

**SOP No:** AHP 59

**SUBJECT:** Generation of a fibrotic mouse model using bleomycin

**REASON FOR USE:** This technique is used to develop a mouse model of scarring, ultimately to reduce or prevent the effects of deep scarring in children.

**POLICY:** This procedure must be performed by an experienced operator.

**PRECAUTIONS:** Gloves, mask, long-sleeve gown, closed in shoes.

**EQUIPMENT:**

- Anaesthetic of choice
- Analgesic
- Scalpel blade
- Scissors
- 25-29G needles
- Gelatin beads
- Isofluorane
- Bleomycin sulphate (Mayne Pharma Ltd) made up to 42,000IU/ml (2.8mg/ml) in sterile saline. Dissolve 10mg bleomycin sulphate in 357ul saline. Make up day before use and store at 4°C.
- Alzet micro-osmotic pumps (Each one time use - model 1004 – 0.11ul per hour 28 days), filled with bleomycin or saline (control) according to manufacturer's directions 24 hours before use (helps prime pumps a little). Keep in 50ml sterile pots at 4°C.

*Note: weigh pump before and after filling to ensure correct volume of solution has gone in.*

**PROCEDURE:**

**METHOD 1:Gelatin Beads repeated i.d. injection:**  
Restrain the mouse. Mark injection site for the gelatin hydrogel beads adsorbed with bleomycin. 200 µl of gelatin beads (200 mg) incubated with 10 mg/400 µl bleomycin sulphate are injected at multiple sites within a 15mm radius approximately every 3 days (range between 2-5 days) with a small bore needle (25G-29G). Gelatin beads are 10-15 µm in diameter and are readily injectable.

**METHOD 2:Micro-Osmotic pump method:**  
Restrain the mouse and insert a bleomycin loaded osmotic pump according to [SOP AHP55](#). Inspect and weigh at 24, 48 and 72 hours then every 2-3 days.

**Record all observations until day 28 when the pump and bleomycin supply will have ceased drug delivery.**

**METHOD 3: Polymer hydrogel block scaffold method:**

**Restrain and anaesthetise the mouse.**

- 1) Mark 1cm incision line across midline of back with permanent marker just below shoulder blades and cut using small scissors or scalpel.**
- 2) Make a pocket either side of the incision extending up the back of the mouse using blunt ended dissection techniques. (Do not make the pocket too large or the 1cmx1cm polymer scaffold will then move too much and end up being mobile throughout the mouse body cavity).**
- 3) Place the 1 cm<sup>2</sup> and 3mm polymer scaffold block (400 mg) incubated/adsorbed with 10 mg/400µl bleomycin sulphate. Place scaffold into pocket, away from the incision lower on the mouse back.**
- 4) Close wound with 3 sutures and tissue glue (e.g. fibronectin-based tissue glue Tisseal).**
- 5) Administer analgesia.**

**Post-mortem**

**At 28 days post bleomycin treatment the animals will be euthanized using CO2 asphyxiation ([SOP AHT36](#)) and biopsies collected for histological wound analysis.**

**Shave back of mouse with electric clippers/razor to expose the skin surface of the insertion and hypertrophic scarring site.**

**Take photograph and use ruler and skin fold calliper next to mouse for calibration.**

**Excise fibrotic lesion/insertion site. Take care as lesion will likely be stuck to pump outlet. Dissect sample and process for scientific assessment e.g. place one third into 10% buffered formalin, and the other either into OCT cooled liquid N<sub>2</sub> for frozen sectioning or 4% glutaraldehyde in PBS buffer for EM.**

**RECOMMENDATIONS:**

**DATE ISSUED: 28 April 2010**

**REVISED:**

## REFERENCES

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