This SOP replaces:	Date: 08.07.2019 Version: A
Reason for Change:	Comments from the veterinary commission
Related SOPs:	SOP-LTK-TRT-18 Injection anesthesia SOP-LTK-RES-3 Stereotactic injection SOP-LTK-RES-4 Implantation of osmotic minipumps
Indication of Use:	Analysis of cerebrospinal fluid
Aim of SOP:	This protocol describes how samples of cerebrospinal fluid are collected from the murine cisterna magna
Distribution:	1. Original: Server 2. Copy: Animal facilities 3. All Module 1 certified Scientists
Attachments:	

Generated	Checked and approved
at: 08.11.2022	at: 08.11.2022
by: Michal Beffinger	by: Johannes vom Berg

Responsible Persons: Researcher with a Module 1 after registration on animal license

Method: Sampling of cerebrospinal fluid (CSF)

Min/Max amount:
The maximum volume of CSF collected is 8 µl / 20 g mouse.

Storage of Material:
Collection tubes (Eppendorf) are found in the animal room.

Material:

1. Scalpel, straight edge razor,
2. Glass capillary,
3. Capillary puller,
4. Shaver,
5. Betadine iodine solution,
6. Cotton swabs,
7. Small animal stereotactic frame,
8. Vitamin A eye ointment,
9. Dissection microscope,
10. Surgical glue.

Safety:
1. General rules for working with sharp tools (scalpels, syringes, scissors) have to be followed.
2. Follow the rules of the animal house

Method Description:

1. Pull a glass capillary and trim it so the inner diameter is about 0.5 mm.
2. Anesthetize the mice according to SOP LTK-TRT-18 Injection anesthesia.
3. Apply vitamin A ointment on the eyes.
4. Shave the neck of the mouse, clean it from cut hair and disinfect using iodine solution.
5. Fix mouse on a suitable stereotactic head holder so the head forms a 135° angle with the body.
6. Using a scalpel make a 1 cm sagittal incision of the skin, inferior to the occiput.
7. Under the dissection microscope separate the subcutaneous tissue. The dura mater of cisterna magna should be visible as on the figure 1.

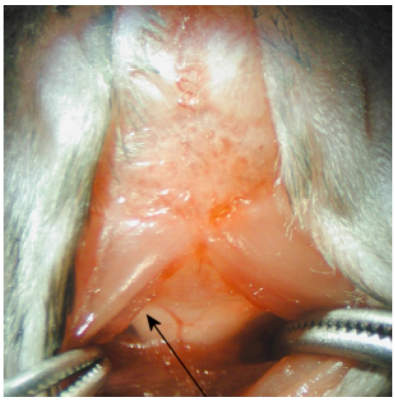

Figure 1. Dura mater of the cisterna magna exposed through the skin incision on the neck. Arrow points the point to be penetrated with the capillary (adapted from: 1)

8. Dry the dura mater with cotton swab.
9. Penetrate the dura mater with the capillary tube and move the tip into the cisterna magna depicted on the figure 1. Capillary is in the cisterna magna once the tissue resistance changes. CSF will flow into the capillary.
10. Remove the capillary and flush the CSF with a syringe into a clean tube.
11. Euthanize animal by decapitation.

Documentation:
Lab Journal.

Problem management:
In case of serious adverse events, contact supervisor, lab head or vet.

Sample storage:
Cerebrospinal fluid samples need to be frozen immediately after collection.

Literature:

1. DeMattos RB, Bales KR, Parsadanian M, O'Dell MA, Foss EM, Paul SM, Holtzman DM; Plaque-associated disruption of CSF and plasma amyloid-β (Aβ) equilibrium in a mouse model of Alzheimer's disease. Journal of Neurochemistry. 2002, 81, 229-236
2. Liu L, Herukka SK, Minkeviciene R, van Groen T, Tanila H; Longitudinal observation on CSF Abeta42 levels in young to middle-aged amyloid precursor protein/presenilin-1 doubly transgenic mice. Neurobiol Dis. 2004 Dec;17(3):516-23.
3. Liu L, Duff K; A Technique for Serial Collection of Cerebrospinal Fluid from the Cisterna Magna in Mouse. J. Vis. Exp. 2008 (21), e960.