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Mouse Water Restriction

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Abstract

This protocol describes a semi-customized workflow to perform water restriction with mice designated for future experiments.

Water restriction is a common method used to increase engagement and motivation of mice performing behavioral experiments that use water as a reward.

Water restriction timeline summary: Mice should have three daily weights collected, which are averaged to determine their baseline weight. The target weight will be a percentage (desired) of their baseline weight. The first day of water restriction begins on the last day of the mouses weight collection and is given 1.0mL of water. The mouse should be weighed and provided at least 1.0mL of water daily while under water restriction. All calculations should be individually determined for each mouse.

Mice under water restriction should be closely monitored for health concerns due to the effects of dehydration. All mice should receive 1.0mL of water daily. Follow IACUC and veterinary requirements for water restricting mice.

Guidelines

Only perform this procedure in accordance with IACUC and veterinary requirements.

Animal Behavior:
Utilize a 12-hour light cycle.
Mice should have access to ad-lib food and water until placed on diet restriction.
Single house mice on diet or water restriction.
Ensure cleanliness of experimental environment.

Materials

Materials:

	Tool / Supply	Manufacturer / Supplier	Part Number
	70% Ethanol (Diluted in house)	Sigma Aldrich	459836
	Kimwipes	Kimberly - Clark Professional	S47299A
	Water filtered through rack filtration system.	-	-
	Nalgene® 125 ml PET Square Media Bottles, Sterile	CP Lab Safety	342040-0125
	1mL Luer-Lok Syringe	Becton Dickinson and Company	309628
	25G 5/8 Blunt Needle *	Advantor	89134-134
	1000mL Nalgene™ Straight-Sided Wide-Mouth Polycarbonate Jars	Fisher Scientific	21161000
	Ohaus® Scout® Portable Balance	Sigma Aldrich	OH30253024
	Water Bowl	Made in-house	0146-000-01
	Cart Cover	Life Science Products	CC-34X19X38Z

Supplies can be substituted with their equivalent.

** = Optional*

Personal Protective Equipment (PPE):

	Suggested PPE
	Gloves
	Disposable lab coat
	Disposable face mask
	Scrubs
	Biohazard sharps disposal container
	Biohazard waste disposal container

Utilize PPE in accordance with IACUC and veterinary requirements.

Safety warnings

Personal Protective Equipment (PPE) should be used at all times while operating this protocol.

Ethics statement

Research focused rodent behavior must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s).

This protocol has been approved by the Allen Institute Animal Care and Use Committee (IACUC).
PHS Assurance : D16-00781
AAALAC : Unit 1854

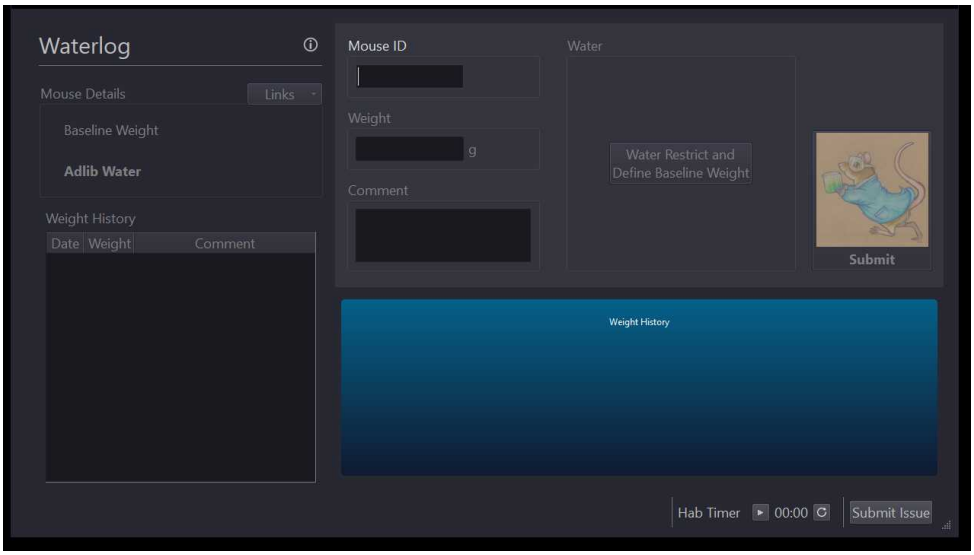
Calculating Baseline Weight and Restriction

1 Open Waterlog via desktop application.

2 Enter your LIMS/SLIMS User ID.

Waterlog entry screen.

