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Lipopolysaccharide intraperitoneal injection in rats and sickness behavior assessment

In 1 collection

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ABSTRACT

This protocol is a quick guide to how to prepare a solution of LPS on the saline vehicle, how to inject a rat for an intraperitoneal injection, and how to monitor the animal after injection. The procedure must be done by trained persons and done according to local Animal Care legislation.

MATERIALS

Equipment:
- Analytical balance.
- Vortex mixer.
- Stirrer

Reagents:
- Lipopolysaccharide (LPS) from *Escherichia coli* O111:B4
- Sterile saline (0.9% NaCl)
- Ethanol (70%)

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1. Lipopolysaccharide (LPS) preparation

1 Before starting, it is better to prepare LPS solution on the same day it will be injected. However, under minimal conditions (that could be acceptable), you can work with LPS from previous days stored at kept at 2-8°C; in this case, shake very well before use.

2 Weigh the animal

3 To prepare a dose of 5 milligrams (mg) per kilogram (kg) of the weight of the rat and obtain an injection volume of 0.5 milliliters (ml) per kg of weight, calculate the amount of reagents that must be prepared as follo

	A	B	C	D
	total mass of rats (Kg)	Minimum mass of LPS required (mg	Mass of LPS to be measured (mg)	Volume of saline solution to dissolve the LPS (ml)
		5mg/Kg*A	B*1.75	C/(mg/ml)

3.1 Weigh the calculated LPS total amount and add the volume of the corresponding saline solution to reach a solution of concentration 1 mg LPS / mL

3.2 mix well the solution with a stirrer for 15 minutes at medium speed (store at 4°C if not immediately used and shake on the vortex shaker for 30 seconds before injection)

2. Intraperitoneal injection

4 Disinfect the lid of the multiple-dose vial with gauze and 70% ethanol

5 Determine the amount of volume per rat to be injected from the LPS solution or sterile saline (0.9% NaCl)

6 With care remove the animal from its homecage, and restrict it appropriately to perform the i.p. injection. Thus, insert the needle with the bevel of the needle facing up into the lower quadrant of the abdomen at an angle of 30-40 degrees.

7 Push the plunger until the entire solution is administered

Note

Be careful:
- The needle must not move inside the abdomen, to avoid lacerating surrounding organs or blood vessels
- When injecting, if a green solution is aspirated, it may be a sign that the intestine has been penetrated; if a yellow liquid comes out, it may indicate that the bladder was punctured; and if blood comes out, it may indicate that a blood vessel was perforated.

8 Once the solution is administered, place the animal back into the homecage

9 carefully, remove the needle from the syringe and discard the syringe in the sharps bin and discard the syringe in the trash

10 The animal should be monitored immediately after the injection, about 10 minutes
• If bleeding occurs, place gauze and apply pressure. Once bleeding stops, clean the site with gauze and water.
• In case of peritonitis, laceration of internal organs and / or infection, consult a veterinarian to assess if the animal can continue in the experiment.

3. Sickness behavior

5m

11 Signs consisting of absent exploration and locomotion, curled body posture, irregular fur, piloerection, and closed eyes are evaluated over time (at least 72h)

11.1 Place animals individually in a transparent cage and score on a four-point scale: 0 = no signs,1 = one sign, 2 = two signs, and 3 = three or more signs. The two experimenter quantifying the sickness signs should be blind to experimental and control conditions

5m

4. Body weight

12 Record animal body weight after each sickness behavior observation and scoring