Procédure Normalisée de Fonctionnement

TITRE : IMPLANTATION INTRACRÂNIENNE DE CELLULES TUMORALES HUMAINES CHEZ LA SOURIS IMMUNODÉFICIENTE	**NUMÉRO :** CHX-1
DESTINATAIRES : Personnel du Service des animaleries et usagers	**Version 1 :** 2007 **Version 5:** 20.05.2016
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BUT : Décrire les différentes étapes de l'implantation intracrânienne chez la souris immunodéficiente.	

MATÉRIEL :

- Female mice Crl: CD-1 *Foxn1*^{nu} 28-42 days from Charles River laboratories
- (ATCC HTB)
- 0.1mg/kg buprenorphine solution (0.3mg/ml concentration diluted 1/5 in saline 0.9%) N.B.: Buprenorphine is a controlled drug by the government.
- BNP (antibiotic ointment)
- Isofluran, induction chamber, stereotaxic mask
- Isofluran gas machine
- Stereotaxic instrument single manipulator
- 10.0µl precision glass syringe (Hamilton #701)
- Cordless micro drill kit
- Wooden block
- Ringer USP injection
- Sterile surgical blades
- Iris scissors
- Curved blunt micro-forceps
- Straight micro-forceps 1/2 teeth
- Mosquito hemostats
- Micro needle holder
- Electric heating pad
- Sterile surgical fields
- Sterile cotton gauzes
- PDS II 5/0 suture sterile individual pack
- Germinator
- Sterile self piercing ear tags
- Sterile tags from National Band and Tag Co #1005-1 MONEL
- Tumor cells suspension
- 70% alcohol
- 1% Proviodine
- 1% Virkon Solution

PROCÉDURES :

1. All material, which may enter in contact with mice during procedures, must be steam sterilized or disinfected with a 1% Virkon solution and washed with 70% alcohol.

2. A 50µl of the buprenorphine solution is given subcutaneously to the mouse (female Crl: CD-1 *Foxn1*^{nu} 20-25g) at least 30 minutes before anesthesia.

3. The mouse (female Crl: CD-1 *Foxn1*^{nu} 20-25g) is anesthetized in the induction cage with a combination of Isofluran (5% concentration) and medical oxygen (1L/minute).

4. When the mouse is asleep, the Isofluran concentration is diminished between 1.5 and 2.5% (oxygen rate stay the same).

5. The mouse is then weigh and identified with a sterile ear tag on the left ear.

6. The mouse is maintained anesthetize with the mask. The mouse is put on her back on the wooden block previously covered with a sterile surgical field. The nose and the mouth of the mouse are introduced inside the anesthesia mask. The head is opposed to manipulator.

7. By pinching posterior paw between the nails, removal reflex is checked (do not squeeze or break bones). If the mouse removes her paw, Isofluran concentration is increased by 0.5% (wait 1 or 2 minutes and check reflex again).

8. The mouse is then correctly positioned on the stereotaxic instrument.

9. A vertical incision is made in the middle of the head with a surgical blade. The incision must not exceed 1cm.

10. The needle is then properly positioned above the incision using the knurls on the stereotaxic instrument and measures on the scales of the stereotaxic instrument are noted.

11. A small hole is made vis-a-vis the needle with the cordless micro drill using the 0.012-inch ball burr in the right frontal zone of the head (make the hole by doing a gentle and careful back-and-forth movement with the drill to avoid brain injuries).

12. 5µl of the tumor cell suspension ($0.5x10^6$ cells/5µl in MEM + 1% methylcellulose) is sucked into the Hamilton #701 syringe. Tumor cell suspension is kept at room temperature during all the procedure.

13. The needle is then slowly inserted in the hole at a depth of 0.35mm.

14. The cells are injected on a 1 minute period.

15. 4 minutes are then allowed before removing the needle to prevent leakage of the cell suspension.

16. The mouse is removed from the stereotaxic instrument and kept warm on the heating pad.

17. The skin incision is closed with a mattress suture using PDS II 5/0.

18. Antibiotic ointment is applied on the wound.

19. A 250µl subcutaneous injection of lactate Ringer is given to the mouse (for hydration purpose).

20. The mouse stays under observation until full recovery.

N.B.: Between each mouse, instruments are cleaned and rinsed with distilled water and dried with gauze. They are then inserted in the Germinator for 15 to 20 seconds. Do not use them immediately because they are extremely hot.

SUIVI POST-IMPLANTATION

Observations and weight variations:

- General behavior and clinical signs of distress are observed and noted when abnormalities appear.
- Mice are weighed three times a week. Weights are compiled in Excel table.
- 3 weight variations are calculated:
    1. from 1st day
    2. from 1st day of treatment
    3. from last day

- Each variation is converted in %.
- When 20% weight loss is observed or signs of distress appear mice are sacrificed and necropsy is performed.

Treatment:

- Treatment begins 7 days post-implantation.
- Mice are randomized in groups where *n*=5 to 8 mice.