LAB_063 Rotarod Test for Rodents (Expiry: June 2029)

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 from females 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.
- Any changes to what is detailed in this SOP must be fully described in project applications and receive AEC approval**.
- All substances administered to rodents as part of this procedure must be clearly detailed as a 'substance' in approved projects **
- Supporting SOPs referenced in this procedure (e.g. handling or substance administration SOPs) must also be described in the approved project ethics **
- Define all Rotarod parameters and maximum trial duration in your project ethics**. Common parameters are listed under 'Procedure', point 2.

III. SAFETY AND COMPLIANCE

- Correct PPE use is essential when handling laboratory rodents
- The person undertaking this task must ensure all relevant approvals are in place, training has been undertaken, and risk assessments have been performed. If unsure, consult your supervisor.
- Facility protocols should be followed.
- Possible risks include mouse/rat bite injury, repetitive task musculoskeletal injury and psychosocial harm.
- All accidents, injury or near misses are to be reported immediately to the Facility Manager and recorded on a UQ OHS Incident Report Form.

IV. TRAINING CONSIDERATIONS

- All unsupervised animal procedures must be performed by appropriately trained personnel who have been deemed competent in the procedure.

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

VI. PREPARATION

- Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work.
- Prepare and disinfect equipment items 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.

VII. PROCEDURE

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

Lux range should be between 20-100 LUX and should remain the same for all rodents within an experiment. This is particularly important for albino mice where light intensity above 400Lux may induce retinal damage.

2. Review all approved Rotarod parameters for testing in your project ethics**. 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. Set the Rotarod using the approved parameters and start the rotation of the rod until the speed reaches the fixed speed or the initial speed.
4. Gently lower 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.
5. 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 trial duration is reached.
6. 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 3-4 times per day 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 trials in 1 day, once a week).
3. **THIS IS NOT AN EXHAUSTION TEST**: If animals reach the maximum trial duration after one or two trials, then there is no motor deficit, and no need to continue with further trials in that test period.

VIII. ANALYSIS

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

IX. REFERENCES

1. Lee JD, McDonald TS, Fung JNT, Woodruff TM. Absence of Receptor for Advanced Glycation End Product (RAGE) Reduces Inflammation and Extends Survival in the hSOD1^{G93A} Mouse Model of Amyotrophic Lateral Sclerosis. Mol Neurobiol. 2020; 57(10): 4143-4155, doi: 10.1007/s12035-020-02019-9. https://pubmed.ncbi.nlm.nih.gov/32676989/
2. 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. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6065374/
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/
4. 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 https://pubmed.ncbi.nlm.nih.gov/9438075/

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: 10.1016/S0006-8993(96)01162-6 https://pubmed.ncbi.nlm.nih.gov/9138718/

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Version #	Reviewing AEC	AEC Review Date	Approval To Date
1.0	Anatomical Biosciences AEC, Health Sciences AEC, Molecular Biosciences AEC and Laboratory Biomedicine AEC	March 2025	12-month extension approved to March 2026
2.0	Anatomical Biosciences AEC, Health Sciences AEC, Molecular Biosciences AEC and Laboratory Biomedicine AEC	June 2026	Approved to June 2029

Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see “AEC Reviewed/Approved” date in this document’s header).