

# Cerebrospinal Fluid Collection from the Cerebellomedullary Cistern in a Gottingen Minipig

Michelle Salerno, Ben Grambo and Nicole Navratil

Marshall BioResources, North Rose, NY

## Introduction:

As the Gottingen Minipig is increasingly used for neurological research, there is a growing need to obtain and test cerebrospinal fluid (CSF). Limited information is available on how to collect CSF from Gottingen Minipigs for experimental purposes. The following procedure was developed at Marshall BioResources for the collection of CSF from euthanized minipigs.

## Supplies used for this procedure:

Clippers, scalpel, tissue forceps, 3 cc luer lock syringes, 20 gauge X 1 ½ " needles, 20 gauge spinal collection needles, collection vials, guaze pads, anesthesia, euthanasia agent

## CSF Collection in an Anesthetized or Euthanized Minipig

It is possible to perform the following collection procedure in a minipig under anesthesia and recover the minipig following collection. If the animal is to be recovered, is it very important sterile technique is used to avoid introducing infection. There is also a risk of trauma to the spinal cord. For the purposes of this paper, collections were performed in euthanized animals.

1. Once the animal has reached an adequate level of anesthesia, or has been completely euthanized confirmed by the absence of a heartbeat through auscultation, then place the minipig belly down on a table with the snout hanging off the table from the shoulders forward. Pull the front legs to the chest and secure the head in a perpendicular position (snout pulled in to the table and pointing toward to the floor) to create a flat midline behind the ears and cranial knuckle, along the spinal column. An assistant can be helpful to hold the snout in place or secure the snout with tape, ties or vet wrap. Shown here using vet wrap.



2. Shave the course hair from the ridge of the neck behind the ears to expose the skin layer.



3. Mark the location of the cerebellomedullary cistern by creating a line with a permanent marker. Pull the ears slightly forward and start from the middle of each ear following onto the neck line.



4. Use the intersection of the lines as a guide to pinpoint entry for the needle.

5. Prior to inserting the needle, prep the skin with an anesthetic wipe, alcohol pad or povidone iodine. The stick will be blind and fairly deep. Slowly insert the spinal collection needle straight in at the intersection point of the marked lines. As you insert past the fat layer and muscle layer, you will be begin to feel more resistance at the spinal column and may feel a pop as you penetrate the spinal column.



6. Pull the center wire from the spinal collection needle and attach the 3 cc luer lock syringe to the needle.



- Pull the plunger up slightly to create a small vacuum. If no fluid is evident slowly continue inserting the needle with a vacuum and watch for the flash of fluid into the syringe.



- Continue to draw the fluid slowly and monitor the color to ensure fluid remains clear and the sample is not tainted with blood. If it is necessary to change syringes, hold the base of the needle using caution not to interrupt the location of the needle as you proceed with the syringe change.

- Open the collection vial, empty the spinal fluid from each syringe into a collection vial, replace the cap and label the vial.



### Viewing the Cut Down

In euthanized animals, the tissues can be cut back to view where the collection is actually taking place. This technique can be useful for bulk collections or in practice before attempting collection in a recovery animal.

- Make sure the animal is completely euthanized, confirmed by the absence of a heartbeat through auscultation. Position the animal as outlined in Step 1 above.
- Make an incision across the neck just in front of the shoulders using a scalpel. Hold the tissue back with forceps and continue to cut through the fat layer and muscle layer but not too deep; avoid entering into the fascia layer above the vertebrae. A number 11 straight blade is recommended.



- Cut back tissues along both sides of the neck and continue to dissect the fat and muscle layers only away from the fascia layer protecting the spinal column until you reach the cranial knuckle.



- If there is any blood left on the fascia layer remove it with a gauze pad prior to proceeding.

- Attach a 20 gauge X 1 1/2" needle to a 3 cc luer lock syringe. Insert the needle straight going just through the fascia layer.



- Pull up slightly on the plunger of the syringe to create a small vacuum and continue inserting the needle until you see a flash of spinal fluid.



- Continue to draw the fluid slowly and monitor the color to ensure the fluid remains clear and is not tainted with blood. Follow the collection instruction detailed above in Step 8. Following the collection, the CSF can be transferred into a collection vial as outlined above in Step 9.