

OF Basal Medium

Intended Use

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

Summary

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

Principle

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. 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.

Formula*

Ingredients	g/L
Casein Enzymic Hydrolysate	2.0
Sodium Chloride	5.0
Dipotassium Phosphate	0.3
Bromothymol Blue	0.08
Agar	2.0
Final pH (at 25°C)	6.8 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability

Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Specimen Collection and Handling

Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 9.38 g of the powder in 1000 mL purified / distilled water.
2. Heat to boiling to dissolve the powder completely.
3. Dispense in 100 mL amounts and sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
4. To first 100 mL of sterile basal medium, aseptically add 10 mL of sterile 10% dextrose solution.
5. To second 100 mL add 10 mL sterile 10% lactose solution.
6. To third 100 mL add 10 mL sterile 10% saccharose solution.
7. Mix and dispense aseptically in 5 mL amounts in sterile tubes in duplicate for aerobic and anaerobic fermentation.

Quality Control

Dehydrated Appearance: Cream to light green, homogeneous free flowing powder.

Prepared Appearance: Green coloured, clear to slightly opalescent semisolid gel forms in tubes.

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

Organism (ATCC)	Only Basal Medium (aerobic)	Only Basal Medium (overlaid with mineral oil)	w/ Dextrose (aerobic)	w/Dextrose (overlaid with mineral oil)
<i>Acinetobacter baumannii</i> (19606)	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
<i>Alcaligenes faecalis</i> (8750)	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
<i>Escherichia coli</i> (25922)	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
<i>Klebsiella aerogenes</i> (13048)	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
<i>Salmonella Enteritidis</i> (13076)	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
<i>Shigella flexneri</i> serotype 2b (12022)	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
<i>Vibrio cholerae</i> (15748)	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

Interpretation of Results

1. 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.
2. Fermentative organisms will produce an acid reaction in both the covered and uncovered medium.
3. Oxidative organisms will produce an acid reaction in the uncovered medium and give slight growth without change in the covered medium.
4. 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.
5. The acidic reaction of oxidative organisms is more apparent at the surface of the medium that gradually spreads throughout the medium.
6. 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.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions / Limitations

1. Dextrose is the most important carbohydrate for use in O F Basal Medium.
2. However, certain organisms may metabolize other carbohydrates even if they are unable to utilize dextrose.
3. Degradation of the carbohydrate to acid is indicated by the pH indicator bromothymol blue which changes its colour to yellow.
4. Oxidative utilization takes place when the medium is exposed to air while fermentative utilization occurs under exclusion of air

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

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.
5. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201150030100	Dehydrated Culture Media	100 g

 Temperature Limit	 Manufacturer	 LOT	 Batch Code	 Date of Manufacture	 This way up	 RO Received on
REF Catalogue Number	 Consult Instructions for use	 Use-by Date	 HC	Hygroscopic keep container tightly closed	OO Opened on	

Revision: 0825/VER-03

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.