Technical Information

Modified Bile Esculin Azide Agar

Product Code: DM 2150

Application: - Modified Bile Esculin Azide Agar is recommended for selective isolation and enumeration of group D Streptococci.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	17.000
Peptic digest of animal tissue	3.000
Yeast extract	5.000
Oxgall	10.000
Sodium chloride	5.000
Sodium citrate	1.000
Esculin	1.000
Ferric ammonium citrate	0.500
Sodium azide	0.250
Agar	13.500
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Group D Streptococci contain the group D lipoteichoic acid antigen in their cell walls. Group D species, are predominant normal inhabitants of the human gastrointestinal tract, are named as faecal streptococci or Enterococci [1]. Meyer and Schonfeld [2] reported the unique ability of Enterococci to split esculin by Enterococci and group D streptococci hydrolyze esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate [3]. The use of esculin hydrolysis in identification of Enterococci was first reported by Rochaix [4]. However, other tests such as salt tolerance should be performed for identifying Enterococci [5]. Modified Bile Esculin Azide Agar was formulated according to Isenberg et al [6], Swan [7], Facklam and Moody [5] and Meyer and Schonfeld [2]. They reported that esculin hydrolysis and bile tolerance permit the isolation and identification of group D streptococci in 24 hours.

Casein enzymic hydrolysate, peptic digest of animal tissue, yeast extract provide all essential growth nutrients. Streptococci hydrolyze esculin to esculetin which reacts with ferric ions to form a dark brown to black coloured complex [3]. Oxgall inhibits most of the gram-positive bacteria other than Enterococci. Sodium azide inhibits gram-negative organism except some *Proteus* species.

Methodology

Suspend 56.25 grams of powder media in 1000 ml distilled water. Shake well and heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Warning: Sodium Azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

Quality Control

Physical Appearance

Cream to brownish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.35% Agar gel

Colour and Clarity of prepared medium

Medium amber coloured clear to slightly opalescent solution with a bluish tinge forms in Petri plates.

Reaction

Reaction of 5.63% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH Range 6.90-7.30

Cultural Response/Characteristics

DM2150: Cultural characteristics observed after an incubation at 35 - 37°C for 18 -24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
Enterococcus faecalis ATCC 29212	50-100	luxuriant	>=50%	Positive reaction blackening of medium around the colony
Proteus mirabilis ATCC 25933	50-100	Fair-goof	30-40%	Negative reaction
Streptococcus pyogenes ATCC 19615	50-100	none-poor	<=10%	Negative reaction
Streptococcus bovis ATCC 27960	50-100	Luxuriant	>=50%	Positive reaction blackening of medium around the colony
Staphylococcus aureus ATCC 25923	50-100	good	40-50%	Negative reaction

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr.,1992, colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed., J. B. Lippinccott Company
2. Meyer K. and Schonfeld H., 1926, Zentralbl Bakteriol Parasitnek Infectionskr. Hyg. Abt Oxig.99:402.
3. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore
4. Rochaix E. R., 1924, Comt Rend Soc. Biol. 90 : 771
5. Facklam R. and Moody M., 1970, Appl. Microbiol. 20 (2): 245
6. Isenberg H. D., Goldberg D., and Sampson J., 1970, Appl. Microbiol., 20 (3): 433
7. Swan A., 1954, J. Clin. Pathol., 7 : 160

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- User must ensure suitability of the product(s) in their application prior to use.
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