Spirit Blue Agar

M445

Intended Use:

For detection and enumeration of lipolytic microorganisms.

Composition**

Ingredients	g/ L
Tryptone	10.000
Yeast extract	5.000
Spirit blue	0.150
Agar	17.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 32.15 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and add 30 ml lipase substrate slowly while agitating to obtain an even distribution. Mix well and pour into sterile Petri plates.

Note : *For proper lipase activity, it is recommended to use glass plates instead of disposable plastic plates.*

Principle And Interpretation

Lipids, including fats and oils, are highly reduced. When a lipid is catabolized, it has the potential to yield more pairs of electrons per gram, and thus more energy, than either carbohydrates or proteins (1). This process is brought about by the enzyme lipase, and the organisms possessing the enzyme lipase are called lipolytic organisms. Growth of lipase-producing microorganisms can contribute to flavour defects in milk and high fat dairy products. Some of the free fatty acids released by the action of lipolytic enzymes have a low flavour threshold and can impart a rancid flavour at low concentrations.

Spirit Blue Agar is prepared according to the formulation of Starr (2) is recommended by APHA (3) for detection and enumeration of lipolytic microorganisms. It is a basal medium to which lipoidal substrate is added for the detection, enumeration and study of lipolytic microorganisms. Formulations in practice before Starr which included dyes as indicators of lipolysis were sometimes inhibitory to the microorganisms. Starr showed spirit blue to be inert and an ideal indicator of lipolysis, visualized as clear halos around colonies.

Tryptone and yeast extract in the medium are sources of carbon, nitrogen, vitamins and minerals. Spirit blue is a dye which acts as an indicator of lipolysis. The lipase reagents recommended as the lipid source are cotton seed meal, cream, olive oil etc. A satisfactory emulsion can be prepared by dissolving 10 gram acacia or 1 ml polysorbate 80 in 400 ml warm distilled water, adding 100 ml cotton seed or olive oil and agitating vigorously to emulsify.

Prepare 1:10 or other suitable dilution of the product to be tested. Spread 0.1 ml of the desired dilutions over the surface of the medium. Incubate at 35-37°C for 24-48 hours. Colonies of lipolytic organisms develop a clear zone and /or a deep blue colour around and under each colony (4).

Type of specimen

Isolated Microorganism from clinical sample and dairy samples.

Specimen Collection and Handling:

For food and 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 :

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 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 media.

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.

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 greenish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.7% Agar gel.

Colour and Clarity of prepared medium

Basal medium yields blue coloured, clear to slightly opalescent gel. With addition of lipase substrate, lavender coloured slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.22% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 48-72 hours with added Lipase substrate.

Organism	Growth	Lipase activity
Proteus mirabilis ATCC 25933	luxuriant	negative reaction, absence of zone around colony
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	luxuriant	positive reaction, clear zone around colony
Staphylococcus epidermidis ATCC 12228 (00036*)	luxuriant	positive reaction, clear zone around colony

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 (4,5).

Reference

1.Nortan C. F., 1986, Microbiology, 2nd Ed., Addison-Wesley Publishing Company.
2.Starr, 1941, Science, 93:333.
3.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
4.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5.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.
6.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C

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IVD ***In vitro*** **diagnostic medical device**

30°C 10°C **Storage temperature**

EC REP

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CE **CE Marking**

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