



AEEC Guidelines Guidelines for Post-operative Management

Objective	To provide guidelines for post-operative management of animals
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Introduction

Post-operative mismanagement can cause significant suffering and impair the validity of research. It is a direct violation of AEEC policies and recommendations in The Guide for the Care and Use of Laboratory Animals, 8th edition. These guidelines provide sound principles to ensure proper post-operative management is applied.

All procedures and aspects of post-operative management must be clearly written in the AEEC protocol.

The principal investigator (PI) is responsible to designate a primary user to conduct surgery, monitor animals, provide timely intervention and implementation of humane endpoints and documentation according to the guideline described below.

The possible sources of pain and distress before, during and after procedures should be considered and opportunities for refinement should be identified to enhance animal welfare. Any procedure that could potentially cause pain or stress to humans is considered to have similar perceptions in animals.

Post-operative care should be adequately provided to the animals under the following conditions:

- the post-procedure period when the animal is still under general anaesthesia,
- the recovery period when the animal return to the home cage,
- before the sutures are removed, or
- the surgical wound has healed completely.

Guidelines

1. Recovery from Anesthesia


Most mortalities occur when recovering from anaesthesia. Close monitoring is required to ensure animal welfare. All animals should be returned to the holding facility when the animals regain full consciousness and no ataxia is noticed.

Environment	<ul style="list-style-type: none"> • A clean cage should be used to prevent infections. • Place animals in a shaded, quiet area for recovery to minimize unnecessary stimulation. • Animals should be recovered in groups under direct monitoring. *separation of the animal may be required to reduce fighting if not under monitoring.
Temperature	<ul style="list-style-type: none"> • Hypothermia during general anaesthesia will cause delayed and poor recovery. • Warming devices such as heat lamps or heat pads are recommended throughout the entire general anaesthesia until the animal regain consciousness. The temperature of these devices should not exceed 40°C. The animal should not be in direct contact with the heating source. Close monitoring is required to prevent burns. • Shredded paper towels can also be provided for recovery in a cage.

2. Post-operative monitoring (until incision is healed)

The frequency of observing animals' well-being depends on the severity of the procedure and the expected rate of changes. It is recommended to attend to the treated animals at least once daily for the initial seven days or until the surgical site has healed completely, followed by a weekly check.

Animal monitoring should include both approaches outlined below:

Home cage assessment	
<p>The home cage should be examined from the exterior.</p> <ul style="list-style-type: none"> • Appetite and fluid intake should be observed and ideally be measured. • Bedding condition is a useful indicator of feed and water intake, which should also be assessed. With bedding materials (e.g. wooden chips) provided, animals generally defecate and urinate in a designated area in a cage, which should separate from the nesting area. Furthermore, if the bedding condition is uniform across the cage, this observation suggests that the animal is unable to move around properly. 	
Clinical Examination	
<p>Animals should be observed from outside the cage to identify any new pain-related abnormal behaviours or loss of normal behaviours. Attitude, body posture, behaviour, gait, and hair coat of the animals should be assessed. Intervention is required if the below signs are observed.</p>	
Signs of pain or distress	<ul style="list-style-type: none"> • Quiet or dull demeanor • Orbital tightening • Nose and cheek bulging • Change in eye and whisker position • Dull hair coat (spiky appearance) – indicating loss of grooming behavior • Abnormal gait – restrictive movement • Porphyrin staining – stress-induced red tears observed around the nose and eyelids • Respiratory distress – e.g., open mouth breathing, pronounced chest movement • Grimace Scale (Appendix I)
	 <p><i>Figure 1 Porphyrin staining</i></p>
Body weight and body condition assessment	<ul style="list-style-type: none"> • $\geq 20\%$ loss of body weight (Baseline weight should be obtained prior to surgery as reference) • Animals should be weighed regularly (at least once a week) and daily as humane end points are near. Weight records should be available for review when requested. • Body condition score (Appendix II).
Hydration status	<ul style="list-style-type: none"> • Animal being sluggish • Skin turgor • Eye clarity • Shape and position of the eye within the orbit • PCV (packed cell volume) (Appendix III)

