Formate Ricinoleate Broth

M123

Intended Use:

Recommended for detection of coliform bacteria in milk, water and other materials of sanitary importance.

Composition**

Ingredients	Gms / Litre
Gelatin peptone	5.000
Lactose	5.000
Sodium formate	5.000
Sodium ricinoleate	1.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 16 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. For inoculum volume greater than one ml, use double strength medium. Distribute in tubes with inverted Durhams tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C.

Principle And Interpretation

The existence of coliforms in dairy products is suggestive of unsanitary conditions or practices during production processing or storage. *Escherichia coli* is a member of the coliform group and is used as an indicator organism, presence of which is suggestive of faecal contamination. This is because coliforms including *E. coli* are normally found in the intestinal tracts of humans and many warm blooded animals (1).

Formate Ricinoleate Broth is used for detecting coliforms in milk, water and other material of sanitary importance. This medium was devised by Stark and England (7) and is recommended for use in the manner specified in Standard Methods for the Examination of Water and Wastewater (4) and in Standard Methods for the Examination of Dairy Products (8).

Ordinarily three to five tubes of medium are used for each specimen. Inoculated cultures are incubated for 48 hours at 35°C. Formation of gas within 48 hours is considered evidence of the presence of coliform bacilli.

Gelatin peptone supply essential nitrogenous nutrients to the coliforms for their growth while lactose is the carbon source. Sodium formate maintains buffering conditions of the medium and also accelerates growth and gas production of *Escherichia coli* and related organisms. Sodium ricinoleate is a sodium salt of 11 Hydroxyheptadec-8-ene-1-Carboxylic acid which suppresses the growth of contaminating organisms especially gram-positive bacteria. It is interesting to note that gas production appears earlier in this medium than in other media under same conditions.

Type of specimen

Dairy samples; Water samples

Specimen Collection and Handling:

For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,8).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(3)

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.This medium is general purpose medium and may not support the growth of fastidious organisms.

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 light brown homogeneous free flowing powder

Colour and Clarity of prepared medium

Whitish opalescent solution with slight precipitate.

Reaction

Reaction of 1.6 % w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Gas
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	good-luxuriant	positive reaction
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	positive reaction
Salmonella Typhi *ATCC 6539*	>=10^4	Inhibited	
Staphylococcus aureus ATCC 25923 (00034*)	>=10^4	Inhibited	
Bacillus subtilis ATCC 6633 subsp. spizizenii (00003*)	>=10^4	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. Use before expiry date on the label.
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 (5,6).

Reference

1. Alcamo E. I., 2001, Fundamentals of Microbiology, 6th Ed., Jones and Bartlett Publishers.

2. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.

3. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

4. Eaton A. D., Clesceri L. S., Rice E. W. and Greenberg A W.(Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.

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

6. 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.

7. Stark and England, 1935, J. Bact., 29:26.

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.

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