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INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

PROTOCOL REVIEW PROCESS

GENERAL POLICY

This policy applies to any vertebrate animal used for research, teaching, or testing, including studies that do not involve a formal grant proposal or are pilot studies.

In accordance with federal regulations and hospital policies, all animal research conducted at, or funded through, the Massachusetts General Hospital (MGH) and the Shriners Hospital for Children-Boston (SHC) must be reviewed and approved by the MGH Institutional Animal Care and Use Committee (IACUC) prior to animal procurement and initiation of the study. The IACUC has the sole authority to approve, require modifications (to secure approval), or withhold approval of research protocols involving the use of animals at MGH and SHC. Protocols are approved for a maximum of three years.

When animal research will be performed in its entirety under the institutional auspices of the MGH or SHC, an IACUC application must be reviewed and approved by the MGH IACUC.

> When the animal research will be performed under the auspices of multiple institutions including the MGH or SHC, the investigator will need to submit an IACUC application, and copies of any relevant outside IACUC approvals. MGH collaborates with institutions that hold an Animal Welfare Assurance (AWA) number (or equivalent, as determined by the IACUC) and USDA registration number, if applicable. AAALAC accreditation is preferred. If the IACUC approves the collaboration, it will oversee only the research component that is performed at MGH or SHC. Any component conducted at an outside institution will be done so under the auspices of that institution's IACUC.

When the animal research will be performed in its entirety under the auspices of an outside institution but the work will be funded in part or whole by or through the MGH or SHC, the MGH IACUC will fully rely on the outside institution's IACUC. The collaboration should be documented through a contract, memorandum of understanding or other agreement. In this circumstance, there is no requirement to submit an IACUC application to the MGH IACUC. When the animal research described above is funded through a Public Health Service (PHS) grant, the MGH will use the IACUC approval date of the institution where the research will be conducted and the MGH AWA number on the face page of the grant.

PROCEDURES

All new proposals for animal research and continuing review of projects must be reviewed by the IACUC. Complete new applications are reviewed in order of receipt at the Office of Animal Welfare Assurance (OAWA). Unless otherwise agreed to, all applications are pre-reviewed by an OAWA administrator and veterinarian. Recommendations and suggested refinements are returned to the investigator for consideration and inclusion into the IACUC protocol. Once the response to the pre-review is received, the IACUC review is conducted as either a *full committee review* (FCR) or a *designated member review* (DMR) for new protocols and triennial review applications. The determination of the level of review is made by the full committee. The MGH IACUC uses guidelines to automatically assign some studies to FCR, e.g., certain studies using USDA-regulated species, survival surgery, category E pain/distress levels, or possible animal welfare issues. All other protocols are reviewed by DMR procedure once all members of the IACUC have been provided an opportunity to call for FCR and no request for FCR has been received.

Review procedures

A. **Full Committee Review (FCR):** The IACUC meets as a full committee once each month. New proposals and triennial review applications are circulated to all members of the IACUC prior to the monthly meeting. Two committee members are assigned, with the IACUC Chair's approval, to serve as primary and secondary reviewers who present the proposal to the full committee for their consideration. The IACUC then discusses the proposal and acts by vote by IACUC members present at that meeting. The IACUC may 1) approve, 2) require modifications (to secure approval), 3) defer or delay decision, or 4) withhold approval/disapprove. Any such action requires a simple majority vote of a properly constituted quorum. If modifications are required to secure approval, the modified proposal may be reviewed by designated member review procedure, at the discretion of the full committee. If a protocol is assigned to more than one designated reviewer, the reviewers must be unanimous in any decision. They must all review identical versions of the protocol and, if modifications are requested by any one of the reviewers, the other reviewers must be aware of and agree to the modifications.

B. **Designated Member Review (DMR):** New proposals and triennial review applications that are eligible for DMR are assigned to the weekly DMR agenda. The agenda is distributed to the IACUC to provide the committee an opportunity to call for FCR. DMR agendas provide a link to the complete application for each transaction under review. The IACUC is provided 48 hours to call for FCR of an application. If no one calls for FCR, DMR of a new protocol or triennial review application is conducted by at least two members of the IACUC. The IACUC Chair

approves review assignments. The reviewers must reach agreement to 1) approve, 2) require modifications (to secure approval), or 3) request full IACUC review.

Following review. the investigator is notified in writing of the action of the IACUC. If the committee action is 'Requires Modifications', the investigator is sent a list of questions and/or concerns that need to be addressed before the proposal is reconsidered for approval. The investigator is asked to submit a point-by-point written response to these questions and concerns, along with a revised IACUC application incorporating the requested modifications. The investigator's response is sent to the DMR reviewers, or FCR, as determined by the committee.

If the IACUC initially withheld approval, the investigator's written response will be reviewed by the full committee, unless otherwise agreed to by a majority of the quorum of the IACUC, at the next monthly meeting scheduled after the response is received by the Office of Animal Welfare Assurance.

All actions taken outside of the convened meeting are reported back to the full committee each month.

Upon approval, the investigator is notified in writing of the action of the IACUC.

Continuing Research

A triennial review application must be reviewed by the IACUC using DMR or FCR every three years. Failure to submit and obtain approval for continuing work prior to the expiration date of the original proposal will result in the automatic inactivation and expiration of the original protocol. Any animals that remain after the expiration date will be reassigned to the institutional holding protocol, an appropriate experimental protocol, or euthanized.

Urgent Situations

MGH Center for Comparative Medicine (CCM) Veterinary Staff may perform emergency veterinary medical procedures to alleviate unnecessary pain or distress and/or prevent the loss of life or limb without prior IACUC approval. The CCM Veterinary Staff are available for direct consultation 24 hours a day, 7 days per week.

IACUC Member Responsibilities

IACUC members are required to attend regularly convened meetings to provide the optimal forum to conduct protocol review and to consider compliance reports or potential suspensions. The committtee members are expected to meet the following protocol review responsibilities:

- Act as a voting member of the IACUC
- Participate as a protocol reviewer and inform the Office of Animal Welfare Assurance (OAWA) promptly if unable to meet review assignment responsibilities
- Participate in all convened monthly meetings, to the extent possible; inform the OAWA of all planned absences in sufficient time so that steps can be taken to ensure quorum
- Be familiar with IACUC policies, procedures and standards and support the Chair and Vice Chair in their application in an equitable and consistent manner as part of protocol review; support the Chair and Vice Chair to ensure the committee are making decisions consistent with all regulations, policies, and standards.
- Maintain committee business and proceedings as confidential

Protocol Review by Videoconference

Videoconferencing will be used for monthly full committee review meetings and ad hoc IACUC meetings. Videoconference meetings proceed as for in-person meetings (attendance is documented, quorum is ensured, etc.). If a member has a conflict of interest with a study under review, the member is placed in a virtual waiting room and the administrative staff re-admits the member to the main meeting room after the study has been reviewed and a decision made by the committee. Meeting materials are distributed and accessed using Insight, our electronic submission and review system, as before.

