Technical methods

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 evenly 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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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 stains3a,4 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.
with other colleagues, found that the most
reliable, when checked for both false posi-
tive and false negative results by using
simultaneous electronmicroscopical exami-
nation, was the phenol auramine tech-
nique; so far, every result has been con-
irmed by electron microscopy.

We report here that the phenol auramine
method still works properly when faeces
that have been previously fixed in 3% cacodylate buffered glutaraldehyde (pH 7-4) are examined. It may also be used to
replace formalin in formol-ether concentra-
tions, and the auramine staining is still
excellent for cryptosporidium, even after
secondary concentration for specimens
with very low cyst content (Figs. 1 and 2).
In general, we found that morphology was
much better preserved using glutaral-
dehyde instead of formalin, both at light
and electron microscopy levels for all these
different techniques.

Safe and adequate fixation is achieved
by using a 10:1 fixative: faecal mass mix-
ture which is shaken and left for one hour
before slow centrifugation to reform a mass
for light or electron microscopy prepara-
tions.

Thus faeces from patients suspected of
having AIDS or those likely to contain
dangerous pathogens can be glutaral-
dehyde fixed and then stained for crypto-
sporidium by the phenol auramine method
without loss of sensitivity, so avoiding the
hazard of potential laboratory infection.

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Bacteraemia secondary to pseudomem-
branous colitis.

In their recent paper Rampling et al
described bacteraemia in nine neutropenic
patients, out of a total of 17, who had Clo-
stridium difficile infection. In the authors'
experience none of the non-neutropenic
patients developed this complication. We
report a case of secondary bacteraemia that
occurred after pseudomembranous colitis
in a non-neutropenic patient.

An 80 year old woman was admitted to
this hospital after a fall in which she frac-
tured her left femur. She received Magna-
pen (fluoxacillin and ampicillin) 500 mg
intra-muscularly preoperatively, which was
continued orally in the same dose four
times a day for one week postoperatively.
On admission her white cell count was 8.9 ×
10⁹/l. Ten days postoperatively she
came febrile and confused. A mid-
stream specimen of urine taken 48 hours
previously had grown 10⁶ organisms/ml of
Klebsiella pneumoniae which were resistant
to ampicillin. There were no pus cells. She
was started on a course of cephalaxin
250 mg four times a day. After 72 hours of
this treatment she developed abdominal
pain and diarrhoea. The white cell count
rose to 19.2 × 10⁹/l with 90% polymorphs.
Over the next four days her haemoglobin concentration fell from 13 g
to 10 g and she became hypoalbuminaemic
(23 g/l) and hypokalaemic (2.3 mmol/
1.6Eg/l). Urea concentration rose to
13 mmol/l (78 mg/100 ml), but bilirubin
concentrations remained within the normal
range. Her condition deteriorated. She
passed seven loose, greenish motions a day,
took little orally, and was more confused
and agitated. On examination her abdo-

Fig. 2  Same preparation further processed and sectioned for electron microscopy examination. Cysts are arrowed. 
× 3250.

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*J Clin Pathol* 1985 38: 1313-1314
doi: 10.1136/jcp.38.11.1313

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