Sellers Differential Agar

M293

Sellers Differential Agar is used for differentiation and identification of gram-negative non-fermentative bacilli especially *Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus* .

Composition**

Ingredients	Gms / Litre
Yeast extract	1.000
Peptic digest of animal tissue	20.000
L-Arginine	1.000
D-Mannitol	2.000
Sodium chloride	2.000
Sodium nitrate	1.000
Sodium nitrite	0.350
Magnesium sulphate	1.500
Dipotassium phosphate	1.000
Bromo thymol blue	0.040
Phenol red	0.008
Agar	15.000
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 44.90 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in test tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 10 minutes. Cool the tubed medium in slanted position. Just before inoculation add 0.15 ml or 2 drops of 50% sterile dextrose solution to each slant by letting it run down the side of the tube opposite the slant.

Principle And Interpretation

Sellers Differential Agar is formulated as described by Sellers (1) for differentiation and identification of non-fermentative gram-negative bacilli especially *Pseudomonas aeruginosa* , *Acinetobacter calcoaceticus* and *Alcaligens faecalis* . The medium is complex with differentiation ability based on oxidation of dextrose, fluorescence, production of nitrogen and pH changes.

Yeast extract and peptic digest of animal tissue are the sources of carbon and nitrogen compounds as well as vitamins and minerals. Oxidation of dextrose by the organisms is readily visible as a yellow band at the slant-butt junction. The dextrose added prior to inoculation diffuses into the medium during incubation period. *P. aeruginosa* exhibits acid reaction from dextrose. However, the reaction is masked by deamination of arginine and high peptone concentration. Most of *Acinetobacter* species produce a yellow band due to glucose oxidation. This band may disappear after 24 hours. D-Mannitol and magnesium sulphate stimulate fluorescence while nitrogen gas production is stimulated by dipotassium phosphate (1, 2). Sodium nitrate and nitrite serve as substrates for the production of nitrogen gas for denitrifying bacteria. Phenol red and bromothymol blue are the pH indicators. Arginine dihydrolase positive reaction is indicated by the formation of blue colour. Inoculation is done by stabbing deep into the butt and streaking the slant.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Green coloured clear to slightly opalescent gel forms in tubes as slants with a butt.

Reaction
Reaction of 4.5% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH
6.50-6.90

Cultural Response
M293: Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Organism	Inoculum (CFU)	Growth	Slant	Butt	Band	Fluorescence(under uv)
Cultural Response						
Acinetobacter baumannii ATCC 19606	50-100	good	blue	green	yellow	negative
Alcaligenes faecalis ATCC 8750	50-100	good	blue	blue-green	none	positive
Pseudomonas aeruginosa ATCC 27853	50-100	good	blue-green	blue-green	blue	positive

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
1. Sellers W., 1964, J. Bacteriol., 87:46.
2. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.

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