Emergency/Ad Hoc Committee Meetings

The Chair may convene an ad hoc meeting to review compliance concerns, considerations for protocol suspension, and conduct protocol review when needed, provided the following can be met:

- All members are given notice that the meeting will be conducted by video conference
- Documents are provided to all members in advance of the meeting
- All members have access to the documents and can participate fully
- A quorum of voting members is convened

- The forum allows for real time verbal interaction equivalent to that occurring in a physically-convened meeting
- If a vote is called for, the vote occurs during the meeting and is taken in a manner that ensures an accurate count of the vote
- Opinions of absent members that are transmitted by mail, telephone, or e-mail may be considered by the convened IACUC members but will not be counted as votes or considered as part of the quorum
- Written minutes of the meeting will be maintained

Requests to Use Animal Tissue and/or Products

An application ("tissue protocol") must be submitted in the Insight system to obtain animal tissues, products, or blood, including whole dead animals and eggs or embryos from egg-laying species, not otherwise approved as part of the investigator's own animal research protocol. Refer to the IACUC Policy on Use and Sharing of Animal Tissues and Products for more information.

Tissue protocols are reviewed administratively by the Office of Animal Welfare Assurance.

REFERENCES

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v1.1, March 2003
v1.2, May 2004
v1.3, August 2006
v1.4, 15 October 2014
v1.5, 18 October 2017
v1.6, 15 November 2017
v1.7, 19 April 2019
v1.8, 17 August 2022

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

AMENDMENT REVIEW PROCESS

GENERAL POLICY

As required by federal regulations and hospital policies, the IACUC must review and approve any changes or modifications to previously approved protocols in advance of implementation.

In August 2014 OLAW released a notice entitled “Guidance on Significant Changes to Animal Activities” (NOT-OD-14-126) that provided direction to the IACUC regarding review of significant changes which are defined as those modifications that have, or have the potential to have, a negative impact on animal welfare.

PROCEDURES

In accordance with this notice, submitted amendments will be assigned to one of three review paths outlined below. The specific assignment will be determined by the Office of Animal Welfare Assurance based on the nature of the revisions proposed in each amendment submission. IACUC members may call for Full Committee Review of any amendment at any time. Each review process and examples of the protocol modifications that will be evaluated via that process are as follows:

A. Designated Member Review (DMR)

Amendments requesting significant modifications must receive review by the IACUC through the DMR process as outlined in the approved MGH IACUC Protocol Review Process policy. Such amendments will be reviewed by at least one appropriately qualified IACUC member, assigned by the IACUC Chairperson, who can take one of three actions:

1. Approval
2. Requires modifications to secure approval; the Principal Investigator (PI) is provided with the reasons for this action and must respond to the feedback to secure approval
3. Call for Full Committee Review (FCR); the amendment will be reviewed through the FCR procedure as detailed in the MGH IACUC Protocol Review Process

Modifications requiring Designated Member Review

- Greater than 20% increase in animal numbers
- Addition of animals to Pain Category E
- Reassignment of animals among pain categories
- Modification of humane endpoints including description of phenotype impacting animal health and care
- Addition of non-standard housing or husbandry practices
- Addition of any new procedure except for animal identification by ear tagging/notching
- Changes that could result in potentially more severe adverse consequences
  - ♦ Changing from non-survival to survival surgery
  - ♦ Reduction in analgesics
- Addition of different classes of experimental drugs/substances
- Addition of new research objectives
- Any modification that impacts personnel safety
- Change of PI
- Other modifications that represent a significant change to the approved protocol as determined by the Office of Animal Welfare Assurance

B. Veterinary Verification and Consultation (VVC)

Some specific significant changes may be handled administratively according to IACUC-reviewed and -approved policies in consultation with a veterinarian authorized by the IACUC. The veterinarian reviewer is not required to be an IACUC member but must be familiar with approved IACUC policies. The veterinarian is not conducting Designated Member Review but is serving as a subject matter expert to verify that compliance with the IACUC-reviewed and approved policy is appropriate for the animals in this circumstance. Specific reference documents available to the veterinarian including guidelines, formularies and policies are included in this policy. Consultation with the veterinarian must be documented via the protocol review process.

The VVC process will be used to make significant modifications to procedures that are already part of the approved protocol and do not increase pain, distress or degree of invasiveness. The veterinarian reviewer should consider the overall welfare of the animals and call for Designated Member Review if the proposed changes will compromise animal welfare. The veterinarian must refer any request that does not meet the parameters of the IACUC-reviewed and -approved policies to Designated Member Review. The veterinarian reviewer can take one of three actions:

1. Accept VVC

2. Decline; the Principal Investigator (PI) is provided with the reasons for this action and must respond to the feedback to secure approval
3. Accept No VVC; the amendment is assigned for Designated Member Review

Modifications that may be reviewed by VVC

- Change in route of substance administration or sample collection
- Addition of a new administered agent that is in the same class as already approved agents
- Change in anesthesia, analgesia or sedation that is consistent with CCM recommendations
- Change to timeline or approach of previously approved surgeries
- Modifications of pre- and post-operative/procedural care
- Change in frequency of approved procedure
- Change in euthanasia to any AVMA approved method (acceptable or conditionally acceptable).
- Increase in frequency of experimental agent administration or sample collection
- Change to timeline of any previously approved experiment
- Addition of IACUC/CCM-approved ear tagging/notching identification procedures
- Addition of any new strain
- Change of sex of animals to be used in approved experiments

C. Administrative Review

Revisions that are considered non-significant in accordance with this policy may be administratively reviewed and approved by an Office of Animal Welfare Assurance representative. The proposed change may be referred for VVC or committee review at the representative's discretion.

Modifications appropriate for Administrative Review

- Less than 20% increase in animal numbers
- Addition or change in funding/sponsor requiring congruency review
- Change/addition of study staff
- Change in performance or housing site involving CCM space or an already approved MGH location
- Change in title of proposal

- Correction of grammatical or typographical errors

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U.S. Department of Health and Human Services. *NOT-OD-14-126: Guidance on Significant Changes to Animal Activities*. Office of Laboratory Animal Welfare, National Institutes of Health: Bethesda, MD, 26 August 2014.

