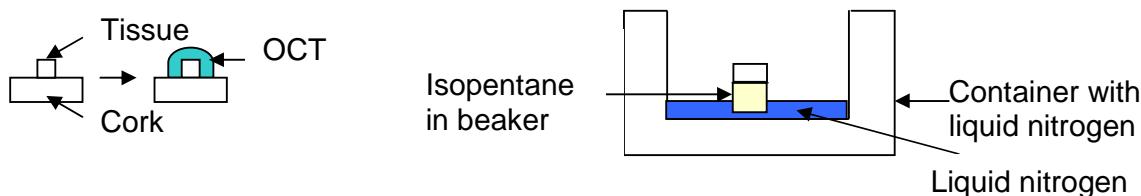


## HOW TO FREEZE DOWN MUSCLE/SKIN BIOPSIES AND CHORIONIC VILLI SAMPLE (CVS) FOR CRYOSECTIONING

There is no single method to freeze samples and this is a suggested protocol that can be easily followed:

### General Procedures:

1. Immerse a 100ml polypropylene or metal beaker of isopentane approximately half-way into liquid nitrogen in a Dewar or polystyrene box and leave until isopentane is frozen (white and solid).
2. Place sample on top of a piece of cork or a small piece of card, and orientate appropriately (see orientation of samples below). Use a dissecting microscope, if necessary.
3. Put a drop of OCT compound (also known as Tissue-Tek, e.g. from Bayer Science, Cat N: 0460 9024) to cover the tissue. In the case of skin and CVS samples, which are usually smaller than a muscle biopsy, put the OCT on the card or cork first and then put the tissue on top of OCT.
4. Remove the beaker with frozen isopentane from the liquid nitrogen and leave it at room temperature until approx 30% of the isopentane is thawed and some solid is still present.
5. Quickly place the cork/card with the tissue into the liquid layer of isopentane using forceps. Once the tissue and OCT have been frozen (white and solid, this can take 10-30 seconds depending on the amount of OCT) drop the OCT tissue block (OCT block) into liquid nitrogen.
6. Store the OCT block in an appropriate container at  $-40^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$ , or liquid nitrogen storage tank.



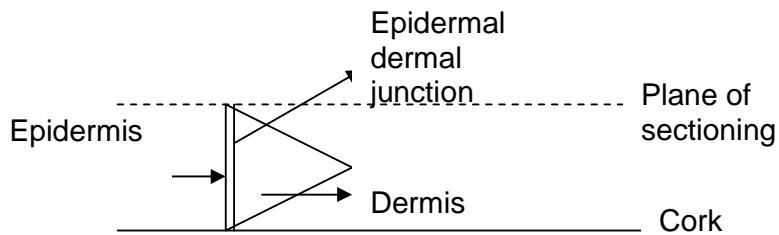
### **Orientations of samples:**

#### **Muscle biopsy**

It is preferable to orientate the muscle in such a way that muscle fibres are sectioned transversally. To do this, first to identify striations of muscle fibres and briefly organise the fibres to lie straight to each other in a similar way with forceps under dissecting microscope. Place the oriented muscle on cork **vertically**, the fibres stand up, before apply the OCT. Carefully apply the OCT around the muscle and make sure the muscle fibres stay **vertically**. After this follow the general procedures step 4.

#### **Skin Biopsy**

It is very important that skin samples are frozen in the orientation represented below to maximise the chances of cutting through the epidermal-dermal junction.



#### **CVS**

The orientation of CVS is not crucial for immunohistochemistry. Place the clean chorionic villi (5-8 branches) carefully on top of a drop of OCT on a piece of cork and make sure they are embedded in OCT.