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	<ul style="list-style-type: none"> • Water level on water bottles may indicate water intake • *Animals with restrictive movement should be monitored daily as they may have difficulties to access water
Physical examination	<p>Gentle and correct animal handling through cupping in the hands or use of a tunnel is advised to minimize stress. Systematic evaluation of the animal and examination of the surgical site for the following symptoms:</p> <ul style="list-style-type: none"> • wound • swelling • infection • bleeding or discharge • breakdown of sutures (commonly in 3-7 days post surgery) • heat or resentment on palpation <p>In case of open wounds, please refer to the following section on <u>wound closure (3a)</u>.</p>

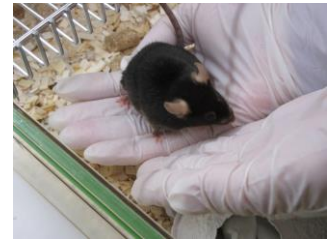


Figure 2 Cup handling

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3. Intervention

The type and timing of intervention required depend on the clinical presentation. The status of the animals can change rapidly; therefore, regular monitoring is essential. Animals may require one, a combination or all of the interventions or supportive measures detailed below.

a. Analgesia

The choice of analgesia, dosage, dosing frequency and duration depends on the anticipated level of pain, invasiveness and location of the surgery, and the drug properties (i.e., mechanism of action, the onset of action and duration of action).

- Common analgesic options include opioids (e.g., buprenorphine) and NSAIDs (e.g., meloxicam, carprofen). Combination of different types of analgesia may be required to provide adequate pain relief. The use of local anaesthetics (e.g., lidocaine, bupivacaine) should also be considered.
- Analgesia details should be clearly written in the AEEC protocol and strictly implemented.
- If animals continue to show signs of pain during analgesia dosing, extra analgesia may be needed. However, it is strongly advised to consult with LASEC veterinarian (39430307/vets@cuhk.edu.hk) to establish an appropriate analgesic protocol.

The veterinarian can support a decision to provide adjunct analgesia pending an amendment of the AEEC protocol.

Examples of analgesic recommendations based on type of procedure			
Invasiveness of Procedure	Example Procedure	Preferred Analgesic Approach	Duration
Minor	Simple skin incision	Local block Or Single dose of an analgesic	1-time dosing followed by monitoring and additional doses if unalleviated pain is noted.
Moderate	Incision with deeper tissue manipulation, but no body cavity involvement (e.g., a vascular cut down and placement of a catheter).	Multimodal analgesia Or Single analgesic given at appropriate intervals	Minimum of 48 hours analgesia followed by monitoring and additional doses if pain is noted.
Severe	Major body cavity manipulation (e.g., laparotomy, manipulation of bone or nerve).	Multimodal Where available: sustained or extended-release formulations	Minimum of 72 hours of analgesia followed by monitoring and additional doses if pain is noted.

Adapted from AALAS Learning Library.

b. Wound closure

Any open wound is likely to become infected and cause pain. Therefore, all sutures or wound breakdowns need to be addressed immediately. Infection and pain impact animal welfare and data quality.

Treatment of an open wound involves:

- Cleaning the area with anti-septic solution
- Wound closure with sutures or surgical staples with or without general anaesthesia
- Additional course of analgesia with or without antibiotics
- Use of head collar to prevent licking or scratching of the surgical site*

For infected and complicated wounds, you should consult LASEC veterinarians for guidance.

*Please consult LASEC veterinarian (vets@cuhk.edu.hk) regarding to the placement and care of head collars on animals

c. Fluid therapy

The cause of dehydration commonly reflects a reduction in water intake, increased loss, or both. Dehydration needs to be corrected promptly and delayed treatment or severe dehydration could lead to death.

