

Protocol -

1.2.11 Porcine lung biopsy processing and staining for TLR 3 and 5

Bronchial biopsies were collected from both lungs at 3 hours in the controls and 3 and 6 hours in the S3/S4 lungs using alligator biopsy forceps through the working channel of the bronchoscope. Samples were placed in 10% neutral buffered formalin for at least 48 hours.

1.2.11.1 Porcine lung tissue processing and slide mounting

Samples were sent to the University of Edinburgh histopathology laboratory (Queen's Medical Research Institute, Edinburgh) where sections were clipped and placed through graded alcohols (0-100%), xylene and embedded in paraffin-wax at 60C using a Leica ASP tissue processor. Using a microtome the samples were then sectioned to 4µm, placed on slides and dried at 37C overnight.

1.2.11.2 Immunostaining for TLR 3 and 5

The slides were dewaxed with xylene (10 minutes) and rehydrated with graded alcohols (70-100%) over 2 minutes. All reagents used in the following steps were from a Novacastra bond polymer refine detection kit, except the primary antibodies. Antigen retrieval was performed in a Ph6 buffer under standard conditions in a pressure cooker.

Slides were loaded onto the Leica Bond Max robot and stained with peroxide (5 mins), primary porcine TLR 3 or 5 antibody (Novus

biologicals) at 1:200 dilution for 120 minutes. The slides were then placed in polymer for 15 minutes, then DAB chromagen for 10 minutes and haematoxylin for 5 minutes.

Slides were removed off the robot, dehydrated with graded alcohols

(70-100%) and placed in xylene. DPX and coverslips were applied before imaging.