Pain Management Regimens for Mice and Rats

v1.1, 19 April 2019
v1.2, 19 February 2020
v1.3, 18 March 2020
v1.4, 20 January 2021

MASSACHUSETTS GENERAL HOSPITAL
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY FOR SHARING ANIMAL TISSUES AND PRODUCTS

GENERAL POLICY

Sharing animal tissues for more than one protocol is encouraged, in order to reduce the total number of animals used in research and teaching at MGH. If only animal-derived tissues but no live animals are needed for an experiment, a full IACUC protocol does not need to be completed. Instead, all research endeavors involving only animal-derived tissues must be described in a Request for Animal Tissues/Products for IACUC approval.

Studies involving only animal blood, blood products (e.g., serum, plasma, platelets, blood cells), or other tissues must also be approved by the IACUC when such material is obtained from sources other than a licensed biological supply company. Such approval is required to identify and avoid infectious disease threats to animals and personnel, confirm that the source is in compliance with federal and state laws and regulations, and to ensure that such use of animal materials is appropriate.

Requests for animal tissues can be made through the Center for Comparative Medicine (CCM). CCM will notify other investigators who may be using animals of that species, sex, age, strain, etc. to see if tissues may be available. Animal tissues may be obtained directly from other MGH investigators or from collaborators from other institutions, or purchased from USDA-inspected abattoirs or other sources, following IACUC approval.

[Most recently revised May 24, 2005]

MASSACHUSETTS GENERAL HOSPITAL

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON PRINCIPAL INVESTIGATOR ELIGIBILITY

GENERAL POLICY

In order to obtain approval to conduct animal research at Massachusetts General Hospital (MGH) or the Shriners Hospitals for Children, Boston (SHC) a Principal Investigator (PI) must be:

- appointed at and employed by MGH, and
- hold a minimum academic rank of Instructor or Lecturer at HMS for the duration of the project.

The PI must meet the requirements set by the Institutional Animal Care and Use Committee (IACUC) with regard to training and completion of the Occupational Health program. The PI responsible for the conduct of the animal research does not necessarily need to be the same as the PI named on the related grant.

Postdoctoral fellows and other licensed professionals (RN, DVM, DDS, PharmD, etc.) may serve as PI when the project/activity is within the scope of their training or licensure, with the approval of the Senior Vice President for Research (or his/her delegate) and the Department Chief. Postdoctoral fellows must include someone who meets the requirements for PI described above as a member of the protocol study staff.

It is the responsibility of the PI to notify the IACUC of a change in employment status or academic rank.

Requests for any other persons to be a PI on an MGH IACUC protocol must be approved by the Senior Vice President for Research.

[Most recently revised September 15, 2021]

MASS GENERAL RESEARCH INSTITUTE
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON RESEARCH SUPPORT TEAMS

GENERAL POLICY

Support teams are groups of defined team members that provide animal support services (e.g., peri-operative, anesthetic, and procedural care performed by Knight Surgical Research Laboratory staff) for animal research studies approved by the MGH IACUC. Such teams must seek approval in advance from the IACUC to be included under this policy. Members of approved animal research support teams may be listed collectively by team name on the protocol. Research support teams are listed as an 'External' group in the Insight system. CCM staff members do not need to be identified in the IACUC protocol if they perform research support services as part of their CCM duties.

It is the responsibility of the team manager to ensure that training and Occupational Health requirements for all team members are up-to-date and to communicate team personnel changes to the Office of Animal Welfare Assurance. The Office of Animal Welfare Assurance will approve the personnel change once training and occupational health requirements have been met. The Office of Animal Welfare Assurance is responsible for maintaining the research support team information, including personnel approved to work on the team, in Insight.

v1.1, 21 August 2013
v1.2, 19 April 2017
v1.3, 21 April 2021

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON ENVIRONMENTAL ENRICHMENT, SOCIAL HOUSING, AND EXERCISE OF LABORATORY ANIMALS

OVERVIEW

In accordance with federal regulations and accreditation standards this policy describes the requirements in effect for all vertebrate laboratory animals at MGH for an enhanced immediate environment and to promote the psychological wellbeing for all laboratory animals under MGH IACUC protocols. The main goal of this policy is to encourage the expression of species-typical behaviors by providing animals with environmental provisions (structural enrichment, sensory stimulation, manipulation devices, and nutritional enrichment options), social interaction and exercise. Such requirements should help prevent or reduce abnormal behaviors, including stereotypical movements, self-aggression and fighting. Enrichment strategies are developed to be compatible with standard laboratory housing/care, animal health, animal welfare, operational efficiency of the animal facility, and research needs.

DEFINITIONS

Social species: Any species known to naturally live and interact with conspecifics (animals of the same species). The majority of laboratory animals housed at MGH are considered social species, including but not limited to the following: most rodents (mice, rats, guinea pigs), rabbits, ferrets, dogs, sheep, pigs, nonhuman primates, and aquatics (frogs and fish).

Social housing: Housing social species in compatible pairs or groups with additional visual, auditory, olfactory, and/or tactile contact of conspecifics housed within the same room.

Single housing: Housing an animal in a primary enclosure by itself with additional visual, auditory, olfactory, and/or tactile contact of conspecifics housed within the same room.

Solitary (or isolated) housing: Housing an animal in a primary enclosure by itself in the absence of any other animals in the same room. Solitary housing is generally not permitted at MGH.

GENERAL POLICY

This policy applies equally to animals used and/or housed both inside and outside of CCM-managed facilities. While the environmental enhancement program is designed on a species-specific level, modifications may be appropriate to accommodate individual animals or phenotypes. Species-specific SOPs or Visual Controls (VCs) document the operational implementation of this policy in the facilities. Species-specific VCs can be found in the IACUC Policies & Guidance section of the IACUC website.

ENVIRONMENTAL ENHANCEMENT PROGRAM PROVISIONS

Social Housing

Social housing or grouping is considered the default method of housing for animals who are known to naturally live and interact with conspecifics. Compatible pairs or groups will be developed based on appropriate sex, age, and behavior and in accordance with the study needs. For periods where social housing is not possible for scientific, veterinary or other reasons, an exemption must be considered (see Exemptions section for more details).

Environmental Enrichment

Environmental enrichment is intended to promote diversity and complexity in the animal's immediate environment. Components of enrichment can be divided into four categories, including 1) structural enrichment, 2) sensory stimulation, 3) manipulation devices, and 4) nutritional enrichment options. All animals should have as many of these components of environmental enrichment as possible based on their species' needs and primary enclosure compatibility.

Exercise

Opportunities for physical and cognitive activity can be facilitated by providing adequate space in the animal's primary enclosure. Additional opportunities may be provided individually or in compatible groups in enlarged enclosures or spaces intended to encourage exercise and/or through positive physical contact with personnel. Dogs will be provided with exercise as required by the Animal Welfare Act and Regulations (AWAR), Exercise for dogs.