3 Enter mouse ID in mouse ID text box and press enter on your keyboard.



Waterlog main page without loaded mouse information.

Note

If "invalid" or a red "X" shows up once inputting the mouse ID, recheck ID number and confirm mouse ID has been entered into the LIMS/SLIMS system. This LIMS/SLIMS entry is required for Waterlog to open with the desired mouse ID.

4 Retrieve mouse from the animal holding room (AHR).

4.1 If transporting mice under reverse light cycle, utilize a cart cover within non-red light rooms.

5 Ensure that mouse has correct enrichment in their cage based on requirements of the experimental design and genotype (i.e. tube, wheel, chewing block, etc.).

6 Collect and record daily weight of mouse on ad-lib water for at least 3 days.

Note

Begin collecting weights on the Wednesday prior to habituation (assuming a weekday schedule).

6.1 Scoop/cup mouse out of cage.

Note

If mouse cage has a tube or other container-shaped enrichment, use as vessel for transportation. This mode of transportation/handling can be less stressful for the mouse.

6.2 Check mouse for correct ID (ear notches and/or tattoo). Check mouse for any other wellness concerns (headframe, prolapse, wounds, etc.).

6.3 Place mouse in weigh container and place on scale. Note the weight.

6.4 Return mouse back to cage.

> **Note**
>
> Use weigh container to return mouse to cage.

6.5 Clean weigh container with 70% Ethanol spray after each use. This is to prevent any additional stress on mice caused by previous mice excrement.

> **Note**
>
> Dry weigh boat with Kimwipes sufficiently after spraying with Ethanol. Ethanol fumes can cause mouse to suffocate.

6.6 Record mouse weight in weight textbox of Waterlog. Click submit button.

7 Place mouse on non-water restriction rack with water spouts and/or attach a filled water bottle to cage for the first two days.

7.1 Toggle water spout prior to placing mouse on rack to confirm that water spout is delivering water.

8 On the third day use Waterlog to calculate the average of your recorded weights to obtain the mouse's baseline weight.

> **Note**
>
> This calculation is typically done on the Friday prior to a habituation week.

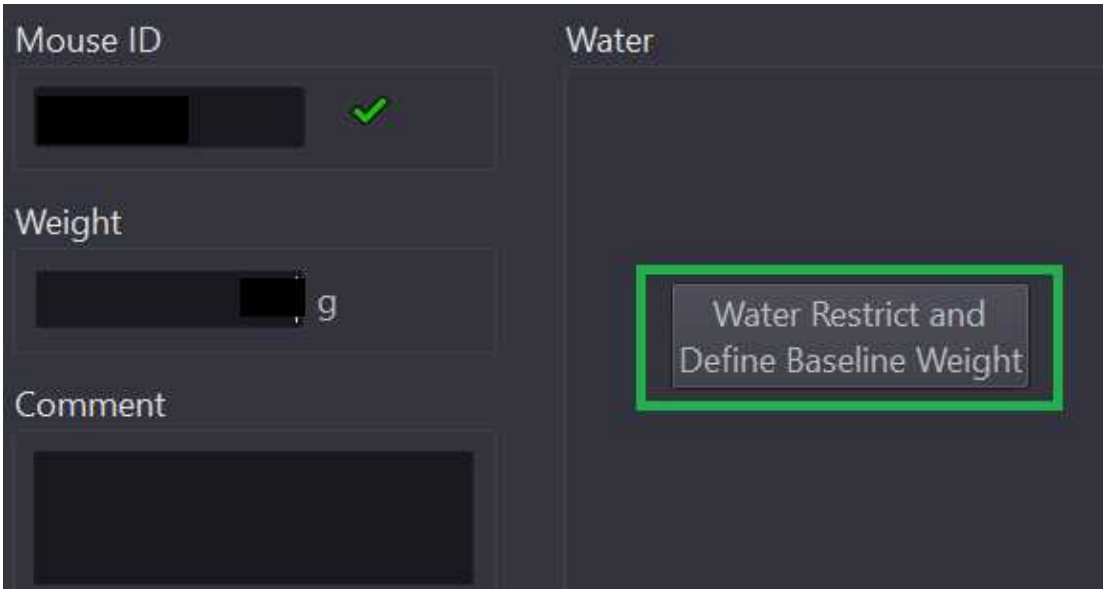
8.1 Open Waterlog and enter LIMS User ID.

