Tests of performance of anaerobic jars

B. WATT, J. G. COLLEE, AND R. BROWN

From the Central Microbiological Laboratories, Western General Hospital, Crewe Road, Edinburgh EH4 2XU and Department of Bacteriology, University Medical School, Teviot Place, Edinburgh

SYNOPSIS This paper briefly reviews methods of assessing the in-use performance of anaerobic jars and outlines a simple system combining a rapid test of catalytic activity with a biological indicator that can detect defects in the jars after incubation.

Since the development of the anaerobic jar as a method for the cultivation of anaerobic bacteria, it has been customary to include an indicator in each anaerobic jar at the time of incubation to check that satisfactory conditions of anaerobiosis are achieved after processing and maintained during incubation of the jars. Two types of indicator have been described, biological indicators and chemical indicator solutions.

Biological Indicators

Several workers have described the use of culture plates seeded with anaerobic bacteria and incubated in the test jar as biological indicators of anaerobiosis. Organisms that have been used include *Clostridium tetani* (see Willis, 1964), and *C. sporogenes* (Wiel-Korstange and Winkler, 1970). More demanding organisms such as *C. oedematiens* (*C. novyii*) type D have been used as indicators of extreme conditions of anaerobiosis in an anaerobic cabinet (Watt *et al.*, 1974).

Biological indicators can provide a useful check on the adequacy of anaerobic procedure and on the performance of anaerobic jars, as a significant defect would result in failure of growth of the indicator organism. However, there are two main disadvantages:

- (1) The results are not known until completion of the incubation period—too late for timely correction of a fault in the anaerobic system; and
- (2) if a very demanding organism is used as the biological indicator, failure of its growth may be due to nutritional factors and not invariably attributable to faulty anaerobic procedure or a faulty anaerobic jar. On the other hand, faulty technique

may be overlooked and maintenance of equipment neglected if an 'easy' organism such as *C. welchii* is used as the indicator organism.

Chemical Indicator Solutions

Indicator solutions of redox dyes such as those described by Fildes and McIntosh (1921) and modified by Parker (1955) can be used to monitor the establishment of anaerobic conditions in anaerobic jars. Methylene blue is commonly used, and other dyes such as resazurin have been recommended (Drollette, 1969). These dyes show a colour change when the gaseous environment has become sufficiently anaerobic; they are used in tubes held inside glass anaerobic jars or attached to the side-arms of metal jars. Preparation of the original indicator solutions was tedious and involved mixing several stock solutions. To overcome this, single-stage indicators were developed; that described by Ulrich and Larsen (1948) was found to deteriorate on storage and to carry a risk of toxicity (Parker, 1955), but a more recent indicator (Lucas, cited by Stokes, 1968) appears to be more satisfactory. Methylene blue, borax, thioglycollic acid, and phenol red are incorporated into semisolid agar, and the complete indicator can then be dispensed into ampoules. The ampoules are sealed until required, when they are opened and connected to the side-arms of anaerobic jars with rubber tubing. Ampoules of this type made by us have not always given consistently good results.

Rutter (1968) considered some of the difficulties associated with indicator solutions. External indicators, as supplied with Baird and Tatlock Ltd (BTL) jars, proved unreliable and the rubber tubing connecting the indicator to the side-arm of the jars was a common source of leaks. For this reason, Rutter prepared indicator solutions that

could be used as internal indicators, ie, placed within the anaerobic jar during incubation.

As internal indicators, like biological indicators, reveal defective jar performance only after incubation for some hours, too late for prompt remedial action to be taken, we sought a rapid method for assessing the activity of the catalyst and the efficiency of the jar before incubation.

The 'Secondary Vacuum' Method of testing Catalytic Activity

Preliminary experiments showed that when BTL anaerobic jars, each equipped with a single room-temperature catalyst, are evacuated to 660 mmHg below atmospheric pressure (ie, 6/7 of the air removed) and then equilibrated to atmospheric pressure with a mixture of 90% H₂ and 10% CO₂, a 'secondary vacuum' of at least 20 mmHg develops after 10 minutes on the bench at room temperature if the catalyst is active. If the catalyst is defective or absent, little or no vacuum develops within 10 minutes. Jars with leaks also fail to develop a secondary vacuum after 10 minutes. Thus the activity of the catalyst can be checked in time to allow replacement of a faulty sachet and reprocessing of the jar before incubation. In our standardized anaerobic procedure for use with the BTL anaerobic jar (Collee *et al*, 1972) we recommend that the secondary vacuum should be checked on a simple mercury manometer after leaving the processed jar for 10 minutes at room temperature. Jars that produce a vacuum of less than 20 mmHg should be checked for leakage, and their catalytic sachets should be renewed.

Our procedure allows us to obtain recoveries of clinically important anaerobes with the BTL anaerobic jar comparable with those obtained with an anaerobic cabinet (Watt *et al*, 1974), and the use of the secondary vacuum method led us to dispense with the external side-arm tubes on the jars so that the side-arms can be sealed and a source of leaks avoided (see above).

Use of a Biological Indicator with the Standardized Anaerobic Procedure

Although the above system has worked well, on occasion a jar giving a satisfactory secondary vacuum after 10 minutes has given suboptimal recovery of a demanding anaerobe such as *C. oedematiens* after incubation. Such a jar was shown to have a slow leak, not detectable after 10 minutes; the jar gave normal recoveries of less demanding organisms such as *C. welchii*.

It was therefore decided that a biological in-

dicator, placed in the jar with the other seeded plates, would serve as an additional check on the establishment and maintenance of adequately anaerobic conditions during incubation. The choice of a test organism presented difficulties: *C. tetani* is an unnecessarily dangerous organism for routine use; *C. sporogenes* is a potent source of persistent contamination; and *Bacteroides* spp are generally not sufficiently demanding. It was therefore decided to try a 'negative' indicator, ie, an aerobic organism that will not grow if conditions are strictly anaerobic but will show growth when conditions of anaerobiosis are suboptimal.