Physical Restraint

Physical restraint is the use of manual or mechanical means to limit some or all of an animals' normal movement for the purpose of examination, collection of samples, drug administration, therapy, or experimental manipulation (*Guide*, p. 29). Restraint devices should be suitable in size, design, and operation to minimize discomfort, pain, distress, and the potential for injury to the animal and the research staff. The IACUC encourages training utilizing positive reinforcement to acclimate an animal, or achieve a cooperative behavior, to a particular environment or procedure.

Prolonged restraint, including chairing of nonhuman primates, should be avoided unless it is essential for achieving research objectives and is specifically approved by the IACUC (*Guide*, p. 29). Non-human primates must not be maintained in restraint devices unless required for health reasons as determined by the AV or scientifically justified in an IACUC-approved protocol. Maintenance under such restraint must be for the shortest period possible. If long-term restraint (restraint longer than 12 hours) is required, the animals must be provided opportunity for unrestrained activity for at least one continuous hour during the restraint period, unless continuous restraint is approved by the IACUC, to be reviewed at least semi-annually (*AWAR*, p. 176). If prolonged restraint is required for veterinary reasons, this exemption must be reviewed at least every 30 days by the AV unless it is a permanent condition.

Special Considerations

Animals identified to have special needs (infant or juvenile animals, animals that show signs of

being in psychological distress through behavior or experience, activity restriction, or housed in isolation) will be evaluated and specific care provided as determined to meet the animal's needs. CCM develops and maintains pre-determined intervention plans/SOPs to address common behavioral concerns or occurrences. Interventions may include increased or modified enrichment, changes in their primary enclosures, increased positive human interactions, changes to feeding strategy, social housing, and/or advanced animal training techniques.

EXEMPTIONS TO THE ENVIRONMENTAL ENHANCEMENT PROGRAM

Exemptions to all or any element of this policy for all or a portion of the study period must be approved by the IACUC or the Attending Veterinarian or his/her delegate. The basis for the exemption shall be reviewed by the IACUC at appropriate intervals as determined by the Committee, but not less than annually. Additionally, program-wide exemptions may be approved by the Attending Veterinarian and the IACUC and documented in the species-specific SOPs.

Exemptions to Social Housing

If single housing of social animals is deemed necessary, it should be limited to the minimum time period necessary and, where possible, visual, auditory, olfactory and, depending on the species, protected tactile contact with compatible conspecifics should be provided. In the absence of other animals, a focus on enriching the environment will be implemented, such as safe and positive interaction with the animal care staff, as appropriate to the species; periodic release into larger enclosures; supplemental enrichment items; and/or the addition of a companion animal in the room or housing area.

A. Husbandry and Veterinary Exemptions. The following general categories of exemptions to social housing have been approved by the IACUC and justification in an animal use protocol, or case-by-case approval by the IACUC is not required:

1. **Standard Animal Husbandry Exemptions:**
   MGH standard animal husbandry and management practices include single housing of social animals. Examples of such situations include, but are not limited to:

   a. The unavailability of another social compatible animal due to:

      i. Research attrition of cage/pen mates

      ii. Aggressive or incompatible conspecifics as determined by CCM veterinary services staff (e.g., adult males of certain species: mice, hamsters, NHP, rabbits, swine)

      iii. Short-term studies (≤ 90 days) for NHPs, swine, or rabbits, where establishment of pairs or resocializing of animals exceeds the time allotted for research conduct, unless the animals were procured in a social grouping or can be easily socially housed on premises.

   b. Breeding and weaning situations, for example:
      i. Pregnant females separated to prevent overcrowding prior to or following birth
      ii. Separation of males and females to prevent unwanted pregnancy
      iii. Only one mouse received, or only one mouse of either gender born in a litter

iv. Single housing to condition males or females prior to or post breeding/weaning

c. Perioperative period – standard practices in managing surgery or other technical procedures including fasting prior to anesthesia and post-operative recovery period that accounts for animals being fasted or until healed from surgery (up to 14 days post-op). In certain species (adult mice, rabbits and hamsters) the separation may be considered permanent when resocialization is not recommended.

2. Veterinary / Clinical Exemptions:

CCM veterinary staff may require individual housing of animals due to medical concerns. The veterinarian will record the basis for the exemption, the period of single housing and frequency of reevaluation in the individual animal medical record (IAMR) or similar health recordkeeping, will monitor the animal as noted and re-house the animal when the clinical condition is resolved. Exemptions for NHPs will be reviewed by a CCM veterinarian at least every 30 days, unless it is a permanent condition.

B. Scientific Exemptions:

When single housing of social species is required for all or a portion of the study period for experimental reasons, or if the perioperative period exceeds 14 days post operatively (see A.1.c. above), a scientific justification must be described in the animal use protocol, submitted for review and approved by the IACUC. The basis for the exemption will be reviewed by the IACUC annually for USDA-regulated species and every 3 years for non-USDA regulated species.

Exemptions to Enrichment

All animals are considered enrolled in the environmental enrichment program.

The Attending Veterinarian may exempt an individual animal from the environmental enrichment program because of health or condition, or in consideration of its well-being. The veterinarian will document the basis of the exemption in the individual animal medical record (IAMR) or similar health recordkeeping, will monitor the animal as noted, and will re-house the animal when the clinical condition is resolved. Exemptions for NHPs will be reviewed at least every 30 days, unless it is a permanent condition.

Experimental procedures requiring exemptions to all enrichment for scientific reasons will be documented in the animal use protocol, approved by the IACUC, and re-evaluated no less than annually for USDA-regulated species and every 3 years for non-USDA regulated species**.**

Use of environmental enrichment other than described in the CCM Species-specific VCs should be done in collaboration with the CCM enrichment personnel and/or veterinarian.

Exemptions to Exercise

The Attending Veterinarian may exempt an individual animal from the dog exercise program because of health or condition, or in consideration of its well-being. The veterinarian will document the basis of the exemption in the individual animal medical record (IAMR) and the exemption will be re-evaluated every 30 days, unless the basis for the exemption is a permanent condition.

Experimental procedures requiring exemptions to the dog exercise policy for scientific reasons will be documented in the animal use protocol, approved by the IACUC, and re-evaluated no less than annually.

