

IACUC Guideline on Use of Experimental Autoimmune Encephalomyelitis Rodent Models

EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS & OTHER DEMYELINATING RODENT DISEASE MODELS

Animal Welfare Regulation 2.33 (b) (5): Each research facility shall establish and maintain programs of adequate veterinary care that includes: (5) Adequate pre procedural and post procedural care in accordance with current established veterinary medical and nursing procedures.

This guideline is designed to provide a single source of information for investigators that use rodent models of experimental autoimmune encephalomyelitis (EAE) and related demyelinating diseases. EAE is a demyelinating disease of the central nervous system (CNS) that serves as a model of CNS autoimmune disease, most often human Multiple Sclerosis. EAE can be induced by administering combinations of CNS components (cells and/or peptides) and Complete Freund's Adjuvant (CFA), often with pertussis toxin or viral/pathogen inoculation. These combinations 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. The following guidelines have been universally accepted by investigators using the rodent EAE model.

This guideline offers direction on the following topics:

- Assessment of affected animals
- Scoring system to provide objectivity to evaluations
- Animal care provisions and notification of veterinary staff
- Recordkeeping

ASSESSMENT

Many investigators use scoring systems for research purposes; often with subtle differences. This set of guidelines focuses on CLINICAL SIGNS & the attendant, necessary CARE. It is important to define levels of care to be provided by the clinical signs in your IACUC animal-use protocol, not necessarily a number. If lab-specific scoring systems are used to assess the animals, clearly defined clinical signs must be assigned to each score (number) and made accessible to the IAS staff.

Common Clinical Signs of EAE

Sign	Description	Onset / Stage of Disease
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or compliance with said guidelines, please contact:**

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Tail Tone (SCORE = 1)	Decreased tail tone or a flaccid tail is often the first noticeable clinical sign in ascending paralysis.	Early
Hind Limb Paresis (SCORE = 2)	Weakness in the hind limbs. Animal is unable to grasp with hind limbs or may have difficulty to right itself if turned on its side or back. Animals may have difficulty moving or appear ataxic or clumsy.	Early
Hind Limb Paraplegia (SCORE=3)	Inability to move one or both hind limbs.	Middle
Urinary Incontinence	Urine leaking. Often in conjunction with atonic bladder. May be seen as wetness or urine scald (dermatitis) around prepuce or vulva.	Middle
Dehydration	Decreased skin turgor. When the skin at the scruff or nape of the neck is gently pinched, it returns very slowly or stays tented.	Middle to Late
Quadriparesis (SCORE=4)	Weakness of forelimbs and hindlimbs (paraparesis) Hind limbs may be paralyzed (paraplegia).	Middle to Late
Atonic Bladder	Urinary bladder is large and thin walled on palpation. More than 2-3 drops of urine expelled on palpation. Indicates inability to urinate voluntarily and thus needs manual expression.	Late
Quadriplegia (SCORE=5)	Animal develops inability to move hindlimbs and forelimbs. Paralysis of all limbs.	Late
Dyspnea	Abnormal or difficulty breathing. May be rapid, slow and deep or erratic.	End stage
Moribund	Animal is not moving. Often hunched or recumbent, dyspneic and sometimes gasping.	End stage

SCORING SYSTEM

Body Condition Score (BCS) should always be used to assess the general physical condition of the animals to effectively follow the progression of disease in the EAE rodent. Scoring systems from 1 (emaciated/wasted) to 5 (obese) are often used. BCSs may be a helpful adjunct to assessment of percentage loss of body weight and evaluating model related clinical signs for overall health.

EAE Scoring System SUMMARY:

Clinical signs and ascending paralysis in EAE are commonly assessed on a six stage scale of 0 – 5 (see below), with 0 being clinically normal and 5 being paralysis of all limbs (quadriplegia). Other scoring systems may be used as long as they are clearly defined in the protocol and made available in close proximity to the animal room.

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- 0 Clinically normal
- 1 Decreased tail tone or weak tail only
- 2 Hind limb weakness (paraparesis)
- 3 Hind limb paralysis (paraplegia) and/or urinary incontinence
- 4 Weakness of fore limbs with paraparesis or paraplegia (quadriparesis) and/or atonic bladder
- 5 Paralysis of all limbs (quadriplegia)

Please refer to the EAE Clinical Signs and Care chart, attached to this guideline.

