MUG Violet Red Bile Agar

M1058

MUG Violet Red Bile Agar is used as a selective medium for the detection and enumeration of coliform organisms by a fluorogenic procedure.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	7.000
Yeast extract	3.000
Bile salts mixture	1.500
Lactose	10.000
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.002
4-Methylumbelliferyl ß-D-glucuronide (MUG)	0.100
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 41.63 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Cool the medium to 45-50°C and pour into sterile Petri plates. DO NOT AUTOCLAVE.

Principle And Interpretation

Escherichia coli is used as an indicator organism to determine unsanitary conditions. A number of selective media are recommended for use in enrichment, presumptive identification and confirmatory procedures for demonstrating the presence of coliforms. These procedures require longer incubation period. Violet Red Bile Agar is recommended by APHA (1, 2) for the detection and enumeration of coliforms in foods and dairy products. Addition of MUG to this medium permits the rapid detection of *E.coli* , when the medium is observed for fluorescence under UV light, requiring no further confirmation (3). *E.coli* possesses the enzyme beta-glucuronidase which specifically cleaves MUG to form a fluorogenic compound 4-methylumbelliferone, which results in visible blue-green fluorescence. MUG Violet Red Bile Agar is therefore recommended for the specific detection of *E. coli* (1, 2-4).

Peptic digest of animal tissue, yeast extract and lactose provide essential nutrients. Crystal violet and bile salts inhibit some gram-positive and gram-negative bacteria. Neutral red acts as a pH indicator and helps to exhibit red colonies in the presence of acid from lactose fermentation. Acidic pH decreases the intensity of fluorescence(8), thus making it difficult to identify fluorescent *E.coli* . The plates after primary identification i.e. red colonies surrounded by bile precipitate were exposed to ammonia fumes to increase fluorescence as suggested by Freir and Hartman (6) The substrate, MUG is hydrolysed by an enzyme beta-glucuronidase, which is present in most of *E. coli* and a few strains of *Salmonella*, *Shigella* and *Yersinia* to yield a fluorescent end product, 4-methylumbelliferone (5). *Proteus vulgaris* in large numbers may suppress gas production by *E. coli* .

Quality Control

Appearance
Light yellow to light pink homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Reddish purple coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.16% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
M1058: Cultural characteristics observed after an incubation at 35-37°C for 20-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Fluorescence under uv *
Cultural Response					
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	>=50%	pinkish red -red	negative
Escherichia coli ATCC 25922	50-100	luxuriant	>=50%	pinkish red -red w/bile ppt.	positive

Key : * - Fluoroscence can be visualized by addition of NaOH solution or exposure to ammonia fumes

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.Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.
2.Marshall, (Ed.), 1985, Standard Methods for the Examination of Dairy Products, 16th Ed., APHA, Washington, D.C.
3.Feng P. C. S. and Hartman P. A., 1982, Appl. Environ. Microbiol., 43 :1320.
4.FDA Bacteriological Analytical Manual, 8th Edi, AOAC International,Gaithersburg
5.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
6.Freir T.A. and Hartman P.A. (1987) Appl. Env. Microbiol. 53. 1246-1250

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