## Explanation of heart valve, heart valve block and heart valve section labelling system.

### (1) Labelling of heart valves

Each implanted and explanted decellularised porcine and ovine heart valve was assigned a unique label (ID) as explained in Table (1)

**Table (1) Labelling of implanted and explanted porcine and ovine pulmonary valves:**

| ID | Sheep number | Group | Graft | Implant date | Explant date |
|---|---|---|---|---|---|
| 1 | 59 | HIC (12M) | Ovine homograft cryopreserved nº1 | 15/08/13 | 04/09/14 |
| 2 | 61 | HIC (12M) | Ovine homograft cryopreserved nº2 | 15/08/13 | 04/09/14 |
| 3 | 56 | HIC (12M) | Ovine homograft cryopreserved nº4 | 22/08/13 | 04/09/14 |
| 4 | 58 | HIC (12M) | Ovine homograft cryopreserved nº3 | 22/08/13 | 04/09/14 |
| 5 | 71 | 12M | Decellularised porcine pulmonary B1 PPV3 | 13/02/14 | 27/02/15 |
| 6 | 72 | 12M | Decellularised porcine pulmonary B2 PPV2 | 13/02/14 | 27/02/15* |
| 7 | 73 | 12M | Decellularised porcine pulmonary B3 PPV3 | 13/02/14 | 27/02/15 |
| 8 | 74 | 12M | Decellularised porcine pulmonary B4 PPV6 | 13/02/14 | 27/02/15* |
| 9 | 75 | 12M | Decellularised porcine pulmonary B4 PPV4 | 20/02/14 | 27/02/15 |
| 10 | 76 | 12M | Decellularised porcine pulmonary B2 PPV4 | 20/02/14 | 27/02/15* |
| 11 | 77 | 12M | Decellularised porcine pulmonary B2 PPV5 | 20/02/14 | 27/02/15 |
| 12 | 79 | 12M | Decellularised porcine pulmonary B1 PPV4 | 20/02/14 | 27/02/15* |
| 13 | 85 | 6M | Decellularised porcine pulmonary B1 PPV2 | 27/02/14 | 04/09/14 |
| 14 | 80 | 6M | Decellularised porcine pulmonary B1 PPV6<br>OBS: Dano na adventicia | 27/02/14 | 04/09/14 |
| 15 | 81 | 6M DEAD | Decellularised porcine pulmonary B4 PPV3 | 27/02/14 | 04/09/14 |
| 19 | 84 | 6M | Decellularised porcine pulmonary B4 PPV2 | 13/03/14 | 04/09/14 |
| 16 | 88 | 6M DEAD | Decellularised porcine pulmonary B2 PPV1 | 27/02/14 | 27/02/14 |
| 17 | 78 | 3M | Decellularised porcine pulmonary B2 PPV3 | 13/03/14 | 11/06/14 |
| 18 | 83 | 3M | Decellularised porcine pulmonary B1 PPV1 | 13/03/14 | 11/06/14 |
| 20 | 86 | 3M | Decellularised porcine pulmonary B4 PPV1 | 13/03/14 | 11/06/14 |

| | | | | | |
|---|---|---|---|---|---|
| 21 | 87 | 3M | Decellularised porcine pulmonary B3 PPV5 | 13/03/14 | 11/06/14 |
| 22 | 158 | 1M | Decellularised porcine pulmonary B3 PPV2 | 20/03/14 | 23/04/14 |
| 23 | 156 | 1M | Decellularised porcine pulmonary B3 PPV6 | 20/03/14 | 23/04/14 |
| 24 | 169 | 1M | Decellularised porcine pulmonary B3 PPV1 | 20/03/14 | 23/04/14 |
| 25 | 176 | 1M DEAD | Decellularised porcine pulmonary B3 PPV3 | 20/03/14 | 20/03/14 |
| 26 | 168 | 1M | Decellularised porcine pulmonary B4 PPV5 | 10/04/14 | 09/05/14 |
| 27 | | NC | Ovine PV10 Non-implanted control | | |
| 28 | | NC | Ovine PV11 Non-implanted control | | |
| 29 | | NC | Ovine PV12 Non-implanted control | | |
| 30 | | NC | Ovine PV13 Non-implanted control | | |

*12 Month decellularised porcine pulmonary valve explants designated for biomechanical testing;

DEAD indicates sheep died prematurely

## (2) **Dissection and fixation of the valves following explantation or preparation**:

Following explantation (or preparation for non-implanted ovine controls), the explanted decellularised porcine pulmonary roots (1 month, 3 months, 6 months and 12 months; sheep 71, 73, 75, 77) and control ovine explanted roots (12 months) were dissected and fixed prior to shipment from Brazil as described in Figure (1).