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v1.7, 19 July 2017
v1.8, 17 March 2021

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON ACCESS TO CENTRALIZED (CCM) AND SATELLITE ANIMAL FACILITIES FOR MGH RESEARCH EMPLOYEES AND VISITORS & VENDORS

GENERAL POLICY

Only authorized personnel are permitted entry into animal facilities that are maintained by the Center for Comparative Medicine (CCM) or managed by the Principal Investigator (PI) with IACUC approval (i.e., satellite housing areas, satellites). This restriction applies at all times and to all persons. For satellite housing areas, access may be obtained via authorized key users or keypad codes. Authorization for centralized animal facility entry is normally provided by the electronic programming of an employee's identification badge by Police & Security (P&S). This programming permits entry through a locked door only into the particular animal facility in which the MGH employee will be regularly working or otherwise needs frequent access. Anyone who may need sporadic access and is not given authorization through the process above will be considered a Visitor or Vendor and will be granted access only with an escort. The processes for obtaining access through either electronic or comparable authorization or for obtaining Visitor and Vendor approval to be in an animal facility are described below.

MGH EMPLOYEES

CCM employees who work in a particular animal facility, research staff listed in an IACUC-approved animal protocol, and specified hospital service personnel who may require occasional access for routine services (e.g., Police & Security, Building & Grounds, Environmental Health and Safety, Research Space Management Group, IACUC staff) are permitted access via programmed identification. MGH employees who need access sporadically for specific reasons (e.g., safety specialists, architects and engineers, IT), will be recognized as a Visitor (see below) and must be escorted by a CCM staff member or other authorized personnel.

Such programmed access may be suspended or revoked if the employee does not observe hospital and animal facility regulations and policies. Examples of situations in which access could be suspended or revoked include: Electronic locks and alarms at entry sites, surveillance cameras, and other devices and strategies that may be necessary are used to secure centralized animal facilities.

- Repeatedly not wearing or displaying one's identificationbadge
- Failure to obtain visitor approval
- Giving an identification badge to unauthorized persons to gain access into an animal facility
- Repeated failure to wear appropriate personal protective equipment (PPE)
- Repeated incidents of non-compliance (per corrective actionplan)
- Involvement in an investigation of an animal welfare concern (as directed by IACUC or AV)

Research staff requiring access to centralized animal facilities must complete the steps outlined below. PI-managed satellite housing areas have comparable steps and are mandated to assure occupational health clearance, CITI training, and protocol inclusion. The majority also require CCM Orientation and Tour through a contractual agreement. Those satellites that do not use the CCM program have a defined training program for new employees; the information can be obtained from the PI or Lab Contact. The information for access to centralized animal facilities may also be found on the IACUC website (http://is.partners.org/aniweb/accessform.htm) and the CCM website (http://intranet.massgeneral.org/ccm/research-community/investigator-orientation/index.asp).

1. Initiate Occupational Health clearance:
   - The staff member must have obtained clearance from the Occupational Health Services (OHS) within the past three years to work with the species indicated on the protocol. If the species indicated is a nonhuman primate, the OHS clearance must be obtained within the past sixmonths.
   - If the employee does not have a current clearance, they must complete the Research Animal Health Screening form and submit it to Occupational Health Services (occhealth@partners.org)
2. Completion of CITI training
   The CITI Training program in Animal Care and Use has been approved by the IACUC to fulfill the training requirements of the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals. Required courses are outlined on the CITI Grid. All courses required for a specific protocol must be completed.
3. Protocol Amendment (only upon completion of steps 1 and2):
   The Principal Investigator must submit an amendment to the IACUC protocol in Insight to include the new researcher as Study Staff. Personnel added as Non-study Staff will not be granted access to centralized animal facilities.

4. Registration Form (only upon completion of steps 1 through 3):
   The employee must complete and submit a Registration Form. The IACUC Office will verify the information on the form and forward the request to the CCM Facility Access Team for processing. Research staff will be approved for access only to the CCM facilities included on the IACUC-approved protocol.
5. Complete CCM Orientation Lecture and CCM Facility Orientation Quiz:
   - The CCM Orientation Lecture is available through HealthStream
   - CCM Facility Orientation Quiz. A minimum grade of 90% is required to pass the quiz.
6. Complete a CCM facility tour:
   Upon completion of steps 1 through 5, the employee will receive an email stating that they are authorized for a facility tour. The email will include instructions on scheduling the facility tour.

Other MGH employees requiring programmed identification access to centralized animal facilities must complete the following steps:

1. Obtain Occupational Health clearance
2. Complete CCM Orientation Lecture and CCM Facility Orientation Quiz
3. Complete a facility tour

VISITORS AND VENDORS

Occasionally, MGH employees not affiliated with the animal program or non-MGH employees may be justified in needing access to an animal facility. Such circumstances may involve representatives from legal, public affairs, engineers and architects, safety specialists, research collaborators visiting from other institutions, municipal emergency responders, regulatory or accreditation site visitors, equipment vendors, or maintenance personnel.

In every case, the Director of CCM or his or her designee must be notified in writing at least one business day in advance of each such instance. Notification should be provided by e-mail and must include:

1. The name of each visitor
2. His/her institution or agency
3. Purpose of the animal facility visit
4. Date(s) involved, and
5. Contact information for the MGH host.

Failure to notify the Director of CCM or his or her designee in a complete and timely manner may result in revocation of animal facility access. If working in BL-2 areas (including non-human primates), visitors must comply with all occupational health and safety requirements and procedures, including evidence of a negative TB test within the previous six months, or must wear a properly fit tested N-95 facemask while in the facility.

All visitors must be accompanied by an MGH employee the entire time they are in an animal facility. Visitors must comply with all applicable policies and procedures required of MGH staff. These restrictions include, but are not limited to, removing documents, records, or animals, and taking photographs or making recordings. Failure to notify the CCM Director prior to the visit; to accompany visitors; or to abide by security and safety precautions may result in revocation of animal facility access.

Non-employees who may need access to animal facilities for extended periods of time will be subject to the same process as new employees to obtain authorization for unescorted access to animal facilities.

TERMINATED EMPLOYEES (CCM STAFF ONLY)

Persons whose employment is terminated will lose their animal facility access privileges and their badges will be deprogrammed by P & S. CCM will be provided a list of such persons on a regular basis to ensure that they do not attempt subsequent entry to animal facilities besides as an approved visitor (see above).

REPORTING ACCESS VIOLATIONS

Any person in an animal facility who is not wearing an identification badge or is not accompanied by an MGH employee authorized in that animal facility will be asked to identify himself/herself and to leave the facility. If the person does not comply, P&S is to be notified immediately. If the person leaves prior to the arrival of P&S, a description of the incident and person in question will be provided to P&S.

REFERENCES

National Research Council of the National Academies. *Guide for the Care and Use of Laboratory Animals,* 8th edition. National Academies Press: Washington, D.C., 2011.

