An improved method for handling tissues for immunofluorescence

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One of the major problems in handling tissues for immunofluorescence is that they must be quick-frozen (Pearse, 1968; Nairn, 1969; Feltkamp-Vroom, 1975). This can be accomplished in a number of ways, but each method has its drawbacks. Liquid nitrogen is commonly used, but this is expensive, not always available, and also hazardous to work with. A special container or thermos flask has to be used to contain it, and this may rupture because of the extremely low temperatures. The liquid nitrogen also evaporates rapidly, producing a concomitant build-up of pressure so that the container cannot be tightly stoppered. An alternative method of obtaining low temperatures is to use dry ice. Again, depending on the supplier, it may not always be available and it, too, is somewhat expensive. If the dry ice is used by itself water may condense on the surface, thus insulating it and resulting in a warmer surface temperature. Another method of using dry ice is to make a slurry with acetone and then freeze the tissue either in a tube or wrapped in foil. This method is somewhat more efficient but there is always the problem of losing the specimen in the slurry, or not having good contact with the chilling mixture with concomitant bad preservation due to slow freezing. Carbon dioxide may also dissolve in the tissues or serum samples, resulting in a lowered pH, and this may have an adverse effect on antibodies.

We have devised a method for overcoming most of these problems that is easy, economical, and very efficient.

The principle of our procedure involves partially filling a one-litre thermos flask with the material used by a number of companies to form refrigerator or ice packs. The nature of this material is a trade secret but apparently it is some sort of plastic and it is stated to be non-toxic and non-corrosive. In the centre of the flask a closely fitting chamber is created, using a template (Fig. 1). For the template, a standard liquid scintillation counting vial (Weaton Glass Co, Brampton, Ontario) wrapped several times with wax paper is used. A one-inch layer of the refrigerant is poured around this and is allowed to solidify in the freezer, thus allowing for expansion before subsequent layers are put on top of this. As can be seen, the top of the vial is left protruding above the surface so that it can easily be removed from the flask. The chamber can also be readily bored with a

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Technical methods

References
Gerlich, W., Stamm, B., and Thomssen, R. (1976). Quantitative standardization in the detection of

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1The trade name of the material is Ice Pak, manufactured by Stanbel Ltd Braided Products Division, 11600 Albert Hudon Blvd, Montreal, Quebec HIG 3K2, Canada.
Technical methods

Fig. 1  Shows a cross-section of the thermos flask and illustrates the depth to which the refrigerant is poured.

drill and bit. For processing tissues for immunofluorescence, the vial is filled with 2 methyl butane (isopentane), put into the chamber, and allowed to cool to a temperature of -75°C or -85°C in an ultra cold freezer. The whole apparatus is kept at this temperature until it is ready to be used. Its ability to maintain a low internal temperature although the flask is standing at room temperature is shown in Figure 2. It can be seen that the critical internal temperature of -20°C will not be reached until at least 7 1/2 hours have passed.

This method has a number of advantages. Most laboratories using the immunofluorescent technique have ready access to ultra low temperature freezers which they use for storing tissue specimens, etc. The apparatus, therefore, can be placed into this freezer where the tissues are stored and thus is always ready at no extra cost. It is also always available, and no time need be spent in obtaining liquid nitrogen or dry ice. There is plenty of time waiting for biopsies and transporting tissues to the laboratories even with considerable delays. The hazards of liquid nitrogen and CO2 are also avoided. The handling of the specimen is minimised because the tissue itself is placed gently into the vial filled with isopentane, which also serves as a storage vessel. The isopentane also serves as a very efficient cooling medium; in this respect it is much better than dry ice. The liquid scintillation counting vials are all uniform in size, and they withstand a low temperature very well. The vials are easily labelled both externally, using a waterproof pen, as well as internally, using a small strip of paper, so that there is no mix-up of specimens. The inside label can also be viewed directly through the isopentane. For storage purposes these vials come in a convenient rack containing 100 vials. These can be stacked vertically and take up a minimum amount of space. Because the vial has a flat bottom, it is quite stable and, when placed into the rack, it will not fall or become mixed up with other vials so that they can be labelled sequentially. They can be located quickly, which is rather important when one is looking for specimens in a very cold freezer. There are no hazards associated with the transport of tissues, and the flask is much more convenient to handle than a container with liquid nitrogen or a bulky package containing dry ice.

