Fixation of brain tissue by perfusion

1. Purpose

The purpose of this method is to rapidly fix tissue and organ with paraformaldehyde by perfusion to optimise preservation of cellular sub-structures and antigens.

2. Procedure

Mice were anaesthetized with a lethal dose of Avertin delivered by intraperitoneal injection. Mice were closely monitored to ensure the animal was completely under the anaesthetic (i.e. unresponsive to stimulus such as no reflex with paw pressure.)

- Place the mouse on a cork or polystyrene board and pin out the body using all 4 extremities. Moisten the fur on the abdomen with Ethanol and using a pair of sharp scissors (Tough Cut scissors) make a median incision opening the abdomen. Cut the skin horizontally just below the rib cage on both sides of the midline.
- Hold the lower large part of the sternum using straight serrated fine forceps, lift the sternum slightly up and cut the diaphragm from the centre outwards horizontally using a small pair of sharp scissors to access the thoracic cavity. Then cut up either side of the rib cage. Do not cut through the top of the sternum as this will damage the great thoracic vessels. The rib cage can then be pinned back also onto the board near the head.
- Cut open the right atrium using a small incision cut with sharp small scissors.
- Hold the heart delicately with straight serrated fine forceps using left hand. Insert a needle immediately into the left ventricle and push through ~ 7.5mls-10mls of PBS slowly (~2.5-3mls/min). The tissue will start to fade in color (lungs go white, liver pales) and this will tell you how well the perfusion is working, adjust needle if this doesn't occur.
- Next change the solution to 4% paraformaldehyde and pump this through as above ~10mls. The mouse should stiffen and the abdominal organs harden and this will allow you to see how well the perfusion is going.
- Once complete remove the brain. Detach head from body using a large pair of tough cut sharp scissors. Remove the brain carefully (work posterior to anterior) by moistening hair with ethanol then cutting away skin, cutting away muscles around skull. Chip off little pieces of skull so as not to damage the brain using F-P micro rongeurs extra fine tips. Cut the cranial and optic nerves, remove tissue and bone surrounding olfactory bulb. Very carefully lift the brain out from the olfactory bulb and using a scoop below the brain cut through the attachments holding the brain in place.
- Fix the tissue further in 4% Para formaldehyde by immersion in ~5mls of the solution for ~2hrs @ 4˚C.
- Cryo preserve the tissue in 30% Sucrose @ 4˚C o/n. Replace the fix solution with ~5-7mls of sucrose in a bijou. The tissue should have sunk to the base of the tube before using further.
- The tissue can be stored longer in the sucrose by storing at -10˚C or by adding 0.02% Sodium Azide into the sucrose solution or by making up a mix of 30% Glycerol + 30% sucrose and storing at -20˚C.

3. Materials

3.1 Preparation of Avertin

- Add 1.25mls Tert amyl alcohol (Sigma/Aldrich - 99% tert-amyl alcohol – T4840-2) using a plastic pipette to 0.625g of 2,2,2-tribromoethanol (Sigma/Aldrich -99% - 75854).
- Cover the beaker in foil and dissolve by swirling the mix under hot water.
- Make the mix up to 50mls with DDW then place in conical flask, cover in foil.
- Swirl under hot water until all has dispersed.
- Aliquot into 5-10mls, cover with foil and store by freezing – only defrost when required and dispose of excess.

3.2 Preparation of 4% Paraformaldehyde Fix

- Mix 20g Para formaldehyde powder (sigma P6148) in 200mls of DDW.
- Heat to 60°C then add 1-2 drops of 2N NaOH (solution should clear).
- Make up to 250mls with DDW. Make up to 500mls with 0.2M PBS.

3.3 Preparation of 30% Sucrose solution.

- Make 130mM Na Phosphate by mixing 72.9mls 1M Na_2HPO_4 + 27.1mls 1M NaH_2PO_4
- Mix 30g Sucrose to 100mls of 130mM Na phosphate use fresh every time - do not store.

3.4 Preparation of 0.2M PB-

1) NaH2PO4.2H2O 1.56gm in 50mls
2) Na2HPO4 5.67gm in 200mls
Mix 32mls of 1) with 168mls of 2).

Dulbecco's phosphate buffered saline (PBS).

Tools for dissection all purchased from Fine Scientific Tools.

- 14090-09 Straight Fine Iris scissors 8.5cm
- 11241-30 Dumont No4 Standard Tip
- 11064-07 Straight Serrated 7cm Fine Forceps
- 10093-13 Scoop
- 14058-11 Tough cut scissors s/s
- 11260-30 Dumont no6
- 14058-09 ToughCut Scissors, 9cm, straight

4. Quality Control

Visually you will be able to tell whether the mouse has flushed properly and also later when dissecting out the brain – if it's red tinged then the flushing hasn't worked well. The tissue will be completely white if flushed well. It will not be used if it is red.

Visually you will be able to tell whether the mouse has perfused properly as the body will be rigid. When dissecting out the brain the tissue will not be soft.

Can also tell visually whether the tissue is cryopreserved. In a bijou it should be left a minimum of o/n to 24 hrs. The tissue will sink once it has absorbed the sucrose.

5. Examples of Data

Not applicable

6. Supporting Information

7. Document History
Created by G2C on 22 February 2008