Preliminary studies showed that *Pseudomonas aeruginosa* (*pyocyanea*) was a suitable organism. Although a few strains grew on blood agar plates even when conditions of anaerobiosis, as assessed by recovery of demanding anaerobes, were optimal, none of four strains tested was able to grow on plates of Oxoid nutrient agar without blood when optimal anaerobic conditions were maintained. This has now been confirmed for all strains of *Ps. aeruginosa* tested. Other workers have noted that the presence of nitrate allows *Pseudomonas* to grow anaerobically (Robinson, 1932); this must be borne in mind in the selection of the medium. Growth on nutrient agar occurred only if the jar or catalyst was faulty. There was excellent correlation between growth of *Ps. aeruginosa* on the indicator plates and the presence of a defect, usually a leak, in the anaerobic jar. The system can be summarized as follows:

1. A nutrient agar (Blood Agar Base No. 2, Oxoid) plate, freshly seeded with a loopful of a broth culture of *Ps. aeruginosa*, is placed in each anaerobic jar along with the other plates for incubation.
 2. The jars are processed, left for 10 minutes at room temperature, and the secondary vacuum is then measured simply by manometry.
 3. Jars showing a satisfactory vacuum are equilibrated with the H₂/CO₂ mixture and are then incubated; jars showing a vacuum of less than 20 mmHg are checked for leaks and their catalyst sachets are replaced.
 4. After incubation, the indicator plate is examined for growth; any jar showing growth of *Ps. aeruginosa* is carefully tested for the presence of leaks.
 5. Leakages, once identified, are repaired and the jar is retested (steps 1-4 above) before being returned for routine use.
- Note Cultures of *Ps. aeruginosa* must be checked periodically for purity; contaminant organisms (eg, *Proteus* spp or *Escherichia coli*) can grow in otherwise satisfactory jars and give erroneous results.

Discussion

Anaerobic procedure tends to be a neglected technique in the busy diagnostic laboratory and anaerobic jars are often faulty. Even with careful procedure and satisfactory jars the catalyst sachets in the jars will fail in time. Thus chemical or biological indicators have been developed that allow checks to be made of the efficiency of anaerobic jars in achieving anaerobiosis. However, in the case of chemical indicators there are specific problems in relating the colour changes observed to the conditions in an anaerobic jar and the E_h at which the indicators change colour. In addition, both chemical and biological indicators can only be checked after incubation. Thus, if reliance is placed solely on one of these methods, it may not be possible to discover faulty catalysts or leaks in jars until after the period of incubation—too late for remedial action to be taken.

We have developed a combined approach to the monitoring of our anaerobic procedure that uses a rapid index of catalytic activity in conjunction with a biological indicator. The monitoring of a secondary vacuum by simple manometry allows detection of faulty sachets or leakages in the jars after only 10 minutes, so that rapid corrective measures can be taken, thus preventing the failure of growth of our anaerobic plates. Any small leakages that are not detected by this method will show themselves after incubation by the presence of the obvious and characteristic growth of *Ps. aeruginosa* on the indicator plates. Such small leakages may allow partial recovery of anaerobic bacteria on culture plates, but the detection of leakages allows them to be remedied before the jar is used again. In addition, the indicator plates will detect faulty anaerobic tech-

nique; 'positive plates' provide a clear indication that improved technique is necessary.

We have used this system in a research laboratory and in a busy diagnostic laboratory for over a year. It has proved simple and convenient to operate and has led to a general improvement in standards of anaerobic procedure. We would recommend its use in laboratories that strive to recover anaerobes satisfactorily from clinical specimens.

J.G.C. acknowledges the support of a Medical Research Council Grant (No. G974/325/B).

References

- Collee, J. G., Watt, B., Fowler, E. B., and Brown, R. (1972). An evaluation of the Gaspak system in the culture of anaerobic bacteria. *J. appl. Bact.*, **35**, 71-82.
- Drollette, D. D. (1969). Anaerobic indicators: a review. *Amer. J. med. Technol.*, **35**, 758-760.
- Fildes, P. and McIntosh, J. (1921). An improved form of the McIntosh and Fildes' anaerobic jar. *Brit. J. exp. Path.*, **2**, 153-154.
- Parker, C. A. (1955). Anaerobiosis with iron wool. *Aust. J. exp. Biol. med. Sci.*, **33**, 33-37.
- Robinson, G. L. (1932). The growth of *B. pyocyaneus* in synthetic media. *Brit. J. exp. Path.*, **13**, 310-317.
- Rutter, J. M. (1968). The occurrence and pathogenicity of *Clostridium oedematiens* in animals. Ph.D. Thesis, University of Edinburgh.
- Stokes, E. J. (1968). *Clinical Bacteriology*, 3rd edition. Arnold, London.
- Ulrich, J. A. and Larsen, A. M. (1948). A single solution indicator for anaerobiosis. *J. Bact.*, **56**, 373-374.
- Watt, B., Collee, J. G., and Brown, R. (1974). The isolation of strict anaerobes: the use of an anaerobic cabinet compared with a conventional procedure. *J. med. Microbiol.*, **7**, 315-324.
- Wiel-Korstange, J. A. van d. and Winkler, K. C. (1970). Medium for differential count of the anaerobic flora in human feces. *Appl. Microbiol.*, **20**, 168-169.
- Willis, A. T. (1964). *Anaerobic Bacteria in Clinical Medicine*, 2nd edition. Butterworth, London.