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Simplified Method of Anaerobic Incubation

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A method is described that obviates the need for vacuum and tanks of gas mixtures for anaerobiosis in the anaerobic incubator.

The use of a tightly sealed container from which the oxygen has been removed by various means (including a catalyst) is a prerequisite for the cultivation of anaerobic organisms (3, 10, 11). These containers range from several different types of jar and lid arrangements to more complex and expensive anaerobic incubators. Currently some investigators (1, 4, 5, 9, 10) state that there are methods now available, such as the Hungate roll tube method or some modification of it, which are better suited for recovery of anaerobic bacteria from diverse sources. However, one of the most practical methods for achieving anaerobic conditions is still the use of the anaerobe jar. From the original principle and application by Laidlaw (6) and the subsequent adaptation by McIntosh and Fildes (7), many modifications of the anaerobe jar, particularly of the McIntosh and Fildes jar, have been introduced. Among the more widely used has been the self-contained, disposable, anaerobic system (GasPak anaerobic jar, BioQuest, Cockeysville, Md.) of Brewer and Allgeier (2). This system uses a safe, disposable hydrogen and carbon dioxide generator envelope plus a disposable anaerobic indicator to provide a practical system for growing most anaerobes from clinical material in any bacteriology laboratory.

In the course of other investigations, we developed a procedure which permitted anaerobic conditions to be produced readily in the incubator (National Anaerobic Incubator, model no. 3630) without resorting to vacuum and tanked gas mixtures. We were using the method of Miller and Finegold (8) and incubating the plates at 37 C in the anaerobic incubator containing an atmosphere of 90% nitrogen and 10% carbon dioxide obtained after six flushings with nitrogen. Because of problems with the excessive amounts of moisture that accumulated during the incubation period, we tried other methods. The problem was resolved when it became apparent that catalysts and GasPaks provided the ideal anaerobic environment needed for growth. In practice, use of three GasPak envelopes (BBL, no. 70304), held upright in a cardboard 1-pint ice cream container, and three catalysts (BBL, no. 70303), in a 50-ml glass beaker, allowed for excellent growth of the anaerobic bacteria with relatively little or no moisture as long as the incubator door and vents were securely tightened during the incubation. [These catalysts may be reactivated after each use by heating them for 2 hr at 160 C (V. R. Dowell, Jr., *personal communication*).] Moreover, this growth was obtained in 48 hr (with the incubator filled to capacity), shortening the previous incubation time by 72 hr.

Presently, several hundred different strains of anaerobic bacteria have been cultivated by this method with excellent results (*Bacteroides* sp., 234; *Fusobacterium* sp., 33; *Peptococcus* sp., 145; *Peptostreptococcus* sp., 72; *Veillonella* sp., 13; *Clostridium* sp., 51; *Propionibacterium* sp., 16; *Catenabacterium* sp., 5; *Eubacterium* sp., 3; and *Ramibacterium* sp., 1). Indeed, our experience has been that, if growth occurs in the GasPak anaerobe jar, growth will occur in the anaerobic incubator when this GasPak procedure is used. Moreover, this procedure permits the use of an incubator of relatively large volume.

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