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ANIMAL CARE AND NOTIFICATION OF VETERINARY STAFF

1. Animals must be weighed at the time of inoculation to determine baseline body weight which must be recorded in an accessible log/treatment book (see Recordkeeping below).
2. At the time of inoculation with a substance intended to elicit an EAE model, each cage must be labeled with the EAE phenotype (pink) card to clearly indicate it is an EAE Model.
This should be communicated to the facility manager and veterinary care staff to ensure that the animal's status and expected progression is known.
The EAE Model labeled cage card should include the inoculation date and name of contact with day/night telephone numbers (if not already listed as the day to day contact).
3. [Score = 1, 2- Flaccid tail, Hind Limb Weakness] When initial clinical signs are noticed (e.g. flaccid tail or paresis), water and food must be made more accessible.
Long sipper tubes are to be added to water bottles.
Gel products that provided water and/or nutrients should be placed on the cage floor daily and recorded in the lab notebook and on the pink cage card.
Semi solid or liquid nutritional supplements may also be placed on the cage floor in a shallow container, such as a petri dish.
Animals in the initial stages of disease must be weighed at least once weekly. Body condition scoring is appropriate.
4. [Score = 3 – Hind Limb Paralysis] Animals must be Checked Daily and at this point an EAE monitoring / score sheet/card must be initiated on the cage card and in the laboratory notebook.
With the onset of urinary incontinence or hind limb paraplegia (hind limb paralysis), the urinary bladder should be monitored three times per week by palpation.
To avoid trauma and ensure access to food and water, paraplegic animals must be separated to a cage with similarly affected animals (preferred) or be singly housed.
Animals must be monitored for lesions secondary to paraplegia such as dermatitis, urine scald, penile prolapse and tail lesions. "Alpha -dri"[®] bedding provides a softer surface and helps prevent or minimize some secondary lesions.
Paraplegic animals must be weighed at least twice weekly, and monitored daily in order to increase the likelihood of noticing quadriplegic or moribund animals as early as possible in the disease course.
Skin turgor should be checked. If dehydrated, 1 ml of sterile saline (or other approved fluid) must be given subcutaneously TWICE DAILY (minimum).
5. [Score = 4 - Fore-Limb Weakness & Hindlimb Paralysis] When weakness in the front limbs is noted with hind limb paralysis then the mice must be evaluated daily for body weight changes (or Body Condition Score); to determine that they can access food & water; that no urine scalding is present; and if the bladder requires manual expression. If an animal develops an atonic urinary bladder, the urinary bladder must be manually expressed at least twice daily. Contact the IAS veterinary staff for assistance with this medical procedure, if needed

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6. [Score = 5] Animals are considered quadriplegic when the hindlimbs are paralyzed and the front legs have become so weak or paralysed that the animals are unable to pull themselves to food and/or water supplies in the cage; or they are unable to right themselves after being placed on their side. When animals progress to quadriplegia, they must be euthanized by the end of that day unless specifically documented and approved by the IACUC how the animals will be maintained in this state. Animals losing 20% body weight from their baseline must be euthanized unless exempted by scientific justification and IACUC approval.
7. Animals found in a moribund state must be euthanized immediately.
8. The assessment, care and documentation for EAE animals are the responsibility of the Principal Investigator. These responsibilities apply 7 days a week,
9. Training on assessment, monitoring and supportive care is available from the IAS veterinary staff upon request.

RECORD KEEPING

1. All daily monitoring, assessments, scoring, weights, supportive care and treatment must be recorded in a monitoring log maintained by the laboratory and accessible to the veterinary staff upon request. All monitoring parameters, conditions and treatments as stated in the IACUC protocol must be recorded in the laboratory notebook.
2. Daily monitoring activities must be recorded at EACH cage on the EAE phenotype “pink card” provided by IAS. Notations on this record must include name (initials), date and time of entry (A.M or P.M), and pertinent information.
3. If special amendments, scoring systems or deviations from these guidelines are approved by the IACUC, they must be included in the notebook.

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EAE CLINICAL SIGNS AND CARE

SCORE/STAGE	CLINICAL SIGNS	CARE TO BE PROVIDED
0	Clinically normal	Baseline weight or body score prior to induction Clearly mark cage with IAS-provided EAE Model "Pink Card"
	Weak tail	Initiate EAE score card or sheet Keep record in a monitoring log in or near animal room Include name, date, and comment for each entry
1	Weak hind limbs	Provide additional water source (e.g. long sipper tubes, gel product etc.) Provide moistened food pellets or nutrient gel on floor of cage Weigh animal weekly
	Paralyzed hind limbs	Monitor animals daily; weigh and record EAE score at least 2 times per week Palpate bladder at least 3 times per week. If bladder is large, it may be atonic, then the mouse becomes Stage 4 Monitor for skin lesions, urine scald, and penile prolapse Monitor for dehydration; provide sterile SQ fluids as needed. Special husbandry <u>considerations</u> include: Change to soft bedding (Alpha Dri) Separate affected mouse from stronger mice to prevent trampling if not already done.
2	Weak front limbs	Monitor animals daily and record EAE score
	Paralyzed front and hind limbs	If the animal develops an atonic urinary bladder, then express bladder twice daily. Contact IAS veterinary staff for assistance as needed. Special husbandry <u>requirements</u> include: Change to soft bedding (Alpha Dri). Separate affected mouse from stronger mice to prevent trampling (if not already done).
3	Weak front limbs	Monitor animals daily and record EAE score
	Paralyzed front and hind limbs	If the animal develops an atonic urinary bladder, then express bladder twice daily. Contact IAS veterinary staff for assistance as needed. Special husbandry <u>requirements</u> include: Change to soft bedding (Alpha Dri). Separate affected mouse from stronger mice to prevent trampling (if not already done).
4	Weak front limbs	Monitor animals daily and record EAE score
	Paralyzed front and hind limbs	If the animal develops an atonic urinary bladder, then express bladder twice daily. Contact IAS veterinary staff for assistance as needed. Special husbandry <u>requirements</u> include: Change to soft bedding (Alpha Dri). Separate affected mouse from stronger mice to prevent trampling (if not already done).
5	Weak front limbs	Monitor animals daily and record EAE score
	Paralyzed front and hind limbs	Mice exhibiting these signs must be euthanized. These animals may be maintained if scientific justification has been provided in an IACUC approved animal protocol only. Mice losing 20% of body weight must be euthanized.
Moribund	Recumbent; +/- abnormal breathing	These animals must be euthanized immediately.

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