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**Standard Operating Procedure  
The Jackson Laboratory Mouse Neurobehavioral  
Phenotyping Facility (JAX-MNBF)**

**Rotarod Assay  
[ROT]**

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<b>Area:</b>	JAX-MNBF
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<b>Controls:</b>	
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## 1. PURPOSE

This SOP addresses the routine procedures used for the evaluation of motor coordination in the rotarod assay in mice including methods for analysis of data, and quality monitoring procedures.

## 2. SCOPE

The SOP applies to The Jackson Laboratory Center for Biometric Analysis and Mouse Neurobehavioral Phenotyping Facility (JAX).

## 3. RESPONSIBILITIES

### 3.1. Laboratory Staff

3.1.1. Remain up to date in training with this SOP

3.1.2. Comply with this SOP

### 3.2. Director of the JAX-MNBF

3.2.1. Ensures that all personnel involved running this SOP are trained to comply with this SOP

## 4. GLOSSARY/DEFINITIONS

Item	Definition
Latency	The cumulative time (seconds) the subject maintains its balance on the rotarod prior to falling off of the rod. The maximum latency is set at 300 seconds per trial.
RPM	Revolutions per minute. The speed of the rod as defined by the number of rotations per minute. For this protocol the subject is placed on the rod at a speed of 4 RPM which accelerates over the course of 300 seconds to 40 RPM (e.g., an increase of 1 RPM is achieved approximately every 8.3 seconds).
Trial	The event period during which the subject is assessed for latency to maintain balance on the rotarod. For this protocol,

	the maximum trial duration is 300 seconds and the subject is tested in 3 consecutive trials.
ITI	Intertrial interval. The period of time that elapses between consecutive trials. For this protocol the ITI is approximately 1 minute in duration which is the time required to sanitize the rod between consecutive trials (within subject).
Session	Each session consists of 3 consecutive trials. A maximum of 5 mice (one per rod lane) can be tested per session. Sessions are consecutive with only instrument sanitization time required prior to the next set of subjects/next session.

## 5. MATERIALS

### 5.1 Instrumentation

5.1.1. Rotarod. A rotating rod, 3 cm in diameter, elevated to a height of 16 cm above the instrument floor, and suitably machined to provide grip for the mice (Ugo Basile, model 47600). Six flanges divide the rod into five equally spaced lanes (5.7cm lane width). Individual trip plates located on the instrument floor, directly below each lane, are used to stop the timer for each lane immediately upon contact with a subject falling from the rod onto the trip plate.

5.1.4. Scale: A scale for weighing animals with 0.1 gram resolution

### 5.2. Consumables

5.2.1. 70% ethanol (ETOH) in water solution: used to sanitize the instrument between subjects

5.2.2. Paper towels

5.2.3. Non-toxic markers: Sharpie® brand (red, blue, black, purple preferred)

## 6. PROCEDURE

### 6.1. Environment

6.1.1. Procedure Room. The dimensions of the procedure room are approximately 14 ft x 10 ft. The rotarod is placed on a laboratory bench

(approximately 5 feet in length, 21 inches depth) on the perimeter wall of the room, adjacent to a laptop to record the data.

- 6.1.2. Temperature. The temperature range in the testing room and anteroom is similar to the housing room at  $72 \pm 2$  F.
- 6.1.3. Humidity. The humidity range in the testing room and anteroom is similar to the housing room at  $50 \pm 20\%$ .
- 6.1.4. Lighting. Room lighting in the testing room and procedure room is by indirect lighting via mounted LED lights with a dimmer switch illuminated to a setting which produces a light level in the testing room of  $\sim 500$  lux.
- 6.1.5. Noise. The ambient background noise levels in the procedure room and anteroom is 60-70dB. Audible timers are not used during this test. The motor from the rod emits a low level noise when the instrument is on and should be turned on and rotating at the start speed of 4 RPM prior to transporting the test subjects into the testing room.
- 6.1.6. Visual Cues. No intended visual cues are used for this test.
- 6.1.7. Time of day. The test is conducted during the light phase of the circadian cycle; beginning at least 60 min after the lights on and concluding at least 30 min prior to lights off.

## 6.2. Subjects

### 6.2.1. Species. Mice

6.2.1.1. Study specific animals (e.g, strain, sex, date of birth) documented

6.2.1.2. Receipt of animals logged (e.g., date of arrival)

### 6.2.2. Sex. Males or females

6.2.3. Age. The test is validated for mice  $\geq 4$  weeks of age.

6.2.4. Housing. Subjects may be group housed or individually housed for this test. Subjects are housed in the MNBF housing room for a minimum of 5 days prior to behavioral testing.

6.2.5. Light Cycle. The light cycle in the MNBF housing and procedure rooms is 12:12 with lights on at 6:00 am and lights off at 6:00 pm.

6.2.6. Husbandry. The test should not be conducted on the same day that a cage change has occurred.

6.2.7. Transport to Procedure Room. Subjects are transported in their home cages from the housing room to the procedure room on a wheeled transport rack.

