiotic	orange	negative	not *C. perfringens*	no growth	negative
Lactobacillus sakei (I)	dairy product	orange	negative	not *C. perfringens*	no growth	negative
Enterococcus faecalis (J)	bath water	orange	negative	not *C. perfringens*	white	negative
E. faecalis (K)	sewage water	orange	negative	not *C. perfringens*	white	negative
E. faecium (L)	outlet water	orange	negative	not *C. perfringens*	white	negative
Weissella viridescens (M)	composite Food	no growth	/	not *C. perfringens*	no growth	negative
Pediococcus pentosaceus (O)	DSMZ strain	no growth	/	not *C. perfringens*	no growth	negative
L. plantarum (Q)	DSMZ strain	blue	negative	not *C. perfringens*	no growth	negative
Enterobacter cloacae (R)	outlet water	no growth	/	not *C. perfringens*	white	negative
Serratia marcescens (S)	pond water	no growth	/	not *C. perfringens*	no growth	positive
Citrobacter freundii (T)	outlet water	no growth	/	not *C. perfringens*	no growth	positive

The change in colour from orange to purplish after 3 to 4 min of acid phosphatase reaction is a modification to the ISO 14189:2017 standard that the operator should be made aware of to avoid misinterpretation of results. As part of the study, the enumeration and confirmation of *C. perfringens* colonies using CHROMagar™ C.perfringens was validated.

Conclusion

The performance of the CHROMagar™ C.perfringens medium has been validated by a series of evaluations. These evaluations included inclusivity and exclusivity studies, as well as analyses of food products and veterinary specimens.

Parameter	Performance of CHROMagar™ C.perfringens
Inclusivity Exclusivity (with bacterial & fungal strains)	100% (n=77) 84% → 99% with acid phosphatase test (n=92)
Detection of *Clostridium* perfringens (in food products and veterinary specimens)	Sensitivity 100% Specificity 100%
Morphological appearance of colonies	Orange
Limit of quantification (poultry faeces)	10^3 CFU/mL

This medium has very good performances, but a few limitations can be pointed out:

- Some strains of Lactobacilli can be detected as false positives (*L. sakei*). Definitive bacterial identification may require additional tests directly on colonies in diary products.
- Some strains of *Clostridium* spp. such as *C. butyricum* and *C. sordellii* can be detected as false positives and can be distinguished by indole, proline or acid phosphatase tests.
- Some granules can be observed in the background of the plates without affecting the medium performance.

In appropriate storage, the shelf life of the powder base and powder supplements is 3 years. Reconstituted supplements are 2 weeks (S1) and 2 months (S2) at 2-8°C. The medium can be prepared and dispensed into bottles (shelf life is 1 month at 2-8°C) which are melted 1 hour at 100°C before use. Good preparation of the medium can be verified by isolating recommended ATCC strains for Quality Control testing.

The results on CHROMagar™ C.perfringens plates are easy to read with the naked eye, advantages in the selectivity and sensitivity on CHROMagar™ C.perfringens plates compared to blood agar or TSC agar plates were reported from laboratories.

Filtration with cellulose nitrate, cellulose ester or nylon membranes give optimal performances, but cellulose acetate, polyethersulfone or polycarbonate membranes are not recommended. The confirmation of *C. perfringens* colonies directly from the filtration method of water samples on CHROMagar™ C.perfringens can be performed with a modification of the acid phosphatase reagent.

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4. Literature

1) ISO 15213-2:2023. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Clostridium* spp. — Part 2: Enumeration of *Clostridium perfringens* by colony-count technique.

2) ISO 14189:2017. Water quality - Enumeration of Clostridium perfringens - Method using membrane filtration.

3) Actalia. 2018. Enumeration medium study of *Clostridium perfringens* in food products. Report, 12 pp. (CHROMagar website).

4) Hustá, M., Ducatelle, R., Haesebrouck, F., Van Immerseel, F., and Goossens, E. 2020. A comparative study on the use of selective media for the enumeration of *Clostridium perfringes* in poultry faeces. *Anaerobe*. **63:** 1-7.

5) Microsept. 2020. Study of an acid phosphatase test for the confirmation of *Clostridium perfringens* directly from CHROMagar™ C.perfringens agar medium. Report, 15 pp. (CHROMagar website).

