PRODUCT SPECIFICATION SHEET

Hugh Leifson Medium (DM370)

Intended Use
Hugh Leifson Medium (DM370) is recommended for detecting aerobic and anaerobic breakdown 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 and Leifson,[1] showed that when an organism is inoculated in duplicate in the Hugh Leifson Medium containing a carbohydrate and prior incubation one of the tubes is covered with melted petrolatum, the patterns of metabolism observed are of differential significance. Oxidative organisms produce only acid reaction in the open tube with little or no growth and no acid formation is observed in the covered tube. Fermentative organisms will produce an acid reaction in both types of tubes. Changes in the covered agar are due to true fermentation, while changes in the open tubes are due to oxidative utilization of the carbohydrate present. If the carbohydrate is not utilized by either method, there is no acid production in either tube.

Principles of the Procedure
Hugh Leifson Medium contains a high concentration of carbohydrate and low concentration of peptic digest of animal tissue to avoid the possibility of utilization of peptic digest of animal tissue by an aerobic organism and producing an alkaline condition which would neutralize slight acidity produced by an oxidative organism. Dipotassium phosphate promotes fermentation and acts as pH controlling buffer. Agar concentration enables the determination of motility and aids in distribution of acid throughout the tube produced at the surface of medium.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	2.00
Sodium chloride	5.00
Dipotassium phosphate	0.30
Glucose	10.00
Bromothymol blue	0.05
Agar	2.00
Final pH: 6.8 ± 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.

Directions
1. Suspend 19.35 grams of the medium in one liter 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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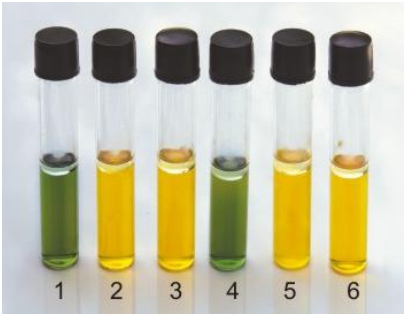
Quality Control Specifications

Dehydrated Appearance	Light yellow to bluish green homogeneous free flowing powder
Prepared Medium	Greenish blue coloured, clear to slightly opalescent gel forms in tubes as butts
Reaction of 1.94% Solution	pH : 6.8 ± 0.2 at 25°C
Gel Strength	Semisolid,comparable with 0.2% Agar gel

Expected Cultural Response: Cultural characteristics observed with paraffin oil overlay, after an incubation at 35-37°C for 18-48 hours

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Motility	Aerobic fermentation	Anaerobic fermentatoin
1.	*Enterobacter aerogenes ATCC 13048*	50 – 100	positive, growth away from stabline causing turbidity	acid and gas production, positive reaction	acid and gas production, positive reaction
2.	*Escherichia coli ATCC 25922*	50 – 100	positive, growth away from stabline causing turbidity	acid and gas production, positive reaction	acid and gas production, positive reaction
3.	*Pseudomonas aeruginosa ATCC 27853*	50 – 100	positive, growth away from stabline causing turbidity	Acid production, negative reaction, no colour change	acid and gas production, positive reaction, yellow colour
4.	*Salmonella Typhi ATCC 6539*	50 – 100	positive, growth away from stabline causing turbidity	acid and gas production, positive reaction	acid and gas production, positive reaction
5.	*Shigella sonnei ATCC 25931*	50 – 100	negative, growth along the stabline, surrounding medium remains clear	acid and gas production, positive reaction, yellow colour	acid and gas production, positive reaction, yellow colour

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



Hugh Leifson Medium (DM370)
(with paraffin oil overlay)

1. Control
2. *Enterobacter aerogenes ATCC 13048*
3. *Escherichia coli ATCC 25922*
4. *Pseudomonas Aeruginosa ATCC 27853*
5. *Salmonella Typhi ATCC 6539*
6. *Shigella sonnei ATCC 25931*

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Test Procedure

1. Using appropriate inoculum, inoculate tubes of media for aerobic and anaerobic fermentation with growth from an 18- to 24-hour old pure culture using an inoculating loop in duplicate.
2. 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.
3. Liquid paraffin tube used should be dry sterilized at 160-170°C for 2 hours. Wet sterilization with high pressure is not sufficient for the purpose.

Results

1. Oxidative organisms produce acid in unsealed tube with little or no growth.
2. No acid formation is observed 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 greenish blue to yellow throughout the medium.

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.

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. The acid reaction produced by oxidative organisms is apparent first at the surface and gradually extends throughout the medium. Where oxidation is weak or slow, an initial alkaline reaction may be observed at the surface of the open tube that may persist for several days but will eventually turn acid.
2. Nonsaccharolytic organisms produce slight alkalinity in the open tube (blue-green color) but the closed tube will not exhibit a color change (green).
3. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
4. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Hugh Leifson Medium
Product Code : DM370
Available Pack sizes : 100gm/ 500gm

References

1. Hugh and Leifson, 1953, J. Bacteriol., 66:24.

Further Information

For further information please contact your local MICROMASTER Representative.

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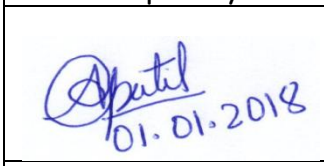	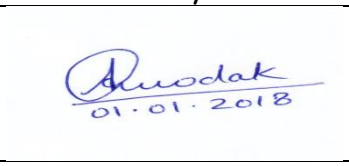	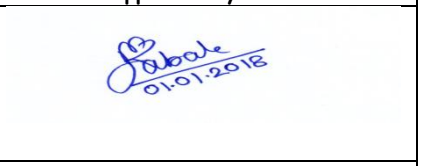
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