Rippey-Cabelli Agar Base

M859

Rippey-Cabelli Agar Base is recommended for differential and selective isolation of *Aeromonas hydrophila* from water samples using membrane filter technique.

Composition**

Ingredients	Gms / Litre
Tryptose	5.000
Trehalose	5.000
Yeast extract	2.000
Sodium chloride	3.000
Potassium chloride	2.000
Magnesium sulphate	0.200
Iron (III) Chloride	0.100
Bromo thymol blue	0.040
Agar	15.000
Final pH (at 25°C)	8.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 16.17 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add 5 ml ethanol and rehydrated contents of 1 vial of Rippey Cabelli Selective Supplement (FD107). Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Aeromonas species are natural inhabitants of aquatic environments worldwide. Their populations are seasonal in all natural waters. Aeromonads cause serious diseases of aquatic animals and represent an economic threat to the aquaculture industry (1). The motile aeromonads have emerged as a serious microbial threat to human populations, especially the immunocompromised (2). Aeromonads can be enumerated in water samples by employing the membrane filter technique. Rippey-Cabelli (RC) Agar, formulated by Rippey and Cabelli (3) is used for this purpose. The medium is differential as it depends on the ability of organisms to ferment trehalose and selective due to the incorporation of selective agents.

Tryptose and yeast extract support the growth of *Aeromonas* species. Bromothymol blue is the pH indicator, which changes from blue to yellow colour under acidic conditions, created due to fermentation of trehalose. Sodium chloride maintains the osmotic equilibrium whereas potassium chloride, magnesium sulphate and ferric chloride provide essential ions. Ampicillin, sodium deoxycholate and ethanol are the selective agents inhibiting gram-positive bacteria, coliforms, *Shigella* species, *Proteus mirabilis* and *Actinomyces* . Ethanol inhibits overgrowth of *Klebsiella* species on the filter (4). Most of the *Enterobacteriaceae* ferment trehalose, therefore it is difficult to distinguish *Aeromonas* from *Enterobacteriaceae* . The medium gives higher specificity and sensitivity when pure cultures are used (4, 5).

However, ampicillin is also unsuitable as a selective agent with Plesiomonas (6).

Membrane filters through which water samples have been filtered are aseptically placed on Rippey-Cabelli Agar Base plates (M859) and incubated at 35-37°C for 24 hours. *Aeromonas* ferments trehalose and forms yellow colonies.

Quality Control

Appearance

Light yellow to pale green homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Dark green coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.23% w/v aqueous solution at 25°C. pH : 8.0±0.2

pH
7.80-8.20

Cultural Response
M859: Cultural characteristics observed with added Rippey-Cabelli Suplement(FD107) after an incubation at 35-37°C for 24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Trehalose fermentation
Aeromonas hydrophila ATCC 7966	50-100	good-luxuriant	>=50%	positive reaction, yellow colour
Escherichia coli ATCC 25922	50-100	none-poor	<=10%	negative reaction,blue green colour
Shigella flexneri ATCC 12022	>=10^3	inhibited	0%	
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%	

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
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3. Rippey S. R. and Cabelli V. J., 1979, Appl. Environ. Microbiol., 38(1): 108.
4. MacFaddin J. F., 1985, Media for Isolation-Identification-Cultivation-Maintenance of Medical Bacteria, Vol. I Williams and Wilkins, Baltimore.
5. Roland, F. P., 1977, Med. Microbiol. Immunol., 163:241.
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