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Bacterial-induced neutrophilic nasal inflammation in mice

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Alba Sánchez Montalvo[1,2], Aaron Ziani Zeryouh[1], Marylène Lecocq[1], Charles Pilette[1,3], Valérie Hox[1,4]

[1]Pole of Pneumology, ORL (airways) and Dermatology (skin) (LUNS), Institute of Experimental and Clinical Research (IREC), Université Catholique de Louvain (UCLouvain), Brussels, Belgium;
[2]Department of Microbiology, Immunology and Transplantation, Allergy and Clinical Immunology Research Group, KULeuven, Leuven, Belgium;
[3]Department of Pneumology, Cliniques Universitaires Saint-Luc, 1200 Brussels, Belgium;
[4]Department of Otorhinolaryngology, Cliniques Universitaires Saint-Luc, Brussels, Belgium

Alba Sánchez Montalvo
Institut de Recherche Expérimentale et Clinique (IREC)

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Abstract

The present surgical procedure provides a step-by-step and detailed protocol with all the critical information to develop a bacterial-induced neutrophilic local inflammation in the nasal mucosa of mice. The protocol is a modified and optimized version of the work previously published by Jacob et al. in 2001. The procedure consists of exposing and drilling out the nasal bones and the upper part of the septum followed by the insertion of a bacterial-inoculated nasal tampon in the nasal cavity which remains in situ until sacrifice.

The day before surgery

1 Prepare the chosen bacteria 1d

1.1 Culture the corresponding bacteria on sheep blood agar plates (BD, Biosciences 254071), according to their specific growth requirements.

1.2 In our hands, bacteria strains used were *Pseudomonas aeruginosa* Vitroids™ (Sigma-Aldrich, VT000256), *Staphylococcus aureus* Lenticules™ (Sigma-Aldrich, CRM06571M) and *Streptococcus pneumoniae* (ATCC®, 49619™). Overnight culture conditions were 37°C for *S. aureus* and *P. aeruginosa* and 37°C in 5% CO_2 for *S. pneumoniae.*

2 Prepare the nasal tampon 4h

2.1 In our hands, a Merocel pope ear wick (Medtronic, MI, USA) was cut at a dimension of 4 mm x 1 mm x 1 mm and sterilized by dry heat 100ºC during 3h.

Pre-surgery

3 Prepare and sterilize your instruments for the surgical procedure

3.1 Stereomicroscope (Motic SMZ-171)
1.5-mm microdrill (Medtronic, MI, USA)
Sterile fields (Hartmann Mediset®)
Temperature monitor (PhysioSuite® Kent Scientific Corporation)
15-mm scalpel blade
5.0 non-resorbable polypropylene sutures (Monosoft™, Covidien)
Scalpel
Forceps
Scissors
Needles
Gloves (CardinalHealth™ Protexis™ PI micro surgical gloves)
Eye drops (Ocry-gel, TVM)
Chlorhexidine digluconate (Hibidil®, Regent Medical).
Tape
IR lamp (IR100 Infrared lamp, MEDISANA®)
Ultrasonic bath (Clifton)

Surgery platform

4 Prepare your bacterial solution 30m

4.1 From the bacterial cultures, take isolated colonies and prepare serial dilutions up to 10^9 CFU/ml in NaCl 0.9% and measure it with a spectrophotometer with a reference optical absorbance at 600nm (OD_{600nm}) = 1.

5 Administration of anesthesia and analgesia to the mouse 30m

5.1 Weight the mouse and administer general anesthesia accordingly: a mix of Xylazin (maximum 15mg/kg) and Ketamin (maximum 80 mg/kg) intraperitoneally.

