

Yersinia Selective HiVegTM Agar Base

MV843

Intended use

Recommended for the selective isolation and enumeration of *Yersinia enterocolitica* from various samples.

Composition**

Ingredients	g / L
HiVeg TM special peptone	20.000
Yeast extract	2.000
Mannitol	20.000
Sodium pyruvate	2.000
Sodium chloride	1.000
Magnesium sulphate	0.010
Synthetic detergent No.III	0.500
Neutral red	0.030
Crystal violet	0.001
Agar	12.500
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29.02 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°C and aseptically add reconstituted contents of 1 vial CTN Selective Supplement(FD034). Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Yersinia enterocolitica is widely distributed in lakes and reservoirs. Epizootic outbreaks of diarrhea, lymphadenopathy, pneumonia and spontaneous abortions occur in various animals. It is the most common species of *Yersinia* recovered from specimens. *Y. enterocolitica* is biochemically more active at room temperature than at 37°C.

This medium is prepared by completely replacing animal based peptones with vegetable peptones to avoid BSE/TSE risks associated with animal peptones. It is used to isolate *Y. enterocolitica* from specimens. The formulation of animal based media is based on CIN Agar of Schiemann (1,2) and is recommended by ISO Committee (3). Schiemann (4) modified his previous formula of CIN medium by replacing bile salts with sodium deoxycholate. In this medium bile salts are replaced by synthetic detergent No. III. The medium differentiates between mannitol fermenting and non-fermenting bacteria. Microorganisms that ferment the sugar mannitol acidify the medium and cause a localized drop in pH around the colonies. In presence of neutral red, the colonies take red colour. Mannitol negative organisms form colorless and translucent colonies. The medium is selective due to the presence of Synthetic detergent No. III and crystal violet, which inhibit gram-positive and a number of gram-negative bacteria. Addition of antibiotic supplement makes it highly selective for *Yersinia*.

Typical colonies of *Y. enterocolitica* will form dark red colonies resembling bulls eye, which are normally surrounded by a transparent border. Colony size, smoothness and ratio of the border to center diameter may vary among different serotypes. For the isolation of *Y. enterocolitica* by direct plating and pour plating, inoculate the specimen directly onto the medium. Incubate at 22-32°C for 24-48 hours or suspend the sample (food, faeces, etc.) in sterile Phosphate Buffer Saline and incubate for upto 21 days (8) at 4°C. Periodically subculture samples onto Agar Plate and incubate as above.

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. *Serratia liquefaciens*, *Citrobacter freundii* and *Enterobacter agglomerans* may resemble *Y. enterocolitica* that can be further identified by biochemical tests.

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 1.25% Agar gel.

Colour and Clarity of prepared medium

Orange red coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 5.8% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed with added CTN Selective Supplement (FD034) after an incubation at 22-32°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	≥10 ⁴	inhibited	0%	
<i>Escherichia coli</i> ATCC 25922 (00013*)	≥10 ⁴	inhibited	0%	
<i>Proteus mirabilis</i> ATCC 25933	≥10 ⁴	inhibited	0%	
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	≥10 ⁴	inhibited	0%	
<i>Yersinia enterocolitica</i> ATCC 27729	50-100	good-luxuriant	≥50%	translucent with dark pink centre & bile precipitate.

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

1. Schiemann D. A., 1979, Can. J. Microbiol., 25: 1298.
2. Weissfeild and Sonnenwirth, 1982, J. Clin. Microbiol. 15 :508.
3. International Organization for Standardization (ISO), 1994, Draft ISO/DIS 10273.
4. Schiemann D. A., 1980, Can. J. Microbiol., 26: 1232.
5. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
6. 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.
7. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
9. 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.

Revision : 02 / 2024

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