TECHNICAL DATA SHEET

REINFORCED CLOSTRIDIAL AGAR

ENUMERATION OF ANAEROBES

1 INTENDED USE

Reinforced Clostridial Agar is a non-selective medium used for the isolation and enumeration of *Clostridia,* other anaerobic bacteria and lactobacilli in biological samples, dairy products, and other food products.

2 HISTORY

This medium was described by Hirsch and Grinsted for the isolation of *Clostridium butyricum*, in a solid medium. Barnes used it to enumerate *Clostridia* in foods. Attenborough & Scarr found an application for this composition in the enumeration of *Clostridium saccharolyticum* in sugar.

3 PRINCIPLES

This is a non-selective media and allows the growth of streptococci and lactobacilli as well as most anaerobes.

Nutritive factors are supplied by Tryptone, meat and yeast extracts, glucose and cysteine, the latter also acting as a reducing substance.

Starch favors spore germination.

Sodium chloride maintains the osmotic equilibrium.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Tryptone ..... 10,0 g
- Meat extract..... 10,0 g
- Yeast extract ..... 3,0 g
- Cysteine (chlorhydrate) ..... 0,5 g
- Glucose ..... 5,0 g
- Soluble starch ..... 1,0 g
- Sodium chloride ..... 5,0 g
- Sodium acetate ..... 3,0 g
- Bacteriological agar..... 15,0 g

pH of the ready-to-use media at 25 °C : 6,8 ± 0,2.

5 PREPARATION

- Dissolve 52,5 g of dehydrated media (BK090) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in tubes or vials.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool and maintain the media in a molten state at 44-47 °C.

✓ **Reconstitution :**
52,5 g/L

✓ **Sterilization :**
15 min at 121°C

6 INSTRUCTIONS FOR USE

- Transfer 1 mL of the test sample and its serial dilutions to empty, sterile Petri plates.
- Pour roughly 15 mL of molten media into each plate.
- Mix well by swirling and let solidify on a cold, flat surface.
- Incubate the plates in an anaerobic jar.
- Incubate at 30, 37 or at 55 °C for 1 to 10 days depending on the analytical protocol being followed.

✓ **Inoculation :**
1 mL in pour plates

✓ **Incubation :**
Dependent on protocol

7 RESULTS

Enumerate by retaining those plates who's colony count is between 15 and 150 colonies.

8 QUALITY CONTROL

Dehydrated media : cream-white powder, free-flowing and homogeneous.
Prepared media : amber agar.

Typical culture response after 48 hours of incubation under anaerobic conditions at 37 °C

Microorganisms		Growth (Productivity Ratio : P_R)
Clostridium perfringens	WDCM 00007	$P_R \geq 70$ %
Clostridium bifermentans	ATCC® 19299	$P_R \geq 70$ %
Clostridium tyrobutyricum	CNRZ 500	$P_R \geq 70$ %

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.
The expiration date is indicated on the label.

Prepared media in vials (*) : 180 days at 2-8 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :
500 g bottle BK090HA

11 BIBLIOGRAPHY

Hirsch, A. and Grinsted, E.. 1954. Methods for the growth and enumeration of anaerobic sporeformers from cheese, with observations on the effect of nisin. Journal of Dairy Research, 21 : 101-110.

Barnes, E.M. and Ingram, M.. 1956. The effect of redox potential on the growth of *Clostridium welchii* strains isolated from horse muscle. Journal of Applied Bacteriology, 19 : 117-122.

Munoa, F.J. and Pares, R.. 1988. Selective medium for isolation and enumeration of *Bifidobactericum* spp. Applied and Environmental Microbiology, 54 : 1715-1718.

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

Document code : RCM AGAR_ENv6.
Creation date : 01-2003
Updated : 05-2016
Origin of revision : General update.