U.S. Department of Agriculture. *Animal Welfare Act and Animal Welfare Regulations*. (Animal Care Blue Book). Code of Federal Regulations (CFR), Title 9, Chapter 1, Subchapter A, Parts 1-4. 2017.

v1.1, February 2005
v1.2, November 2005
v1.3, 20 September 2017

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON HOUSING OF LIVE VERTEBRATE ANIMALS OUTSIDE OF CCM CENTRALIZED FACILITIES

OVERVIEW

Animals used in research, training and teaching at MGH locations must be housed and used in facilities that meet Federal, State, and local requirements. Most centralized animal research facilities at MGH are under the oversight of the Center for Comparative Medicine (CCM) and should be used whenever possible. However, there are circumstances that require animal housing in MGH locations outside of the CCM centralized facilities. This policy outlines the process for review and approval of non-CCM centralized and satellite housing facilities. This policy also details the roles and responsibilities of the IACUC, CCM and the research staff for oversight and management of these locations.

DEFINITIONS

Animal: The PHS Policy on Humane Care and Use of Laboratory Animals defines animal as, “any live, vertebrate animal used or intended for use in research, research training, experimentation or biological testing or for related purposes. Furthermore, OLAW interpretation of PHS policy considers the offspring of egg-laying vertebrate species, including larval forms of amphibians and fish, as "live, vertebrate animals" at hatching.

CCM-managed facilities: facilities assigned to and overseen by Center for Comparative Medicine (CCM).

Centralized Facility: animal facility in which the support, care and use areas are adjacent to the animal housing space.

PI-managed Facilities: facilities assigned to and overseen by a Principal Investigator or research department.

Satellite Facility: an IACUC-approved animal facility in a space outside of the centralized facilities in which animals are housed for greater than 12 hours.

Laboratory Animal Use Area: an IACUC-approved space in a laboratory where animals may be used for up to 12-hours for experimental purposes.

GENERAL POLICY

PI-managed Centralized and Satellite Animal Housing Facilities

Animals must be housed in the CCM centralized facilities whenever possible. Housing of live vertebrate animals outside of CCM centralized facilities may be permitted in PI-managed centralized facilities and PI-managed satellite facilities only when CCM cannot accommodate animal housing needs or there is rigorous scientific justification for the exception. PI-managed housing facilities must be approved in advance by the IACUC and must meet the same standards required of a CCM facility, as outlined in the Guide for the Care and Use of Laboratory Animals (*Guide)*. PI-managed housing facilities (centralized and satellite) are subject to the following conditions:

1. The PI must provide a justification for housing outside the CCM centralized facilities in the IACUC-approved protocol(s) covering animals housed in the PI-managed facilities. IACUC approval will be provided only if there is a demonstrated need to house animals outside to CCM centralized facilities for scientific purposes. Convenience of proximity or cost savings are not considered a demonstrated need.
2. The PI is responsible for ensuring that all Federal, State, and local regulations and IACUC policies for the housing, care, and use of laboratory animals are met in their facility, equivalent to the standards of the CCM facilities. The PI may contract with CCM to help meet these obligations as outlined below.
3. The facility must pass an inspection by the IACUC, Attending Veterinarian or designee, Research Space Management, Environmental Health & Safety, and other applicable administrative entities prior to use. Renovations needed to meet requirements for an animal housing facility are the responsibility of the PI, unless otherwise agreed to. Final approval of the facility is at the discretion of the IACUC and the Attending Veterinarian.
4. The PI is responsible for the maintenance and upkeep of the animal facility, unless otherwise agreed to.
5. PI-managed animal facilities are part of the MGH animal care and use program.
    a. A current Laboratory Animal Services (LAS) Agreement must be in place between the PI and CCM which defines the responsible party for animal husbandry, daily recordkeeping, environmental sanitation and monitoring, security, and other items.
    b. The Attending Veterinarian has responsibility for all animals at MGH, including those housed at PI-managed locations. The LAS agreement will document veterinary services provided by CCM veterinary staff at PI-managed locations.
    c. PI-managed facilities are subject to inspection by the IACUC, as part of the semiannual facility inspection, and other regulatory bodies.
    d. The IACUC and the CCM Veterinary staff must have access to PI-managed locations at all times.
    e. The PI or their designee, such as a laboratory manager, is responsible for:
        i. Serving as the primary contact for the IACUC, CCM, Buildings & Grounds, Police & Security, and other institutional departments.
        ii. Overseeing the facility access process to include documenting who has access and providing training/ensuring all required training is current.

iii. Establishing an emergency response plan if the space is outside a CCM facility and training relevant parties on the response plan.

iv. Implementing changes to the animal care and use program as required based on communications from the IACUC and CCM.

v. Reporting animal welfare concerns or non-compliance events to the IACUC.

vi. Serving as a liaison for internal and external regulatory reviews.

vii. Ensuring compliance with approved protocols and IACUC policies and guidelines.

viii. Implementing corrective action plans issued by the IACUC, Department, or any oversight body.

ix. Providing census information to the IACUC on request.

6. An Emergency Contact List, including contact information for emergency veterinary assistance (e.g., phone number for the CCM veterinarian-on-call) must be posted in the immediate vicinity of the animals.

7. A copy of the Reporting Animal Welfare Concerns sign must be posted in the area.

8. A copy of the "IACUC Approved Housing" poster must be posted on the door to the Facility, or other appropriate location. This poster must be obtained directly from the Office of Animal Welfare Assurance office.

9. Personal protective equipment appropriate for the work conducted in the facility (such as gloves, masks, lab coat or gown, etc.) must be made available in area.

10. All cages must be identified properly and completely with cage cards attached, as in the centralized facilities.

11. If animal use ceases, or there are changes to the facility, the Principal Investigator must notify the IACUC. The IACUC can be notified of a closure of a facility by contacting the Director, Animal Welfare Assurance or the Attending Veterinarian directly. Revisions to the research protocols accommodated within the space and changes to room numbers, etc., must be captured in the approved IACUC protocol. Revisions to the PI or laboratory contact for PI-managed facilities must be communicated directly to the Director, Animal Welfare Assurance and the Attending Veterinarian.

12. This policy presumes routine housing of animals for greater than 12 hours in the PI-managed housing facility. On occasion, there may be a scientific need to retain animals in a laboratory animal use area for greater than 12 hours for a single experiment. If this work cannot be accommodated in the centralized or satellite housing facilities, a request may be submitted to the IACUC via the IACUC protocol to retain animals in the laboratory for greater than 12 hours. These requests will be considered by the IACUC as part of protocol review on a case-by-case basis. The IACUC will require a justification why the work cannot be conducted in an approved

housing location and the scientific basis for needing to retain the animals in the laboratory for > 12 hours.

