Semisolid Rappaport Vassiliadis Medium, Modified

M1282

Intended Use

Used for the detection of motile *Salmonella* species from food, faeces and environmental specimens.

Composition**

Ingredients	g / L
Tryptose	4.590
Tryptone	4.590
Sodium chloride	7.340
Potassium dihydrogen phosphate	1.470
Magnesium chloride, anhydrous	10.930
Malachite green oxalate	0.037
Agar	2.700
Final pH (at 25°C)	5.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.66 grams in 1000 ml distilled water. Heat to boiling with frequent agitation to dissolve the medium completely. **DO NOT AUTOCLAVE.** Cool to 45-50°C and aseptically add the rehydrated contents of 1 vial of NO 20 Selective Supplement (FD290) or 4 vials of NO 5 Selective Supplement (FD096). Mix well and pour into sterile Petri plates. Air dry the plated medium at room temperature for at least one hour.

Principle And Interpretation

Semisolid Rappaport Vassiliadis Medium, Modified is prepared as per the formulation described by DeSmedt et al (1), for the detection of motile *Salmonella* species from food and environmental specimens. This medium detects more *Salmonella* positive samples than the routinely used enrichment procedures (2,3,4).

Tryptose, tryptone provide the nitrogenous and carbonaceous substances and other essential growth nutrients. Phosphate gives good buffering capacity to the medium. Malachite green oxalate and Novobiocin inhibits many gram positive bacteria. The working of medium is based on the ability of *Salmonella* species to migrate in the selective medium by competing with the other motile organisms, thus producing opaque halos of growth. This medium can be used in combination with direct culture and Selenite F Broth (M052A) enrichment for isolation of *Salmonella* species from faeces and subculturing on XLD Agar (M031) or Mannitol Lysine Agar (M1071) results in higher recovery rates (5). This medium is not suitable for the detection of non-motile strains of *Salmonella* (6). Inoculate 3 drops (0.1 ml) of pre-enrichment culture (16-20 hours old) in separate spots on the air-dried medium surface. Incubate the plates in an upright position at 42°C for upto 24 hours. The motile bacteria will show a halo or zone of growth originating from inoculation spot. *Salmonella* species show straw coloured colonies. Sub-cultures can be carried out from the outside edge of the halo to confirm purity and for further biochemical and serological tests.

Type of specimen

Clinical samples - Faeces, Food samples : meat and meat products

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (9).

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. 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. Due to variable nutritional requirements, some strains show poor growth on this medium.
2. The plates should be incubated in upright and steady position as medium contains less agar.
3. Air dried plate should be used, or it may give erroneous results.

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 blue homogeneous free flowing powder

Gelling

Semisolid, comparable with 0.27% Agar gel.

Colour and Clarity of prepared medium

Greenish blue coloured clear to slightly opalescent semisolid gel forms in Petri plates which may have a slight precipiate.

Reaction

Reaction of 3.16% w/v aqueous solution at 25°C. pH : 5.2±0.2

pH

5.00-5.40

Cultural Response

Cultural characteristics observed after an incubation at 42-43°C for 18-24 hours with added NO 20 Selective Supplement (FD290) or NO 5 Selective Supplement (FD096).

Organism	Inoculum (CFU)	Growth at 42±1°C	Motility
Citrobacter freundii ATCC 8090 (00006*)	50-100	none-poor	negative reaction, no colour change
Escherichia coli ATCC 25922 (00013)*	50-100	none-poor	negative reaction, no colour change
Salmonella Typhi ATCC 6539	50-100	good-luxuriant	positive reaction, colourless to light pink zone
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant	positive reaction, colourless to light pink zone
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	positive reaction, colourless to light pink zone

Key : *Corresponding WDCM numbers.

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 (7,8).

Reference

1. De Smedt J.M., Balderdijk R., Rappold H. and Lautenschlaeger D., 1986, J. Food Prot., 49:510.
2. De Smedt J.M., Balderdijk R., 1987, J. Food Prof., 50:658.
3. De Zutter L. et al, 1991, Int. J. Food Microbiol., 13:11.
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5. Holbrook R. et al, 1989, Lett. Appl. Microbiol., 8:139.
6. Aspinall S.T., Hindle M.A. and Hutchinson D.N., 1992, Europ. J. Clin. Microbiol. Inf. Dis., 11:936.
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
8. 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.
9. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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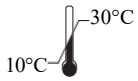
IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE Marking

Do not use if package is damaged

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