HYA Agar

M601

Intended Use:

Recommended for differentiation of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* on the basis of colony morphology.

Composition**

Ingredients	Gms / Litre
HM peptone B #	1.000
Proteose peptone	10.000
Dextrose (Glucose)	2.500
Galactose	2.500
Lactose	5.000
Agar	15.000
Final pH (at 25°C)	6.8±0.2

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

Directions

Suspend 36.0 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 20 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Yoghurt is a fermentable milk product in which *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are the essential microbial species and are active in a symbiotic relationship. The large number of media proposed for lactic acid bacteria, particularly for Streptococci and lactobacilli is indicative of the difficulties encountered in growing some strains of these organisms. The choice of medium is governed to some extent by the particular strains under study and therefore by products or habitat. In general, lactic acid bacteria are tolerant to low pH, they can be very sensitive to other adverse conditions. Samples to be examined for numbers of viable lactic acid bacteria should not be frozen prior to analysis (4).
Porubcan and Sellars (3) described this medium on which *L. bulgaricus* grow as diffuse, low mass colonies (2-10 mm in diameter) and *S.thermophilus* as discrete high mass colonies (1-3 mm in diameter). To obtain optimum consistency, flavour and odour, many investigators claim that the two species should be present in about equal numbers in the culture. Dominance by either species can cause defects. Because of the emphasis on maintaining balance between coccus and rods, techniques are needed to determine the relative proportion of *S.thermophilus* and *L.bulgaricus* when grown together in milk culture. Differentiation of two species on HYA Agar is based on colony morphology. Also this media is recommended by APHA (4). HM peptone B and proteose peptone provides necessary nitrogenous nutrients required for growth of two species. The sugars dextrose, galactose, lactose serve as energy sources.

Type of specimen

Pure isolate

Specimen Collection and Handling:

For samples, follow appropriate techniques for sample collection and processing as per guidelines (1,2).
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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further biochemical and serological tests must be carried out for further identification.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

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

Yellow coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.6% 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 24-48 hours .

Organism	Growth
Lactobacillus bulgaricus ATCC 11842 (00102*)	luxuriant
Streptococcus thermophilus ATCC 14485	luxuriant

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. 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.
3. Porubcan R. S., and Sellars R. L., 1973, J. Dairy Sci., 56: 634.
4. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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