Technical methods

A new anaerobic jar

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In clinical microbiology laboratories the anaerobic jar provides the standard means of obtaining an oxygen-free atmosphere for the isolation and culture of obligately anaerobic bacteria. Although anaerobic jars have been used with considerable success over the years, some contemporary modifications of design may lead to deficiencies in the anaerobic system.

This paper describes a new vented anaerobic jar (Don Whitley Scientific) which overcomes many of the technical problems met with in other commercially available jars.

The Whitley anaerobic jar

The jar is cast in a corrosion-resistant aluminium alloy and finished inside and out with an epoxy resin. The lid, which has a locating flange on the underside, is held in place by an alloy clamp. A gas-tight seal is ensured by a replaceable rubber 'O' ring which is recessed in a groove into the flange of the jar. There is no side-arm attachment for an external indicator of anaerobiosis. The lid is domed so that the catalyst sachet is recessed on its underside. The jar holds 11 standard 9 cm plastic petri dishes.

VENTING

Venting of the jar is provided by two special Schraeder valves mounted in the lid. These valves are of the type used for aircraft tyres and are designed to withstand a much wider range of pressures than is encountered in an anaerobic jar. The valve consists of a body and a valve core which is easily and cheaply replaced. The valves are actuated by chuck fittings which are attached to the end of the vacuum, manometer, and gas lines. When the chuck is clipped onto the valve body, the core pin is automatically depressed and the valve is fully opened, thus permitting the free flow of gases in either direction. The valve automatically re-seals when the chuck is removed.

CATALYST

The catalyst sachet is a twin capsule made from fine mesh stainless steel gauze and charged with 4 g of catalyst ('D' catalyst, Englehard Industries). It is held in place in the recess of the lid by a steel finger spring.

Operation of the jar

The jar has been built to operate on the evacuation-replacement principle or with an internal disposable hydrogen/carbon dioxide generator of the GasPak (Becton Dickinson Ltd) or GasKit (Don Whitley Scientific) type.

USE OF AN EXTERNAL GAS SOURCE

The jar is connected to the manometer and vacuum lines by clipping the chucks to the valves. Air is evacuated until there is a 'negative pressure' in the jar of about 30 cm Hg. The vacuum line is then disconnected. If there are any leaks in the system the manometer will register an increasing internal pressure in the jar, ie, the manometer reading will fall. A low-pressure hydrogen/carbon dioxide line is attached to the other valve to allow the gas mixture to enter the jar; it is disconnected when the internal pressure has risen to that of the atmosphere. If the catalyst is fully active a 'negative pressure' begins to register on the manometer within a few seconds. This 'negative pressure' should be in excess of 5 cm

Figure The Whitley anaerobic jar.

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Hg in 2 minutes and increase to about 20 cm Hg in 5 to 10 minutes. If these pressure changes occur more slowly, or do not occur at all, the catalyst is either partly or completely inactivated.

USE OF AN INTERNAL GAS GENERATOR

Before use, the integrity of the jar should be tested manometrically under 'negative pressure' as outlined above. In addition, a test under positive pressure should also be applied; this is conveniently effected with a bicycle pump attached to one of the Schraeder valves. When the clamp is fingertight, air starts to escape from around the 'O' ring seal at an internal pressure of about 25 cm Hg above atmospheric. In use, the activated gas generator (GasPak or GasKit) is placed in the jar and the lid is secured immediately. The manometer chuck is clipped to one of the valves and the pressure changes are observed. With the GasKit generator evolution of hydrogen is prompt; there is a rapid increase in pressure to about 16 cm Hg by 6-8 minutes. Thereafter the pressure falls quickly as catalysis occurs, reaching about 12 cm at 10 minutes. Catalysis is virtually complete after about 20 minutes (Ferguson *et al.*, 1976).

With the GasPak generator, slower evolution of hydrogen leads to the development of a substantial 'negative pressure' as catalysis occurs; complete anaerobiosis is achieved in about one hour (Brewer and Allgeier, 1966).

Culture of obligate anaerobes

The efficiency of the Whitley jar was examined by performing duplicate surface viable counts (Miles and Misra, 1938) on a selection of obligate anaerobes (*Bacteroides fragilis*, *Bacteroides capillosus*, *Fusobacterium varium*, and *Peptostreptococcus* species) for incubation in both the Whitley jar and in the standard anaerobic jars used in this laboratory. In all cases similar counts were obtained in both types of jar.

