



Reinforced Clostridial Agar

Medium for detection of clostridia and other anaerobic bacteria from clinical and nonclinical specimens.

DESCRIPTION

Reinforced Clostridial Agar is a medium used for the cultivation and enumeration of anaerobes, especially *Clostridium* spp.

This medium is a solid version of Reinforced Medium for Clostridia which is recommended in the harmonized chapters of the Pharmacopoeia (USP/EP/JP) for the microbiological examination of non-sterile products.

TYPICAL FORMULA

	(g/l)
Beef Extract	10.0
Peptone	10.0
Yeast Extract	3.0
Soluble Starch	1.0
Glucose Monohydrate	5.0
Cystine Hydrochloride	0.5
Sodium Chloride	5.0
Sodium Acetate	3.0
Agar	15.0
Final pH 6.8 ± 0.2 at 25°C	

METHOD PRINCIPLE

Beef extract and peptone provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Yeast extract is a source of vitamins, particularly of B-group. Glucose provides energy. Cystine is a reducing agent. Sodium chloride maintains the osmotic balance of the medium. Sodium acetate acts as a buffer. Agar is the solidifying agent.

PREPARATION

<u>Dehydrated medium</u>	Suspend 52.5 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Sterilize in autoclave at 115°C for 15 minutes.
<u>Medium in bottles</u>	Melt the content of a bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool to 45-50°C, mix well avoiding foam formation before pouring.

TEST PROCEDURE

To inoculate Reinforced Clostridial Agar, the overlay method can be used: transfer 1 ml test sample or 1 ml initial suspension to duplicate plates or tubes, add the melted medium and mix carefully. Repeat the procedure with further dilutions. After the agar has solidified, pour the same medium as an overlay. Incubate anaerobically at 30-35°C for 24-48 hours. If thermophilic bacteria are suspected, prepare a second set of plates (or tubes) and incubate at 50-55°C.

INTERPRETING RESULTS

Count colonies. Report the count as CFU per ml of sample allowing for dilution factors.

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, light beige.
Prepared medium: slightly opalescent, light amber.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles: 2 years.

QUALITY CONTROL

The medium is inoculated with the microbial strains indicated in the QC table.

Inoculum for productivity: 50-100 CFU.

Incubation conditions: 24-48 h at 30-35°C in anaerobic atmosphere.

QC Table.

Microorganism		Growth
<i>Clostridium perfringens</i>	ATCC® 13124	Good
<i>Clostridium sporogenes</i>	ATCC® 19404	Good

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended *in-vitro* diagnostic use and must be used by properly trained operators only.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.








BIBLIOGRAPHY

1. European Pharmacopoeia 9.0 (2010) 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms.
2. United States Pharmacopoeia 40-NF 35 (2017) <62> Microbiological examination of non-sterile products: Test for specified microorganisms.
3. Japanese Pharmacopoeia 4.05 (2016) Microbiological examination of non-sterile products: Test for specified microorganisms.
4. ISO 6461-1:1986. Water quality -- Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) -- Part 1: Method by enrichment in a liquid medium.

PRESENTATION	Category	Packaging	Ref.
Reinforced Clostridial Agar	Tubes - Bottles	6 x 500 ml bottles	470270 ♦
Reinforced Clostridial Agar	Dehydrated media	500 g of powder	610096
Reinforced Clostridial Agar	Dehydrated media	100 g of powder	620096

♦, not CE marked

TABLE OF SYMBOLS

LOT Batch code	IVD <i>In vitro</i> Diagnostic Medical Device	 Manufacturer	 Use by	 Fragile, handle with care
REF Catalogue number	 Temperature limitation	 Contains sufficient for <n> tests	 Caution, consult Instruction For Use	 Do not reuse



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