Streptococcus agalactiae Selective Agar Base

M1257

Intended Use:

Recommended for selective isolation of *Streptococcus agalactiae* from dairy products.

Composition**

Ingredients	g / L
Peptone	10.000
HM extract #	5.000
Sodium chloride	5.000
Esculin	1.000
Thallous sulphate	0.333
Crystal violet	0.0013
Agar	13.000
Final pH (at 25°C)	7.4±0.2

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

Directions

Suspend 34.34 grams in 940 ml purified / distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and add 60 ml defibrinated blood and 25 ml Staphylococcus β toxin. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Streptococcus agalactiae is a gram-positive *Streptococcus* characterized by the presence of group B Lancefield antigen. *S. agalactiae* exhibits beta haemolysic reaction. On Blood agar plate, it forms zones of haemolysis that are slightly bigger than the size of colonies formed. Group B streptococci hydrolyze sodium hippurate and give a positive response in the CAMP test. *S. agalactiae* is also sensitive to bile and will lyse in its presence. Streptococcus Agalactiae Selective Agar was formulated by Hauge and Kohler-Ellingsen (1) for the isolation of *S. agalactiae*, the causative agent of mastitis in cattle. Differentiation between *Streptococcus* species is done on the basis of esculin hydrolysis seen as dark brown colour due to formation of an esculin-thallium complex. Thallous sulphate and crystal violet inhibit the accompanying bacterial flora. *Staphylococcus* ß-toxin attacks the erythrocytes present in the medium in such a way that they may be completely haemolyzed. *S. agalactiae* is not haemolytic on simple blood agar. Thus *S.agalactiae* can be distinguished from obligate, non-haemolyzing colonies.Peptone and HM extract serves as a source of nitrogeneous and carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients.

S. agalactiae forms dove-blue coloured smooth colonies surrounded by zones of haemolysis. Further identification is done by using biochemical and serological methods, but primarily by using CAMP test (2).

Type of specimen

Dairy samples

Specimen Collection and Handling:

For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4). 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. Further identification is done by using biochemical and serological methods, but primarily by using CAMP test (2).

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 1.3% Agar gel.

Colour and Clarity of prepared medium

Basal medium forms light purple coloured, clear to slightly opalescent gel. On addition of blood, red coloured opalescent gel forms in Petri plates

Reaction

Reaction of 3.43% 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 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Blue colony	Haemolysis
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%		
Enterococcus faecalis ATCC 29212 (00087*)	50-100	good-luxuriant	>=50%	variable reaction	alpha
Pseudomonas aeruginosa ATCC 27853 (00025*)	>=10^4	inhibited	0%		
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^4	inhibited	0%		
Streptococcus agalactiae ATCC 13813	50-100	luxuriant	>=50%	positive	beta
Streptococcus agalactiae ATCC 27956	50-100	luxuriant	>=50%	positive	beta
Streptococcus cremoris ATCC 19257	50-100	luxuriant	>=50%	variable reaction	alpha
Streptococcus pneumoniae ATCC 6301	50-100	luxuriant	>=50%	negative	alpha
Streptococcus pyogenes ATCCC 19615	50-100	luxuriant	>=50%	negative	beta
Streptococcus lactis ATCC 19435 (00016*)	>=10^4	inhibited	0%		

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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 (5,6).

Reference

1. Hauge S. T. and u Kohler-Ellingsen J., 1953, Nord. Vet. Med., 5:539.
2. Christie R., Atkins N. E. and Munch-Petersen E., 1944, Aust. J. Exp. Biol. Med. Sci., 22:197.
3. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
4.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
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.

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