8.2 Enter mouse weight into the current weight text box. Enter any behaviorally relevant information (behavior program title, animal performance notes, hardware/software issues, etc.) in the comment text box.

Note

Confirm mouse information (ID, baseline weight, target weight fraction, weight history) are correct. This should be congruent to mouse behavior schedule, vertical water restriction card and horizontal cage card.

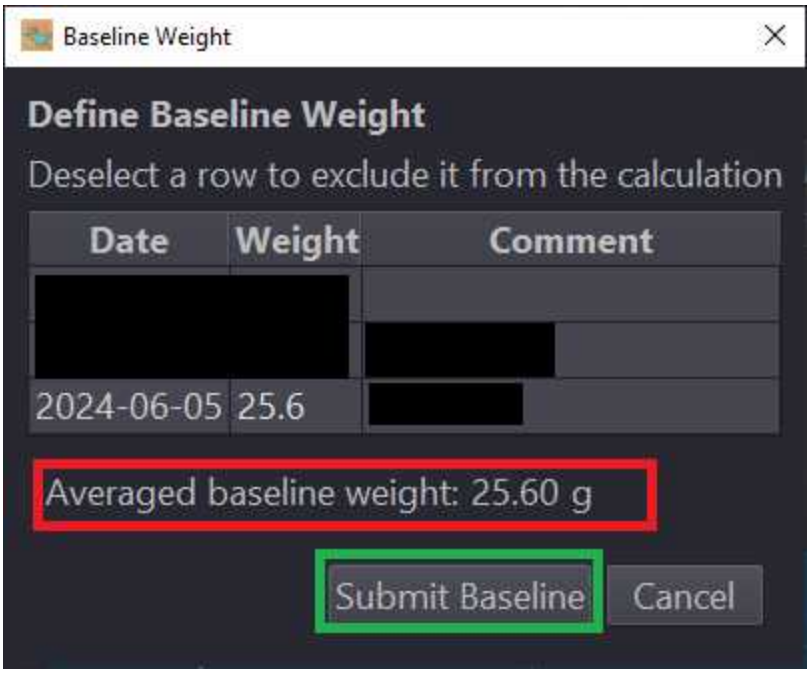
8.3 Place mouse on water restriction within waterlog.



Waterlog water section
Water restriction button outlined in green.

8.4 Click the "Water Restrict and Define Baseline Weight" button (outlined in green above).

8.5 Confirm that the calculated baseline weight appears to be correct (average weight over 3 days).



Calculated baseline weight outlined in red.
Submit baseline button outlined in green.

8.6 Confirm mouse ID, sex, pedigree and protocol between mouse horizontal cage card, "links" dropdown menu in Waterlog, and personal schedule.
Confirm baseline weight, target ratio, and target weight calculations in Mouse Details section of Waterlog page.

Waterlog main page with loaded mouse ID (water restricted).
Weight entry textbox outlined in top green.
Session comment textbox outlined in bottom green.
Earned water reward outlined in top red.
Water delivered checkbox outlined in bottom red.

Note

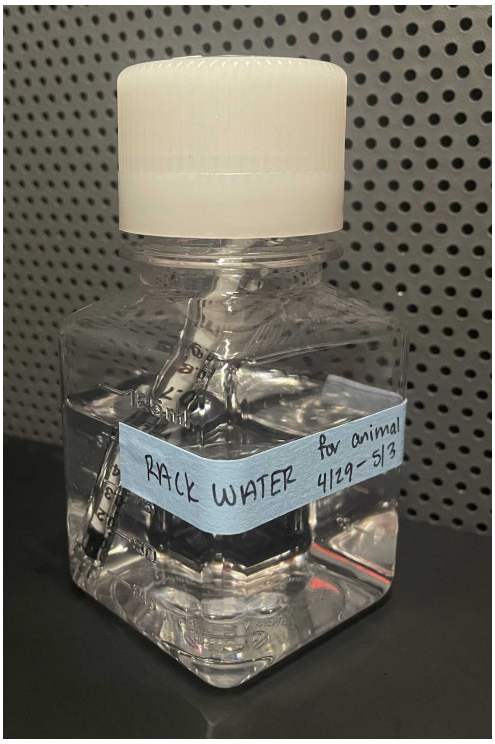
It is common for water restriction of less than 85% of body weight to negatively impact the mouse's health.
Follow all IACUC and veterinary requirements.

