PRODUCT SPECIFICATION SHEET

OF Basal Medium (DM187)

Intended Use
OF Basal Medium (DM187) is recommended for differentiation of gram-negative bacteria based on their carbohydrate oxidation and fermentation reactions.

Product Summary and Explanation
Hugh and Leifson Hugh and Leifson who described the taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by gram-negative bacteria, developed OF Medium to study oxidative and fermentative metabolism of carbohydrates by gram-negative bacteria. This criterion is used during taxonomic studies of *Enterobacteriaceae*.[1] They illustrated that when an organism is inoculated into two tubes of OF Basal Medium containing a carbohydrate and the medium in one of the tubes is covered with mineral oil prior to incubation, the patterns of metabolism are of differential significance. The low concentration of agar permits the determination of motility and aids in the even distribution of any acid produced at the surface of the medium.[2]

Principles of the Procedure
OF Basal Medium contains casein enzymic hydrolysate which provides the necessary carbon and nitrogen, vitamins etc required for bacterial growth. A carbohydrate whose fermentation reaction is to be studied is added separately. Phosphate buffers the medium and the low agar concentration determines motility and dispersion of the acid produced on the surface. Bromothymol blue acts as the pH indicator. The low concentration of agar permits the determination of motility and aids in the even distribution of any acid produced at the surface of the medium. Motility is observed as diffused zone of flaring out from the line of inoculation. Non-motile organisms grow along the line of inoculation. Dextrose is the most important carbohydrate for use in OF Basal Medium. However, certain organisms may metabolize other carbohydrates even if they are unable to utilize dextrose. Degradation of the carbohydrate to acid is indicated by the pH indicator bromothymol blue which changes its colour to yellow. Oxidative utilization takes place when the medium is exposed to air while fermentative utilization occurs under exclusion of air.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	2.00
Sodium chloride	5.00
Dipotassium phosphate	0.30
Bromo thymol blue	0.08
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 9.38 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Dispense in 100 ml amounts.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. To first 100 ml of sterile basal medium, aseptically add 10 ml of sterile 10% dextrose solution.
6. To second 100 ml add 10 ml sterile 10% lactose solution.
7. To third 100 ml add 10 ml sterile 10% saccharose solution.
8. Mix and dispense aseptically in 5 ml amounts in sterile tubes in duplicate for aerobic and anaerobic fermentation.

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Quality Control Specifications

Dehydrated Appearance	Cream to greenish yellow homogeneous free flowing powder
Prepared Medium	Green coloured clear to slightly opalescent gel forms in tubes
Reaction of 0.94% Solution	pH : 6.8 ± 0.2 at 25°C
Gel Strength	Semisolid, comparable with 0.2% Agar gel

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Sr. No.	Organisms	Results to be achieved				
		Inoculum (CFU)	Only Basal Medium	Only Basal Medium	w/ Dextrose	w/Dextrose
			aerobic	overlayed with mineral oil	aerobic	overlayed with mineral oil
1.	*Acinetobacter baumannii ATCC 19606*	50 – 100	alkaline reaction, green colour of the medium	alkaline reaction, green colour of the medium	acidic reaction, yellowing of the medium	alkaline reaction, green colour of the medium
2.	*Alcaligenes faecalis ATCC 8750*	50 – 100	alkaline reaction, green colour of the medium	alkaline reaction, green colour of the medium	alkaline reaction, green colour of the medium	alkaline reaction, green colour of the medium
3.	*Escherichia coli ATCC 25922*	50 – 100	alkaline reaction, green colour of the medium	alkaline reaction, green colour of the medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium with gas formation
4.	*Enterobacter aerogenes ATCC 13048*	50 – 100	alkaline reaction, green colour of the medium	alkaline reaction, green colour of the medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium with gas formation
5.	*Pseudomonas aeruginosa ATCC 9027*	50 – 100	alkaline reaction, green colour of the medium	alkaline reaction, green colour of the medium	acidic reaction, yellowing of the medium	alkaline reaction, green colour of the medium
6.	*Salmonella enteritidis ATCC 13076*	50 – 100	alkaline reaction, green colour of the medium	alkaline reaction, green colour of the medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium with gas formation
7.	*Shigella flexneri ATCC 12022*	50 – 100	alkaline reaction, green colour of the medium	alkaline reaction, green colour of the medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium with gas formation
8.	*Vibrio cholera ATCC 15748*	50 – 100	alkaline reaction, green colour of the medium	alkaline reaction, green colour of the medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium with gas formation

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

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Test Procedure

1. Prepare the medium with 1% dextrose and without 1% dextrose.
2. Two tubes of each carbohydrate are used per organism and inoculated by stabbing.
3. One of the inoculated tubes of each carbohydrate medium is covered with 2 ml of sterile mineral oil and the other is left uncovered.
4. The tubes are incubated at 35-37°C for 18-48 hours or longer.
5. The results are read after 48 hours.
6. Do not discard as negative until after 4 days of incubation.

Results

1. Fermentative organisms will produce an acid reaction in both the covered and uncovered medium.
2. Oxidative organisms will produce an acid reaction in the uncovered medium and give slight growth without change in the covered medium.
3. Organisms which are not classified either as oxidative or fermentative show no change in the covered medium and an alkaline reaction in the uncovered medium.
4. Aerobic fermentation reaction in both covered and covered tube is indicated as a colour change from green to yellow or acid production along with gas production.
5. Both oxidation and fermentation reaction in both covered and covered tube is indicated as a colour change from green to yellow or acid production with or without gas production.

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 at the surface and gradually spreads throughout the medium. If the oxidation is weak or slow, however, an initial alkaline reaction at the surface of the open tube may persist for several days and eventually convert to an acid reaction.
2. If an organism is unable to grow on OF Basal Medium, Cowan recommends adding either 2% serum or 0.1% yeast extract to each carbohydrate tube.
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 : OF Basal Medium
Product Code : DM187
Available Pack sizes : 100gm/ 500gm

References

1. Hugh R. and Leifson E., 1953, J. Bacteriol. 66:24.
2. MacFaddin J. F., 1985, Media for the Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.

Further Information

For further information please contact your local MICROMASTER Representative.

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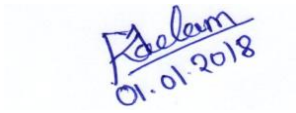	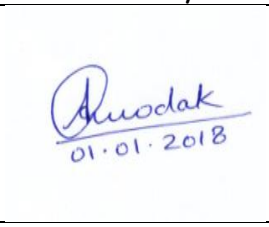	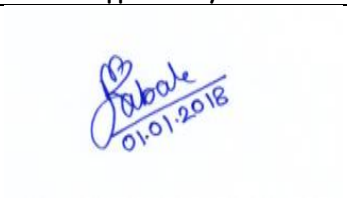
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