Table 1 *Depletion of oxygen by combustion of methylated spirit in jars containing varying numbers of culture plates*

	No of plates				
	2	4	6	8	10
Oxygen lost by heating (%)	3·8	3·6	3·7	3·1	2·4
Oxygen lost by burning (%)	6·7	6·7	5·8	6·0	4·6
Final concentration of oxygen (%)	10·5	10·7	11·5	11·9	14·0

Table 2 *Growth of three campylobacter species in jars prepared in different ways*

	Log10 values of colony counts with given jar preparation				
	Candle burn	Methylated spirit burn	Vacuum* plus added carbon dioxide		
			(a)	(b)	(c)
C laridis	No growth	6·8	6·7	6·6	6·6
C coli	4·7	6·0	5·7	6·5	6·2
C jejuni	5·7	7·4	7·0	7·7	7·3

*380 mm Hg.
(a) by reaction of citric acid with sodium bicarbonate.
(b) by candle burn before drawing vacuum.
(c) by expired air.

carbon dioxide. Even more striking than the improvement in viable counts by the use of these simple alternatives to the candle jar was the much greater size of the colonies obtained.

Discussion

The combustion of methylated spirit provides an atmosphere much more suitable for the growth of "thermophilic" campylobacters than a candle jar, and with little extra trouble or expense. Some variation in the level of oxygen is inevitable according to the loading of the jar, but if this does not exceed eight plates in a standard anaerobic jar satisfactory results can be expected.

If a jar with a tap and means of drawing a vacuum are available levels of oxygen can be controlled exactly, irrespective of loading. Three equally effective ways of providing the necessary supplement of carbon dioxide have been described.

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Requests for reprints to: Dr CD Ribeiro, Consultant Medical Microbiologist, Public Health Laboratory, University Hospital of Wales, Heath Park, Cardiff CF4 4XW, Wales.

An easy way to orientate small muscle biopsy tissue

PC SO *Department of Pathology, University of Hong Kong, Hong Kong*

With the advent of various histochemical techniques for studying human skeletal muscle the diagnostic value of muscle biopsy to help establish the major categories of muscle diseases has grown considerably. Undoubtedly, an open surgical biopsy can show a greater area of the diseased muscle and with better precision. Percutaneous needle biopsy of skeletal muscle, on the other hand, is rapid and relatively atraumatic: complications are rare. In addition, minimal preparation of the patient is required, and biopsy can be carried out in the outpatient clinic as well as on the hospital ward. Specimens obtained by means of the needle biopsy technique, however, are usually small, which makes them difficult to orientate adequately for cutting true transverse sections. I describe a method that enables well orientated transverse cryostat sections to be obtained easily and essentially free of ice crystal artefacts.

Accepted for publication 19 June 1985

Material and methods

The various pieces of muscle tissue obtained by percutaneous needle biopsy were placed on to a blank

glass slide and examined under a dissecting microscope to check orientation. The tissue was kept moist with the minimal amount of physiological saline if required. A suitable block was selected for histochemical study and rolled in talcum powder until evently coated. Meanwhile, a small piece of aluminium foil was folded to provide a groove on to which was applied a small amount of Tissue-Tek II OCT embedding compound (Lab-Tek). The coated muscle tissue was then placed into the OCT compound at one end, care being taken to ensure that the muscle fibres were running in a parallel direction with the groove. The aluminium foil was held in a pair of forceps and immediately plunged into a Dewar of liquid nitrogen and vortex mixed.

When large bubbles stopped forming the aluminium foil, together with the frozen tissue, was transferred into the cryostat at −20°C. The frozen strip of OCT compound with the muscle block embedded in it was easily freed from the aluminium foil. Excessive OCT compound was then trimmed away using a precooled razor blade at the end opposite to the tissue to provide a flat base for embedding. A tissue holder was then placed on to the freezing stage of the cryostat and a small blob of OCT compound added. When it began to solidify the block of frozen muscle was carefully picked up with a pair of precooled fine forceps and immediately embedded flat end first into the OCT compound. Attachment was facilitated by a cryospray.

Results and discussion

This method allows well orientated transverse sections to be easily obtained, using the antiroll device of the cryostat, although slight adjustment of the tissue holder may sometimes be required. Rapid freezing of the muscle tissue is essential to avoid ice crystal artefacts, and this can be achieved by using talcum powder.[1 2] Ice crystals can still occur, however, if the frozen tissue is allowed to thaw again during the stage of embedding. With the technique described here only the previously frozen strip of OCT compound is fused with the newly applied OCT embedding medium, leaving the tissue practically untouched. Therefore, sections cut from this final preparation are essentially free of any ice crystal artefacts. Finally, although this technique is primarily designed for percutaneous needle biopsy specimens, it applies equally well to samples obtained at open surgical biopsy.

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Requests for reprints to: Mr PC So, Histopathology Laboratory, Department of Pathology, University of Hong Kong, Queen Mary Hospital Compound, Hong Kong.

Letters to the Editor

Safe method for identifying cryptosporidium cysts in the faeces of patients with suspected AIDS, or those infected with other serious concomitant pathogens

The first case of human cryptosporidiosis was reported in 1976.[1] Since then this parasite has been associated with outbreaks of diarrhoea in children and occasionally adults and, recently, as a serious complication in some patients with the acquired immune deficiency syndrome (AIDS).[2] Although faeces sent for examination from patients in these first two groups pose no special safety problems, specimens from patients with suspected AIDS need to be handled with special precautions.

Recent work suggested that the Ziehl-Neelsen (hot or cold) and phenol auramine fluorescence stains[3a b] are among the best methods for identifying cryptosporidium cysts in faecal smears.[4] We tried these and other methods over a wide range of medical and veterinary specimens and,

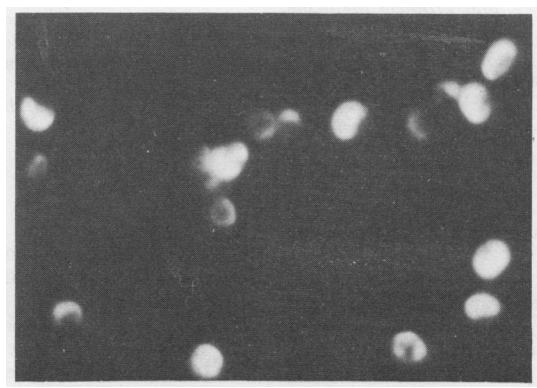

Fig. 1 *Concentrated faecal preparation after Glutaraldehyde fixation of cryptosporidium cysts, showing fluorescence after phenol auramine staining.* × *3000.*