Edwards Medium Base, Modified

M748

Edwards Medium Base, Modified is a selective medium for the rapid isolation of *Streptococcus agalactiae* and other streptococci associated with mastitis and also from other clinical samples.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Beef extract	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

Directions

Suspend 41.33 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at D 115°C for 20 minutes. Cool to 50°C and aseptically add 5 to 7% v/v sterile sheep blood. Mix well and pour into sterile Petri plates.

(D 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).

Peptic digest of animal tissue and beef extract 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.

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 .

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Cultural Response				
Enterococcus faecalis ATCC 29212	50-100	good-luxuriant	>=50%	black
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%	
Streptococcus agalactiae ATCC 13813	50-100	good-luxuriant	>=50%	colourless, w/ haemolysis

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
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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.

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