67438 C. perfringens Millichrome™ plus Agar Base

For detection and enumeration of *Clostridium perfringens*.

Composition:

Ingredients	Grams/Litre
Peptones and yeast extract	25.0
NaCl	6.0
Chromogenic and Selective mix*	1.4
Growth factors	3.5
Agar	15.0

Final pH 7.6 +/- 0.2 at 25°C

* confidential mix with chromogenic substrates and selective agents

Store prepared media below 8°C, protected from direct light (max. 1 month). Store dehydrated powder, in a dry place, in tightly sealed containers at 2-25°C.

Preparation:

Step 1 (Preparation of the base C. perfringens Millichrome™ plus Agar)
- Disperse slowly 50.9 g of powder base in 1 L of purified water.
- Stir until agar is well thickened.
- Heat and bring to boiling (100 °C) while swirling or stirring regularly.
- AUTOCLAVE at 121 °C during 15 min.
- Mix when removing it from the autoclave.
- Cool in a water bath to 45-50 °C, swirling or stirring gently.

Step 2 (Preparation of the Supplement 1)
- In a transparent vessel, add 2 g of C. perfringens Millichrome™ plus supplement 1 in 20 mL of purified water.
- Swirl well until complete dissolution.
- Filter to sterilize at 0.45 µm.

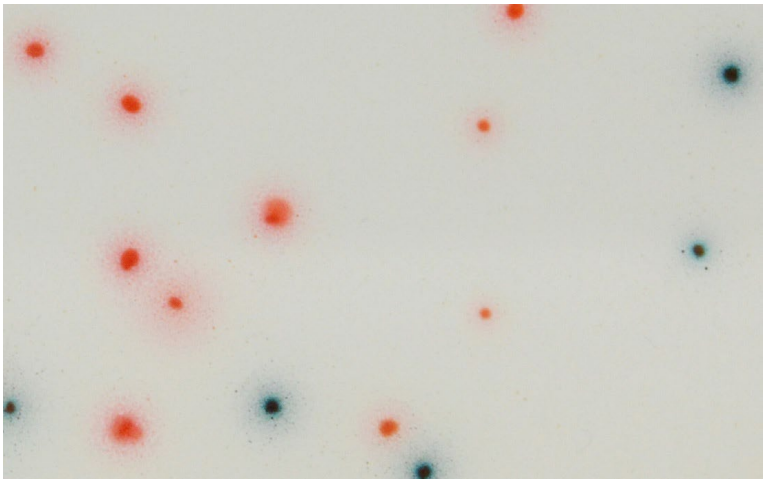
Step 3 (Preparation of the Supplement 2)
- Add 0.12 g of C. perfringens Millichrome™ plus supplement 2 in 1 mL of purified water.
- Filter to sterilize at 0.45 µm

Step 4 (Mixing of the prepared base and the supplements 1 and 2)
- Add 20 mL/L of the supplement 1 solution to the melted base at 45-50 °C.
- Add 1 mL/L of the supplement 2 solution to the melted base at 45-50 °C.
- Swirl or stir gently to homogenize.
- Pour into sterile Petri dishes
- Let it solidify and dry.

Inoculation:

Samples are inoculated by direct streaking on the plate, or a filter can be placed on the plate.
- If the agar plate has been refrigerated, allow to warm to room temperature before inoculation.
- Streak sample onto plate.
- Place filter without air bubbles on the plate (use Cellulose Nitrate, Cellulose Ester or Nylon membranes for optimal performance)

Principle and Interpretation:
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. 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.[1]
Open surface water is a natural source of *C. perfringens* and is often used to check in the water testing as it builds spores and survives several treatments, not like *E. coli*.
C. perfringens Millichrome™ plus Agar can be used with pouring or surface method (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. Compared to TSC medium it is specific for *C. perfringens* and detects not all sulfate-reducing bacteria. The orange color of the of *C. perfringens* colonies makes the differentiation easy and the color is stable not like on TSC agar where the color is fading after a while. Most other organisms show blue/green colors or are inhibited.



- Clostridium perfringens (orange colonies)
- Other organisms (blue colonies)

Peptone and yeast extract provide nitrogenous nutrients for growth and other essential growth factors. Sodium chloride is needed for the osmotic balance. Chromogenic and selective mix contains chromogenic substrates for the color differentiation based on the ability to cleave the substrate by characteristic enzymes. This mix contains as well selective agent which allows *C. perfringens* to grow while other organisms are inhibited. C. perfringens Millichrome™ plus supplement 1 and 2 gives additional selectivity to this medium. The medium contains as well some special grow factors for the growth of *C. perfringens*. Agar is the solidifying agent.

Limitation and further testing

- Definite identification may require additional testing.
- Some strains of *C. sordelii* can be detected as false positives and can be distinguished by biochemical tests like indole or proline.
- Some globules can be observed on the background of the media. They don't change its performance.

Quality control:
Cultural characteristics after 24 h at 37°C under anaerobic conditions.

Organisms (ATCC/WDCM)	Growth	Colony color
C. perfringens (3624/-)	+++	orange
C. perfringens (12920/NCTC 8679)	+++	orange
C. perfringens (12916/00080)	+++	orange
Enterococcus faecalis (29212/0008)	-	
Escherichia coli (25922/00013)	-	

References:

1. CDC - Centers for Disease Control and Prevention
2. M. Husta et al., A comparative study on the use of selective media for the enumeration of Clostridium perfringens in poultry faeces, Anaerobe Volume 63 (2020)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.