6) NF V08-061:2009. Microbiology of food and animal feeding stuffs - Anaerobic enumeration of sulfito-reducing bacteria by colony count technique at 46 °C.

Annexes

Annex 1. Website information about CHROMagar™ C. perfringens.

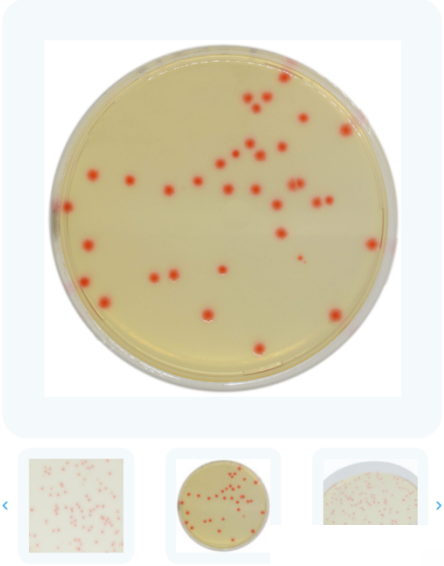
Our Company | Products | Technical documents | CHROMagar™ The Chromogenic Media Pioneer | Find your distributor | Contact | EN

Clinical Microbiology
A complete range of culture media to help in clinical diagnosis

Food Industry
A complete range of culture media for the food industry

Water Testing
A complete range of culture media for water analysis

Veterinary Microbiology
A complete range of culture media for the veterinary sector



CHROMagar™ C. perfringens

For detection and enumeration of *Clostridium perfringens*

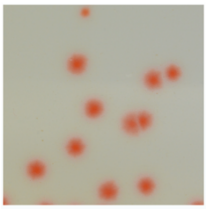
Order References

Please use these references when contacting your local distributor:

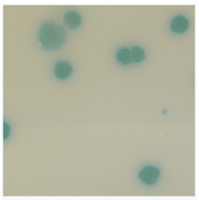
5000 mL Pack PF652

Included : base PF652(B) + supplement PF652(S1) + supplement PF652(S2)

Colony appearance



Clostridium perfringens → orange



Clostridioides. difficile → *green-blue to green*

Composition

Powder Base	Total ..... 50.9 g/L Agar ..... 15.0 Peptones and yeast extract ..... 25.0 NaCl ..... 6.0 Chromogenic and selective mix ..... 1.4 Growth factors..... 3.5 Storage at 15/30 °C - pH: 7.6 +/- 0.2 Shelf Life ..... > 12 months
+	
2 Supplements (included in the pack)	1st : Powder..... 2 g/L Storage at 2/8 °C Shelf Life ... > 12 months 2nd : Powder..... 0.12 g/L Storage at 2/8 °C Shelf Life ... > 18 months

Usual Samples	Industrial: Food, water, environmental samples
Procedure	Direct streaking. Incubation 24 h at 37 °C Anaerobic conditions.

Performance

Clostridium perfringens is involved in food poisoning and animals' infections.Beef, poultry, gravies, and dried or pre-cooked foods are common sources of *C. perfringens* infections. *C. perfringens* infection often occurs when foods are prepared in large quantities and kept warm for a long time before serving.

Although *C. perfringens* may live normally in the human intestine, illness is caused by eating food contaminated with large numbers of *C. perfringens* bacteria that produce enough toxin in the intestines to cause illness.

Everyone is susceptible to food poisoning from *C. perfringens*. The very young and elderly are most at risk of *C. perfringens* infection and can experience more severe symptoms that may last for 1 to 2 weeks. Complications, including dehydration, may occur in severe cases.

CDC - Centers for Disease Control and Prevention

1. To be used with pouring or surface méthode (by direct streaking, spreading or filtration technique) whereas with TSC medium bacteria have to be placed between two layers of agar in order to grow in black colonies.

2. Specific medium for *Clostridium perfringens* while TSC medium detects sulfate-reducing bacteria, including the non pathogens.

3. The orange coloration makes the vizualization very easy on the other hand, the spread of the colonies black color and the fact that they faint after a while in TSC medium (as described in the ISO 14189) makes the colony count difficult.