

LAB_063 Rotarod Test for Rodents (Expiry: March 2026)

I. OBJECTIVE

To describe the procedure for measuring motor coordination using a rotarod.

NB: The use of (*) indicates this statement is dependent on the facility procedures NB: The use of (**) indicates this statement is dependent on AEC Approvals

II. COMMENTS / RECOMMENDATIONS

- Behavioural assessments are ideally performed in a dedicated behavioural suite.
- The environment should be free from uncontrolled external stimuli that may influence the animal's behaviour such as human traffic, unnecessary noise, intense lighting. Similarly, it is important that assessments are controlled for those stimuli which cannot be removed, such as such as time of day and light or dark phase.
- Male and female rodents should be tested separately, with one sex in the room at a time. Where possible
 males should be tested first, preferably on separate days but with at least thorough cleaning between the
 sexes. This is unless rodents are already housed within wire top cages or equivalent and both sexes are
 present in the home room.

III. EQUIPMENT

PPE*

Minimum PPE is gloves and gown, additional PPE may be required based on facility or additional risk e.g. working with infectious animals.

- Appropriate trolley for transporting cages.
- Disinfectant*, little brush, and paper towel for cleaning equipment.
- Rotarod An apparatus consisting of a horizontally oriented, rotating cylinder (rod) suspended above a cage floor, which is low enough not to injure the animal, but high enough to induce avoidance of fall. Ideally commercially available Rotarod systems should be used, and the dimensions of the apparatus should be within the ranges specified in the table below.



Apparatus dimensions	Mouse	Rats
The diameter of the rod (cm)	3 - 9	6 - 8
The width of the lanes (cm)	5 - 6	7 - 9

Conditions:

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The diameter of the flanges (cm)	20 - 30	30- 40
The height to fall (cm)	15 - 30	25 - 35

IV. PREPARATION

- Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work.
- Prepare equipment items including disinfecting prior to first use.
- Bring rodents into the room (with lighting levels pre-set at the level required for the experiment) for at least 30 mins prior to start of experiment.

Length of habituation time in the testing room should be consistent for all rodents within an experiment.

V. PROCEDURE

1. Record light levels in the middle of the arena, for reproducibility and consistency.

Lux range should be between 80-100 LUX and should remain the same for all rodents within an experiment.

2. Define all parameters for testing. Common parameters are listed below:

Fixed speed: set up one speed for the testing, which can range between 5 - 40 rpm. One trial should last between 3 to 5 min.

Accelerating speed: set up initial speed (2 - 5 rpm), final speed (20 - 40 rpm), and accelerating duration or ramp (1 - 5 min). One trial should last between 3 to 5 min.

Reverse (rarely used): set up initial speed (2 - 4 rpm), highest speed (20 - 40 rpm), accelerating duration or ramp (3 - 5 min), decelerating duration (3 - 5 min) and final speed (2 - 4 rpm).

Custom setting: some experiments require a stepped increase that includes both acceleration and fixed speed. One trial lasting between 3 to 5 min.

- 3. Start the rotation of the rod until the speed reaches the fixed speed or the initial speed.
- 4. Handling of rodents as per: <u>LAB_006 Handling and Restraint in Mice and Neonates</u> LAB_039 Handling and Restraint in Rats and Neonates
- 5. Gently swing the rodents onto the top or the back of the rod, making sure they start walking instead of just holding onto the rod and cartwheeling.
- 6. As soon as all the rodents are up and walking, start the protocol and begin timing. Record the duration that the animals stay on the rod as the latency to fall. Each trial ends when all the animals drop, or the maximum time is reached.
- 7. Remove scat and thoroughly disinfect the apparatus and allow to dry completely before proceeding to the next trial.

NOTES:

1. Initial testing in an animal may be repeated daily for up to 4 days. On the first testing day, a maximum of four trials are recommended. On each of the following days, three trials are recommended.

2. Testing may be repeated over time to examine longitudinal changes in motor function. The frequency of testing over prolonged periods is dependent upon animal strain but can be as often as weekly (i.e. 3 trails in 1 day, once a week).

3. **THIS IS NOT AN EXHAUSTION TEST**: If animals reach the maximum time after one or two trials, then there is no motor deficit, and no need to continue their trials.

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VI. ANALYSIS

The longest latency to fall for each animal obtained from all training sessions should be recorded and plotted.

VII. REFERENCES

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- Zhao J, Cooper LT, Boyd AW and Bartlett PF. Hippocampal Gamma Rhythms During Y-maze Navigation in the Juvenile Rat. Sci Rep. 2018; 8: 11393, doi: 10.1038/s41598-018-29845-1. <u>https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6065374/</u>
- 3. Deacon RMJ. Measuring Motor Coordination in Mice. J Vis Exp. 2013; (75): 2609, doi: 10.3791/2609. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3724562/
- Rozas G, Guerra MJ, Labandeira-García JL. An automated rotarod method for quantitative drug-free evaluation of overall motor deficits in rat models of parkinsonism. Brain Research Protocols. 1997; 2(1): 75-84. doi: 10.1016/s1385-299x(97)00034-2 <u>https://pubmed.ncbi.nlm.nih.gov/9438075/</u>
- 5. Rozas G, Garcia JL. Drug-free evaluation of rat models of parkinsonism and nigral grafts using a new automated rotarod test. Brain research. 1997; 1; 749(2): 188-99, doi: <u>10.1016/S0006-8993(96)01162-6</u> <u>https://pubmed.ncbi.nlm.nih.gov/9138718/</u>

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