This SOP replaces:	Version: C
Reason for Change:	Adjustments in response to Vet Office review
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 SOP-LTK-RES-3 -EN Stereotactic injection
Indication of Use:	Continous intracranial delivery of biologically active (cytokines, small molecules, peptides, antibodies, chemotherapy) and inactive (tracers) substances via osmotic minipumps
Aim of SOP:	This procedure describes how to implant Alzet osmotic minipumps for the continuous intracranial (i.c.) application of liquids containing biologically active or inactive compounds into anatomical structures at defined three dimensional coordinates
Distribution:	1. Server 2. Animal Facility 3. Group vom Berg
Attachments:	

Generated	Checked and approved
at: 17.10.2019	at: 18.10.19
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Responsible Persons: Any person with Module 1 and registered on a particular animal permit

Method: microsurgery

Principle of Method:

ALZET pumps operate because of an osmotic pressure difference between a compartment within the pump, called the salt sleeve, and the tissue environment in which the pump is implanted. The high osmolality of the salt sleeve causes water to flux into the pump through a semipermeable membrane which forms the outer surface of the pump. As the water enters the salt sleeve, it compresses the flexible reservoir, displacing the test solution from the pump at a controlled, predetermined rate. Because the compressed reservoir cannot be refilled, the pumps are designed for single-use only.

The **ALZET Brain Infusion Kits** are designed specifically for use with ALZET pumps for targeted delivery to the central nervous system. They can be used in two ways:

Infusion into the cerebral ventricles exposes a wide variety of brain regions to the infusate via the cerebrospinal fluid which bathes the brain.

Direct microperfusion of discrete brain structures results in localized distribution of infusate in the target tissue.

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"**

Material to be used:

2 weeks - Alzet® #1002 0.25 µl/h

2 weeks - Alzet® #2002 0.5 µl/h

4 weeks - Alzet® #2004 0.25 µl/h

6 weeks - Alzet® #2004 0.15 µl/h

Alzet® Brain infusion Kit III #0008851

All suitable for mice heavier than 10g body weight (6 week old C57/BL6 usually weighs between 17-23 g)

Surgical tools: scalpel, scissors (small and large), forceps

Betadine Iodine solution

Sterile cotton swabs

Dental cement / high-viscosity acrylamide glue

0.4 mm nylon / metal clamps / tissue glue (Indermil®, Henkel®)
Electrical heating mat, small animal electrical hair trimmer
Vit A eye ointment / Humigel

Material acquisition: Alzet is distributed by Charles River

Calibration: Pumps are ready to use, variation is lot dependent, so only use pumps from the same lot (all info in the accompanying package insert)

Storage of Material: pumps and infusion kit at RT

Machine: Regular small animal stereotactic frame e.g. from DKI or Stoelting

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

Method Description:

1. Sterilize all material, tools and solutions prior to surgery
2. Adjust catheter to 1.5-2 cm length
3. Fill pump, catheter and needle with compound solution according to technical data sheet, make sure that no air bubbles are trapped and assemble the implant
4. Incubate implant in PBS at 37°C for 6-24h (depending on pump model, see respective technical data sheet) to start the osmotic process ("priming")
5. Animals are anesthesized (**following SOP-LTK-TRT-18-EN Injection anesthesia**), shaved (use a small animal hair trimmer, shave the head area) and mounted onto a stereotactic frame (**SOP-LTK-RES-3-EN Stereotactic injection**)**.** The skin on the head is disinfected, eyes are being protected from drying out by application of eye ointment.
6. The scalp is incised by 1 cm long median-sagittal cut.
7. The skull is dried and cleaned of periost connective tissue with a sterile cotton swab
8. Take a closed arterial clamp and – starting from the skin incision - form a little pocket for the pump at the height of the scapulae by opening the clamp carefully.
9. Find bregma (the intersection of the coronal and sagittal sutures)
10. Place the drill over bregma, move 2 mm lateral and 1 mm frontal, drill a hole until you reach the dura.
11. Place the cannula of the implant (Alzet brain infusion Kit III) at the desired Z position in the head (usually 3 mm below the dura) and fix it with dental cement (or high-viscosity acrylamide glue) on the skullcap
12. Put the pump into the skin pocket at the neck over the scapulae, this way the mouse has full freedom of movement.
13. Suture the skin incision using a 0.4 mm nylon or metal clamps and/or tissue glue (Indermil®, Henkel®)
14. Apply analgesia according to **SOP-LTK-TRT-17-EN Post-surgery analgesia**
15. Apply antidote according to **SOP-LTK-TRT-18-EN Injection anesthesia**
16. Move animal into the 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.
17. Check for postoperative complications after 1-2 h, re-apply analgesia at 9 pm. Check latest at 9 am the next day and re-apply analgesia if necessary (**SOP-LTK-TRT-17-EN Post-surgery analgesia**). Monitor mice according to **SOP-LTK- RES-5-EN Scoring and withdrawal criteria of i.c. tumors**
18. **For experiments that exceed the indicated maximal working time of the pump model used** (check technical data sheet and pump specifications), **explant pump**: Perform steps 1 and 5 of this SOP, then make a 1 cm midline incision on the scalp, carefully lift the disc of the brain infusion kit (it might be necessary to loosen the cement / glue using the forceps) and slowly take out the infusion cannula. Now remove the actual pump together with the silicon tubing. Clean contact area where the disc was glued to the skull and with a sterile cotton swab and cover burr hole with bone wax. Then follow steps 13 - 16 of this SOP.

Controls:
After pump removal, check remaining volume and check whether or not tubing was clogged

Factors influencing outcome:
Low temperature reduces the osmotic process - which is designed for 37°C - also during surgery keep pumps at 37°C (e.g. in a small incubator), remove them only just before implantation

Documentation:
Lab book and Score sheet according to **SOP-LTK-RES-5-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 or vet, In case there is arterial bleeding (strong and pulsating bright-red bleeding), euthanize the animal by immediate overdose of injection anesthesia (**SOP-LTK-TRT-18-EN Injection anesthesia**)

Literatur:
Intratumoral IL-12 combined with CTLA-4 blockade elicits T cell-mediated glioma rejection.
Vom Berg J, Vrohlings 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.