THE FROZEN ARK - INVERTEBRATE PRESERVATION METHODS

A) Cell Preservation Method [Preferred Method]

1) Cover the tissue slice in 100ul slicing medium and finely slice the tissue into tiny pieces using a sterile scalpel blade.

2) Transfer the sliced tissue to a sterile 2ml screw-top eppendorf tube containing 2ml freezing medium.

3) To freeze the cells slowly (in order to preserve the cells), place the tubes inside a MrFrosty containing 250ml Isopropanol (following manufacturers instructions) and place in a -80°C freezer overnight.

4) Transfer tubes to liquid nitrogen.

Transportation: Samples must be transported frozen either in liquid nitrogen or on dry ice. Samples could potentially be kept at -80°C for a short time before transport to a liquid nitrogen facility. It would also be possible to place the MrFrosty (step 3) in dry ice rather than in a -80°C and then post directly to the liquid nitrogen facility.

B) Freezing Tissue for DNA Preservation

1) Place tissue slice in a 2ml screw-top eppendorf tube (or alternatively whole specimen in an appropriate tube/ container).

2) Transfer tubes to freezer, ideally at -80°C but if this is not possible -20°C will suffice.

Transportation: Once frozen, samples must be kept permanently frozen so transportation must be undertaken on dry ice, in liquid nitrogen or in a portable freezer.

C) Ethanol Preservation of Tissue for DNA Preservation

1) Place tissue slice in a 2ml screw-top eppendorf tube containing approximately 2ml 95-100% ethanol (or alternatively whole specimen in an appropriate tube/ container filled with ethanol). It is important that the tube contains plenty of ethanol – a ratio of 1 part specimen to approximately 9 parts ethanol will suffice. If the specimen contains a lot of liquid/mucus etc it is desirable to replace the ethanol with fresh ethanol to ensure that the ethanol concentration remains high.

2) Tubes should then be transferred to a -80°C or -20°C freezer (but can be stored at room temperature short term).

Transportation: Ethanol effectively pickles the sample making it stable at room temperature therefore allowing the sample to be transported at room temperature.

Alternatives: RNA Later could be used as an alternative and is suitable for preserving high quality RNA and DNA. However it is expensive! There are also various CTAB/Salt solutions that can be used to preserve tissue.

D) Whatman FTA Cards [Good alternative for blood/ haemolymph]

1) Place a maximum of 125µl of blood/haemolymph onto a Whatman paper. Avoid 'puddling' the sample as it will overload the chemicals on the card – dispense blood slowly and evenly across the card. Also do not rub or smear the blood/haemolymph onto the card.
2) Allow the card to dry (approximately 1 hour but this will depend on conditions), label the card and then store in a dry place.

Transportation: Cards can be sent through normal post at ambient temperature.

Sample Preparation:

It is essential that aseptic conditions are used during sample preparation. In particular, it is extremely important that fresh sterile scalpel blades, tips etc. are used for each specimen when taking samples of tissue in order to avoid cross contamination.

Specimens must be fresh. DNA will degrade very rapidly as soon as the specimen is dead.

Number of Replicates:

For each specimen, several replicates should be obtained where possible (the more the better!)

Number of Specimens:

Ideally, each species should be represented by samples from several individuals. The ideal is 10 to 15 for maximum genetic diversity. If there are samples of the species from several localities, it is desirable to have the localities equally represented. A single sample from an endangered species is better than none, but multiple samples are highly desirable.

Labelling Specimens:

Specimens in tubes should be labelled in two ways:
1) Using a permanent marker pen on the exterior of the collection tube
2) Using a pencil (or Indian Ink marker) with the label written on paper and stored with the specimen inside the tube.

NB. Permanent marker will wash off in ethanol and can be rubbed off quite easily when handling tubes.

Whatman FTA cards should be labelled directly in the space on the front of the card.

Voucher Specimens:

It is highly desirable to obtain voucher specimens. Taxonomic identifications are often inaccurate (particularly for invertebrates!) and even where identifications are reliable, revisions to the taxonomy in future years make the acquisition of voucher specimens a valuable resource. Where it is not possible to obtain voucher specimen then digital photographs should be considered as an alternative.

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