RELATED POLICIES

Policy on Reporting Animal Welfare Concerns
Policy for Transportation of Animals

REFERENCES

Bartlett, D.H.; Silk, S.B. Office of Laboratory Animal Welfare Comments. *Zebrafish*. 2016, 13(6), 1-2.

National Research Council of the National Academies. *Guide for the Care and Use of Laboratory Animals,* 8th edition. National Academies Press: Washington, D.C., 2011.

U.S. Department of Agriculture. *Animal Welfare Act and Regulations* (Animal Care Blue Book). Code of Federal Regulations (CFR), Title 9, Chapter 1, Subchapter A, Parts 1-4. 2020.

U.S. Department of Health and Human Services. *Public Health Service Policy on Humane Care and Use of Laboratory Animals.* Office of Laboratory Animal Welfare, National Institutes of Health: Bethesda, MD, 2015.

U.S. Department of Health and Human Services. PHS Policy on Humane Care and Use of Laboratory Animals FAQ A5: Does the PHS Policy apply to larval forms of amphibians and fish? Office of Laboratory Animal Welfare, National Institutes of Health: Bethesda, MD, 2021.

v1.1, February 2005
v1.2, May 2006
v1.3, September 2011
v1.4, 16 November 2011
v2.1, 15 September 2017
v2.2, 21 July 2021

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON HOUSING AND USE OF VERTEBRATE RESEARCH ANIMALS OUTSIDE OF MGH FACILITIES

OVERVIEW

Animals housed and used in IACUC-approved facilities at MGH are covered by MGH IACUC protocols. Circumstances may require that animals be housed or used at locations not part of the MGH animal care and use program ("off-site locations"). These include field studies, additional housing needs for the program, the conduct of research by or under the direction of MGH personnel in connection with his/her/their MGH responsibilities, and/or when MGH is identified in research funding applications. This policy outlines the institutional responsibility and requirements for the care and oversight of MGH animals housed or used at these off-site locations.

DEFINITIONS

Off-site location: any location not owned, operated, or leased by MGH where animals are housed or used for the purpose of research, testing, or teaching.

Off-site animal activity: any housing or use of animals for the purposes of research, testing, teaching, or related purposes that is conducted in whole or in part in the field or at a non-MGH institution, company, contracting laboratory, contract research organization, or similar facility.

GENERAL POLICY

Field studies.

An MGH IACUC protocol should be submitted for any study conducted by MGH personnel on free-living wild animals regardless of where it will be performed. that involves procedures that may harm or materially alter the behavior of the animals under study (*i.e.*, trapping/capture, physical/chemical restraint, and/or invasive procedures causing stress, including removal from nest and habitat for short durations). The MGH IACUC reserves the right to rely on another IACUC if appropriate and when an MOU is in place that decribes the terms of the reliance agreement. The MGH IACUC may invite local experts to participate in the review as a consultant and will work with the principal investigator to determine the best mechanism to use for the conduct of semiannual, or other, inspections. This may include questionnaires, videos or photographs, local experts, or in-person inspections. For regulated species, all inspections will be completed by two members of the IACUC. The principal investigator is required to be aware of, and obtain, all permits and licenses required at the Federal, State and local level prior to the conduct of the research, and must meet the requirements of all regulations governing the conduct of field research (see references). The investigator must describe which permits and licenses are required in the IACUC protocol and must be able to provide a copy of these permits and licenses to the IACUC during inspection. Other considerations include appropriate euthanasia methods,

occupational health concerns, and the safety of research personnel and other animals. Shipping and receiving of animals or specimens must be conducted in accordance with federal safety and importation guidelines and regulations.

Off-site housing and use facilities.

At times, the MGH Animal Care and Use Program (ACUP) may use off-site facilities for quarantine, holding, research, or other purposes. Animals may be housed or used off-site entirely for, or at times throughout, a research project. An animal maintenance agreement and assurances addendum will typically be put into effect for these facilities. Any investigator requesting or requested to use these off-site facilities may do so only with the approval of the MGH IACUC. The MGH IACUC, together with the Attending Veterinarian, determines which off-site facilities may be approved as an MGH ACUP off-site facility. Investigators, conducting research in connection with his/her/their MGH responsibilities, may not utilize these off-site facilities for purposes other than those outlined in the agreement without prior approval of the MGH IACUC.

MGH Investigators may anticipate collaborative research in research grants, proposals and as part of their research projects. This may include situations where all the live animal work is conducted at MGH, all the live animal work is conducted at a collaborator's facility, or live animal work is conducted at both MGH and the collaborator's facility. In general, the institution where the animals are housed will be responsible for animal welfare and full regulatory compliance, unless otherwise agreed to by MGH and the collaborating institution(s). The housing institution's IACUC will have oversight of the animal research protocol and the conduct of the research, as well as have the responsibility to meet all applicable Federal, State, and other regulations and standards; veterinary care of the animals will also be the responsibility of the institution where the animals are housed. For collaborations where work will be conducted in part at MGH, and at part at a collaborating research facility, the MGH IACUC protocol will detail the work conducted at MGH and reference the transfer of animals to/from the collaborating research facility and generally describe the work conducted at the collaborating research facility and how it relates to the animal work conducted at MGH. A copy of the approved protocol from the collaborating site will be requested as part of the MGH IACUC review. The details of these collaborations will be captured in a grant subcontract, animal use agreement, memorandum of understanding or similar document, in most instances. When possible, investigators should consult with the MGH IACUC before entering into an agreement with off-site research facilities.

In situations where animals are housed or used at off-site facilities, although the MGH IACUC will rely on the collaborating institution for oversight and care of the animals, the MGH IACUC will ensure that the collaborating institutions meet the federal and state laws and regulations and current accreditation standards, when applicable, regardless of the animals' location. MGH's responsibilities are met by the following means, unless otherwise approved:

- Any institution that provides housing or research resources for MGH animals must be licensed by the USDA if regulated species are involved. In addition, the institution must have an approved Letter of Assurance from the NIH Office of Laboratory Animal Welfare. If the site does not have an assurance with OLAW, a special exception must be granted by

the Institutional Official, before the MGH IACUC will permit housing or use at that facility. Accreditation by the Association for Accreditation of Laboratory Animal Care International (AAALAC) is preferred.

- Before the MGH IACUC will rely on a collaborating site for research oversight and regulatory compliance, MGH reserves the right to require that the MGH Center for Comparative Medicine (CCM) conduct a site visit on behalf of the IACUC in advance of any MGH animals arriving at an off-site institution, or at any time during the research collaboration. In lieu of a site visit, the MGH IACUC may request the collaborating site complete an animal welfare questionnaire as part of MGH's due diligence to ensure the highest standards of animal welfare, particularly for MGH-funded research. In general, the MGH IACUC will rely on the semiannual facility inspection and program review of the collaborating institution, but may conduct site visits as part of the MGH semiannual facility inspection cycle.