- The recommended fluid choices are Lactated Ringers, 0.9% saline and glucose-saline (if an energy supplement is also required).
- The volume of fluid administered depends on the severity of dehydration, daily maintenance requirement and estimated ongoing loss.
 - 1-2ml for 25g mouse per day
 - 5-10ml for 250g rat per day
 - 30-50ml for 3kg rabbit per day
 - 30-50ml for 2kg ferret per day
- The route of administration is orally (once or twice daily) or via subcutaneous injection (glucose-saline must be given orally or intravenously).
- The fluid should be warmed to body temperature before administration to prevent rapid loss in body temperature.
- For long surgical periods or periods of extended food restriction, intra-operative fluids should also be considered.
- The use of long drinking nipples or placement of watergel (e.g. HydroGel®) into the cage allows easier access to the water by the animals with **restrictive movement**.

d. Nutritional support

Adequate nutritional support enhances recovery and is important for post-operative care to prevent complications.

- High-protein and high-fat diet is recommended (Please contact LASEC staff if require any)

- Peanut butter
- Baby rice cereal
- High protein drinks such as Ensure
- Chocolate milk
- High calorie dietary supplements such as DietGel®Boost
- Alternatively, you may soften the feed with water and put it directly into the cage. This increases the digestibility of the feed, allows easier access to the feed and lessens the chewing efforts required.
- Additional nutritional support for animals with restrictive movement should be considered to promote recovery.



Figure 3 Soften feed placed in cage

4. **Record Keeping**

Clear and detailed record keeping is critical for animal monitoring between individual users and allowing the principal investigator, LASEC staff and veterinarians to oversee the projects. Each cage of animals that underwent surgery should have BOTH an Animal Holding Card (cage card) and a Post-Operation Care Card displayed. The Post-Operation Care Card should be displayed until the animals have been euthanized. The following details should be included in each Post-Operation Care Card (Refer to **Appendix IV** as example):


- Basic cage card information (e.g., name of PI and user, AEEC number, contact number)
- Operation or procedure name
- Site or location of the surgery
- The date of surgery performed
- Details of the drug given include the drug name, frequency, and duration. It should be clearly signed off by the user in charge every time a medication is administered to the animals.
- Any remarks (e.g., abnormalities of the animals, sutures, or staples removal date, etc.)

Users are also responsible to keep their own record of animal body weight and other monitoring items as stated in their application. The AEEC and veterinarians may request to review such records when necessary.

References

- National Centre for the Replacement Refinement & Reduction of Animals in Research (2021) Grimace scales
- Quesenberry K.E. and Carpenter J.W. (2012) Ferrets, Rabbits, and Rodents: Clinical Medicine and Surgery
- Flecknell P. (2016) Laboratory Animal Anaesthesia

Appendix I Grimace Scale



**NC
3Rs**
















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The Mouse Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in mice.

The specific facial action units shown below have been used to generate the Mouse Grimace Scale. These action units increase in intensity in response to post-procedural pain and can be used as part of a clinical assessment.

The action units should only be used in awake animals. Each animal should be observed for a short period of time to avoid scoring brief changes in facial expression that are unrelated to the animal's welfare.

	Not present "0"	Moderately present "1"	Obviously present "2"
Orbital tightening <ul style="list-style-type: none"> • Closing of the eyelid (narrowing of orbital area) • A wrinkle may be visible around the eye 			
Nose bulge <ul style="list-style-type: none"> • Bulging on the bridge of the nose • Vertical wrinkles on the side of the nose 			
Cheek bulge <ul style="list-style-type: none"> • Bulging of the cheeks 			
Ear position <ul style="list-style-type: none"> • Ears rotate outwards and/or backwards, away from the face • Ears may fold to form a 'pointed' shape • Space between the ears increases 			
Whisker change <ul style="list-style-type: none"> • Whiskers are either pulled back against the cheek, or pulled forward to 'stand on end' • Whiskers may clump together • Whiskers lose their natural 'downward' curve 			

National Centre for the Replacement Refinement & Reduction of Animals in Research (2021) Grimace scales, [NC3Rs Mouse Grimace Scale Poster \(EN\).pdf](#).