5.2 Administer analgesia: Temgésic (maximum 0,05 mg/kg) subcutneously.

6 Wait 15-20 minutes for the aesthesia and analgesia to do their effect before starting the surgical intervention.

Surgical procedure 10m

7 Shave the snout over the intervention area and fix the mouse on a sterile field (Figure 1, A).

8 Monitor and maintain the intraoperative body temperature of the mouse during the procedure.

9 Disinfect the intervention area with Chlorhexidine digluconate and use eye drops to prevent eyes from drying.

10 Use a 15-mm scalpel blade to make a 8-mm midline incision over the nasal dorsum to the snout. Raise skin flaps laterally to expose the nasal bone (Figure 1, B).

11 Use a 1.5-mm microdrill to drill the nasal bone over the nasal fossa and remove the upper part of the septum. Be extremely careful not to drill completely the bucco-sinusal bone communication. Clear out any bleeding to prevent aspiration (Figure 1, C).

12 Inoculate the pre-cut nasal tampon with 10 µL of the corresponding bacterial solution or saline for controls and carefully place it into the nasal cavity (Figure 1, D-F).

13 Suture skin flaps with 5.0 non-resorbable polypropylene sutures and dettach the mouse from the surgical field (Figure 1, G).

14 Place the mouse in a cage softly heated laterally by an IR lamp for recovery after surgery. Make sure the lamp is not too close or too far from the cage (Figure 1, H).

