Edwards Medium Base, Modified

M748

Intended Use:

Recommended for selective and rapid isolation of *Streptococcus agalactiae* and other Streptococci associated with bovine mastitis

Composition**

Ingredients	g / L
Peptone	10.000
HM peptone B #	10.000
Esculin	1.000
Sodium chloride	5.000
Crystal violet	0.0013
Thallous sulphate	0.330
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef extract

Directions

Suspend 41.33 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at Δ115°C for 20 minutes. Cool to 45-50°C and aseptically add 5 to 7% v/v sterile sheep blood. Mix well and pour into sterile Petri plates.
(Δ corresponds to 10lbs pressure)

Principle And Interpretation

Streptococci are gram-positive facultatively anaerobic bacteria, which constitute normal commensal flora of mouth, skin, intestine and upper respiratory tract of humans. Group B Streptococci are an important cause of systemic infections in infants and occasionally of bacterial endocarditis (1). Mastitis is a disease of cattle caused by the organisms *Streptococcus agalactiae*. It belongs to the Lancefield group B Streptococci .

The most common selective agents used for selective isolation of Streptococci are crystal violet and thallium salts. A selective medium containing crystal violet was used by Haxthausen to isolate skin Streptococci (2). Subsequently it was observed that Streptococci from milk were able to grow on Gentian Violet Blood Agar whereas the other saprophytic milk bacteria were inhibited on this medium (3). An Esculin Blood Agar containing crystal violet was used by Edwards to isolate the causative agent of mastitis (4). A similar medium containing thallous acetate was also used to isolate the causative agent of mastitis (5).

Peptone and HM peptone B serve as sources of carbon, nitrogen and other essential nutrients. Esculin helps to differentiate esculin-positive (group D Streptococci) organisms from esculin- negative (*S. agalactiae*) organisms. Sodium chloride helps to maintain the osmotic equilibrium of the medium. Crystal violet and thallous sulphate serve as the selective agents for Streptococci. Supplementation with blood provides additional nutrients in addition to serving as an indicator of haemolysis. Mastitis Streptococci show alpha, beta or gamma type of haemolysis. Esculin differentiates esculin- positive group D Streptococci (black colonies) from esculin-negative *Streptococcus agalactiae* (blue to colourless colonies). Centrifuged test milk sample is directly inoculated on the surface of the medium plate. Esculin-negative (blue to colourless) *S. agalactiae* organisms are further subcultured for identification tests.

Type of specimen

Clinical samples - faeces, vaginal swabs, ; Dairy samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (8,9).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. For professional use only. 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 clinical 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.
Further biochemical and serological 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

Cream to yellow homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Basal medium :Amber coloured, clear to slightly opalescent gel. After addition of 5-7% v/v sterile defibrinated sheep blood : Cherry red coloured opaque gel forms in Petri plates

Reaction

Reaction of 4.13% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed with added 5-7%v/v sterile defibrinated sheep blood after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Enterococcus faecalis ATCC 29212 (00087*)	50-100	good-luxuriant	>=50%	greyish blue
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^4	inhibited	0%	
Streptococcus agalactiae ATCC 13813	50-100	good-luxuriant	>=50%	greyish blue to colourless w/ haemolysis

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

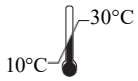
Reference

1. Cruickshank R., Duguid J. P., Marmion B. P. , Swain R. H. A., (Eds.), 1975, Medical Microbiology, The Practice of Medical Microbiology , 12th Edition, Vol. II , Churchill Livingstone.
2. Haxsthausen H., 1927, Ann. Derm. Suph., 8.201.
3. Bryan C. S., 1932, Am. J. Public Health, 22. 749.
4. Edwards S. J., 1933, J. Comp. Path. Therap., 46:211-217.
5. McKenzie D. A., 1941, Vet. Rec., 53:473-480.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7. 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.
8. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
9. 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 : 04/2024

HiMedia Laboratories Pvt. Limited,
Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

IVD *In vitro* diagnostic medical device



Storage temperature

EC REP

CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE **CE Marking**

Do not use if package is damaged

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.