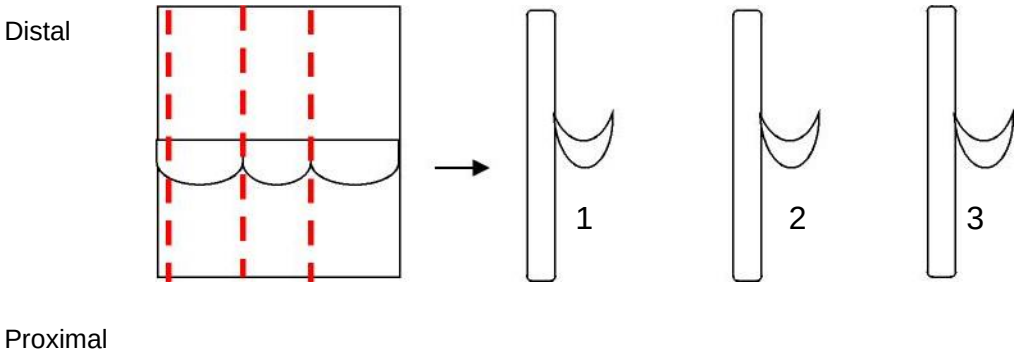



**Figure (1) Dissection of explanted pulmonary roots for fixation and shipment.** Roots were dissected longitudinally to produce three samples each incorporating muscle, junction, leaflet and pulmonary artery wall. Samples 1 and 2 were fixed in neutral buffered formalin (F1 and F2) and sample 3 was fixed in zinc fixative (3Z).

Each pulmonary root was dissected longitudinally into three samples, each comprising ventricular muscle, proximal suture line, junction, leaflet pulmonary wall and distal suture line as shown in Figure (1). Samples (1) and (2) were placed in pots and fixed in 25 ml 10% (v/v) neutral buffered formalin (NBF) for 24 hours. The samples were then stored in 25 ml 70% (v/v) ethanol in labelled pots prior to

and during shipment to Leeds. Sample (3) was placed in a pot and fixed in 25 ml zinc fixative for 24 hours and then transferred to 25 ml 70% (v/v) ethanol for storage and shipment back to Leeds. All of the explanted pulmonary valve samples were carefully labelled with the date, sheep identification number, months (1, 3, 6 or 12) in vivo and fixation method, as illustrated in Figure (2).

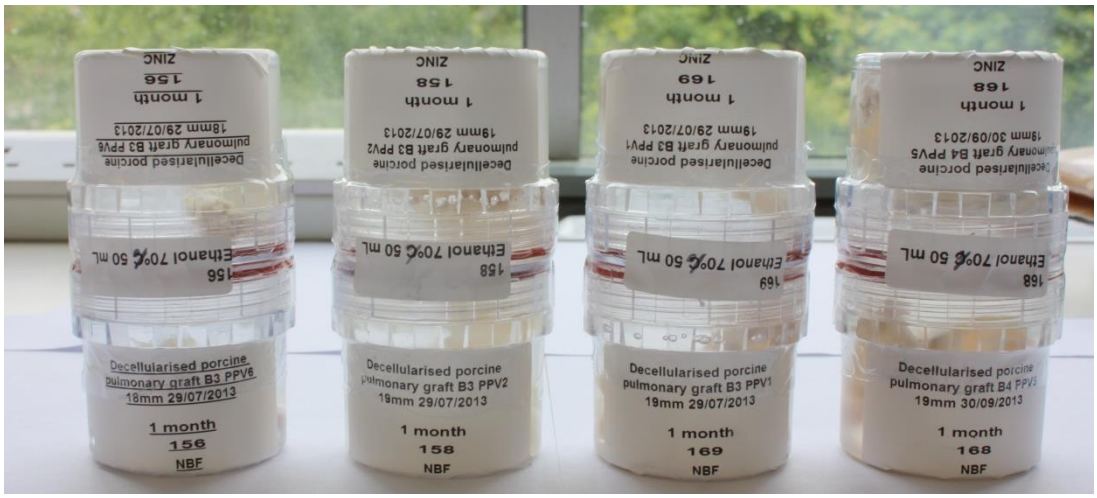



**Figure (2) Labelling of pots in which samples of pulmonary root explants were stored and shipped in 70% ethanol**

The samples were shipped to Leeds by courier in four shipments. Shipment (1) comprised the 1 month decellularised porcine explants, shipment (2) the 3 month decellularised porcine explants, shipment (3) the 6 month decellularised porcineexplants and the cryopreserved ovine homograft control 12 month explants (HIC) and shipment (4) the 12 month decellularised porcine explants. This was facilitated through appropriate export (Ministerio da Fazenda, Brazil) and import (DEFRA) licences.

