

Cecal Ligation and Puncture (CLP).v1

This protocol aims to set up mouse model of sepsis (Wen H. Methods Mol Biol. 2013;1031:117-24, 23824895)

Procedures:

1. Perform the surgical procedure at about 3 pm in the afternoon.
2. Prepare all of sterile surgical equipment, including the scissors, forceps, and wound clips, 18-gauge needle, Vetclose tissue glue, 3-0 silk suture.
3. Mark the mouse by ear puncher and weigh and record it.
4. Anesthetize the mouse using the isoflurane system in the induction chamber.
5. Take the mouse out of the induction chamber and put it's nose into a nosecone to maintain anesthesia by switching the gas to the nosecone.
6. Tape the four legs of the mouse with plastic tape to the table.
7. Sterilize the abdomen of the mouse with Betadine once and 70% ethanol twice.
8. Make a 1-cm midline incision to the ventral surface of the abdomen and expose the cecum.
9. Ligate the middle cecum(1.0 cm from the tip) with a 3-0 silk suture.
10. Grasp the tied off part of the cecum with a pair of blunt forceps and pierce it all the way with a 18-gauge needle in the middle part and remove the needle.
11. For sham operation, perform the same procedures, but don't ligate and pierce the cecum.
12. Place all innards back inside the peritoneal cavity of the mouse.
13. Use Vetclose tissue glue to the insicion to close the peritoneal cavity.
14. Apply two surgical wound clips to secure the incision closure.
15. Immediately inject 1 ml of sterile saline subcutaneously to each mouse to fluied resusciation.
16. Put the mouse in a face-down position in a bedding-free, pre-warmed fresh cage placed over a heating pad for recovery from anesthesia and monitor for 30 min.
17. Euthanize the mice under anesthesia and collect blood, heart, lung, liver, kidney and skeletal muscle for fresh frozen blocks, protein and mRNA.