RS Medium Base

M576

Rimler-Shotts (RS) Medium Base is used for selective isolation, cultivation and presumptive identification of *Aeromonas hydrophila* .

Composition**

Ingredients	Gms / Litre
Yeast extract	3.000
Maltose	3.500
L-Cysteine hydrochloride	0.300
L-Lysine hydrochloride	5.000
L-Ornithine hydrochloride	6.500
Sodium thiosulphate	6.800
Ferric ammonium citrate	0.800
Sodium deoxycholate	1.000
Sodium chloride	5.000
Bromothymol blue	0.030
Agar	13.500
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 45.43 grams in 990 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and aseptically add rehydrated content of 1 vial of Novobiocin Supplement (FD096). Mix well and pour into sterile Petri plates.

Principle And Interpretation

RS Medium was formulated by Rimler and Shotts (2) based on the principle of Xylose-Lysine (XL) Agars (3, 4). It is used for selective isolation and presumptive identification of *Aeromonas hydrophila* and other gram-negative bacteria based on their ability to decarboxylate lysine and ornithine, maltose fermentation and H2S production (1).

Yeast extract acts as a source of nutrients. Sodium thiosulphate, L-cysteine hydrochloride and ferric ammonium citrate are the indicators of H2S production. The medium contains maltose, which is mostly fermented by all *Aeromonas* . Maltose fermentation is indicated by bromothymol blue. Sodium deoxycholate and novobiocin inhibit gram-positive bacteria and *Vibrio* species. *Citrobacter freundii* usually produce H2S but occasionally negative strains exist. The medium contains L-cysteine and L-ornithine, which are often decarboxylated by enteric bacteria to give alkaline products. Lysine positive and ornithine positive strains of *Aeromonas* may not have the typical strong yellow colour because of alkaline products produced during decarboxylation of the amino acids. Results are interpreted within 24 hours since after 26 hours slow reversion of yellow colour to a basic (green) colour occurs. Medium should be incubated at 35°C, which will eliminate possible growth of *Aeromonas salmonicida* , which may grow at reduced temperatures giving false-positive reaction. Test the yellow colonies with or without black centers (of H2S) for oxidase to rule out *Citrobacter* species. *Proteus mirabilis* is inhibited on this medium.

Quality Control

Appearance

Light yellow to light green homogeneous free flowing powder

Gelling

Firm, comparable with 1.35% Agar gel.

Colour and Clarity of prepared medium

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

Reaction
Reaction of 4.54% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 24 hours with added Novobiocin Supplement(FD096)

Cultural Response

Organism	Inoculum (CFU)	Growth	Maltose fermentation	Lysine/ Ornithine decarboxylation	H2S
Aeromonas hydrophila ATCC 7966	50-100	good	positive reaction, yellow coloured colonies	negative reaction	negative reaction
Citrobacter freundii ATCC 8090	50-100	good	negative reaction	variable reaction	positive, black centered colonies
Escherichia coli ATCC 25922	50-100	good	negative reaction	variable reaction	negative reaction
Proteus vulgaris ATCC 13315	50-100	good	positive reaction, yellow coloured colonies	negative reaction	positive, black centered colonies
Salmonella Typhi ATCC 6539	50-100	good	positive reaction, yellow coloured colonies	negative reaction	negative reaction

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

Reference
1.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
2.Shotts E. B. Jr. and Rimler R., 1973, Appl. Microbiol., 26(4):550.
3.Taylor W. I. and Harris B., 1965, Am. J. Clin. Pathol., 44:476.
4.Taylor W. I., 1965, Am. J. Clin. Pathol., 44:471.

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