Kanamycin Esculin Azide Agar

M510

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 (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 44.67 grams in 1000 ml distilled water. Heat to boiling 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 off the disposables .

Principle And Interpretation

Enterococci may be considered an essential part of the autochthonous microflora of humans and animals. Faecal streptococci bearing 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 is formulated as per Mossel et al (1, 2) to detect Enterococci in foodstuffs. Mossel et al (3) used it for the dip slide technique for bacteriological monitoring of foods.

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 M463) and decimal dilutions are prepared. The decimal dilutions are inoculated in Kanamycin Esculin Azide Broth (M776) and incubated at 35-37°C for 16-24 hours. If blackening of medium occurs, streaking is done on agar (M510) 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 all isolates should have their identity confirmed with further tests.

Quality Control

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
6.80-7.20

Cultural Response
M510: 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%	
Enterococcus faecalis 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
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.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, 25:393.
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.

Revision : 1 / 2011

CE