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# Reinforced Clostridial Medium

Reinforced Clostridial Medium is used for cultivating and enumerating clostridia, other anaerobes, and other species of bacteria from foods and clinical specimens.

| | | | |
|---|---|---|---|
| REF: BS.1/RCM01.100.0100 | 100 Gram | REF: BS.1/RCM01.250.0250 | 250 Gram |
| REF: BS.1/RCM01.500.0500 | 500 Grams | | |

## CLINICAL SIGNIFICANCE

Reinforced *Clostridial* Medium is a semisolid medium. It is recommended for the cultivation and enumeration of anaerobes, particularly *Clostridium* and other microorganisms, in foods and clinical specimens. It was formulated by Hirsch and Grinstead in 1954.Their work demonstrated that the medium outperformed other media in supporting the growth of Clostridium from small inoculum and produced higher viable cell counts.

## METHOD PRINCIPLE

Reinforced Clostridial Medium contains peptone and beef extract as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate source. Sodium chloride maintains the osmotic balance. In low concentrations, soluble starch detoxifies metabolic by-products. Cysteine HCl is the reducing agent. Sodium acetate acts as a buffer. The small amount of agar makes the medium semisolid.

| Item | Formula per liter of medium |
|---|---|
| Bacteriological agar | 0.5 gm |
| Glucose monohydrate | 5 gm |
| Peptone | 10 gm |
| Cysteine hydrochloride | 0.5 gm |
| Beef extract | 10 gm |
| Yeast extract | 3 gm |
| Sodium acetate | 3 gm |
| Soluble starch | 1 gm |
| Sodium chloride | 5 gm |

## MEDIA COMPOSITION

***Final pH 6.8 ± 0.2 at 25°C***

## PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  ***S56:*** dispose of this material and its container at hazardous or special waste collection point.
  ***S57:*** use appropriate container to avoid environmental contamination.
  ***S61:*** avoid release in environment.

For further information, refer to the MacConkey Agar material safety data sheet.

## STORAGE AND STABILITY

***BioScien*** Reinforced *Clostridial* medium should be stored between 10-30°C in a firmly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

## PREPARATION

- Suspend 38.00 grams in 1000 ml distilled water.
- Adjust pH to *6.8 ± 0.2 at 25°C.*
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 lbs. pressure (121°C) for 15 minutes.
- Pour into appropriate containers

### *Deterioration*

The color of ***BioScien*** Reinforced Clostridial medium is Cream to yellow homogeneous free flowing powder. Prepared Media is Upon cooling, amber and becomes more opalescent. If there are any physical changes for powder or signs of deterioration (shrinking, cracking, or discoloration), and contaminations for hydrated media, discard the medium.

## SPECIMEN

- Clinical samples (e.g., faeces, blood, and wounds).
- Pharmaceutical samples
- Food sample (milk and other dairyproducts)

## EQUIPMENT REQUIRED NOT PROVIDED

• Inoculating loops, swabs, collection containers
• Incubators
• Petri dish

## Expected Results

After appropriate incubation time and temperature, subculture each tube or bottle to two Columbia Agar plates. Incubate under both aerobic and anaerobic conditions for 48 hours at 30-35°C to confirm the presence of anaerobic growth. After incubation of these plates, if isolates grow anaerobically only (with or without endospores) and are catalase negative, this indicates the presence of Clostridium sp.1 Perform other confirmatory biochemical testing as necessary

## PERFORMANCE CHARACTERISTICS

| **Positive Controls for Reinforced Clostridial Medium (CM0149)**<br><br>Incubation Conditions: 24h @ 37°C aerobic | |
|---|---|
| *Clostridium perfringens* ATCC® 13124™ | Turbid growth and gas |
| *Clostridium sporogenes* ATCC® 11437™ | Turbid growth and gas |
| *Clostridium sporogenes* ATCC® 19404™ | Turbid growth and gas |
| *Clostridium tetani* ATCC® 9441™ | Turbid growth and gas |
| *Clostridium sphenoides* ATCC® 19403™ | Turbid growth and gas |

## QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control still out of range please contact ***BioScien*** technical support.

## REFERENCES


1. Public Health England. 2015a. 'Identification of Anaerobic Cocci'. UK Standards for Microbiology Investigations. ID 14 (3). https://www.gov.uk/government/publications/smi-id14-identification-of-anaerobic-cocci
2. Public Health England. 2015b. 'Identification of Anaerobic Gram-Negative Rods'. UK Standards for Microbiology Investigations ID 25 (2). https://www.gov.uk/government/publications/smiid-25-identification-of-anaerobic-gram-negative-rods
3. Brook, I., 2016. 'Spectrum and Treatment of Anaerobic Infections'. Journal of Infection and Chemotherapy22(1):1-13. https://doi.org/10.1016/j.jiac.2015.10.010.


| **SYMBOLS IN PRODUCT LABELLING** | |
|---|---|
| IVD For in-vitro diagnostic use | Number of <n> test in the pack |
| LOT Batch Code/Lot number | Caution |
| REF Catalogue Number | Do not use if package is damaged |
| Temperature Limitation | Consult Instruction for use |
| Expiration Date | |
| Manufactured by | |