We have used this apparatus to transport tissues from bed to laboratory as well as from one hospital to another. It may also be advantageous to ship the flask packed in dry ice from one city to another although we personally have not done this as yet. If one wants to collect many samples as, for example, in the postmortem room, a styrofoam box can be adapted to hold many vials for ready access, labeling, etc. Indeed, a large chamber can be made so that a whole organ, for example, a heart or a kidney, can be placed into it and taken to the freezer.

This method of handling tissues, therefore, has much to recommend it; the apparatus is readily made, is very cheap, always available, and very efficient, and can be used repeatedly. There is less handling of the tissues and no expenses are incurred for supplies of, and containers and space for, liquid nitrogen or CO2.

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Application of a modified Attwood’s stain to the study of decalcified bone sections

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Using routine histological procedures, it is difficult to distinguish osteoid from calcified bone and woven from lamellar bone in decalcified preparations of bone. Although several alternatives are available to overcome the first difficulty, differential staining of woven and lamellar bone has not been described.

In 1958, Attwood modified Lendrum’s (1947) phloxine-tartrazine stain to identify fetal amniotic squames in maternal pulmonary vessels. During a study of the organisation of amniotic debris in the middle ear cavity of newborn infants (deSa, 1977) it was noted that the osseous components of the middle ear and the petrous temporal bone were staining in an irregular fashion. By examination of the sections with polarised light it was seen that the ‘lamellar’ bone stained selectively with phloxine. It was decided to investigate this phenomenon further.

Material and methods

Formalin-fixed, paraffin-embedded sections from patients with osteoid osteoma, osteogenic sarcoma, Paget’s disease of bone, fracture callus, subperiosteal fibrous defect (periosteal desmoid), and costochondral junctions from infants with severe growth retardation and from infants with older pseudorachitic changes were selected. They were stained with a modification of alcian green, phloxine, and tartrazine (Attwood’s stain) using prior oxidation with 0.5% potassium permanganate (Table). Phloxine B solutions were prepared both with and without calcium chloride. Some sections were treated with periodic acid before staining.

Results

In sections stained with prior oxidation with potassium permanganate and stained with phloxine B solution containing calcium chloride, a clear distinction between the phloxine positive lamellar bone and the tartrazine positive woven bone was demonstrated (Figs 1 to 4). In particular, the nidus of an osteoid osteoma could be clearly distinguished from the surrounding sclerotic bone, and fracture callus with its woven bone could be readily distinguished from the mature bone of the cortex of a rib. In all cases, cartilage present in the section stained strongly with alcian green.

Differential staining was altered if calcium chloride was omitted from the phloxine B solution. In these sections even the lamellar bone lost much of its affinity for phloxine. Pretreatment of sections with periodic acid resulted in all bone, whether woven or lamellar, staining positively with phloxine.

In other randomly selected bone specimens, it was noted that the osteoid seams on the growing surface of bone trabeculae were stained preferentially with tartrazine and could be distinguished from the older lamellar layers of bone which stained selectively with phloxine (Fig. 1).

Changing Staining Characteristics of Maturing Bone

It became apparent that in older areas of fracture callus and in the central areas of some tumours, the staining characteristics of woven bone changed. An increasing number of phloxine-positive areas could be identified in the maturing segments of bone, and as callus matured its staining characteristics could be seen to change dramatically. This feature could be used as an extremely valuable control of the relative sensitivity of the staining technique, if appropriate sections were selected as a control.
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