



Stereotactic Injection

LTK-RES-3-B-EN

Version: B

This SOP replaces: Date: None
Version: None

Reason for Change: None

Related SOPs:
SOP-LTK-TRT-13- EN Isoflurane anesthesia
SOP-LTK-TRT-18- EN Injection anesthesia
SOP-LTK-RES-5- EN Scoring and withdrawal criteria of i.c. tumors
SOP-LTK-RES-6 - EN In vivo imaging
SOP-LTK-TRT-17- EN Post-surgery analgesia
SOP-LTK-TRT-19 -EN Tail bleeding

Indication of Use: Implantation of intracranial (i.c.) (tumor-) cell suspensions, application of biologically active (cytokines, small molecules, chemical drugs, peptides, antibodies, viral particles) and inactive (tracers) substances

Aim of SOP: This procedure describes how to perform stereotactic surgery for the intracranial (i.c.) application of liquids containing biologically active or inactive compounds, tumor cell suspensions, lymphocytes, viral particles or tracer substances into anatomical structures at defined three dimensional coordinates

Distribution:
1. Server
2. Animal facility
3. Group vom Berg

Attachments:

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Responsible Persons: Any person with Module 1 and registered on a particular animal permit

Safety:

1. General rules for working with sharp tools (scalpels, syringes, scissors) have to be followed.
2. Only in the case of viral particles or chemotherapeutic agents additional biosafety rules have to be obeyed
3. Follow the rules of the animal house

Material to be used:

Surgical tools: scalpel and forceps
 Betadine® Iodine solution
 Sterile cotton swabs
 Dental cement / high-viscosity acrylamide glue
 0.4 mm nylon / metal clamps / tissue glue (Indermil®, Henkel®)
 Small animal stereotactic frame (e.g. Stoelting or DKI)
 A suitable syringe (for tumor cells a blunt needle, 26 gauge, gas tight, Hamilton, Reno, NV)
 Electrical heating mat, small animal electrical hair trimmer
 Vit A eye ointment / Humigel

Principle of Method:

By fixing the head in all three dimensions, exact anatomical regions within the brain can be reached by using manipulator arms. Stereotactic coordinates for specific structures can be found in stereotactic atlases, such as "The Mouse Brain in Stereotaxic Coordinates" by George Paxinos, Keith B. J. Franklin



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Procedure Description:

1. Anesthetise the mouse using injection anesthesia (**SOP-LTK-TRT-18-A-EN Injection anesthesia**)
2. Shave the head of the animal by using an electric hair trimmer for small animals
3. Disinfect skin of the head with Betadine® Iodine solution, cover eyes with eye ointment
4. Using a scalpel, make a 1 cm skin incision along the midline
5. Fix mouse on a suitable stereotactic head holder
6. Find the intersection of the coronal and sagittal sutures (bregma), place the drill over bregma, Drill a hole using a stereotactic drill at the desired XY coordinates (for the implantation of tumors in the striatum 1.5 mm lateral and 1 mm anterior to Bregma) until you reach the dura mater.
7. exchange the drill for a suitable syringe (for tumor cells a blunt needle, 26 gauge, gas tight, Hamilton, Reno, NV) and move it to the desired XY coordinates. Slowly lower the syringe into the burr hole to the desired Z coordinates (For the implantation of tumors in the striatum, lower it 4mm and retract it 1mm. Inject 2 µl of solution (cells in DMEM or PBS) over a 2 min period. Apply biologically active or inactive compounds or cells at maximal volume of 5 µl and a maximal infusion rate of 1 µl/min. Retract the needle, wash the site of surgery with 0.9% NaCl (sterile) and suture the skin with a 0.4 Nylon (alternative: use metal clamps instead of Nylon or surgical glue such as Indermil® Henkel).
8. Apply analgesia according to **SOP-LTK-TRT-17-A-EN Post-surgery analgesia**
9. Apply antidote (**SOP-LTK-TRT-18-A-EN Injection anesthesia**)
10. Move animal into wake up cage (a regular cage placed on a 37°C electrical heating mat, covered with a surgical cloth), only put fully awake animals back to the housing cage.
11. Check for postoperative complications after 1-2 h and the next day and re-apply analgesia if necessary (**SOP-LTK-TRT-17-A-EN Post-surgery analgesia**). Monitor mice according to **SOP-LTK- RES-5-A-EN Scoring and withdrawal criteria of i.c. tumors**

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Documentation:

Lab book, Surgery Protocol and Score sheet according to **SOP-LTK-RES-5-A-EN Scoring and withdrawal criteria of i.c. tumors**

The mice have to be placed in the respective experiment and project in iRATS. The actual severity has to be recorded in iRATS at the end of the experiment for each mouse.

Problem management:

Report any adverse event to your supervisor, In case there is arterial bleeding (strong and pulsating bright-red bleeding), euthanize the animal by an overdose of injection anesthesia immediately (5x times the regular dose)

Literatur:

[Intratumoral IL-12 combined with CTLA-4 blockade elicits T cell-mediated glioma rejection.](#)

Vom Berg J, Vrohling M, Haller S, Haimovici A, Kulig P, Sledzinska A, Weller M, Becher B. J Exp Med. 2013 Dec 16;210(13):2803-11. doi: 10.1084/jem.20130678. Epub 2013 Nov 25

"The Mouse Brain in Stereotaxic Coordinates" by George Paxinos, Keith B. J. Franklin