67683 Campylobacter Millichrome™ plus Agar Base

For detection of thermotolerant Campylobacter.

Composition:

Ingredients	Grams/Litre
Peptone and yeast extract	25.0
NaCl	9.0
Chromogenic and selective mix*	2.2
Agar	15.0

Final pH 7.4 +/- 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 Campylobacter Millichrome™ plus Agar Base):

- Disperse slowly 51.2 g of powder base in 1L of purified water.
- Stir until agar is well thickened.
- Heat and bring to boil (100 °C) while swirling or stirring regularly.

DO NOT HEAT TO MORE THAN 100 °C. DO NOT AUTOCLAVE AT 121 °C.
Warning: If using an autoclave, do so without pressure.
Advice: For the 100 °C heating step, mixture may also be brought to a boil in a microwave oven: after initial boiling, remove from oven, stir gently, then return to oven for short repeated bursts of heating until complete fusion of the agar grains has taken place (large bubbles replacing foam). • Cool in a water bath to 45-50 °C. Swirl or stir gently to homogenize.

Step 2 (preparation of the supplement):

- In a transparent vessel, add 210 mg of Campylobacter Millichrome™ plus Supplement in 10 ml of purified water.
- Swirl well until complete dissolution.
- Filter to sterilize at 0.45 µm.
- Add the 10 ml of the supplement solution to the melted base (Step1) at 45-50 °C.
- Swirl or stir gently to homogenize.

Step 3 (pouring):

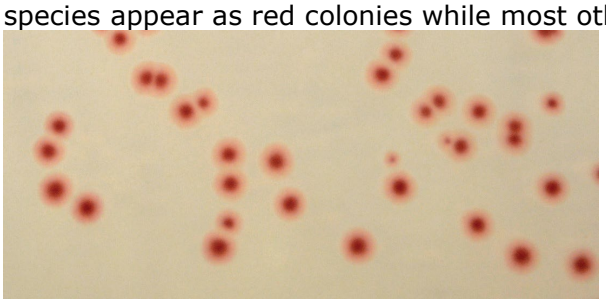
- Pour into sterile Petri dishes.
- Let it solidify and dry (longer than usual).

Inoculation:

Samples are inoculated by direct streaking on the plate, or after enrichment step.

- If the agar plate has been refrigerated, allow to warm to room temperature before inoculation.
- Streak sample onto plate.

Principle and Interpretation:
*Campylobacte*r bacteria are the major cause of foodborne diarrhoea it causes more cases than *Salmonella*. The high incidence of *Campylobacter* diarrhoea, as well as its duration and possible sequelae, makes the testing highly important. In developing countries, *Campylobacter* sometimes causes death of children under the age of two years.[1]
Campylobacter spp. are fastidious and sensitive bacteria that may be difficult to recover due to suboptimal specimen transport and/or storage conditions and lack of proper culture procedures. Several culture media formulations have been developed, they are normally blood-based or include charcoal. But all of these media have shown to be a poor compromise between specificity and sensitivity.
Campylobacter Millichrome™ plus Agar is a highly selective chromogenic medium used in the qualitative direct detection, differentiation and presumptive identification of thermotolerant *Campylobacter*. This medium can be used in the detection of *Campylobacter* in the analyses of food products for human consumption, animal feed and in environmental samples in accordance with the ISO 10272-1 (selective medium free of joice).
Results can be interpreted after 36-48 h of micro-aerophilic incubation at 42 °C. Campylobacter species appear as red colonies while most other organisms show blue or green color or are inhibited.


Campylobacter 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 *Campylobacte*r spp. to grow while other organisms are inhibited. Campylobacter Millichrome™ plus Supplement gives additional selectivity to this medium. Agar is the solidifying agent.

Limitation and further testing
A lack of growth or the absence of colonies on Campylobacter Millichrome™ plus Agar does not preclude the presence of *Campylobacter*. This agar is not intended to diagnose infection nor to guide nor monitor treatment for infections.

- Final identification may require complementary tests such as hippurate hydrolisis, directly from the plate.
- Other final identification tests can be done from a subculture on blood agar (oxydase, acetate test, ...).
- *C. fetus* might not grow in this medium.

Quality control:
Cultural characteristics after 36-48 h at 42°C under micro-aerophilic conditions.

Organisms (ATCC/WDCM)	Growth	Colony color
Campylobacter jejuni (33291/00005)	+++	red
Campylobacter coli (33559/00072)	+++	red
Campylobacter lari (35221/00204)	+++	red
Enterococcus faecalis (29212/00087)	-	
Candida albicans (60193/-)	-	
Escherichia coli (25922/00013)	-	

References:

1. World Health Organisation (WHO) – fact sheet N°255
2. L. De Zutter et al., Comparison of four different selective media for the quantification of Campylobacter in poultry meat and rapid confirmation of suspect colonies, Ghent University, Faculty of Veterinary Medecine (2017)
3. M. J. Sylte et al., Evaluation of different Campylobacter jejuni isolates to colonize the intestinal tract of commercial turkey poults and selective media for enumeration, Poultry Science Association 97:1689-1698 November (2017)

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.