Improved Anaerobic Indicator

JOHN H. BREWER, DANIEL L. ALLGEIER, AND C. BAXTER McLAUGHLIN

Baltimore Biological Laboratory and Hynson, Westcott & Dunning, Inc., Baltimore, Maryland

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Methylene blue is the most extensively used chemical indicator of anaerobiosis. Its use as such an indicator was reported early in the literature (Smith, Wilder Quarter Century Book, p. 187, 1893). Techniques such as hanging strips of methylene blue-impregnated gauze inside an anaerobic jar were described by van Reimsdijk (Ned. Tijdschr. Geneesk. 66:1423, 1922).

Present-day microbiologists often incorporate reduced methylene blue solutions as an integral part of their oxygen detection systems. All too often, however, in the rush of clinical or industrial research, time is not taken to prepare such indicators. In cases where negative results are obtained, and no anaerobic indicator was used to monitor the effectiveness of the system, the results are often attributed to an inability of the test object to function anaerobically. In reality, however, the equipment used to create anaerobiosis may have functioned improperly or not at all. The importance of establishing the state of anaerobiosis, and of employing an anaerobic indicator in certain biological systems, therefore, is of considerable importance.

We now describe a convenient means for determining anaerobiosis within a Brewer anaerobic jar (J. Lab. Clin. Med. 24:1190, 1939) which contains an electrically heated catalyst, as well as in a new Brewer cold catalyst jar (in press). The indicator unit is easy to operate and may be discarded after use.

The unit (Fig. 1) consists of a heat-sealable plastic sachet which contains a methylene blue solution. The sachet is affixed to a polyethylene-coated card which is placed upright inside the vessel for easy viewing. The film used is Teflon F. E. P., a commercially available transparent fluorocarbon which is extremely permeable to oxygen transfer. There is little water vapor loss because of a high moisture barrier. In addition, it is not adversely affected by prolonged exposure to ethylene oxide gas sterilization. The sachets may be effectively autoclaved, but there is some loss in color contrast.

Each sachet contains 1.0 ml of a solution composed of equal parts of a 60% tris(hydroxy-methyl) aminomethane (Tris), 4% dextrose, and 0.02% methylene blue. The Tris should be heated to solution, and all solutions should be at room temperature before being combined. The sachets are filled and heat-sealed. A 0.5-inch overlap allows the 1 by 2 inch sachets to be stapled to a 2 by 6 inch polyethylene card; the card is plastic-coated to prevent absorption of the condensate which collects in the jar. Prior to attachment to the card, the sachets may be

![Diagram of Anaerobic Indicator Unit](http://aem.asm.org/)

**Fig. 1.** Diagrammatic representation of anaerobic indicator. (1) Plastic-coated cardboard. (2) Teflon bag. (3) Indicator solution.
sterilized by autoclaving, or preferably by ethylene oxide gas sterilization. Ethylene oxide sterilization may be effected by exposure for 1.5 hr at 20 lb of pressure to Oxyfume 80, a commercially available ethylene oxide-carbon dioxide mix.

Methylene blue acts as a hydrogen acceptor and loses its color in the reduced form. One atom of hydrogen is taken up by the double-bonded nitrogen, converting the blue-colored solution into the colorless or leuco form. This reaction is readily reversed by exposure to air or by addition of oxygen. Dextrose is the reducing sugar; Tris buffers the solution.

For use, the card is placed between the jar contents and the glass side. The units are at a convenient height in the jar to be readily observed. In an efficient system, progressive anaerobiosis may be detected within 1 hr by the gradual decolorization of the solution. Complete loss of color occurs within several hours, depending on the effectiveness of the system. If minute amounts of oxygen remain in the system, they may be readily observed as streaks of blue color in the sachet. The units are reusable but should be stored in a dark area when not in use.