
REINFORCED CLOSTRIDIAL MEDIUM

DETECTION OF CLOSTRIDIA

1 INTENDED USE

Reinforced Clostridial Medium is a non-selective medium used for the growth and enumeration of spores of total gas producing *Clostridia* in dairy products, canned foods, semi-preserved foods and other food products. It is also used for the detection of *Clostridia* in the microbiological examination of non-sterile products according to US and European Pharmacopeia.

The typical composition of the broth corresponds to the formula described in the European and US Pharmacopeia.

2 HISTORY

This medium was described by Hirsch and Grinsted for the enumeration of anaerobes, especially *Clostridium butyricum*, using the most probable number method.

3 PRINCIPLES

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.

The medium is non-selective. It allows the culture of lactobacilli as well as the majority of other anaerobic bacteria.

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.....	0,5 g

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

5 PREPARATION

- Dissolve 38,0 g of dehydrated media (BK094) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense while hot into tubes at 10 mL per tube.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool to room temperature.

✓ **Reconstitution :**
38,0 g/L

✓ **Sterilization :**
15 min at 121 °C

NOTE :

If the media has been prepared in advance, it is necessary to regenerate the media by heating it to 100°C for 20 minutes, then cooling to room temperature.

6 INSTRUCTIONS FOR USE

Detection of *Clostridia* in pharmaceutical products

- Heat 10 mL of the stock solution for 10 minutes at 80 °C in order to destroy the vegetative forms and activate the spores, then rapidly cool.
- Do not heat the second part of the stock solution in order to search for the vegetative forms.
- Inoculate 1 mL of sample into each tube of media.
- Incubate under anaerobic conditions for 48 hours at 30-35 °C
- Proceed to subcultures on Columbia agar (BK019).

✓ **Inoculation :**
1 mL per tube

✓ **Incubation :**
48 h at 30-35 °C

NOTE : According to the protocol used, anaerobic conditions may be obtained by the addition of 2 mL of sterile liquid paraffin per tube.

The media can also be used within the context of the MPN method, for the enumeration of anaerobic microorganisms.

7 RESULTS

Consider as positive, tubes that present turbidity (growth).

In the presence of paraffin, consider as positive the tubes presenting escaping gas bubbles and gas release that raises the solid paraffin plug.

8 QUALITY CONTROL

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

Prepared media : amber, semi-solid media.

Typical culture response after 24-48 hours of incubation in anaerobic conditions at 30-35 °C, inoculum ≤ 10² microorganisms

Microorganisms		Growth
<i>Clostridium sporogenes</i>	WDCM 00008	Positive

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.

The expiration date is indicated on the label.

Prepared media in tubes or vials (*) : 180 days at 2-8 °C. Regenerate the tubes before use.

(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :

500 g bottle BK094HA

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.

Gibbs, B.M. and Freame, B.. 1965. Methods for the recovery of *Clostridia* from foods. Journal of Applied Bacteriology, **28** : 95-111.

Pharmacopée Européenne. Chapitre 2.6.13. Contrôle microbiologique des produits non stériles : Recherche de microorganismes spécifiés.

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.

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