



TECHNICAL DATA SHEET

AS-1339 | Reinforced Clostridial Medium (RCM)
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Reinforced Clostridial Medium (RCM) — Technical Data Sheet

Catalogue Number: AS-1339 | acc. harm. EP/USP/JP

Product Identification

Product Name	Reinforced Clostridial Medium (RCM)
Catalogue Number	AS-1339
HS Code	3821.00.00
Medium Type	Non-selective enriched reducing semi-solid medium — Clostridia and anaerobe cultivation
Pharmacopoeial Harmonisation	EP 2.6.13 USP <62> JP Chapter 4.05
Physical Form	Dehydrated powder
Colour (powder)	Beige to pale brown
Colour (prepared medium)	Clear to yellowish semi-solid
Also known as	RCM, Reinforced Clostridial Broth (RCB when without agar)
Related product	Reinforced Clostridial Agar (RCA) — solid format with 15 g/L agar

Mode of Action

RCM is free from selective inhibitors. **L-Cysteine hydrochloride** scavenges dissolved oxygen, maintaining a low oxidation-reduction potential (Eh) suitable for obligate anaerobes. **Sodium acetate** provides pH buffering and supplementary carbon for acetoclastic organisms. **Soluble starch** absorbs toxic metabolic byproducts. The semi-solid agar (0.5 g/L) retards O₂ diffusion, creating an anaerobic gradient at depth without gelling. Polymyxin B may be added post-sterilisation at 0.02 g/L to inhibit Gram-negative organisms selectively.

Composition (per litre of prepared medium)

Ingredient	CAS Number	Function	Amount (g/L)
Meat Extract	8001-31-8	Meat peptides, amino acids, glycogen, growth factors	10.0



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Peptone	73049-73-7	Nitrogen and carbon source — amino acids and peptides	10.0
Yeast Extract	8013-01-2	B-vitamins, nucleotides, coenzymes	3.0
D(+)-Glucose	50-99-7	Fermentable carbohydrate; energy source for anaerobic metabolism	5.0
Soluble Starch	9005-84-9	Absorbs toxic metabolic byproducts	1.0
Sodium Chloride (NaCl)	7647-14-5	Osmotic balance	5.0
Sodium Acetate (C ₂ H ₃ NaO ₂)	127-09-3	pH buffer; supplementary carbon source	3.0
L-Cysteine Hydrochloride	52-89-1	Primary reducing agent — O ₂ scavenger	0.5
Agar (semi-solid)	9002-18-0	Retards O ₂ diffusion; anaerobic gradient	0.5

Total: 38.0 g/L | Final pH: 6.8 ± 0.2 at 25 °C

Physical & Chemical Specifications

Appearance (powder)	Beige to pale brown homogeneous powder
Appearance (prepared)	Clear to yellowish semi-solid (not gelled)
pH (prepared medium, 25 °C)	6.8 ± 0.2
Loss on Drying (moisture)	≤ 5.0%
Solubility	Completely soluble with gentle heating
Agar concentration	0.5 g/L — semi-solid, not a gel; retards convection
Reducing agent	L-Cysteine HCl 0.5 g/L — no colour interference
Optional supplement	Polymyxin B 0.02 g/L — added post-autoclave for Gram-negative inhibition

Performance / Quality Control

Inoculum: ≤100 CFU per test organism. Incubation: anaerobic (paraffin overlay or anaerobic jar).

Organism	ATCC No.	Inoculum (CFU)	Incubation	Expected Result
Clostridium sporogenes	11437	≤ 100	48 h, 30–35 °C, anaerobic	Growth
Clostridium sporogenes	19404	≤ 100	48 h, 30–35 °C, anaerobic	Growth
Clostridium perfringens	10543	≤ 100	48 h, 30–35 °C, anaerobic	Growth
Clostridium novyi	17861	≤ 100	48 h, 30–35 °C, anaerobic	Growth
Escherichia coli (facultative)	25922	≤ 100	48 h, 30–35 °C, aerobic	Growth
Bacillus subtilis	6633	≤ 100	48 h, 30–35 °C, aerobic	Growth

Pharmacopoeial QC: Tests for Clostridium sporogenes must be performed as specified in EP 2.6.13, USP <62>, or JP Chapter 4.05 for non-sterile product testing compliance.

Preparation Protocol

1. Dissolve 38.0 g per litre in purified water with gentle heating and agitation.
2. Dispense into test tubes (8–10 mL per tube).
3. Autoclave at 121 °C for 15 minutes.
4. Cool to room temperature. Appearance: clear, yellowish semi-solid.
5. Optional Polymyxin B: add 0.02 g/L sterile aqueous solution aseptically post-autoclave.
6. For strict anaerobes: heat in boiling water bath 15 min; cool rapidly; inoculate immediately; overlay with sterile paraffin or 1.5% agar.

Key Limitations

- Not a selective medium — non-target organisms will grow alongside Clostridia
- Semi-solid format — subculture to solid RCA required for colony count and morphology
- Polymyxin B supplement required for selective Gram-negative inhibition
- Strict anaerobes require additional oxygen-exclusion measures (paraffin overlay, anaerobic jar, glove box)
- For pharmacopoeial compliance testing — follow exact EP/USP/JP method specifications



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Storage & Stability

Dehydrated powder	15–25 °C, tightly sealed, dry, protected from light and moisture
Prepared tubes	15–25 °C upright, sealed; use within 3 weeks
Shelf life	As per labelled expiry date

Regulatory & Quality Standards

EP 2.6.13	European Pharmacopoeia — Microbial examination of non-sterile products: Tests for specified microorganisms
USP <62>	United States Pharmacopoeia — Tests for specified microorganisms
JP Chapter 4.05	Japanese Pharmacopoeia — Microbial Limit Test II
Country of Manufacture	Australia

Literature & References

Hirsch, A. & 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(1), 101–110.

European Pharmacopoeia (EP), 11th Ed., Chapter 2.6.13 — Microbiological examination of non-sterile products: Tests for specified microorganisms.

United States Pharmacopoeia USP 38 NF 33 (2015). <62> Microbiological examination of non-sterile products: Tests for specified microorganisms.

Japanese Ministry of Health, Labour and Welfare (2011). *Japanese Pharmacopoeia*, 16th Ed., Chapter 4.05.

Willis, A.T. (1977). *Anaerobic Bacteriology: Clinical and Laboratory Practice*, 3rd Ed. Butterworths, London.

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