PRODUCT SPECIFICATION SHEET

Hugh Leifson Glucose Medium (DM369)

Intended Use

Hugh Leifson Glucose Medium (DM369) is recommended for differentiation of *Staphylococci* from *Micrococci* on the basis of anaerobic fermentation of glucose.

Product Summary and Explanation

Hugh Leifson Medium was formulated by Hugh and Leifson[1] who described the taxonomic significance of fermentative and oxidative metabolism of carbohydrates in gram-negative intestinal bacteria. There are two ways of utilizing carbohydrates by microorganisms, namely fermentation and oxidation. This property may be frequently used for the differentiation of some bacteria. Hugh Leifson Glucose Medium is prepared as described by FDA[2] for differentiation of Staphylococci from Micrococci.

Principles of the Procedure

Hugh Leifson Glucose Medium contains a high concentration of glucose as a carbohydrate source and low concentration of peptic digest of animal tissue to avoid the possibility of an aerobic organism utilizing peptic digest of animal tissue and producing an alkaline condition which would neutralize slight acidity produced by an oxidative organism.[3,4] Agar concentration enables the determination of motility and aids in distribution of acid throughout the tube produced at the surface of medium. High salt concentration thus it is used for the identification of pathogenic and halophilic organisms and for testing aerobic and anaerobic breakdown of glucose by Staphylococci and Micrococci.[5]

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	2.00
Yeast extract	0.50
Sodium chloride	30.00
Glucose	10.00
Bromocresol purple	0.015
Agar	3.00
Final pH: 7.4 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

sDirections

1. Suspend 45.52 grams of the medium in one litre of distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Dispense into test tubes in duplicate for aerobic and anaerobic fermentation.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Cool the tubed medium in an upright position.

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Quality Control Specifications

Dehydrated Appearance	Light yellow to bluish grey homogeneous free flowing powder
Prepared Medium	Purple coloured, clear to slightly opalescent gel forms in tubes as butts
Reaction of 4.55% Solution	pH : 7.4 ± 0.2 at 25°C
Gel Strength	Semisolid, comparable with 0.3% Agar gel

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Colour of Medium (Aerobic)	Colour of Medium (Anaerobic)
1.	*Micrococcus luteus ATCC 10240*	50-100	good-luxuriant	yellow	pink-purple
2.	*Staphylococcus aureus ATCC 25923*	50-100	good-luxuriant	yellow	yellow

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

a) Oxidation and Fermentation Reaction :

1. The tubes for aerobic and anaerobic fermentation are inoculated and the agar surface of one tube of duplicate is covered with layer of sterile paraffin oil, about 25 mm thickness and incubated at 37°C.
2. Oxidative organisms produce acid in unsealed tube with little or no growth and no acid formation in sealed tube while fermentative organisms produce acid in both sealed and unsealed tubes.
3. If acid is produced, it is indicated by change in colour from purple to yellow throughout the medium.
4. Liquid paraffin tube used should be dry sterilized at 160-170°C for 2 hours.
5. Wet sterilization with high pressure is not sufficient for the purpose.

b) Differentiation of Staphylococci from Micrococci :

1. Inoculate the culture under test into two tubes of the medium by stabbing throughout their length with a long wire loop.
2. Cover one tube of the pair with layer of sterile liquid paraffin and incubate at 37°C.
3. Read yellow colouration as acid production from glucose.
4. Staphylococci produce acid by fermentation throughout the depth of the medium both in the anaerobic tubes sealed with paraffin and the aerobic unsealed tube.
5. Micrococci either fail to produce acid in either tube or produce it only by oxidation in the upper part of the aerobic tube.

Refer appropriate references for standard test procedures.

Results

Refer appropriate references and test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

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Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Hugh Leifson Glucose Medium

Product Code : DM369

Available Pack sizes : 500gm

References

1. Hugh and Leifson, 1953, J. Bacteriol., 66:24.
2. Bacteriological Analytical Manual, 1995, 8th Ed., Food & Drug Administration, AOAC International, USA.
3. MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol.I, Williams and Wilkins, Baltimore.
4. Finegold S. M., Martin W. J., and Scott E. G., 1978, Bailey and Scotts Diagnostic Microbiology, 5th Ed., The C.V. Mosby Co., St. Louis.
5. Baird Parker, 1966, International subcommittee on Staphylococci and Micrococci.

Further Information

For further information please contact your local MICROMASTER Representative.

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