OF Basal Medium

M395

Intended use

Recommended for differentiation of gram- negative bacteria from clinical and non-clinical samples on the basis of fermentative and oxidative metabolism of carbohydrates.

Composition**

Ingredients	g / L
Tryptone	2.000
Sodium chloride	5.000
Dipotassium hydrogen 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 purified/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 5 ml amounts aseptically into 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). Tryptone 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).

Type of specimen

Isolated Microorganism from clinical and non-clinical samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines(7,8). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.Since the medium is pH dependent, pH must be monitored.
2.Certain fastidious organisms may not grow and require supplementation of 2% serum or yeast extract (0.1%) (5).

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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

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

Organism	Only Basal Medium (aerobic)	Only Basal Medium (overlayed with mineral oil)	w/ Dextrose (aerobic)	w/Dextrose (overlayed with mineral oil)
Acinetobacter baumannii ATCC 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
Alcaligenes faecalis ATCC 87500	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 (00013*)	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
# *Klebsiella aerogenes* ATCC 13048 (00175*)	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 paraeruginosa* ATCC 9027 (00026*)	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 (00030*)	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 (00126*)	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	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

Key : * Corresponding WDCM numbers,

Formerly known as *Enterobacter aerogenes* ^ Formerly known as *Pseudomonas aeruginosa*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

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4. Cowan, 1974, Cowans and Steeles Manual for the Identification of Medical Bacteria, 2nd Ed., Cambridge University Press,Cambridge, Mass.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
7. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
8. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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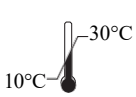
IVD ***In vitro*** **diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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www.cepartner4u.eu

CE **CE Marking**

Do not use if package is damaged

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