- 6.2.8. Body Weight. Body weights are recorded to the nearest 0.1 gram prior to the start of the acclimation period which precedes the beginning of the testing period.
- 6.2.9. Subject Identification. The tail of each test subject is identified with an ID number for the test day, written on each subject's tail using a non-toxic marker (Sharpie® brand marker in red, blue, black, or purple color) which also facilitates selecting each mouse at their scheduled test time/repeated trial without having to restrain (e.g. to confirm ear notch or chip ID).
- 6.2.10. Acclimation. Prior to transporting the mice to the procedure room, the rotarod is turned on and set to a fixed speed of 4.0 RPM such that the test subjects can acclimate to the noise emitted from the rod's motor, prior to testing. Upon arrival to the procedure room, subjects are briefly handled and assessed for welfare concerns that may result in exclusion from testing (e.g., fight wounds or bite marks), weighed, identified by a tail label (see 6.2.9 above) and then left undisturbed to acclimate for 60 min prior to testing.
- 6.2.11. Randomization and counterbalancing. Subjects are counterbalanced across all lanes of the rod and across sessions within the testing day. Subjects are not returned to their homecage until all cagemates within a homecage have been tested and instead are placed in a separate clean empty transition cage until all testing has completed. A run sheet in excel format is created prior to the test that lists the test date, environmental variables, subject IDs, test sessions, run times, body weights, test lane with subject IDs, and a comments session to note any unexpected observations.

### 6.3. Testing

- 6.3.1. Sanitization. Prior to placing the mice on the rod, and between subjects, the rod is thoroughly sanitized with 70% ETOH solution (in water), gross urine and feces are removed, and the maze is wiped dry with clean paper towels.
- 6.3.2. Recording Software set up.
- 6.3.2.1. Create new experiment. In the Rotarod program double click the box that reads Label 1 and name your experiment with a unique identifier.
- 6.3.2.2. Select Speed mode. Click Accelerated Speed Mode. When prompted with "Set Ramp Duration?" Use Up/Down buttons to adjust test duration in minutes. Set 300 seconds. Press ESC to enter. When prompted for "With Reverse?", select No.

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6.3.2.3. Select Speed Settings. Under Speed, click Set Max Value. Use Up/Down buttons to set max speed value to 40 RPM. Press ESC to enter. Repeat for Set Min Value to 4 RPM. Press ESC to enter.

6.3.2.4. Create Subject ID list. Double click box in bottom right of main menu, and enter animal IDs with respect to lane number. Click OK to save. Note this must be entered for each set of test subjects after the 3 consecutive trials is completed for a set of mice.

6.3.2.5. Confirm configurations. Select File from menu bar and click Configuration and OK. Then select File from menu bar again and click Acquired Data. Delete any previous data using the green burn bucket icon. Check box that says "Refresh Each Measure".

6.3.3. Placement onto the rod.

6.3.3.1. Move the cage(s) of mice to be tested in the current trial to the bench on the left side beside the rotarod. This should be done after the rod has been sanitized as in 6.3.1 above.

6.3.3.2. Prior to placing the subjects onto the rod, confirm the trip plates are in the active position, the rod is rotating at the start speed of 4 RPM and the reset button has been clicked.

6.3.3.3. Place the mice onto the rotating rod in sequential order of rod lanes from lane 1 on the far left of the rod to lane 5. Immediately upon all mice being placed onto the rod, press the START button on the instrument panel to start the timers.

6.3.3.4. The technician does not move away from the rod or sit down during testing and should remain standing facing the rod in the same position as they were during placement of the mice on the rod. This enables the technician to quickly transfer mice that fall back to their cage between trials (see 6.3.4.1 below).

6.3.4. Test duration. As the mice fall from the rod, each lane's trip plate will stop the timer and display the latency in seconds. Confirm the computer captures the correct value on the display; however do not reset the trip plate or the speed setting until all mice have fallen from the rod (completed the trial).

6.3.4.1. Mice that fall from the rod upon placement, but prior to the technician pressing start, are given a "0" for that trial (0 second latency to fall) and returned to their cage until their next consecutive trial.

6.3.4.2. Mice that maintain their balance on the rotarod for the maximum time of 300 seconds are removed and placed into their cage during the ITI until their next consecutive trial.

- 6.3.5. Once all mice in a trial have fallen and placed into their cages as in 6.3.4.2 above, the technician removes all gross urine and feces with a dry paper towel.
- 6.3.6. Repeat steps 6.3.3 through 6.3.6 for the next subsequent set of subjects; ensuring each subject is placed back in the same lane on the rod and same sequential order of placement for all 3 trials.
- 6.3.7. At the conclusion of the 3 consecutive trials, the subjects are returned to the housing room and the instrument is thoroughly sanitized with 70% ETOH as in 6.3.1 above.
- 6.3.8. At the conclusion of all testing for the day, the subjects are returned to the housing room and the instrument is thoroughly sanitized as in 6.3.1 above and turned off.

#### 6.4. Data Analysis and QC

##### 6.4.1. Data QC.

6.4.1.1. Confirm all chambers recorded data as expected for all endpoints and timebins. Identify any technical issues with chambers not reporting data. Remove data from chambers that did not contain subjects “empty chambers” which would output “0” values. Identify and reconcile errors (e.g., subject placed in wrong chamber or incorrect subject ID number was identified with incorrect chamber).

6.4.1.2. Mice that fall or jump onto the floor or escape from the rod or testing bench area should be excluded from analysis.

##### 6.4.2. Resulted analyzed

6.4.2.1. Data are analyzed by a technician blind to treatment prior to revealing the code. Data are analyzed over time bins as latency to fall (sec) over each trial, as well as the average latency across the 3 trials (as defined in the glossary 4.0 above).