IACUC Guideline
EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS
& OTHER DEMYELINATING MOUSE DISEASE MODELS

Introduction
The University of Pennsylvania’s Institutional Animal Care and Use Committee (IACUC) and the Attending Veterinarian (ULAR) are charged with ensuring that all research animals are provided with adequate veterinary care. This guideline is designed to provide information for investigators using rodent models of experimental autoimmune encephalomyelitis (EAE) and other demyelinating models.

Background
• EAE is one of the oldest and most frequently used model systems for studying multiple sclerosis (MS) in laboratory animals, in which immunization against CNS-specific antigen results in central nervous system inflammation.
• These models may result in a complex spectrum of acute, chronic and relapsing-remitting disease courses that most often result in varying degrees of progressive ascending paralysis.
• Due to the extreme variability in the onset and progression of clinical signs and disease course, close monitoring and provision of supportive care are necessary for EAE animals.
• If signs of EAE are expected to be unusual in your model, please discuss this with a ULAR veterinarian.

Clinical Assessment and Care of Affected Mice
1) EAE Scoring
• Clinical signs and ascending paralysis in EAE are commonly assessed on a six-stage scale of 0 – 5 (see below and Table 1 on pg. 4), with “0” being clinically normal and “4” being paralysis of all limbs (quadriplegia). The scoring results should be documented in the monitoring logbook by laboratory members as described below.

  0 - Clinically normal
  1 - Decreased tail tone or weak tail only (up to tail paralysis)
  2 - Hind limb weakness (paraparesis)
  3 - Hind limb paralysis (paraplegia) and/or urinary incontinence
  4 - Complete paralysis (tetraplegia) of hind limbs and front limbs
  5 - Moribund

• Other scoring systems may be preferable for certain types of research, and may be used as long as they are clearly defined in the approved protocol and made available in close proximity to the animal room where it can be readily viewed by the Animal Care staff.

2. Body Condition Scoring
• Body condition score (BCS) should also be used by ranking an animal with BCS of “1” for an emaciated appearance, and up to “5” for an extremely obese animal. Body condition scores should be documented in the monitoring logbook as described below.

  BCS of “3” is assigned to a clinically normal animal of appropriate body weight.

  Please see BCS diagram (Figure 1) of this document.
3. Recordkeeping

- All daily monitoring, assessments, scoring, weights, supportive care and treatments must be recorded in a monitoring logbook/record maintained by the laboratory.
- Notations in the record must include name of observer, date and time of entry, and pertinent information related to EAE scoring and BCS observations.
- If alternative scoring systems or deviations from these guidelines are approved by the IACUC, they should be included in the notebook.
- The log/record for experimental animals should be kept in the animal room (or another area accessible to ULAR veterinary staff). Please contact the ULAR veterinary staff to establish the site for placement of the logbook.
- An example EAE logbook sheet can be viewed as an editable attachment (EAE Log.docx)

4. Animal Care

- At the time of inoculation, label each cage card with “EAE”, inoculation date, and name of contact with relevant telephone numbers (if different than “contact” listed on cage card). This should be a 24 hour active emergency contact number (cell phone number preferred).
  - Inform the facility manager and ULAR veterinarian about the presence and initiation of experiments to induce any EAE in mice.
  - Modify aspects of the cage to ensure comfort for the animals as the EAE disease course progresses.
    - Complete “Special Requirements Card” for alpha-dri bedding; this softer bedding may decrease skin trauma secondary to paralysis.
    - Apply a ‘No nestlet’ opt-out sticker (provided within the facilities) to the cage card. (Nestlet fibers may entrap and strangulate weak limbs/tails).
    - Other enrichment substrates are required instead of Nestlet material. Work with the Facility Manager for other enrichment options.
  - When clinical signs are expected to initiate, lab staff should monitor mice at least daily, including weekends and holidays.
  - Training on assessment, monitoring and supportive care is available through ULAR trainers or veterinary staff upon request.

- When initial clinical signs are noted (flaccid tail or hind limb weakness, EAE Score 1-2):
  - Separate affected animals to another cage, so that they are not injured by unaffected animals; affected animals may be housed with similarly affected animals (preferred), or singly housed. Please submit an exemption from social housing request to IACUC for mice exhibiting clinical signs of EAE. Social housing will be considered the default state until clinical signs occur.
  - Long sipper tubes should be requested from the Facility Manager to place onto water bottles.
  - If the cage has been reported by the Animal Care Staff, all treatments and monitoring performed by the laboratory must be recorded in the Sick Animal Logbook.
  - Place gel substrate for fluid supplementation (e.g., HydroGel® or NapaNectar™) on the floor of the cage. Replenish as needed or at least each Monday, Wednesday, Friday.
  - Place pelleted rodent chow from the food hopper onto the floor of the cage.
  - Animals in the initial stages of disease must be weighed and have BCS determined at least twice weekly. If BCS is 2 or less, or if animal has lost over 10% of its baseline weight, or has a prolonged skin tent over the dorsum, the laboratory should initiate the following:
    - Add gel or dough diet (eg DietGel 76A®, Bacon Softies™, NutraGel™, Transgenic Dough Diet TM) to the floor of the cage each Monday, Wednesday, Friday.
Give 1 ml sterile 0.9% saline administered subcutaneously once daily.

