



Kanamycin Esculin Azide Agar Base

M510A

Intended Use:

Recommended for selective isolation and identification of group D Streptococci in foodstuff.

Composition**

Ingredients	g / L
Tryptone	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
Agar	10.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 21.32 grams in 500 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45 50°C and aseptically add rehydrated contents of one vial of KS Selective Supplement (FD146). Mix well before pouring into sterile petri plates.

Principle And Interpretation

Kanamycin Esculin Azide media are formulated as per Mossel et al (1,2) to detect Enterococci in food stuffs. Mossel et al (3) used it for the dip slide technique for bacteriological monitoring of foods.

Tryptone, yeast extract provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients for Enterococci. Kanamycin sulphate and Sodium azide are the selective inhibitory components. Esculin and Ferric ammonium citrate together form indicator system to detect esculin - hydrolysing Streptococci and imparts black zones around the colonies.

Mossel et al (4) adopted the following procedure as - 1gm or 1ml mixed food is added to prechilled diluent (Tryptone water M463) and decimal dilutions are prepared. The decimal dilution are inoculated in Kanamycin Esculin Azide Broth (M776) and incubated at 35°C for 16 -24 hours.

Type of specimen

Food and dairy samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. The media is intended for primary isolation, further confirmatory tests to be done for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to light brown coloured homogeneous free flowing powder

Gelling

Firm, comparable with 1.0% 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 4.26% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed with added KS Selective Supplement (FD146) , after an incubation at 35-37°C or 42°C for 18-24 hours .

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
<i>Enterococcus bovis</i> ATCC 27960	50-100	luxuriant	≥50%	Positive reaction, blackening of medium around the colony
<i>Enterococcus faecium</i> ATCC 19434 (00010*)	50-100	luxuriant	≥50%	Positive reaction, blackening of medium around the colony
<i>Escherichia coli</i> ATCC 25922 (00013*)	≥10 ⁴	inhibited	0%	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

- Mossel D.A.A., Bijker, P.G.H. and Eelderink I., 1978, Arch. Lebensmittel - Hyg., 29:121.
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- Mossel D.A.A., Harrenwijn G.A. and Elzebroek B.J.M., 1973, UNICEF Geneva.
- Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015. Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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- Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
- Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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