Bushnell Haas Agar

M349

Intended Use:

Recommended for examination of fuels for microbial contamination and for studying hydrocarbon deterioration by microorganisms.

Composition**

Ingredients	Gms / Litre
Magnesium sulphate	0.200
Calcium chloride anhydrous	0.020
Potassium dihydrogen phosphate	1.000
Dipotassium hydrogen phosphate	1.000
Ammonium nitrate	1.000
Ferric chloride	0.050
Agar	20.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 23.27 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. Mix well and pour into sterile Petri plates. A white precipitate prior to sterilization becoming yellow to orange after sterilization is normal.

Principle And Interpretation

Bushnell Haas Agar is prepared as per the formula of Bushnell and Haas (2) and recommended for the microbiological examination of fuels by the SIM Committee on microbiological deteriorations of fuels (1). These media contain all nutrients except carbon source, necessary for the growth of bacteria. Only those bacteria that are able to decompose hydrocarbon will grow in these media. Specific carbon source i.e. hydrocarbon can be added to this medium and their utilization by different microorganisms can be studied.

These bacteria can decompose a variety of hydrocarbons like kerosene, mineral oil, paraffin wax and gasoline. For liquid hydrocarbon the hydrocarbon is layered on the surface of inoculated agar. For testing volatile hydrocarbons such as gasoline the Petri-plates containing the medium are inverted and the hydrocarbon is poured into the lid. Magnesium sulphate, calcium chloride and ferric chloride provide trace elements. Ammonium nitrate is a nitrogen source while monopotassium phosphate and potassium phosphate buffers the medium.

Type of specimen

Petroleum industry samples.

Specimen Collection and Handling

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 biochemical and serological tests must be carried out for further identification.

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
White to cream homogeneous free flowing powder

Gelling
Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium
Light amber coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 2.33% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed after an incubation at 25-30°C within 1 week.

Organism	Inoculum (CFU)	Growth (Plain)	Growth w/ minerals
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	poor	good-luxuriant
Pseudomonas aeruginosa ATCC 9027 (00026*)	50-100	poor	good-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 (3,4).

Reference

1. Allred, DeGray, Edwards, Hedrick, Klemme, Rogers, Wulf and Hodge, 1963, Proposed Procedures for Microbiological Examination of Fuels, SIM Special Publications, No. 1. Merck, Sharp & Dohme Research Laboratories, Rahway, N.J.
2. Bushnell and Haas, 1941, J. Bacteriol., 41:653.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. 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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