OF Basal Medium

M395

OF (Oxidation Fermentation) Basal medium is used for the determination of oxidative and fermentative metabolism of carbohydrates by gram-negative bacteria.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	2.000
Sodium chloride	5.000
Dipotassium phosphate	0.300
Bromo thymol blue	0.080
Agar	2.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 9.38 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in 100 ml amounts and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. To first 100 ml of sterile basal medium, aseptically add 10 ml of sterile 10% dextrose solution. To second 100 ml add 10 ml sterile 10% lactose solution. To third 100 ml add 10 ml sterile 10% saccharose solution. Mix and dispense aseptically in 5 ml amounts in sterile tubes in duplicate for aerobic and anaerobic fermentation.

Principle And Interpretation

Hugh and Leifson 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).

Casein enzymic hydrolysate in the medium 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 (2). 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 (3). 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.

The authors Hugh and Leifson showed that when a gram-negative organism is inoculated in this medium containing a carbohydrate in duplicate, of which one tube is covered with mineral oil to exclude oxygen and the second tube is uncovered; reactions of differential value can be observed. Fermentative organisms will produce an acid reaction in both the covered and uncovered medium. Oxidative organisms will produce an acid reaction in the uncovered medium and give slight growth without change in the covered medium. 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 (3).

Prepare the medium with 1% dextrose and without 1% dextrose. Two tubes of each carbohydrate are used per organism and inoculated by stabbing. One of the inoculated tubes of each carbohydrate medium is covered with 2 ml of sterile mineral oil and the other is left uncovered. The tubes are incubated at 35-37°C for 18-48 hours or longer. The results are read after 48 hours.

The acidic reaction of oxidative organisms is more apparent at the surface of the medium that gradually spreads throughout the medium. If the oxidation reaction is weak or slow, an initial alkaline reaction at the surface of the uncovered tube may persist for several days and eventually convert to an acid reaction.

OF Basal Medium can be supplemented with 2% serum or yeast extract (0.1%) to make the medium more nutritious for the growth of bacteria (4).

Quality Control

Appearance
Cream to greenish yellow homogeneous free flowing powder

Gelling
Semisolid, comparable with 0.2% Agar gel.

Colour and Clarity of Prepared medium
Green coloured clear to slightly opalescent gel forms in tubes.

Reaction
Reaction of 0.94% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
M395: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Only Basal Medium (aerobic)	Only Basal Medium (overlayed with mineral oil)	w/ Dextrose (aerobic)	w/Dextrose (overlayed with mineral oil)
Cultural Response					
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
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
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 with gas formation	acidic reaction, yellowing of the medium with gas formation
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 with gas formation	acidic reaction, yellowing of the medium with gas formation
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
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 with gas formation	acidic reaction, yellowing of the medium with gas formation
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
Vibrio cholerae ATCC 15748	50-100	alkaline reaction, green	alkaline reaction, green	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium

colour of the medium	colour of the medium

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Hugh R. and Leifson E., 1953, J. Bacteriol. 66:24.
2. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott , Williams & Wilkins, Baltimore, Md .
3. MacFaddin J. F., 1985, Media for the Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore
4. Cowan, 1974, Cowans and Steeles Manual for the Identification of Medical Bacteria, 2nd Ed., Cambridge University Press, Cambridge, Mass.

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