15

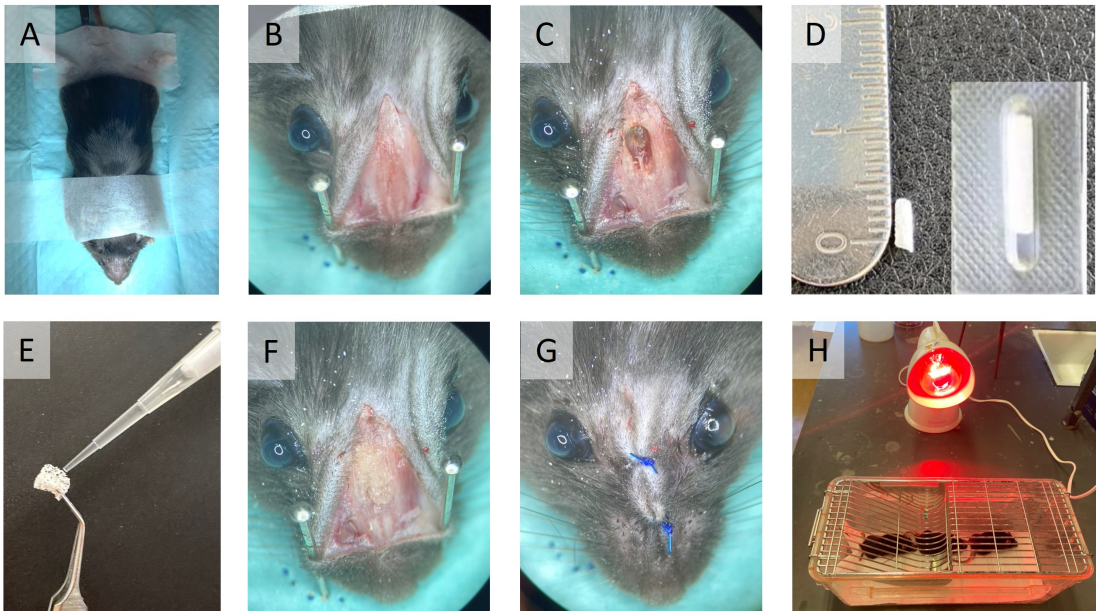
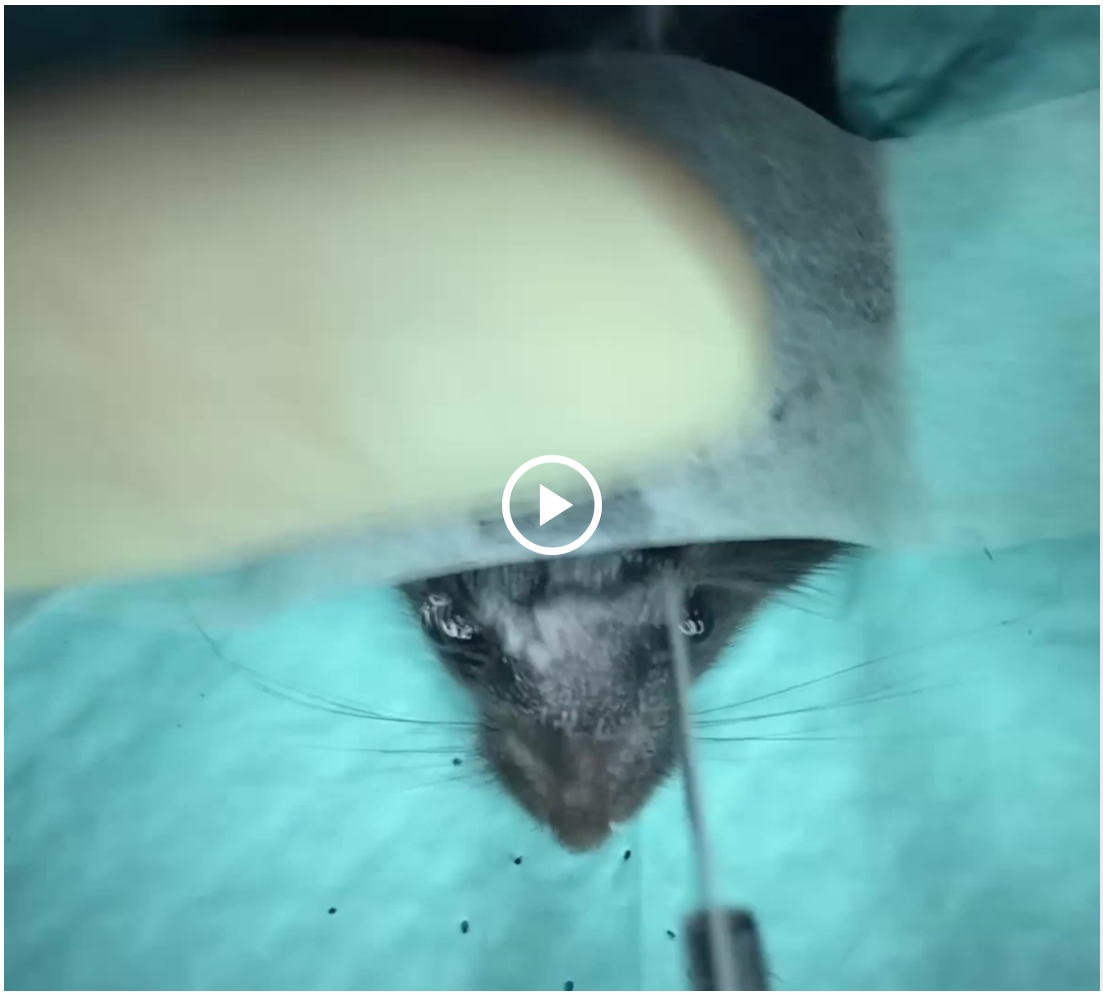


Figure 1. Overview of the surgical procedure. *(A) The mouse is fixed in the surgical area. (B) Skin flaps are opened and (C) the nasal bones are drilled to expose the nasal mucosa. (D-E) A pre-cut sterile nasal tampon is inoculated with a saline or bacterial solution and (F) inserted into the nasal cavity of the mouse. Last, (G) skin is sutured with non-resorbable sutures and (H) the mouse is placed in a warm environment to recover.*



Video: surgical intervention.

Post-surgery care

16 Once awake and active, place the mouse into an ABSL-2 facility.

17 To overcome the possible post-operative pain, administer Temgésic (maximum 0,05 mg/kg) subcutaneously to the mice every 12h for 24h after surgery.

18 Closely watch the animals every day for at least one week to ensure their well-being. According to humane endpoints, mice reaching a score of non well-being or losing more than 20% of their body weight must be euthanized.

Protocol references

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