OF Basal Medium • OF Medium with Carbohydrates

Intended Use

OF (Oxidation Fermentation) media are used for the determination of oxidative and fermentative metabolism of carbohydrates by gram-negative rods on the basis of acid reaction in either the open or closed system.

Summary and Explanation

OF Medium was developed by Hugh and Leifson who described the taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by gram-negative bacteria.[1] They showed 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 melted petrolatum prior to incubation, the patterns of metabolism are of differential significance. Oxidative organisms only produce an acid reaction in the open tube with little or no growth and no acid formation in the covered tube. Fermentative organisms will produce an acid reaction in both types of tubes.

Changes in the covered agar are considered to be 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

The medium contains a high concentration of added carbohydrates relative to the peptone concentration to avoid the utilization of peptone by an aerobic organism and the resultant production of an alkaline reaction which would neutralize slight acidity produced by an oxidative organism.[2] The dipotassium phosphate adds buffering capacity to the medium. The agar permits the determination of motility and aids in the even distribution of any acid produced at the surface of the medium.[3]

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. Prepared tubed media containing arabinose, dextrose, dulcitol, fructose, galactose, lactose, maltose, mannose, raffinose, rhamnose, salicin, sorbitol, sucrose and xylose are provided.

Formula

Difco™ OF Basal Medium

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	2.0	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	0.3	g
Bromthymol Blue	0.08	g
Agar	2.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ OF Basal Medium

Dehydrated Appearance:	Light beige with green tinge, free-flowing, homogeneous.
Solution:	0.94% solution, soluble in purified water upon boiling. Solution is green, clear to very slightly opalescent.
Prepared Appearance:	Green, clear to very slightly opalescent.
Reaction of 0.94% Solution at 25°C:	pH 6.8 ± 0.2

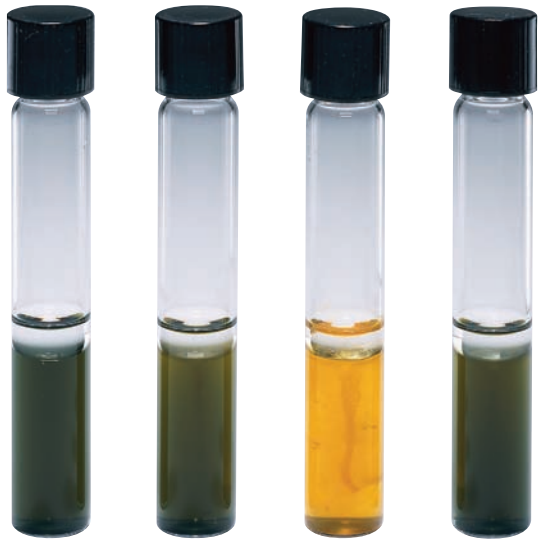
Cultural Response

Difco™ OF Basal Medium

Prepare the medium per label directions without (plain) and with 1% dextrose. Inoculate tubes in duplicate with fresh cultures using an inoculating needle and add an overlay of mineral oil to one set of tubes. Incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	PLAIN OPEN	PLAIN CLOSED	WITH DEXTROSE OPEN	WITH DEXTROSE CLOSED
Acinetobacter calcoaceticus	19606	K	K	A	K
Enterobacter aerogenes	13048	K	K	A, G	A, G
Escherichia coli	25922	K	K	A, G	A, G
Pseudomonas aeruginosa	27853	K	K	A	K
Shigella flexneri	12022	K	K	A	A

K = alkaline reaction, green medium
A = acid reaction, yellow medium
G = gas production



Uninoculated Tube | *Acinetobacter calcoaceticus* ATCC™ 19606 | *Escherichia coli* ATCC™ 25922 | *Pseudomonas aeruginosa* ATCC™ 27853

Tubes above are closed, with Dextrose

Directions for Preparation from Dehydrated Product

1. Suspend 9.4 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Add 1% carbohydrate before or after autoclaving depending on heat lability.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate a pair of OF tubes of each carbohydrate used with each organism being tested. The tubes should be stabbed to approximately 1/4 inch from the bottom using an inoculating needle and a light inoculum. Overlay one tube of each pair with sterile mineral oil. Incubate tubes at 35 ± 2°C in an aerobic atmosphere for 48 hours. Do not discard as negative until after 4 days of incubation.

Expected Results

Record results as acid (A) or alkaline/no change (–). Also record whether or not the organism is motile as evidenced by the appearance of growth away from the line of inoculation. Typical reaction patterns are as follows.[2-4]

Enteric OF Carbohydrate Utilization Patterns

REACTION	TUBE WITH REACTION	OPEN TUBE	COVERED TUBE
Oxidation (O)	Open	Yellow (A)	Green (–)
Fermentation (F)			
Anaerogenic	Covered	Yellow (A)	Yellow (A)
Aerogenic	Covered	Yellow (AG)	Yellow (AG)
Neither Oxidation nor Fermentation (–)	Neither*	Blue or Green (–)	Green (–)
Both Oxidation and Fermentation (O/F)	Both	Yellow (A or AG)	Yellow (A or AG)

A = acid production
G = gas production
– = no change or alkaline
** = Uninoculated carbohydrate control reading; no change in color.*

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[5] recommends adding either 2% serum or 0.1% yeast extract to each carbohydrate tube.

References

1. Hugh and Leifson. 1953. J. Bacteriol. 66:24.
2. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
4. Shigei. 1992. *In* Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
5. Cowan. 1974. Cowan and Steele's manual for the identification of medical bacteria, 2nd ed. Cambridge University Press, Cambridge, Mass.

Availability

Difco™ OF Basal Medium

BAM CCAM

Cat. No. 268820 Dehydrated – 500 g

BBL™ OF Basal Medium

Cat. No. 221326 Prepared Tubes, 5 mL (K Tubes) – Pkg. of 10*

BBL™ OF Basal Medium with Carbohydrates

Cat. No.
297783 Prepared Tubes with Arabinose – Pkg. of 10*
221328 Prepared Tubes with Dextrose – Pkg. of 10*
221329 Prepared Tubes with Dextrose – Ctn. of 100*
297784 Prepared Tubes with Dulcitol – Pkg. of 10*
297366 Prepared Tubes with Fructose (Levulose) – Pkg. of 10*
297785 Prepared Tubes with Galactose – Pkg. of 10*
221330 Prepared Tubes with Lactose – Pkg. of 10*
221332 Prepared Tubes with Maltose – Pkg. of 10*
221334 Prepared Tubes with Mannitol – Pkg. of 10*
297786 Prepared Tubes with Mannose – Pkg. of 10*
296374 Prepared Tubes with Raffinose – Pkg. of 10*
297368 Prepared Tubes with Rhamnose – Pkg. of 10*
297365 Prepared Tubes with Salicin – Pkg. of 10*
297367 Prepared Tubes with Sorbitol – Pkg. of 10*
221336 Prepared Tubes with Sucrose – Pkg. of 10*
221338 Prepared Tubes with Xylose – Pkg. of 10*

**Store at 2-8°C.*