OF BROTH

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.

PRINCIPLE

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.

REAGENTS (FORMULA)

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
Deionized Water	1000.0	ml

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]

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.

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
Escherichia coli ATCC 25922	35 ± 2°C for 18-48 hours	Plain open (K), Plain closed (K), With dextrose open (A,G), With dextrose close (A,G)

ATCC® is a registered trademark of American Type Culture Collection.
K=alkaline reaction, green medium
A=acid reaction, yellow medium
G=gas production

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4. Shigei. 1992. In Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

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