RS Medium Base (Rimler-Shotts Medium Base) M576

Intended use

Used for selective isolation, cultivation and presumptive identification of *Aeromonas hydrophila* from clinical and nonclinical samples.

Composition**

Ingredients	g / L
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 purified/distilled water. Heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE**. Cool to 45-50°C and aseptically add rehydrated content of one vial of NO 5 Selective Supplement (FD096). Mix well and pour into sterile Petri plates.

Principle And Interpretation

RS Medium was formulated by Rimler and Shotts (1) based on the principle of Xylose-Lysine (XL) Agars (2,3). 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 H_2S production (4).

Yeast extract acts as a source of nutrients. Sodium thiosulphate, L-cysteine hydrochloride and ferric ammonium citrate are the indicators of H_2S 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 H_2S 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 H_2S) for oxidase to rule out *Citrobacter* species. *Proteus mirabilis* is inhibited on this medium.

Type of specimen

Clinical samples - Stool, Food samples; Water samples.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (7).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (8). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. The media should be handled by trained personnel only. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Pure isolate must be used.
2. Results are interpreted within 24 hours since after 26 hours slow reversion of yellow colour to a basic (green) colour occurs.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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 with added NO 5 Selective Supplement (FD096) after an incubation at 35-37°C for 24 hours .

Organism	Growth	Maltose fermentation	Lysine/ Ornithine decarboxylation	H_2S
Aeromonas hydrophila ATCC 7966 (00063*)	good	positive reaction, yellow coloured colonies	negative reaction	negative reaction
Citrobacter freundii ATCC 8090	good	negative reaction	variable reaction	positive, black centered colonies
Escherichia coli ATCC 25922 (00013*)	good	negative reaction	variable reaction	negative reaction
$ Proteus hauseri ATCC 13315	good	positive reaction, yellow coloured colonies	negative reaction	positive, black centered colonies
Salmonella Typhi ATCC 6539	good	positive reaction, yellow coloured colonies	negative reaction	negative reaction

Key : * Corresponding WDCM numbers $ Formerly known as *Proteus vulgaris*

Storage and Shelf Life

Store between 10-30°C in a tightly 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 prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in 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.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

References

1.Shotts E. B. Jr. and Rimler R., 1973, Appl. Microbiol., 26(4):550.
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3.Taylor W. I., 1965, Am. J. Clin. Pathol., 44:471.
4.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
5.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
7.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
8.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

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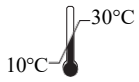
IVD *In vitro* diagnostic medical device



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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CE **CE Marking**

Do not use if package is damaged