

Oxidative-Fermentative Test Protocol

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Information

History

The oxidative-fermentative (OF) test was developed by Hugh and Leifson and outlined in their 1953 paper (4). Prior to this time microbiologists “had observed that some bacteria produced acid from carbohydrates only under aerobic conditions and others produced acid both under aerobic and anaerobic conditions” (4). Production of acid from the metabolism of carbohydrates in aerobic and anaerobic metabolism was at this time defined as fermentation. Hugh and Leifson were the first to refer to the production of acid from carbohydrates under aerobic conditions only, as oxidative (4). It was noted that the amount of acid produced by bacteria using carbohydrates under aerobic conditions was less than the amount

the media.

Theory

The oxidative-fermentative test determines if certain gram-negative rods metabolize glucose by fermentation or aerobic respiration (oxidatively) (6, 8). During the anaerobic process of fermentation, pyruvate is converted to a variety of mixed acids depending on the type of fermentation. The high concentration of acid produced during fermentation will turn the bromthymol blue indicator in OF media from green to yellow in the presence or absence of oxygen (6,8).

Certain nonfermenting gram-negative bacteria metabolize glucose using aerobic respiration and therefore only produce a small amount of weak acids during the Krebs cycle and Entner Doudoroff (glycolysis) (8). The increased concentration of glucose in the medium enhances the production of these weak acids to a level that can be detected by

OF basal medium is commercially available in a premixed form from biological supply companies. The carbohydrate source is not included and must be added as stated above.

PROTOCOL

I. Oxidative-fermentative test using OF media with glucose

A. Inoculation of media

Two tubes of oxidative-fermentative medium are inoculated by stabbing "half way to the bottom" (8) or $\frac{1}{4}$ -

See Atlas images for other fermentative results

2.

FIG. 3. Oxidative-fermentative test inoculated with *Alcaligenes faecalis*. No color change in the oil-covered tube and color change to alkaline in the open tube indicates a negative result. *A. faecalis* cannot use glucose fermentatively or oxidatively. The blue at the top of the open tube is due to amine production resulting from the metabolism of protein in the media.

See Atlas images for other negative results

II. The oxidative-fermentative test using carbohydrates other than glucose

Nonfermenting gram-negative rods that have been shown to give an oxidative result in an OF glucose test can be further tested for their ability to metabolize other carbohydrates oxidatively. The glucose is replaced by maltose, lactose, mannitol, or sucrose in the medium and only one tube per carbohydrate is inoculated. A heavy inoculum should be used, as many of these nonfermenters are slow growing (6). As the result being detected is based on aerobic respiration no mineral oil or agar layer is used. A positive result is indicated by an acid production and a cha

2. If screw cap tubes are used, do not close them too tightly.
3. Use uninoculated controls with and without oil that are incubated and not incubated as OF media may change color during exposure to incubation temperatures.

REFERENCES

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2. **Blair, J., E. Lennette, and J. Truant.** 1970. Manual of clinical microbiology. American Society for Microbiology, Washington, DC.
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