8.7 Within the AHR, locate the hose for the external cage rack water supply. This is typically behind the cage racks.

Note

"Rack water" has gone through reverse osmosis. Do not use deionized or non-potable water.

8.8 Prepare a bottle with rack water and a 1mL syringe.

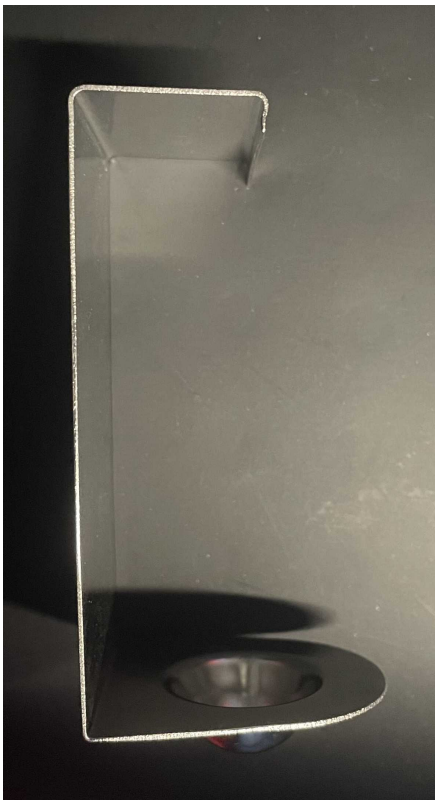


Bottle filled with rack water and a 1mL syringe.

Note

Write the date of making, date of expiration, and your initials on the bottle. Follow all IACUC and veterinary requirements.

8.9 Obtain a water dish.



Side view of water dish.

9 If Waterlog is not accessible, manually calculate the mouse's baseline and target weight.

9.1 Calculate the average weight of the mouse with a minimum of three recently collected weights. This is your baseline weight.

9.2 Multiply your baseline weight by the desired water restriction percentage (as specified by your IACUC protocol).

For example:

- Mouse has a baseline weight of 25.00 grams.
- Desired water restriction at 85% of weight.
- 25.00 grams x 0.85 = 21.25 grams.
- Maintain mouse at target weight of 21.25 grams.

Note

It is common for water restriction of less than 85% of body weight to negatively impact the mouse's health.
Follow all IACUC and veterinary requirements.

10 Provide mouse with suggested water amount.

Suggested supplemental water amount in top red outline.
Water delivered checkbox outlined in bottom red.
Submit button outlined in green.

10.1 Using the water bottle with syringe, pull up 1.0mL of water and provide to mouse via water dish in cage.

Note

If using water reward bottle and syringe for multiple mice, avoid touching mouse, dish, or other possible contaminates/excrement in cage.

Note

Mice are typically provided 1mL of water on their first day to begin lowering their weight down to target weight.

Note

Mice should receive 1mL of water a day at minimum.
Follow all IACUC and veterinary requirements.

10.2 Select the "delivered" checkbox and click the "submit" button to the right to submit the mouses watering information for the day.