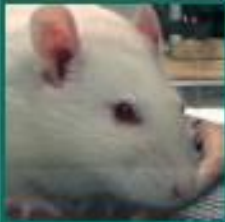






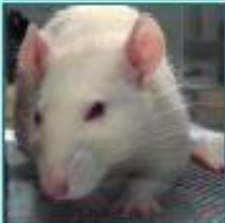

NC 3R^s National Centre for the Replacement Refinement & Reduction of Animals in Research

The Rat Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in rats.

The specific facial action units shown below have been used to generate the Rat Grimace Scale. These action units increase in intensity in response to post-procedural pain and can be used as part of a clinical assessment.

The action units should only be used in awake animals. Each animal should be observed for a short period of time to avoid scoring brief changes in facial expression that are unrelated to the animal's welfare.

	Not present "0"	Moderately present "1"	Obviously present "2"
Orbital tightening <ul style="list-style-type: none"> Closing of the eyelid (narrowing of orbital area) A wrinkle may be visible around the eye 			
Nose/cheek flattening <ul style="list-style-type: none"> Flattening and elongation of the bridge of the nose Flattening of the cheeks (potentially sunken look) 			
Ear changes <ul style="list-style-type: none"> Ears curl inward and are angled forward to form a 'pointed' shape Space between the ears increases 			
Whisker change <ul style="list-style-type: none"> Whiskers stiffen and angle along the face Whiskers may 'clump' together Whiskers lose their natural 'downward' curve 			

Read the original paper:
Bocsi AI, Boggs RE, Zakaria A, Tuttle AH, Martin LJ, Westcott JS, Meppelbeck JCS, Wei P, Zhan S, Zhang S, McDougall JJ, King OD, Magil JS. 2011. The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Molecular Pain* 7:55. doi:10.1186/1745-0213-7-58

For guidance on using the Rat Grimace Scale, research papers that describe this technique and for grimace scales in other species, visit: www.nc3rs.org.uk/rat-grimace-scale. To request copies of this poster, please email: enquiries@nc3rs.org.uk. The NC3Rs provides a range of 3Rs resources at www.nc3rs.org.uk/resources. Images kindly provided by Dr Jeffrey Magil, Northumbria University.

National Centre for the Replacement Refinement & Reduction of Animals in Research (2021) Grimace scales, [NC3Rs Rat Grimace Scale Poster \(EN\).pdf](#).

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The Rabbit Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in rabbits.

The specific facial action units shown below comprise the Rabbit Grimace Scale. These action units increase in intensity in response to post-procedural pain and can form part of a clinical assessment alongside other validated indices of pain.

The action units should only be used in awake animals. Each animal should be observed for a short period of time to avoid scoring brief changes in facial expression that are unrelated to the animal's welfare.

	Action units		
	Not present "0"	Moderately present "1"	Obviously present "2"
Orbital tightening <ul style="list-style-type: none"> Closing of the eyelid (narrowing of orbital area) A wrinkle may be visible around the eye 			
Cheek flattening <ul style="list-style-type: none"> Flattening of the cheeks. When 'obviously present', cheeks have a sunken look. The face becomes more angular and less rounded 			
Nostril shape <ul style="list-style-type: none"> Nostrils (nares) are drawn vertically forming a 'V' rather than 'U' shape Nose tip is moved down towards the chin 			
Whisker shape and position <ul style="list-style-type: none"> Whiskers are pushed away from the face to 'stand on end' Whiskers stiffen and lose their natural, downward curve Whiskers increasingly point in the same direction. When 'obviously present', whiskers move downwards 			
Ear shape and position <ul style="list-style-type: none"> Ears become more tightly folded / curled (more cylindrical) in shape Ears rotate from facing towards the source of sound to facing towards the hindquarters Ears may be held closer to the back or sides of the body 			