The non-implanted ovine control valves were subject to the same dissection and fixation procedure at the University of Leeds.

## (3) Preparation and labelling of histology blocks

One by one, each tissue sample was removed from 70% ethanol. Images of the fixed tissue samples were captured as illustrated in Figure (3).

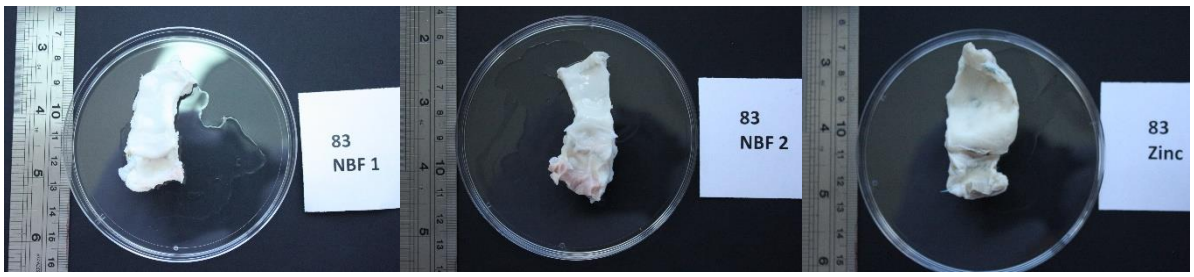
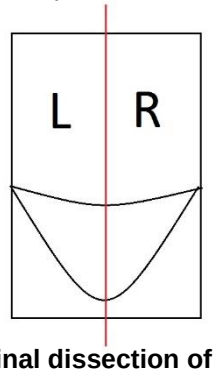



**Figure (3) Representative images of the samples of an explanted, dissected and fixed valve (valve ID 83 showing the three longitudinal samples, each comprising a whole leaflet, muscle, wall, proximal and distal suture lines).**

Those formalin fixed samples designated NBF 2 (F2) were then stored in 70% ethanol until utilised for calcium analysis.

Those formalin fixed samples designated NBF1 (F1) and zinc fixed samples (Z3) were then bisected longitudinally using a scalpel as illustrated in Figure (4a).

(a)




(b)

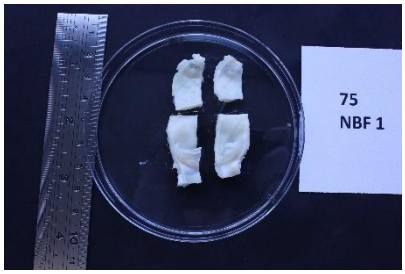



**Figure (4) Longitudinal dissection of fixed tissue samples (F1 and Z3) into right and left hand portions through the centre of the leaflet (a) and representative image of the two portions (F1-L and F1-R) cut into two sub-portions for preparation of histology blocks (b).**

Each portion (F1-L; F1-R; Z3-L; Z3-R) was placed into a separate histology cassette with the leaflet facing upwards for tissue processing, paraffin wax embedding and subsequent sectioning. Due to the size of the samples, the tissues were divided into smaller sub-portions for the preparation of histology blocks as illustrated in Figure (4b), with all sub-portions originating from eg. F1-L or F1-R embedded within the same block. Hence four histology blocks were prepared from each valve.

Histology blocks were labelled as follows:


For example, valve number 9; decellularised porcine explanted after 12 months; formalin fixed sample (F1); right hand leaflet portion was labelled

**9-M12-F1-R**

And, valve number 28; non-implanted ovine control; zinc fixed sample, left hand leaflet portion was labelled

**28-NC-Z3-L**

### (4) Labelling of tissue sections/ slides

Beyond blocks, slides generated from each block retained the block label system but with the addition of another label indicating the section number from 1 counting upwards for each section taken from the block.

For example Valve number 11, 12 month explanted decellularised porcine, formalin fixed sample (F1) left hand leaflet portion, section number 93 was labelled:

**11-M12-F1-L-93**

And, valve number 3, cryopreserved ovine homograft explanted at 12 months, formalin fixed sample (F1) right hand leaflet portion, section number 33 was labelled:

**3-HIC-F1-R-33**