Technical Information

Kanamycin Esculin Azide Agar

Product Code: DM 1510

Application: - Kanamycin Esculin Azide Agar is used for isolation of Group D Streptococci in foodstuffs.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	20.000
Yeast extract	5.000
Sodium chloride	5.000
Sodium citrate	1.000
Esculin	1.000
Ferric ammonium citrate	0.500
Sodium azide	0.150
Kanamycin sulphate	0.020
Agar	12.000
Final pH (25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Enterococci may be considered an essential part of the autochthonous microflora of humans and animals. Faecal streptococci having the group D Lancefield antigens are grouped as Enterococci. Lancefield Group D-Streptococci constituting the faecal Streptococci are contaminants of various food commodities, especially those of animal origin. Kanamycin Esculin Azide Agar devised by Mossel et al [1, 2] is used to detect Enterococci in foodstuffs. They used this media for the dip slide technique for bacteriological monitoring of foods [3].

Casein enzymic hydrolysate and yeast extract provides essential nutrients for Enterococci. Kanamycin sulphate and sodium azide are the selective inhibitory components. Esculin and ferric ammonium citrate together forms the indicator system to detect esculin-hydrolyzing Streptococci, which form black zones around the colonies. The black zones are produced from the formation of black iron phenolic compounds derived from esculin-hydrolysis products and ferrous ions. Mossel et al [4] described the following procedure - 1gm or 1ml mixed food is added to 9 ml of pre-chilled diluent (Tryptone water DM1463) and tentold dilutions are prepared. The tentold dilutions are inoculated in Kanamycin Esculin Azide Broth (DM1776) and incubated at 35-37°C for 16-24 hours. If blackening of medium occurs, streaking is done on agar (DM1510) and after incubation confirmatory tests are carried out.

Kanamycin Esculin Azide Agar has been used successfully for the isolation of glycopeptide-resistant Enterococci from clinical specimens and foods [5, 6]. There is no universal medium that will isolate all strains of Enterococci [7]. Unless a presumptive count is acceptable the identity of all isolates should be confirmed with other tests.

Methodology

Suspend 44.67 grams of powder medium in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Dispense as desired.

Caution: Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush of the disposables.

Quality Control

Physical Appearance
Cream to yellow w/greenish tinge homogeneous free flowing powder

Gelling
Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium
Medium amber coloured, clear to slightly opalescent gel with purplish tinge forms in Petri plates.

Reaction
Reaction of 4.47% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH Range 6.80-7.20

Cultural Response/ characteristices
DM 1510: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
Enterococcus bovis ATCC 27960	50-100	good-luxuriant	>=50%	positive, blackening of medium around the colony
Enterococcus faecium ATCC 19434	50-100	good-luxuriant	>=50%	positive, blackening of medium around the colony
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	
Enterococcusfaecalis ATCC 29212	50-100	good-luxuriant	>=50%	positive, blackening of medium around the colony
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%	

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^{o} in sealable plastic bags for 2-5 days.

Further Reading

1. Mossel D. A. A., Bijker P. G. H. and Eelderink I., 1978, Arch. Lebensmittel - hyg., 29: 121.
2. Mossel D. A .A. el al, 1978, In : `Streptococci., Skinner F. A. and Quesnel L. B. (Eds.), SAB Symposium, Series No.7, Academic Press, London.
3. Mossel D. A. A. et al, 1976, Lab. Practice, 25:3 93.
4. Mossel D. A. A., Harrenwijn G. A. and Elzebroek B. J. M., 1973, UNICEF, Geneva.
5. Chadwick P. R., Brown D. F. J., Wilcox M. H. et al, 1997, Clin. Microbiol., Inf. 3. 559-563.
6. Van den Braak N., Van Belkum A., Van Keulen. M et al, 1998, J. Clin. Microbiol., 36. 1927-1932.
7. Reuter G., 1985, Inter. J. Food. Microbiol., 2.103-114.

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