



Technical Data

Kanamycin Esculin Azide HiVeg® Agar

MV510

Intended Use:

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

Composition**

Ingredients	g / L
HiVeg® 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 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. Dispense as desired.

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.

Kanamycin Esculin Azide HiVeg® Agar is prepared by completely replacing animal based peptone with vegetable peptones to avoid BSE/TSE risks associate with animal peptones. HiVeg® 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 Broth HiVeg® MV463) and decimal dilutions are prepared. The decimal dilutions are inoculated in Kanamycin Esculin Azide HiVeg® Broth and incubated at 35-37°C for 16-24 hours. If blackening of medium occurs, streaking is done on agar and after incubation confirmatory tests are carried out. Kanamycin Esculin Azide HiVeg® 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.

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 (8,9,10). 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.

Please refer disclaimer Overleaf.

Limitations :

1. Further biochemical and serological tests must be carried out for further 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

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

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

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
<i>Enterococcus bovis</i> ATCC 27960	50-100	good-luxuriant	≥50%	positive, blackening of medium around the colony
<i>Enterococcus faecium</i> ATCC 19434 (00010*)	50-100	good-luxuriant	≥50%	positive, blackening of medium around the colony
<i>Escherichia coli</i> ATCC 25922 (00013*)	≥10 ⁴	inhibited	0%	
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	50-100	good-luxuriant	≥50%	positive, blackening of medium around the colony
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	≥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 20-30°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 (11,12).

Reference

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