BBL™ OF Basal Medium
BBL™ OF Medium with Dextrose

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

OF Basal Medium, when supplemented with an appropriate carbohydrate, is used to determine the oxidative and fermentative metabolic activities of gram-negative bacilli.

II PERFORMANCE TEST PROCEDURE

1. Loosen caps and boil the medium in a boiling water bath* for approximately 2 min prior to use. Cool to room temperature.

*__NOTE:__ Use of a microwave oven is not recommended.

2. Inoculate representative samples with the cultures listed below.
   a. Inoculate two tubes of each medium by stabbing the medium once with a straight needle, stabbing almost to the bottom of the tube. Use 18- to 24-h **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) cultures as inocula.
   b. Cover one tube of each pair with 1.0 mL of sterile mineral oil. Include uninoculated control tubes (covered and uncovered).
   c. Incubate tubes at 35 ± 2 °C in an aerobic atmosphere.
3. Examine tubes after 18–24 and 42–48 h for growth and reactions.
4. Expected Results

			Reaction	
Medium	**Organisms**	**ATCC®**	**Uncovered**	**Covered with Oil**
OF Basal Medium	**Acinetobacter baumannii*	19606	–	–
	Pseudomonas aeruginosa	10145	–	–
	**Shigella sonnei*	9290	–	–
OF with Dextrose	**Alcaligenes faecalis*	19018	–	–
	**Enterobacter aerogenes*	13048	A	A
	**Pseudomonas aeruginosa*	10145	A	–
	**Shigella sonnei*	9290	A	A

Key: A = acid (yellow), – = unchanged (green) or alkaline (blue)

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under “Product Deterioration.”
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 6.8 ± 0.1 for OF Medium with Dextrose and 6.8 ± 0.2 for OF Basal Medium.
4. Incubate uninoculated representative tubes at 20–25 °C and 30–35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV 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.

V 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.

VI 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.

VII REAGENTS

OF Basal Medium

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	2.0 g	Agar	2.5 g
Sodium Chloride	5.0 g	Bromthymol Blue	0.03 g
Dipotassium Phosphate	0.3 g		

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

OF Medium with Dextrose, contains the above ingredients with, per liter, 10.0 g of dextrose.

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. Prior to discarding, sterilize prepared tubes, specimen containers and other contaminated materials by autoclaving.

Storage Instructions: On receipt, store tubes in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

This product is not intended for use directly with specimens or mixed cultures. The organism to be tested must first be in pure culture.

IX PROCEDURE

Material Provided: OF Basal Medium or OF Medium with Dextrose

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Loosen caps and boil the medium in a boiling water bath* for approximately 2 min prior to use. Cool to room temperature.

Inoculate a pair of OF tubes 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 1.0 mL sterile mineral oil. Include uninoculated control tubes (covered and uncovered).

Incubate tubes at 35 ± 2 °C in an aerobic atmosphere for 48 h. Do not discard as negative until after 4 days of incubation.

***NOTE:** Use of a microwave oven is not recommended.

User Quality Control: See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

A single electrode of sufficiently small size to fit into the tubes should be used to determine the pH potentiometrically of tubed media. The tip of the electrode should be positioned in the central portion of the agar mass in semisolid media.

X RESULTS

Record results as acid (A), or alkaline or 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:[2,3]

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 (A)	Yellow (A)
Neither Oxidation Nor Fermentation(–)	Neither*	Blue or Green (–)	Green (–)
Both Oxidation and Fermentation (O/F)	Both	Yellow (A)	Yellow (A)

A = acid production

– = no change or alkaline

* = Uninoculated carbohydrate control reading; no change in color.

XI LIMITATIONS OF THE PROCEDURE[4]

The acid reaction produced by oxidative organisms is apparent first at the surface and gradually extends throughout the medium. Where oxidation is weak or slow, an initial alkaline reaction may be observed at the surface of the open tube that may persist for several days but will eventually turn acid.

Nonsaccharolytic organisms produce slight alkalinity in the open tube (blue-green color) but the closed tube will not exhibit a color change (green).

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[2,5-7]

XII PERFORMANCE CHARACTERISTICS

OF Basal Medium

Prior to release, all lots of OF Basal Medium are tested for specific performance characteristics. Samples are inoculated directly by stabbing two tubes of the medium with cultures of *Acinetobacter baumannii* ATCC 19606, *Shigella sonnei* ATCC 9290 and *Pseudomonas aeruginosa* ATCC 10145, grown for 18–24 h on **BBL Trypticase** Soy Agar with 5% Sheep Blood. One tube for each organism is covered with 1 mL of mineral oil. Tubes are incubated with loose caps at 35–37 °C for 2 days in an aerobic atmosphere. No color change occurs with any of the organisms with or without mineral oil.

OF Medium with Dextrose

Kantor et al. developed a rapid and simplified scheme, using a minimum of three tests and a maximum of seven tests, that could be used routinely by laboratories to identify nonfermentative gram-negative bacteria. A total of 229 unknown nonfermentative gram-negative organisms and 14 reference strains were identified using this scheme. OF Basal Medium with Glucose (Dextrose) was used, along with oxidase and motility, as the primary tests to subdivide the nonfermenters. OF glucose was used to aid in the speciation of *Acinetobacter*. For non-motile, oxidase-positive organisms, OF glucose was used to aid in the differentiation of *Moraxella* sp. from *Flavobacterium* sp. OF glucose was used to differentiate *Pseudomonas alcaligenes* from other oxidase-positive, motile *Pseudomonas* spp.[8]

XIII AVAILABILITY

Cat. No.	Description
221326	**BD BBL™** OF Basal Medium, Pkg. of 10 size K tubes
221328	**BD BBL™** OF Medium with Dextrose, Pkg. of 10 size K tubes
221329	**BD BBL™** OF Medium with Dextrose, Ctn. of 100 size K tubes

XIV REFERENCES

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6. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
7. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schrekenberger, and W.C. Winn, Jr. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott-Raven, Philadelphia.
8. Kantor, L.T., S.D. Kominos, and R.B. Yee. 1975. Identification of nonfermentative gram-negative bacteria in the clinical laboratory. Am. J. Med. Technol. *41*:3-9.

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