



# Kanamycin Esculin Azide Broth

# Intended Use:

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

## **Composition\*\***

Ingredients	Gms / Litre
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
Kanamycin sulphate	0.020
Final pH ( at 25°C)	7.0±0.2

\*\*Formula adjusted, standardized to suit performance parameters

## Directions

Suspend 32.67 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flask as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

# **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 Broth is formulated as per Mossel et al (3,4) to detect Enterococci in foodstuffs. Mossel et al (5) used it for the dip slide technique for bacteriological monitoring of foods.

Tryptone 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.

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.

## Type of specimen

Food samples

### **Specimen Collection and Handling**

Mossel et al (6) 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 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.

## 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. Further biochemical and serological tests must be carried out 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.

**M776** 

## **Quality Control**

#### Appearance

Cream to yellow w/greenish tinge homogeneous free flowing powder

#### Colour and Clarity of prepared medium

Medium amber coloured, clear solution in tubes.

#### Reaction

Reaction of 3.27% w/v aqueous solution at 25°C. pH : 7.0±0.2

#### pН

6.80-7.20

#### **Cultural Response**

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

Organism	Inoculum	Growth	Esculin
	(CFU)		Hydrolysis
Enterococcus bovis ATCC	50-100	good-luxuriant	positive,
27960			blackening of
			medium
Enterococcus faecium ATCC	50-100	good-luxuriant	positive,
19434 (00010*)			blackening of
			medium
Escherichia coli ATCC	>=104	inhibited	
25922 (00013*)			
Enterococcus faecalis ATCC	50-100	good-luxuriant	positive,
29212 (00087*)		e	blackening of
			medium
Staphylococcus aureus	>=104	inhibited	
subsp. <i>aureus</i> ATCC			
25923 (00034*)			

Key: (\*) Corresponding WDCM numbers.

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°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 (1,2).

### Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

- 2. 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.
- 3. Mossel D. A. A., Bijker P. G. H. and Eelderink I., 1978, Arch. Lebensmittel hyg., 29:121.

4. 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.

- 5. Mossel D. A. A. et al, 1976, Lab. Practice, 25:393.
- 6. Mossel D. A. A., Harrenwijn G. A. and Elzebroek B. J. M., 1973, UNICEF, Geneva.
- 7. Reuter G., 1985, Inter. J. Food. Microbiol., 2.103-114.

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#### Disclaimer :

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