

Reinforced Clostridial Medium (NCM0102)

Intended Use

Reinforced Clostridial Medium is used for the cultivation of anaerobes from food. This medium conforms to Harmonized USP/EP/JP Requirements and is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Reinforced Clostridial Medium (RCM) is recommended by the Harmonized Pharmacopoeia for the selective enrichment of *Clostridia* from non-sterile samples and conforms to USP/EP/JP performance specifications. Peptone, beef and yeast extract provide a source of nitrogen, essential vitamins and amino acids. Starch aids the detoxification of harmful metabolites and glucose is a fermentable carbohydrate. Sodium chloride provides osmotic balance and sodium acetate acts as a buffer. L-Cysteine hydrochloride acts as a reducing agent to create an anaerobic environment and maintain a low Eh. This is aided by the low level of agar which reduces the oxygen permeability through the medium. According to the Harmonized Pharmacopoeia, Reinforced Medium for Clostridia is used as a selective enrichment broth, with subculture performed onto Columbia Agar.

Typical Formulation

Beef Extract	10.0 g/L
Peptone	10.0 g/L
Yeast Extract	3.0 g/L
Soluble Starch	1.0 g/L
Glucose Monohydrate	5.0 g/L
Cysteine Hydrochloride	0.5 g/L
Sodium Chloride	5.0 g/L
Sodium Acetate	3.0 g/L
Agar	0.5 g/L

Final pH: 6.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Preparation

1. Suspend 38 grams of powder in 1 liter of purified water.
2. Heat with frequent agitation to completely dissolve the medium, if necessary.
3. Autoclave at 121°C for 15 minutes.

Test Procedure

For *Clostridium* spp. and other anaerobic bacteria refer to specific procedures in appropriate references.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is clear to slight haze, with no precipitate, and yellow.

Expected Cultural Response and USP/EP/JP Growth Promotion Testing: Cultural response in Reinforced Clostridial Medium inoculated with the organisms listed on the next page. Cultures were incubated aerobically at 30-35°C and examined for growth at 24 - 48 hours.



Technical Specification Sheet



Microorganism	Approx. Inoculum (CFU)	Expected Results
<i>Bacteroides fragilis</i> ATCC® 25285	10-100	Growth
<i>Clostridium novyi</i> ATCC® 7659	10-100	Growth
<i>Clostridium perfringens</i> ATCC® 13124	10-100	Growth
<i>Clostridium sporogenes</i> ATCC® 19404	10-100	Growth
<i>Lactobacillus fermentum</i> ATCC® 9338	10-100	Growth

The organisms listed are the minimum that should be used for quality control testing.

Results

Observe for organism growth. Further tests are required to identify *Clostridium* spp. and other anaerobic bacteria. Refer to appropriate references.

Expiration

Refer to expiration date stamped on the container. Dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. Further tests are necessary for confirmation of *Clostridium* spp.

Storage

Store dehydrated culture media at 2 – 30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. European Pharmacopoeia 10th Edition (2020)
2. United States Pharmacopoeia National Formulary 2018: USP 41 NF 36
3. Japanese Pharmacopoeia 17th Edition (2017)