- When hind limb paralysis or urinary incontinence (EAE score 3) is noted, the laboratory should:
  - Gently palpate bladder each day. Gently press on the caudal abdomen to assist urinating.
  - If animal is not able to urinate on own (likely due to atonic bladder), express bladder twice daily.
  - Monitor for dermatitis, urine scald, penile prolapse (if male) and tail lesions. Alert ULAR veterinary staff if any of these or other clinical abnormalities are observed.
  - Continue to weigh animals and assess BCS at least twice weekly.
  - Continue treatment with gel/dough diet and sterile saline as outlined above in #3.

5. Euthanasia:

- When an animal becomes quadriplegic (EAE Score 4; paralyzed in all 4 limbs), it must be euthanized by the end of the workday, unless the approved IACUC protocol states otherwise.
- When an animal has lost a maximum of 20% of its body weight from baseline levels, or has a BCS of 1, it must be euthanized by the end of the day.
- Animals that are moribund (EAE Score 5), have increased respiratory effort, or are quadriplegic and dull to obtunded, must be euthanized immediately.
- If other problems arise, please consult with the ULAR veterinarian. If the ULAR veterinarian mandates euthanasia for humane reasons, the animal must be euthanized.

References:


Figure 1: Body Condition Scoring (BCS) chart for mice

<table>
<thead>
<tr>
<th>BCS</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mouse is emaciated.</td>
</tr>
<tr>
<td></td>
<td>- Skeletal structure extremely prominent;</td>
</tr>
<tr>
<td></td>
<td>little or no flesh cover.</td>
</tr>
<tr>
<td></td>
<td>- Vertebrae distinctly segmented.</td>
</tr>
<tr>
<td>2</td>
<td>Mouse is underconditioned.</td>
</tr>
<tr>
<td></td>
<td>- Segmentation of vertebral column evident.</td>
</tr>
<tr>
<td></td>
<td>- Dorsal pelvic bones are readily palpable.</td>
</tr>
<tr>
<td>3</td>
<td>Mouse is well-conditioned.</td>
</tr>
<tr>
<td></td>
<td>- Vertebrae and dorsal pelvis not prominent;</td>
</tr>
<tr>
<td></td>
<td>palpable with slight pressure.</td>
</tr>
<tr>
<td>4</td>
<td>Mouse is overconditioned.</td>
</tr>
<tr>
<td></td>
<td>- Spine is a continuous column.</td>
</tr>
<tr>
<td></td>
<td>- Vertebrae palpable only with firm pressure.</td>
</tr>
<tr>
<td>5</td>
<td>Mouse is obese.</td>
</tr>
<tr>
<td></td>
<td>- Mouse is smooth and bulky.</td>
</tr>
<tr>
<td></td>
<td>- Bone structure disappears under flesh and</td>
</tr>
<tr>
<td></td>
<td>subcutaneous fat.</td>
</tr>
</tbody>
</table>

A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e., ...2+, 2, 2...).
# Table 1: EAE CLINICAL SIGNS AND CARE

<table>
<thead>
<tr>
<th>SCORE/STAGE</th>
<th>CLINICAL SIGNS</th>
<th>CARE TO BE PROVIDED</th>
</tr>
</thead>
</table>
| 0           | Clinically normal | - Baseline weight or body score prior to induction  
- Clearly mark “EAE Model” on each cage card, initiate EAE score card or sheet, and contact ULAR veterinarian and facility manager.  
- Place “No Nestlet” sticker on cage-card, and discuss alternative enrichment strategy with facility manager.  
- Complete Special Requirements card for Alpha-dri bedding. |
| 1-2         | Weak tail and/or hind limbs (paresis) | - Separate affected animals to another cage.  
- Provide long sipper tubes.  
- Place fluid gel (eg HydroGel®) on the floor of the cage. Replenish as needed or at least every Monday, Wednesday, Friday.  
- Provide food pellets on floor of cage.  
- Twice weekly, weigh animal and determine BCS. If BCS is 2 or less, or if animal has lost over 10% of its baseline weight, add gel or dough diet (eg DietGel 76A®, Transgenic Dough Diet™, Bacon Softies™) to the floor of the cage each Monday, Wednesday and Friday. In addition, give 1 ml sterile 0.9% saline subcutaneously once daily until weight or BCS returns to ~3 or clinically normal.  
- If BCS is 1 or animal has lost 20% of its body weight, it should be euthanized by the end of the day. |
| 3           | Paralyzed hind limbs | - Continue above care.  
- Palpate bladder at least once daily. Gently press on the caudal abdomen to assist urinating. If atonic (very large) bladder occurs, express bladder twice daily.  
- Monitor for dermatitis, tail lesions, urine scald, and penile prolapsed (if male). Alert ULAR veterinary staff if seen. |
| 4           | Paralyzed front and hind limbs | - Quadriplegic mice must be euthanized by the end of the day, unless the approved IACUC protocol states otherwise. |
| 5           | Recumbent; +/- abnormal breathing | - These animals must be euthanized immediately. |