Violet Red Bile HiVeg® Agar

MV049

Intended use

This medium is prepared by completely replacing animal based peptones with vegetable peptones. Recommended for selective isolation, detection and enumeration of coli-aerogenes bacteria in water, milk other dairy, food products

Composition**

ISO specification :
Crystal violet neutral red bile lactose (VRBL) agar

Ingredients	g / L
Enzymatic digest of animal tissues	7.000
Yeast extract	3.000
Sodium chloride	5.000
Bile salts mixture	1.500
Lactose	10.000
Neutral red	0.030
Crystal violet	0.002
Agar	15.000
Final pH (at 25°C)	7.4±0.2

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HiVeg® peptone $	7.000
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**Formula adjusted, standardized to suit performance parameters
$ - Equivalent to Enzymatic digest of animal tissues

Directions

Suspend 41.53 gram in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE**. Cool to 45-50°C and immediately pour into sterile Petri plates containing the inoculum. If desired, the medium can be sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

The coliform group consists of several genera of bacteria belonging to the family *Enterobacteriaceae*. The historical definition of this group has been based on the method used for detection i.e. lactose fermentation. This group is defined as all aerobic and facultative anaerobic, gram-negative, non-spore-forming rod shaped bacteria that ferment lactose with gas and acid formation within 48 hour at 35°C (1,2). Examination of foods, ingredients and raw materials, for the presence of marker groups such as coliforms is the one of the common tests.

Violet Red Bile Agar, a modification of MacConkey's original formulation (1) is used for the enumeration of coli-aerogenes bacterial group. It relies on the use of the selective inhibitory components crystals violet and bile salts and the indicator system lactose, and neutral red. Thus, the growth of many unwanted organisms is suppressed, while tentative identification of sought bacteria can be made. Organisms, which rapidly attack lactose, produce purple colonies surrounded by purple halos. Non-fermenters or late lactose-fermenters produce pale colonies with greenish zones (3). VRBA is recommended by APHA (4,5). Selectivity of VRBA can be increased by incubation under anaerobic conditions and/ or at elevated temperature, i.e. equal to or above 42°C (6-8). It is also recommended by ISO (9,10).Violet Red Bile HiVeg® Agar is same as Violet Red Bile Agar except that the animal based peptones are completely replaced with vegetable peptones to avoid the BSE/TSE risks associated with animal peptones. HiVeg® peptone and yeast extract serve as sources of carbon, nitrogen, vitamins and other essential growth nutrients. Lactose is the fermentable carbohydrate, utilization of which leads to the production of acids. Neutral red indicator detects the acidity so formed. Crystal violet and bile salts mixture help to inhibit the accompanying gram-positive and unrelated flora. Sodium chloride maintains the osmotic equilibrium. Violet Red Bile Agar is not completely specific for enteric; other accompanying bacteria may give the same reaction. Further biochemical tests are necessary for positive identification (5).

Type of specimen

Food and dairy samples; Water samples

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3-5,9,10). For water samples, follow appropriate techniques for sample collection, processing as per guidelines & local standards (11).

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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Further biochemical 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.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

Reddish purple coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 4.15% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Productivity : Cultural characteristics observed after an incubation at 30 ± 1°C for 24 ± 2 hours. Recovery rate is considered as 100% for bacteria growth on Reference Medium - Tryptone Soya Agar

Selectivity : Cultural characteristics observed after an incubation at 30 ± 1°C for 24 ± 2 hours.

Specificity : Cultural characteristics observed after an incubation at 30 ± 1°C for 24 ± 2 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Productivity				
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=50%	purplish red colonies with or without precipitation
Escherichia coli ATCC 8739 (00012*)	50-100	luxuriant	>=50%	purplish red colonies with or without precipitation
Selectivity				
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibited		
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited		
Selectivity				
Pseudomonas aeruginosa ATCC 27853 (00025*)	10^3-10^4	good		colourless to beige colonies

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 (12,13).

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