Dehydrated Culture Media

REINFORCED CLOSTRIDIAL MEDIUM (RCM)

Code: CM0149

A semi-solid medium for the enumeration and cultivation of clostridia and other anaerobes occurring in food and pathological specimens. It is the basal medium for Differential Reinforced Clostridial Medium.

Typical Formula*

	gm/litre
Yeast extract	13.0
Peptone	10.0
Glucose	5.0
Soluble starch	1.0
Sodium chloride	5.0
Sodium acetate	3.0
Cysteine hydrochloride	0.5
Agar	0.5
pH 6.8 ± 0.2	

*** Adjusted as required to meet performance standards**

Directions
Suspend 38 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
A semi-solid medium for the enumeration and cultivation of anaerobes. Recommended for the isolation and cultivation of anaerobic organisms occurring in a variety of habitats, including food and pathological specimens.

Reinforced Clostridial Medium (RCM) was designed by Hirsch & Grinstead[1] for the cultivation and enumeration of clostridia. They showed that the medium was more fertile and enabled growth to be initiated from small inocula more readily than five other media tested. In a further comparison, the highest viable count obtainable was the criterion used, and again, RCM proved superior. Compared with the spleen infusion medium of Mundt & Jones[2], RCM gave somewhat higher counts (Gibbs & Hirsch[3]).

Reinforced Clostridial Medium can be made differential for sulphite-reducing clostridia by the addition of sodium sulphite and ferric citrate[4]. Differential Reinforced Clostridial Medium is recommended for detection of sulphite-reducing clostridia and *Clostridium perfringens* in drinking water[5].

Preparation of differential reinforced clostridial medium
Make separate solutions of 4% sodium sulphite (anhydrous) and 7% ferric citrate in distilled water. Heat the ferric citrate solution to dissolve. Sterilise both solutions by filtration. The solutions may be stored at 4°C for up to 14 days.

On the day of use mix equal volumes of the two solutions. Add 0.5 ml of the mixture to each 25 ml volume of single- strength freshly steamed and cooled Reinforced Clostridial Medium. To each 10 ml and 50 ml volume of double-strength medium add 0.4 ml and 2 ml respectively of the mixed solutions.

All cultures showing blackening must be sub-cultured for confirmatory tests.

Weenk, Fitzmaurice and Mossel[6] modified Differential Reinforced Clostridial Medium by increasing the iron content to 1 gram/litre of ferric ammonium citrate and accurately adjusting the sulphite concentration to 0.05% disodium sulphite heptahydrate. The time required for sulphite-reducing clostridium colonies to blacken was significantly shorter than that when using iron sulphite agar. The modified medium to a great extent suppressed the formation of black colonies by hydrogen sulphide-positive *Bacillus* spp. Resistance to metronidazole and growth on aerobically incubated tryptone soya agar are reliable criteria for recognising *Bacillus* spp. colonies that develop.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder
Prepared medium: Straw coloured solution

Quality control

Positive control:
Expected results
Clostridium perfringens ATCC® 13124 Turbid growth.
Negative control:

Uninoculated medium
No change.

Precautions
Further identification tests must be carried out on organisms isolated from this medium.

References
1. Hirsch A. and Grinstead E. (1954) *J. Dairy Res*. 21. 101-110.
2. Mundt J. O. and Jones V.W. (1952) *Bact. Proc*. p. 106.
3. Gibbs B.M. and Hirsch A. (1956) *J. Appl. Bact*. 19. 129-141.
4. Gibbs B.M. and Freame B. (1965) *J. Appl. Bact*. 28. 95-111.
5. The Microbiology of Water 1994 Part 1 - *Drinking Water. Report on Public Health and Medical Subjects Number 71: Methods for the Examination of Waters and Associated Materials. HMSO London*.
6. Weenk G., Fitzmaurice E. and Mossel D.A.A. (1991) *J. Appl. Bact*. 70. 135-143.