Further comparisons were made on a semi-quantitative basis in which the growth of pure cultures of a variety of anaerobes, including *Clostridium tetani* and *Clostridium haemolyticum*, and the growth of anaerobes in clinical specimens were compared. In no instance was there any obvious difference in the quality or quantity of bacterial growth in the two types of jars.

Discussion

The two essential criteria for the development and maintenance of anaerobiosis in anaerobic jars are (1) the integrity of the jar, and (2) the presence of an adequate quantity of active palladium catalyst. A

manometric check of these must be performed before incubation if failures of anaerobiosis are to be avoided.

The integrity of an anaerobic jar is most likely to fail at the 'O' ring seal, at the vents, and at the side arm of jars designed to carry an external chemical indicator of anaerobic conditions. There appears to be no way of improving the 'O' ring seal which is common to all modern anaerobic jars. The benefit of a side arm facility is small compared with the risk of leaks developing at this site. Indeed, few laboratories make use of this indicator attachment, and many have sealed the side arm permanently. Ill-fitting or maladjusted vents are a common cause of failure of anaerobic jars. Conventional needle valves mounted on the lid of the jar are a potential source of leaks, which may occur when the valve is in either the open or closed position. The points of leakage are the leather packing washers, which become worn through normal use, the needle ends, and the valve seats, both of which are easily damaged if the valve is overtightened. Replacement needle valves are expensive. In polycarbonate jars, the vent consists merely of a nozzle to which is attached a piece of rubber tubing; closure is effected by means of a gate clamp. This type of venting system is clumsy in operation and has the inherent faults of any pressure system that utilises rubber tubing.

The use of Schraeder valves in anaerobic jars overcomes the disadvantages of conventional venting systems. Evacuation, replacement, and manometry of internal pressures are easily and quickly effected by means of Schraeder valves and their chuck attachments. This valve system has been subjected to exhaustive testing in this laboratory over a period of 12 months and has been found to be superior to conventional needle valves for reliability and ease of operation.

A useful innovation of design in the Whitley jar is the twin-chambered catalyst sachet, which holds 4 g of palladised alumina pellets. The rate of development of anaerobiosis in the modern anaerobic jar is a function both of the surface area of active palladium catalyst, and of the initial proportions of hydrogen and oxygen in the jar atmosphere. Thus, both inadequate amounts of catalyst and hydrogen 'starvation' may greatly extend the time taken for anaerobiosis to be achieved. The studies of Watt *et al.* (1973) showed that the recovery of demanding anaerobes was greatly improved when the amount of catalyst used in a standard anaerobic jar was increased from 1 g to 3 g. The double-compartment design of the new sachet ensures increased surface area exposure of 4 g of palladised alumina pellets for prompt and rapid catalysis in the presence of suitable concentrations of hydrogen and oxygen.

The steel finger spring facilitates rapid change of the catalyst as required.

In routine use the Whitley anaerobic jar has proved entirely reliable and has performed satisfactorily both with external cylindered gas and with gas provided internally from both GasPak and GasKit generators.

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A method for standardised resuspension of lymphocyte E-rosettes

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In recent years the number of requests to immunology laboratories for enumeration of T (thymus dependent) and B (bone marrow derived) lymphocytes in peripheral blood has increased considerably. T lymphocytes can be identified by their ability to form non-immune rosettes with sheep red blood cells (Fröland, 1972; Jondal *et al.*, 1972); although the enumeration of E-rosettes is relatively simple, the results may be invalidated by disruption during resuspension of the pellet. Since the widely used method of manual Pasteur pipette resuspension produces variable disruption, depending on the experience of the worker, various attempts at standardisation of the procedure have been proposed, such as the use of a Matburn rotary mixer (Papamichail *et al.*, 1972) or manual rolling of the tubes (Roszman *et al.*, 1977), but these have proved unsatisfactory in this laboratory. To overcome this technical problem we designed a simple and inexpensive piece of apparatus (Figure) which, regardless of the operator's experience in the technique, gives reproducible results.

Material and methods

APPARATUS

This consists of a synchronous motor and gearbox driving an arm bearing a free-running roller at 6 rpm within a U-shaped base plate. As the arm rotates, the roller has a peristaltic effect on plastic tubing which is open to the atmosphere at one end and at the other is connected to a Pasteur pipette held with a clip at a constant position within a plastic test tube (LP3, Luckham Ltd, Burgess Hill, Sussex) containing the cell pellet. During the first half of each cycle of the rotor, a standard volume of supernatant is slowly and gently drawn into the Pasteur pipette by compression of the tubing; the speed of rotation is such that during the second half

Figure Apparatus for resuspension of cell pellet.

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