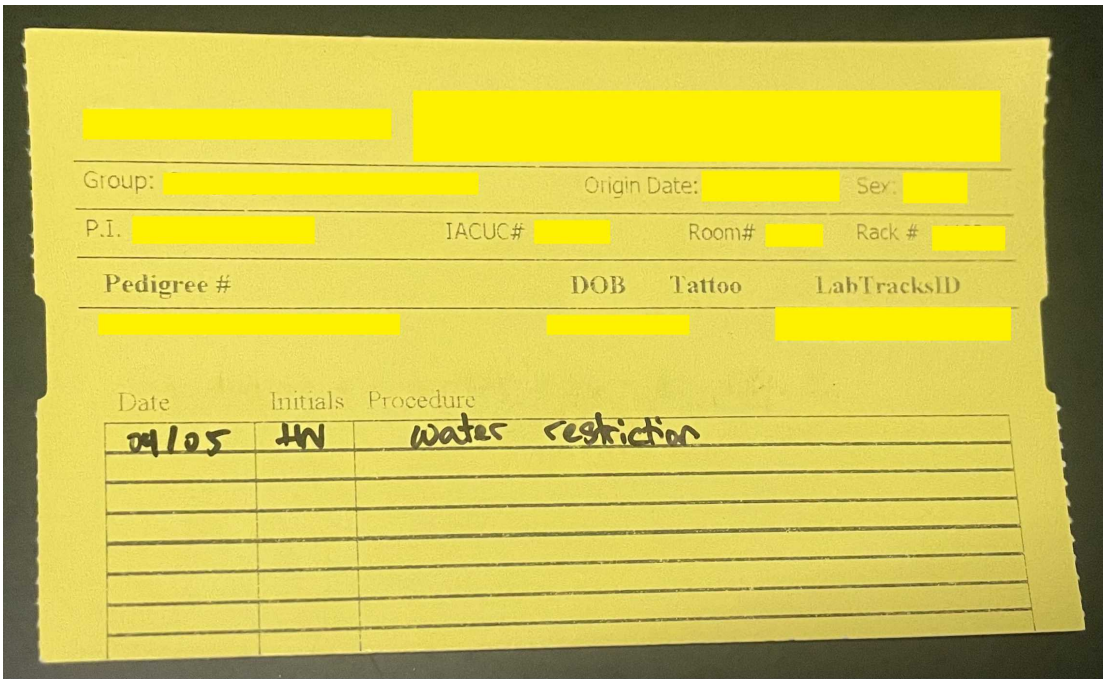
11 Record necessary information on physical cards on mouse cage.

11.1 Record mouse information on on vertical water restriction card including:
- Trainer name
- Watering time (AM or PM)
- Mouse ID
- Workflow
- Baseline weight

Record daily mouse watering information including:
- Date
- Time
- Total water provided
- Initials

Recording information on water restriction card (in red).

11.2 Record that animal has been placed on water restriction on horizontal animal cage card. Include your initials and the date placed on water restriction.



Horizontal cage card.

12 Return mouse to water restriction rack (racks with detached water valves) in AHR.

Maintaining a Mouse on Water Restriction

13 For each day the mouse is on water restriction, the mouse will need to be given their calculated water atollment each day.

14 Retrieve mouse cage from AHR. Confirm mouse ID on all cage cards.

15 Open Waterlog and access mouse record by repeating go to step #1 through step #5

16 Confirm mouse ID, sex, pedigree and protocol between mouse horizontal cage card, "links" dropdown menu, and personal schedule.
Confirm baseline weight, target ratio, and target weight calculations in Mouse Details section of Waterlog

Waterlog main page with loaded mouse ID (water restricted).
Weight entry textbox outlined in top green.
Session comment textbox outlined in bottom green.
Earned water reward outlined in top red.
Water delivered checkbox outlined in bottom red.

17 Weigh mouse for each day on water restriction to maintain mouse at target weight.

Note

Weigh mouse after any behavior or experimentation.

17.1 Scoop/cup mouse out of cage.

Note

If mouse cage has a tube or other container-shaped enrichment, use as vessel for transportation. This mode of transportation/handling can be less stressful for the mouse.

17.2 Check mouse for correct ID (ear notches and/or tattoo). Check mouse for any other wellness concerns (headframe, prolapse, wounds, etc.).

17.3 Place mouse in weigh container and place on scale. Note the weight.

17.4 Return mouse back to cage.

Note

Use weigh container to return mouse to cage.

17.5 Clean weigh container with 70% Ethanol spray after each use. This is to prevent any additional stress on mice caused by previous mice excrement.

Note

Dry weigh boat with Kimwipes sufficiently after spraying with Ethanol. Ethanol fumes can cause mouse to suffocate.

17.6 Record mouse weight in weight textbox of Waterlog.

18 Enter water amount received prior to weighing in "already earned" textbox.

19 Provide mouse with [now] calculated water allotment as written in "suggested" textbox.

Note

Mice should receive 1mL of water a day at minimum.
Follow all IACUC and veterinary requirements.

20 If Waterlog is not accessible, manually calculate the mouse's water allotment.

20.1 Water allotment calculation (no previous water rewards):
- Mouse weight is 20.00 grams.
- Baseline weight is 21.25 grams.
- 21.25 grams - 20.00 grams = 1.25 grams.
- 1.0mL = 1.0 grams.
- Provide mouse 1.25 mL of water.

