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Cerebrospinal Fluid (CSF) Collection in mouse models

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We use this protocol and it's working

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Abstract

This protocol describes a method for the collection of Cerebrospinal fluid (CSF) from a mouse able to be used for downstream analysis.

Materials

Large Scissors
Small Scissors
70% EtOH
Fine Forceps (Dumont Curved)
Capillary tube made of polished borosilicate glass with filament, size O.D.:1.0mm, I.D.: 0.78mm.
Dissection microscope

Troubleshooting

Terminal Cerebrospinal Fluid (CSF) Collection

1 Apply 70% ethanol (EtOH) to the surgical area to wet down the hair of the mouse.

2 Using a #10 blade, make a midline skin incision over the occiput to the second cervical vertebrae (C2).

3 Incise between the cervical muscles on the midline over the occiput to C2, using sharp dissection. The cervical muscles rhomboideus cervicis, the cervical part of the trapezius, splenius capitis, semispinalis capitis, and erector spinae muscles will all need to be separated at midline to expose the cisterna magna; however, the deeper muscles which are difficult to visualize with the eye, may be separated under the dissecting microscope in step 6 using blunt dissection techniques.

4 Place the ventral surface of the thorax of the mouse on the weight boat under the microscope.

5 Stabilize the mouse, ensuring that the skull is hyper flexed for maximum access to the cisterna magna. See Fig. 1

Fig. 1 Mouse Positioning to obtain CSF. Anesthetized mouse positioned for cerebrospinal fluid (CSF) collection, demonstrating hyperflexion in the cranial-occipital region. The mouse is secured with orange tape to maintain the necessary posture for effective CSF extraction and hyperflexion is induced via a weigh boat and tape roll or any object that will hold the correct stabilization and placement of the head.

6 Bluntly dissect and separate at midline the interior muscles remaining from step 3 down to the cisterna magna to visualize a transparent membrane. This is the arachnoid

membrane covering the cisterna magna, which is a large pocket of CSF within the subarachnoid space. If hemorrhage from the musculature occurs, blot it with a cotton swab prior to puncturing the arachnoid membrane as it is critical to avoid blood contamination in the CSF.

7 Once the arachnoid membrane is visualized, use small thumb forceps to move the muscles laterally if needed for complete visualization.

8 With the capillary tube, puncture the arachnoid membrane at a 45° angle, using a gentle spinning motion.

Note

Using a micropipette puller, make a needle from a 10 cm length capillary tube made of polished borosilicate glass with filament, size O.D.:1.0mm I.D.: 0.78mm. The end of the needle will need to be cut to a diameter of 0.2mm, as the larger diameter will allow the CSF to flow into the tube by capillary action. See Fig 2.

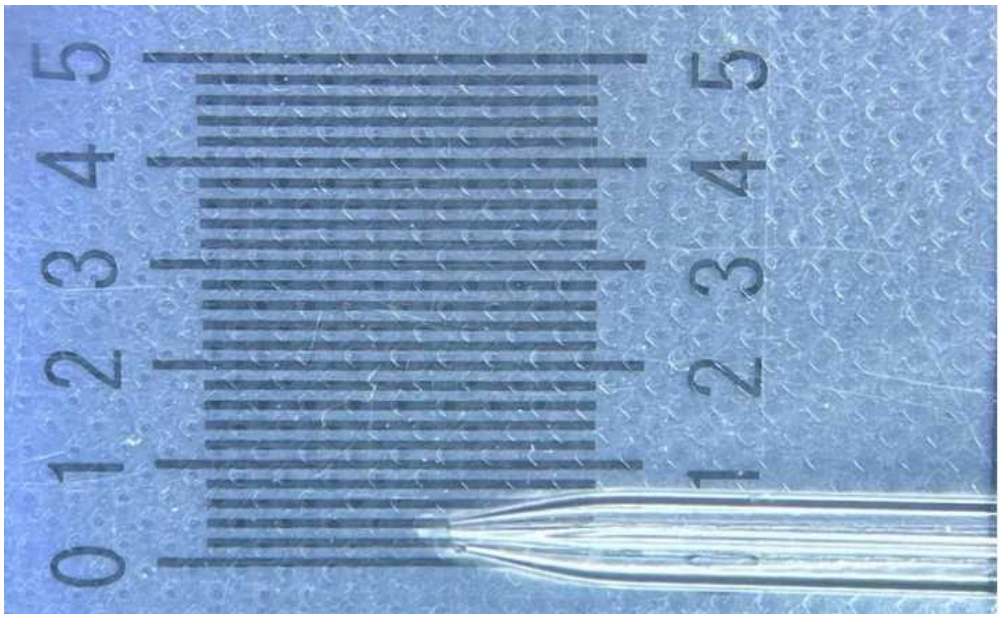

Fig.2 Capillary tube recommendations. Borosilicate glass with filament [O.D.: 1.0mm, I.D.: 0.78mm, 10cm length] capillary tube pulled into a needle and cut leaving a 0.2mm diameter opening for puncture of the arachidonic membrane.

9 Allow CSF to flow into the tube by capillary action. The approximate amount of CSF collected is between 4-10µL. See Fig 3.

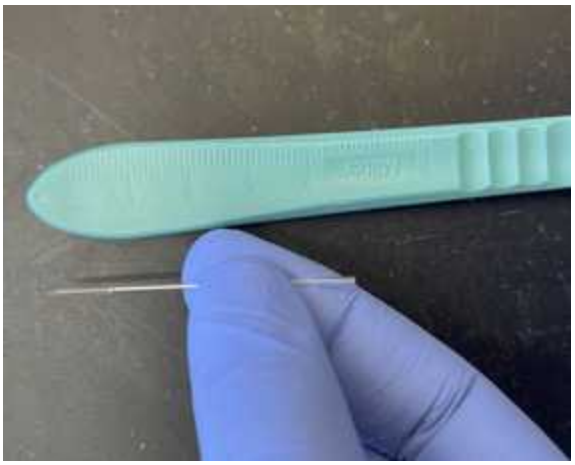

Fig. 3 *Capillary tube containing CSF*.

10 Place the blunt end of the capillary tube into the rubber bulb capillary dispenser instrument and expel the sample into the desired sample tube. See Fig. 4

Fig. 4 *Transference of Collected CSF*. Using a rubber bulb capillary dispenser into a storage vial in a laboratory setting. This process ensures the integrity and sterility of the sample for subsequent analyses.

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