TM 955 – EDWARD'S MEDIUM BASE, MODIFIED

INTENDED USE

For isolation of *Streptococcus agalactiae* and other Streptococci associated with bovine mastitis.

PRODUCT SUMMARY AND EXPLANATION

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. 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. 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. An Esculin Blood Agar containing crystal violet was used by Edwards to isolate the causative agent of mastitis. A similar medium containing thallous acetate was also used to isolate the causative agent of mastitis.

COMPOSITION

Ingredients	Gms / Ltr
Peptone	10.000
Beef extract	10.000
Esculin	1.000
Sodium chloride	5.000
Crystal violet	0.0013
Thallous sulphate	0.330
Agar	15.000

PRINCIPLE

The medium consists of Peptone and Beef extract which 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 sub cultured for identification tests.

INSTRUCTION FOR USE

- Dissolve 41.33 grams in 1000 ml purified / distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 10 psi pressure (115°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.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.

Appearance 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.

pH (at 25°C) : 7.4 ± 0.2

INTERPRETATION

Cultural characteristics observed with added 5-7%v/v sterile defibrinated sheep blood after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Colour of colony	Incubation Temperature	Incubation Period
Enterococcus faecalis	29212	50-100	Good-luxuriant	>=50%	Black	35-37°C	24-48 Hours
Escherichia coli	25922	>=10^3	Inhibited	0%	-	35-37°C	24 -48 Hours
Staphylococcus aureus subsp. aureus	25923	>=10^3	Inhibited	0%	-	35-37°C	24-48 Hours
Streptococcus agalactiae	13813	50-100	Good-luxuriant	>=50%	Colourless, w/ haemolysis	35-37°C	24 -48 Hours

PACKAGING:

In pack size of 100 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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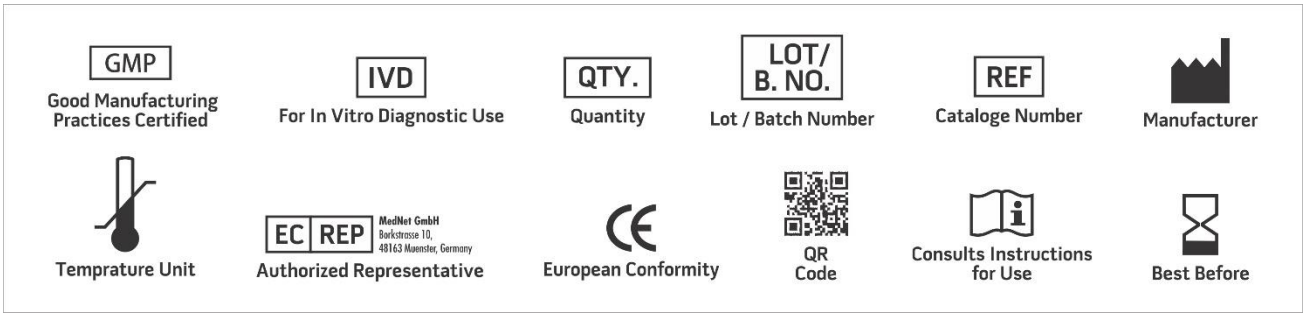
3. Edwards S. J., 1933, J. Comp. Path. Therap., 46:211-217.

4. Haxsthausen H., 1927, Ann. Derm. Suph., 8.201.
5. McKenzie D. A., 1941, Vet. Rec., 53:473-480.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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