



Modified Semisolid RV Medium Base

M1482

A semisolid medium for detection of motile *Salmonella* species from food and environmental specimens

Composition**

Ingredients	Gms / Litre
Peptone, special	8.250
Yeast extract	0.920
Sodium chloride	7.330
Potassium dihydrogen phosphate	1.470
Magnesium chloride, anhydrous	12.370
Malachite green oxalate	0.037
Agar	2.570
Final pH (at 25°C)	5.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 32.95 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 115°C for 15 minutes. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial of IMRV/ RV Selective Supplement (FD193). Mix well and dispense as desired (butts or Petri plates)

Note: The motility of *Salmonellas* can be drastically reduced when the agar surface becomes too dry. Hence the plates should be well dried before use. If visible moisture occurs on the lid of the plates or the surface of agar, it must be removed. While incubation, incubate the plates aerobically in an upright position for no longer than 24 hours at respective temperatures.

Principle And Interpretation

Salmonella species have been isolated from humans and almost all animals throughout the world. They cause many types of infections from mild, self-limiting gastroenteritis to life threatening typhoid fever (1).

Successful isolation of *Salmonella* is often both time-consuming and complex. As over 90% of all *Salmonella* species are motile, a number of isolation procedures have been based on motility. Use of Modified Semisolid RV Medium Base provides a method for isolating motile *Salmonella* species from clinical, food and environmental samples (2,3,4,5).

Peptone special provide the nitrogenous and carbonaceous substances and other essential growth nutrients. Yeast extract serves as a source of B complex vitamins. Phosphate gives good buffering capacity to the medium. Malachite green serves as a selective agent. Addition of novobiocin as a supplement (FD193) in the medium selectively inhibits most gram-positive organisms. Sodium chloride maintains the osmotic equilibrium of the media. *Salmonella* generally survives a little high osmotic pressure (due to MgCl₂ in the medium), grows at slightly low pH and are resistant to malachite green compared to other bacteria. All these factors make the medium selective for the isolation of *Salmonella*. These medium enrich *Salmonella* and the semi-solid nature of the medium helps to differentiate the motile *Salmonella* from non-motile ones.

The working of these media is based on the ability of *Salmonella* species to migrate in the selective medium competing other motile organisms, thus producing opaque halos of growth. The motile bacteria will show a halo or zone of growth originating from inoculation spot. These media can be used in combination with direct culture. Selenite F Broth (M052) can be used for enrichment, while for isolation of *Salmonella* species, XLD Agar(M031) can be used. Subculturing on XLD Agar (M031) or Mannitol Lysine Agar (M1071) results in higher recovery rates (6). These medium is not suitable for the detection of non-motile strains of *Salmonella* (7).

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.

Quality Control

Appearance

Light yellow to light blue homogeneous free flowing powder

Gelling

Semisolid, comparable with 0.26% Agar gel.

Colour and Clarity of prepared medium

Blue coloured clear to slightly opalescent gel forms in Petri plates or in tubes as butts.

Reaction

Reaction of 3.29% w/v aqueous solution at 25°C. pH : 5.5±0.2

pH

5.30-5.70

Cultural Response

M1482: Cultural characteristics observed after an incubation at different temperatures for 18-24 hours with added IMRV/RV Selective Supplement (FD193).

Organism	Inoculum (CFU)	Growth at 35-37°C	Growth at 42±1°C	Motility
Cultural Response <i>Salmonella Paratyphi B</i> ATCC 8759	50-100	good	good	positive, opaque halos of growth originating from inoculation spot in Petri plates or opaque growth dispersing away from stabline in butts.
<i>Salmonella Typhi</i> ATCC 6539	50-100	fair-good	good	positive, opaque halos of growth originating from inoculation spot in Petri plates or opaque growth dispersing away from stabline in butts.
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	good-luxuriant	luxuriant	positive, opaque halos of growth originating from inoculation spot in Petri plates or opaque growth dispersing away from stabline in butts.

Storage and Shelf Life

Store below 30°C and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

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