Technical methods

Simple economical anaerobiosis

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The usual technique for the identification of anaerobes from clinical material involves inoculation of solid media to produce isolated colonies and subsequent incubation in one of several types of anaerobic jar. These methods necessitate the use of a vacuum system and a source of hydrogen. The Gaspak system (BBL), which dispenses with evacuation, whilst providing an atmosphere of carbon dioxide, has recently been evaluated (Collee, Watt, Fowler, and Brown, 1972). For reasons of space, economics, or specimen transit, the above methods may be inconvenient, especially in small laboratories.

A method of anaerobiosis which overcomes these problems has been used for the culture of *Clostridium butyricum* (Parker, 1955). This method has been adapted for use in the clinical laboratory by using readily available plastic bags.

Materials and Methods

Although the proportions of the ingredients appear to be flexible, the choice of plastic bag was found to influence the effectiveness of the system. The following has been adopted for routine use.

The materials required are 2-4 g of the cheapest iron wool which is not pre-soaped or rust resistant; domestic vinegar or 1% acetic acid mixed with a wetting agent such as 0.25% sodium lauryl sulphate or some brands of commercial household detergents; a small pledge of absorbent cotton wool soaked in a saturated solution of sodium bicarbonate; a 15 cm by 30 cm bag made from laminated plastic or polyvinylidene chloride, a bulldog clip, and the lid of a glass or plastic Petri dish.

The pledget of iron wool is placed in the Petri dish and soaked with sufficient acid-detergent mixture to moisten but not supersaturate the wool, thus avoiding excess fluid flooding into the dish. The absorbent

cotton wool soaked in the bicarbonate solution is positioned alongside but not touching the iron wool. Insert into the plastic bag the inverted test plate, a control plate culture of a strict anaerobe such as *Clostridium tetani*, or if preferred, a semi-solid visual indicator of anaerobiosis (Baker, Silverton, and Luckcock, 1966) and the iron wool-bicarbonate system. Press the bag to remove excess air, fold over the end of the bag several times, clamp with the bulldog clip, thus sealing the system. The system must be maintained in a level position in the incubator to avoid any excess fluid spilling. When several plates had to be incubated simultaneously, the small plastic bags were replaced by larger bags. Under these conditions it has proved advisable to increase the amount of iron wool used, otherwise anaerobiosis may be delayed due to the extra volume of air.

Results

The type of bag recommended was selected by comparing viable counts from 24-hour broth cultures of *Clostridium tetani*, *Clostridium sporogenes*, and *Clostridium welchii*, diluted in peptone water by the method of Miles and Misra (1938) using blood agar plates as the culture substrate. In the case of *Clostridium tetani* the agar strength was increased to form firm agar plates; the plate surfaces were well dried and spread with 0.1 ml of tetanus antiserum (Willis and Williams, 1970). The commercially available bags tested were made from various plastics as listed in table I and were compared with a conventional anaerobic jar.

A difference of means ($P < 0.05$) was found between *Clostridium tetani* in low density polyethylene and the other containers; in all other comparisons $P > 0.05$.

For several months the recommended procedure was used in parallel with Oxoid thioglycollate broth medium for the routine culture of suitable specimens. On the limited specimens available *Clostridium perfringens*, *Bacteroides* species, anaerobic streptococci, and *Actinomyces israeli* were grown by both methods, in either pure or mixed culture from 21 individual patients. The specimens included pus from peritoneal abscesses, high vaginal swabs, and swabs from lesions of feet. A hundred and fifty

Container	Oxygen Permeability (ml (STP)/M ² /24 hr/ cmHg) Measured at 25°C/65% RH	Mean Count of Five Tests		
		<i>Clostridium tetani</i>	<i>Clostridium sporogenes</i>	<i>Clostridium welchii</i>
		Dilution 1/100 000	Dilution 1/1000	Dilution 1/100 000
Anaerobic jar	0.00	28	71	55
Low density polyethylene (LDPE)	120.00	0.016	60	49
Polyvinylidene chloride (PVDC)	00.11	24	66	56
Laminated plastic composed of LDPE and nitro-cellulose	<00.11	26	67	54

Table Mean counts of five tests

other specimens which were checked proved negative for anaerobes by both methods.

On one occasion when due to the distances involved, a delay between collection of the specimen and incubation was expected, this method was used to transport the inoculated plates under anaerobic conditions from the patient's bedside to the laboratory, thus reducing the possibility of the loss of oxygen-sensitive organisms.

Comment

Using the anaerobic system suggested, the onset of anaerobiosis is rapid due to the formation of ferric acetate from the ferrous salt formed between the acetic acid and iron wool. Minute air leaks are combated and anaerobiosis is maintained by the formation of complex ferric salts from the unused iron, similar to the normal rusting process.

It has been noted that the colour of the treated wool acts as a supplementary indicator of anaerobiosis because when there is an air leak an excess of red ferric salts is formed, whereas under normal anaerobic conditions the iron wool is predominantly black.

The results of the *Clostridium tetani* culture in the low density polyethylene bag do not confirm that anaerobiosis is maintained as $P < 0.05$. The bags used were packing from plastic Petri dishes and it was found that the walls of the bags were weakened due to the Petri dishes rubbing on the plastic. On checking further bags, both by the ether method (Baker *et al*, 1966) and visually, it was found that some bags contained minute holes, probably due to rough handling on their 2000 mile journey from manufacturer to consumer. In emergencies this

defect has been overcome by a visual check for wear and by using two bags positioned to superimpose the intact area of the inner bag next to the possibly damaged area of the outer bag. When the two-bag system was compared with the other methods tested previously, it was found that $P > 0.05$.

The size of discrete colonies varied between the methods and commencing with the smaller colonies they were in the following order: LDPE < 2 LDPE < PVDC < laminated plastic $\approx$ anaerobic jar.

The problem of water condensate and jar contamination leading to routine disinfection procedures is eliminated because the system is completely disposable. The method is sufficiently convenient and economical to allow incubation of each anaerobic plate immediately following inoculation.

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