Read the original paper: Keating SCJ, Thomas AA, Flecknell PA, Leach MC (2012) Evaluation of EMLA cream for preventing pain during tattooing of rabbits: Changes in physiological, behavioural and facial expression responses. PLOS ONE 7(9): e44437. doi:10.1371/journal.pone.0044437

For guidance on using the Rabbit Grimace Scale, additional images of each action unit, research papers that underpin this technique, and for grimace scales in other species, visit: www.nc3rs.org.uk/grimacescales

To request copies of this poster, please email: enquiries@nc3rs.org.uk
The NC3Rs provides a range of 3Rs resources at www.nc3rs.org.uk/resources

Images kindly provided by Dr Matthew Leach, Newcastle University

The Rabbit Grimace Scale would not have been developed without the continuing work of the Pain and Animal Welfare Sciences Group (PAWS) at Newcastle University

National Centre for the Replacement Refinement & Reduction of Animals in Research (2021) Grimace scales, [Grimace scale: Rabbit | NC3Rs](#).

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Appendix II. Body Condition Score



BC 1

Mouse is emaciated.

- *Skeletal structure extremely prominent; little or no flesh cover.*
- *Vertebrae distinctly segmented.*



BC 2

Mouse is underconditioned.

- *Segmentation of vertebral column evident.*
- *Dorsal pelvic bones are readily palpable.*



BC 3

Mouse is well-conditioned.

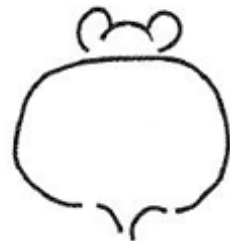
- *Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.*



BC 4

Mouse is overconditioned.

- *Spine is a continuous column.*
- *Vertebrae palpable only with firm pressure.*



BC 5

Mouse is obese.

- *Mouse is smooth and bulky.*
- *Bone structure disappears under flesh and subcutaneous fat.*

A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e. ...2+, 2, 2-...)

Ullman-Cullere et al. (1999) Body Condition Scoring: A rapid and accurate method for assessing health status in mice. *Laboratory Animal Science* 49(3):319-23

Appendix III Reference Range for Hematologic Values in Common Laboratory Species

Value	Mice	Rats	Rabbits
Hematocrit (%)	34-50	33.0-47.0	33-50
Hemoglobin (g/dL)	12.8-16.1	11.2-15.9	10.0-17.4
Red blood cells (x10 ⁶ /μL)	7.5-9.7	6.4-8.2	5.1-7.9
White blood cells (x10 ³ /μL)	4.5-9.1	4.7-9.4	5.2-12.5
Neutrophils (%)	21-57	7.0-32.0	20-75
Lymphocytes (%)	49-82	57.0-91.0	30-85
Monocytes (%)	2-8	2.0-5.0	1-4
Eosinophils (%)	0-3	0-4.0	1-4
Basophils (%)	0-3	0-3.0	1-7
Platelets (10 ³ /μL)	421-733	411-626	250-650

Quesenberry K.E. and Carpenter J.W. (2012) Ferrets, Rabbits, and Rodents: Clinical Medicine and Surgery, pp. 177, 346 & 349.

Appendix IV Post-Operative Card

Post-Operation Care 手術後護理							
Principal Investigator: Prof. Jacky Chan				AEEC No.: 14/000/MIS-5-C			
User: Jon Li				Tel.: Your mobile number			
Operation 手術名稱: OVX				Site 部位: Dorsal (L & R)			
Date 手術日期: 01/12/2021				Stitches Removal Date 拆線日期: 15/12/2021			
Observation / Treatment							
Date 日期:		1/12	2/12	3/12			
Drug 藥物	Buprenorphine	√ √	√ √	√ √			
Sign 簽名	Researcher	JL	JL	JL			
	LASEC's Staff						
Remarks:							