*Movie script for:* **Technique of veno-venous extracorporeal membrane oxygenation in a mouse**

Here we present a protocol describing the technique of veno-venous extracorporeal membrane oxygenation in a non-intubated, spontaneously breathing mouse. This murine model of ECMO can be effectively implemented in experimental studies of acute and end-stage lung diseases.

1. **Preparation**

Prepare the priming solution (**Table 1**). Include 30 IU/mL heparin and 2.5% v/v of an 8.4% solution of NaHCO3. Refrigerate this solution at 4 ºC until ready to use. Prime the circuit with 500 uL of priming solution.

Place the outflow cannula into the priming solution and fill the ECMO machine by switching on the peristaltic pump. Continue to circulate the priming solution through the machine for the next 30 min at a flow rate of 1 mL/min.

Give 0.5 L/min of 100% oxygen to the oxygenator.

**2. Anesthesia**

Place the animal in an induction chamber filled with 2.5% v/v isoflurane/oxygen mixture. Provide 0.5 L/min of 100% oxygen to the vaporizer.

Use a warming pad to maintain the body temperature at 37 oC.

Perform inhalation mask anesthesia using an isoflurane vaporizer and inject 5 mg/kg carprofen subcutaneously.

Regularly observe the spontaneous breathing and adjust the concentration of isoflurane so that it is between 1.3 to 2.5%.

**3. Surgery**

Expose the left jugular vein.

Once the left jugular vein is exposed, ligate the distal part using an 8-0 silk suture with the help of micro-forceps.

Insert a 2 Fr polyurethane (PU) cannula into the proximal part of the jugular vein, rotating it slightly while pushing it to a depth of 4 cm. In doing so, the iliac bifurcation of inferior vena cava (IVC) will be reached.

Secure the cannula with 8-0 silk knots using microforceps.

Now expose the right jugular vein.

Cannulate the right jugular vein with a 1 Fr PU cannula and gently move it 5 mm in the direction of right atrium.

Catheterize the left femoral artery with another 1 Fr PU cannula and use it for invasive pressure monitoring as well as blood sampling for blood gas analysis.

Insert electrocardiogram needles connected to a data acquisition device subcutaneously into both forelimbs and into the left thoracic wall.

Insert a rectal thermometer connected to a data acquisition device.

**4. Veno-venous extracorporeal membrane oxygenation and blood gas analysis**

Initiate ECMO on the animal by turning on the pump with an initial flow rate of 0.1 mL/min. Adjust the flow rate of the pump within the next 2 min to 3 – 5 mL/min.

Under stable flow, continue to monitor in real-time mode all vital parameters via the data acquisition device.

Collect any blood leaking from wounds and return it to the ECMO circuit via the air-trapping reservoir.

For BGA, use a blood sampling cartridge to collect approximately 75 µL of arterial blood at the following time points and from the following locations:

* 10 min after the initiation of ECMO, collect blood from the IVC before the oxygenator, directly after oxygenator (control), and from the femoral artery
* 30 min after the initiation of ECMO, collect blood from the femoral artery
* 1 h after the initiation of ECMO from the femoral artery
* 2 h after the initiation of ECMO from the oxygenator, IVC, and the femoral artery

After 2 h, reduce the flow rate on the pump gradually (over the course of 5 min), thereby stopping ECMO.

Physiological parameters were recorded every 10 min and data from a representative ECMO experiment are presented in **Figure 2**. BGA data from a successful ECMO are shown in **Table 1**.