Water allotment calculation (previous water rewards):
- Mouse weight is 20.00 grams.

- Baseline weight is 21.25 grams.
- Mouse received 0.75mL of water reward.
- 21.25 grams - 20.00 grams = 1.25 grams.
- 1.0mL = 1.0 grams.
- 1.25 mL - 0.75mL = 0.5mL.
- Provide mouse 0.5 mL of water.

Note

1 milliliter of water equals 1 gram because the density of water is 1g/mL.

Note

Mice should receive 1mL of water a day at minimum.
Follow all IACUC and veterinary requirements.

21 Record mouse watering information on vertical water restriction card including:
- Date
- Time
- Total water provided
- Initials

22 Return mouse to water restriction rack (racks with detached water valves) in AHR.

Taking a Mouse off Water Restriction

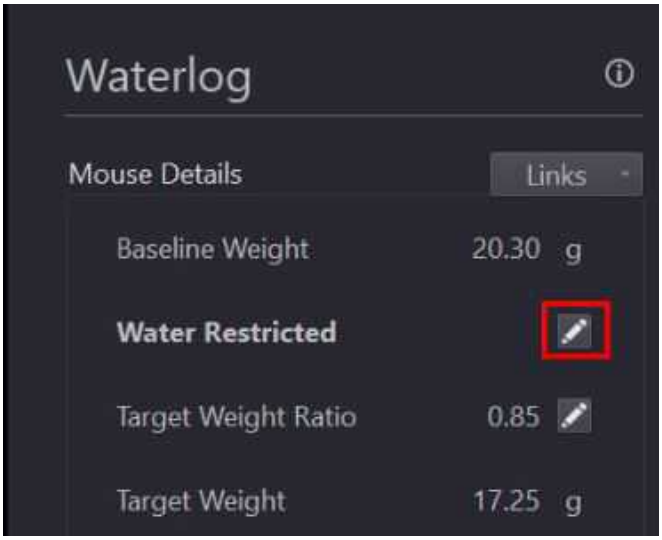
23 Repeat section "Maintaining a Mouse on Water Restriction". Specifically
go to step #14 step #21 .

Note

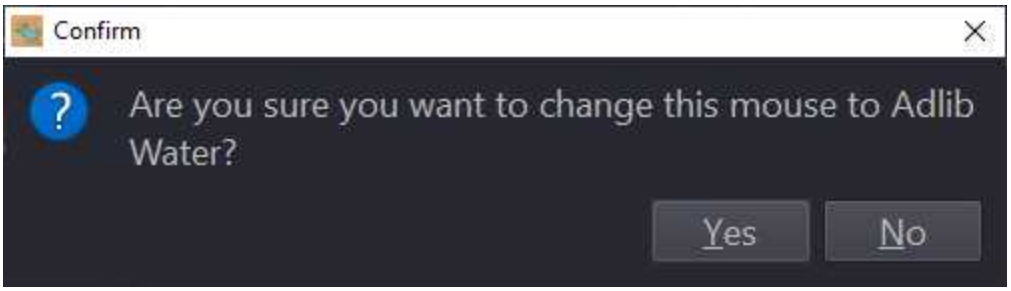
Taking a mouse off of water restriction is typically done on the last day of behavioral training/experimentation, provided it is under standard/non-urgent procedures.
Follow all IACUC and veterinary requirements.

24 Remove mouse from water restriction in Waterlog.

24.1 Click the edit/pencil icon button to remove mouse from water restriction. Confirm this change within the popup window.



Waterlog Mouse Details section.
Water restriction editing button outlined in red.



Confirmation popup window for moving a mouse back to Adlib water.

25 Confirm that mouse is to be removed from water restriction.

26 Allow the mouse to drink it's alloted water amount, and then remove water dish from cage.

Note

This ensures that that mouse has received it's daily water and is given some time to remember about the lickspouts on the non-water restriction rack.

27 Record on horizontal animal cage card that animal has been placed on adlib water. Include the date.

28 Retrieve the "placed back on adlib" cage flag and place on cage over cage cards. Write the date.

29 Place mouse on non-water restriction rack with water spouts and/or attach a filled water bottle to cage.

29.1 Toggle water spout prior to placing mouse on rack to confirm that water spout is delivering water.

30 Place used water dish in dirty bin of AHR.

Protocol references

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