Department of Microbiology
BBL™ OF Media Procedure

I. Purpose and Test Principle

OF media are used for the determination of oxidative and fermentative metabolism of carbohydrates by gram-negative rods on the basis of acid production in either the open or closed system. The medium contains a high concentration of carbohydrates and bromthymol blue as a pH indicator. When carbohydrates are metabolized, the acid byproducts result in a pH shift in medium that is visible by a change in color from green to yellow. For the determination of glucose utilization, two tubes are inoculated with a test isolate. One tube is overlaid with mineral oil to create an anaerobic environment and the other is left uncovered for an aerobic environment. After incubation, a facultative glucose fermenter will produce a yellow color change in both tubes. A glucose oxidizer will only produce a color change in the open tube. A glucose non-oxidizer will not produce a color change in either tube.

II. Specimen Information

The organism to be tested must be in pure culture.

III. Reagents & Equipment

- OF Basal Medium
- OF Medium with Dextrose
- OF Medium with Lactose
- OF Medium with Maltose
- OF Medium with Mannitol
- OF Medium with Sucrose
- OF Medium with Xylose

  Storage: On receipt, store media at 2-8°C. Avoid freezing or overheating. Tubes must be boiled with loose caps for approximately 2 min prior to use. Do not agitate tubes after boiling. Tubes should be allowed to equilibrate to room temperature prior to inoculation.
- Mineral oil
- Inoculation needle and Bunsen burner

IV. Procedure

A. Using an inoculation needle, touch a well isolated colony of the test isolate and stab each medium to within about a ¼ inch from the bottom of the tube. A pair of dextrose tubes and one of each of the other carbohydrates and a basal medium should be inoculated.
B. Overlay one of the dextrose tubes with at least 1 mL of mineral oil.
C. Make sure the lids are left slightly loose to allow for air exchange within the tubes.
D. Incubate the tubes in an aerobic (non-CO_2) incubator at 35 ± 2°C.
E. Examine the tubes for color change daily. Do not discard tubes as negative until after a minimum of 4 full days of incubation.

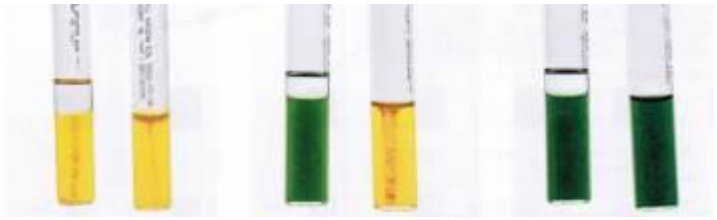
V. Interpretation and Reporting

Examine the tubes for color change. It is helpful to use transmitted light to detect weak reactions. There should be no color change in the base tube.

A. Glucose Fermentation
   A yellow color in the dextrose tube overlaid with oil indicates that the isolate is fermentative. No color change in the overlaid tube indicates that the organism is not a fermenter.

B. Oxidation
   A yellow color in the non-overlaid, open dextrose tube or any of the other carbohydrate tubes indicates that the isolate is oxidative.

C. Asaccharolytic Reaction
   No color change in the open tubes indicates that the organism is not an oxidizer of the specific carbohydrate.



Fermentation Oxidation Non-saccharolytic

VI. Quality Control

Each new lot or shipment of media should be examined for product deterioration and tested with the following control strains by following the procedure outlined above. Incubate tubes at 35 ± 2°C in an aerobic atmosphere (non-CO_2).

Tube	Control strain	Expected Results
Base	*K. pneumoniae* 13882	Green
	M. catarrhalis 25238	Green
Dextrose w/oil	*K. pneumoniae* 13882	Yellow
	P. aeruginosa 27853	Green
Dextrose Lactose Maltose Mannitol Sucrose Xylose	*K. pneumoniae* 13882	Yellow
	M. catarrhalis 25238	Green

VII. Limitations

A. 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.

B. Nonsaccharolytic organisms produce a slight alkalinity in the open tube (blue-green) but the overlaid tube will not exhibit a color change (green).
C. For identification, organisms must be in pure culture.

VIII. Verification of Test Method

Two OF products were evaluated for use. This included BD BBL™ OF Media and OF King Media from Remel. Both media were inoculated with a variety of clinically isolated, previously characterized non-fermenting gram-negative rods. These isolates were obtained from respiratory cultures of cystic fibrosis patients and from other body sites from non-CF patients. The test isolates included 2 *Alcaligenes* spp., 4 *Burkholderia cepacia,* 1 *Chryseobacterium indologenes,* 1 *Flavobacterium orizyhabitans,* 1 *Pseudomonas aeruginosa,* 1 *Ralstonia* spp., 1 *Rhizobium radiobacter,* 1 *Sphingobacterium multivorans,* and 1 *Sphingomonas paucimobilis*. The tubes were inoculated and incubated as recommended by the manufacturers. However, initially the tubes from BD were not boiled for 2 min prior to use as suggested in the package insert. The tubes were incubated in a non-CO_2 incubator and examined for color changes at 24 and 48 h of incubation. The Remel tubes produced color changes for several isolates at 24 h that were not apparent in the BD tubes until 48 h of incubation. However, the phenol red pH indicator in the Remel tubes was much more difficult to interpret than the bromthymol blue indicator in the BD tubes. Because of the potential for ambiguous reactions with the Remel products, the BD OF Media were selected for routine use. However, the BD OF media did produce some false-positive fermentation reactions. Additional testing was performed to determine the cause of these results. Due to the slightly liquid form of the BD media when it is received, the media was boiled for 2 min to help eliminate air bubbles that may have been introduced into the media during shipment. Additional testing using 3 *B. cepacia* isolates revealed that false fermentation was also eliminated by using at least 1 mL of oil to overlay the fermentation tube. Using less oil and not boiling the media prior to use seemed to contribute to the false fermentative reactions previously seen.

IX. References

A. Package insert: BBL™ OF Basal Medium, BBL™ OF Medium with Dextrose L007484, Rev. 08, March 2007.
B. Package insert: OF Basal Medium, BBL™ OF Medium with Lactose, BBL™ OF Medium with Maltose, BBL™ OF Medium with Mannitol, BBL™ OF Medium with Sucrose, BBL™ OF Medium with Xylose L009476, Rev. 00, March 2006.

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Updates and Revisions: