Harvest of Large Animal Tissues

1 Purpose / Introduction

1.1 To collect tissues and fluids from large animals during post mortem for subsequent extraction of RNA/DNA and isolation of immune cell types.

2 Equipment/Reagents/Materials

2.1 15ml Falcon tubes containing 5ml RNALater solution.

Cryotubes for snap freezing tissues.

Liquid Nitrogen dewars filled with liquid nitrogen.

No.11 Swan Morten Scalpels

No. 22 Swan Morten Scalpels

Acidic Citrate Dextrose (ACD)

Sterile Plastic Forceps

Sterile Scissors

Sterile PBS in Litre bottles and 50ml falcon tubes

Beakers

3 Litre Jug

50ml and 10ml Syringes and 18G needles.

Dry Ice

Wet Ice

Ziplock bags

Chemical Waste Bags

Sterile Funnel

1.5ml screw top eppendorfs

50ml Falcon Tubes

Petri Dishes

Cable ties

Sharps Bins

3 Principle

3.1 A veterinarian and at least two farm technicians carry out each post mortem. Tissues are handed to those on the tissue collection team to cut into small pieces (<0.5cm diameter). For preservation of RNA the chopped up tissues are either snap frozen in liquid nitrogen or transferred to a 15ml falcon tube containing 5ml of RNALater solution.

4 Procedure

4.1 All surfaces should be cleaned with disinfectant then RNAlater and all participants should wear gloves and ensure they regularly change these gloves throughout the harvest.

4.2 Tissues should arrive from the post mortem team on a plastic petri dish. The tissue should be cleaned of any excess blood or gut contents (for GI tract tissues only) by dipping in sterile PBS using plastic forceps. A No. 22 Swan Morton disposable scalpel should then be used to chop the tissue into small pieces that are <0.5cm diameter in any direction. To secure the tissue for chopping either a No. 11 Swan Mortem Scalpel or sterile plastic forceps can be used. A new set of scalpels should be used for each tissue. For lipid rich tissues such as brain and adipose pieces should be placed singly and evenly in cryovials, which are immediately snap frozen in liquid nitrogen. All other tissues after being chopped finely are placed in a 15ml falcon tube containing 5ml of RNALater solution. At the end of the post mortem the snap frozen tissues are transferred carefully onto dry ice using a slotted spoon for transport up to the Roslin Institute and filing into the -155°C freezer. The RNALater tubes are transported to Roslin at room

temperature then placed in the 4°C cold room for a minimum of 12 hours and a maximum of one month.

4.3 In addition, some members of the tissue collection team may be involved in dissection of the animal. These individuals will use scissors and scalpels to dissect out the desired tissues.

4.4 Blood is collected into a 3 litre jug containing ACD (10% of collected blood volume) by exsanguination by a farm technician or by the tissue collection team via the heart using an 18G needle and 50ml syringe. After collection blood is decanted into 50ml falcon tubes under a laminar flow hood (see protocol ROSLIN_SOP_Isolation of macrophages from the lungs, blood and bone marrow of large animals_20160413).

4.5 Lungs are removed by a farm technician, flushed using sterile PBS and the contents decanted into 50ml falcon tubes or 500ml flasks depending on the volume involved. Washing of the lungs also takes place in the laminar flow hood (see protocol ROSLIN_SOP_Isolation of macrophages from the lungs, blood and bone marrow of large animals_20160413).

4.6 In pregnant animals the allantoic and amniotic fluid is sampled using a 10ml syringe and 18G needle. The needle is used to penetrate the amniotic and allantoic sacks and draw up the fluid, which is then decanted into 1.5ml screw top eppendorf tubes and immediately snap frozen.

4.7 Ribs are removed by the farm staff and are placed in a ziplock bag filled with PBS on ice before transport for processing (see protocol ROSLIN_SOP_Isolation of macrophages from the lungs, blood and bone marrow of large animals_20160413).