

## Lauryl Sulphate HiVeg® Broth(Lauryl Tryptose HiVeg® Broth) MV080

### Intended use

Recommended for detection and enumeration of coliform bacteria in water, waste water, dairy products and other food samples.

### Composition\*\*

Ingredients	g / L
HiVeg® hydrolysate No. 1	20.000
Lactose	5.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	2.750
Potassium dihydrogen phosphate	2.750
Sodium lauryl sulphate (SLS)	0.100
Final pH ( at 25°C)	6.8±0.2

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

### Directions

Suspend 35.6 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Distribute into tubes containing inverted Durham's' tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C.

### Principle And Interpretation

Lauryl Tryptose HiVeg® Broth is prepared by replacing tryptose with HiVeg® hydrolysate No.1, making the medium free of BSE/TSE risks. This can be used as an alternative for Lauryl Tryptose Broth which was formulated by Mallmann and Darby (1) and is recommended by APHA and ISO committee (2) for the presumptive detection of coliforms in water, effluent or sewage by MPN test and for the detection of coliforms in foods (3). Coliforms are considered to be members of *Enterobacteriaceae*, which grow in presence of bile salts and produce acid and gas from lactose within 48 hours at 37°C. They generally show β-galactosidase activity (4). Cows (5) demonstrated that inclusion of sodium lauryl sulphate (SLS) makes the medium selective for coliform bacteria (1). Aerobic spore bearers are completely inhibited. This media has also been reported to provide a higher colon index than the confirmatory standard methods media and the gas production in this not only acts as a presumptive test, but also as a confirmatory test for the presence of coliforms, in the routine testing of water. Aerobic spore-bearers are completely inhibited in this medium.

HiVeg® hydrolysate No.1 provides essential growth substances, such as nitrogen and carbon compounds, sulphate and trace ingredients. The potassium phosphates provide buffering system, while sodium chloride maintains osmotic equilibrium. For inoculum of 1 ml or less, use single strength medium. For inocula of 10 ml or more, double strength or proportionate medium should be prepared. After inoculation, incubate the tubes at 35-37°C for 24 to 48 hours. For every tube showing fermentation (primary fermentation), inoculate two tubes of Lauryl Tryptose HiVeg® Broth from the tube showing primary fermentation and incubate these tubes at 35-37°C and 44°C respectively. If there is fermentation in the tube incubated at 44°C after 8 to 24 hours, perform indole test by adding Kovac's reagent. A positive indole test in a broth tube showing gas production at 44°C indicates the presence of *Escherichia coli*. If no fermentation occurs in the tube incubated at 35-37°C after 24 hours, the primary fermentation is assumed to be due to organisms other than coliforms. Broth becomes cloudy or forms precipitate if stored at 2 - 8°C, but it should get cleared at room temperature.

### 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 (9,10). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(3,6). 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. Due to nutritional variations, some strains may show poor growth.
2. Further Biochemical and serological test needs to be carried out for confirmation.

## 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 homogeneous free flowing powder

### Colour and Clarity of prepared medium

Light yellow coloured, clear solution without any precipitate

### Reaction

Reaction of 3.56% w/v aqueous solution at 25°C. pH : 6.8±0.2

### pH

6.60-7.00

### Cultural Response

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

Organism	Inoculum (CFU)	Growth	Gas Production	Indole production (44°C)
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	positive reaction	positive reaction, red ring at the interface of the medium
<i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50-100	luxuriant	positive reaction	negative reaction, no colour development / cloudy ring
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	≥10 <sup>4</sup>	inhibited		
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	negative reaction	negative reaction, no colour development / cloudy ring
<i>Staphylococcus aureus</i> subsp <i>aureus</i> ATCC 25923 (00034*)	≥10 <sup>4</sup>	inhibited		

Key : (\*) corresponding WDCM numbers

(#) Formerly known as *Enterobacter aerogenes*

## 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 (9,10).

## Reference

1. Mallmann W. C. and Darby C. W., 1941, Am. J. Public Health, 31:127
2. International Organization for Standardization (ISO), 1991, Draft ISO/DIS 4831.
3. Lipps WC, Braun-Howland EB, Baxter TE, eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
4. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill, Livingstone.
5. Cows P. B., 1938, J. Am. Water Works Assoc., 30:979.
6. Department of Environment, Department of Health and Social Security, Public Health Laboratory Service, 1982, Methods for the Examination of Water and Associated Materials, The Bacteriological Examination of Drinking Water Supplies, 1982, Her Majestys Stationary Office, London.
7. Salfinger Y., and Tortorello M.L. 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 01/2025

### Disclaimer :

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