The MGH IACUC may, at any time temporarily or permanently suspend their reliance on another institution's animal care and use program if regulatory violations are discovered or if animal welfare is determined to be unreasonably compromised. The MGH Institutional Official will be notified of any suspensions. If an MGH protocol is also suspended in concert with this action, regulatory authorities and AAALAC will be similarly notified, per standard MGH IACUC procedure and policy (Policy on Reporting Animal Welfare Concerns).

Animals housed at off-site collaborating institutions that need to be transferred to the MGH may require the same procedures as animals imported from non-approved sources, including quarantine and health assessment, before they can be placed in MGH animal housing areas. The Attending Veterinarian will determine the necessary procedures in such cases.

REFERENCES

Beaupre SJ, Jacobson ER, Lillywhite HB, and Zamudio, K. Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research (viewable at Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research, 2nd edition. American Society of Ichthyologists and Herpetologists: Lawrence, KS, 2004.

Fair JM, Paul E, Jones J, Clark AB, Davie C. and Kaiser G. Guidelines to the Use of Wild Birds in Research, The Ornithological Council: Washington DC, 2010.

National Research Council of the National Academies. Guide for the Care and Use of Laboratory Animals, 8th edition*. National Academies Press: Washington, D.C., 2011.*

Nickum, JG, Bart HL, Bowser PR, Greer IE, Hubbs C, Jenkins JA, MacMillan JRm Rachlin JW, Rose JD, Sorensen PW, and Tomasso JR. Guidelines for the Use of Fishes in Research. American Fisheries Society: Bethesda, MD, 2004

Sikes RS, Gannon WL, and the Animal Care and Use Committee of the American Society of Mammologists. Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research. *Journal of Mammology*. 2011, 92(1):235-253.

U.S. Department of Agriculture. *Animal Welfare Act and Regulations* (Animal Care Blue Book). Code of Federal Regulations (CFR), Title 9, Chapter 1, Subchapter A, Parts 1-4. 2020.

v1.1, 16 February 2005
v1.2, 20 September 2017
v2.1, 16 June 2021

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
POLICY ON TRANSPORTATION OF ANIMALS

GENERAL POLICY

All animal transportation within an MGH animal facility, between MGH animal facilities, and to and from MGH must comply with federal, state, and local laws and regulations and with current accreditation standards. A Transportation form should be included in the IACUC protocol if live animals will be transported to facilities outside of MGH or if live animals will be moved within or between MGH facilities, including between housing facilities and laboratory or imaging areas.

Transportation of research animals within MGH buildings must be done in a discreet and protected manner, to minimize contact between the animal and non-research staff, patients, and the public. All animals must be enclosed completely in a container or covered while on a stable transport device approved by the Director of the Center for Comparative Medicine (CCM) or their designee. Contact the CCM facility manager to discuss appropriate methods of transportation. Larger species (e.g., dogs, livestock, nonhuman primates) and smaller yet possibly vocal species (e.g., guinea pigs, rabbits) must be transported in a manner that minimizes apparent sound or motion and strong consideration should be given to using sedation to facilitate transport within MGH buildings. Transport of NHPs outside of CCM facilities must be conducted using CCM transport boxes or the PI/designee must provide CCM with a plan for transport to the laboratory destination that minimizes NHP exposure to other MGRI personnel.

Transportation of research animals between MGH campuses and to and from other destinations outside MGH should be captured in the approved IACUC protocol and be consistent with the CCM transport process. A request for CCM animal transportation services can be made by submitting an Animal Transfer Form to the CCM Transfer Import Export Office (ccmtie@partners.org). In cases involving frequent transport of larger species for training or imaging sessions, research staff may perform such transportation using a CCM vehicle only after clearance has been obtained and proper training has been completed. Information on CCM transportation van use by non-CCM staff can be found on the Animal Ordering & Transfers page of the CCM website under Live Animal and Equipment Transportation. Private vehicles, public transit, or MGB shuttles must never be used to transport live animals. Transports that require the use of controlled substances for sedation must follow the process outlined in Appendix A of the Policy on Use of Controlled Substances in Non-Human Research.

REFERENCES

Policy on Use of Controlled Substances in Non-Human Research - Appendix A

v1.1, February 2005
v1.2, May 2006
v1.3, 15 September 2021
v1.4, 16 February 2022

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON ANESTHESIA AND ANALGESIA

GENERAL POLICY

The Animal Welfare Act and Regulations and the Public Health Service Policy on Humane Care and Use of Laboratory Animals require the use of appropriate sedation, analgesia, and anesthesia for procedures that might cause more than momentary or slight pain or distress, unless scientifically justified and approved by the IACUC. Anesthetic and analgesic regimens appropriate to the species and procedure will be developed in consultation with Center for Comparative Medicine (CCM) veterinarians and must be approved by the IACUC. Species-specific recommendations can be found on the Veterinary Guidance page of the CCM website (https://mghresearch.partners.org/ccm/).

The IACUC requires that any person administering and/or monitoring anesthesia must be listed in the approved protocol. The Principal Investigator (PI) is responsible for ensuring that all staff administering and monitoring anesthesia are qualified to perform the intended activities on the species covered in the protocol. Experience and training may be obtained from the PI, through the CCM, from relevant outside training opportunities, or other experienced third-party whose proficiency is documented on their own protocol or equivalent. Staff who provide anesthesia for non-rodent species may have their proficiency assessed periodically by IACUC-designated personnel as described in the Perioperative/Periprocedural Support section of the Policy on Surgery and Other Experimental Procedures.

INVESTIGATOR RESPONSIBILITIES: USDA-COVERED NON-RODENT SPECIES

1. For surgical manipulations that are expected to last longer than 20 minutes, at least one person, exclusive of the person(s) performing the actual procedure on the animal subject, must be present during the entire procedure to provide anesthetic and peri-operative support.

2. Anesthetic administration, monitoring, and documentation must be followed as described in the IACUC-approved protocol. The appropriate physiological parameters (e.g., heart rate, respiratory rate, body temperature) must be monitored and documented every 15 minutes, or more frequently, as stated in the protocol. The documentation requirement may be satisfied by manually recording the information on a Animal Anesthesia Monitoring Form, or by manual notation or tagging of a continuous print-out generated by monitoring equipment.

3. The animal's body temperature should be supported periprocedurally by applying external heat sources, such as commercially available warming devices (e.g., a water-circulating heating pad or Bair Hugger) and/or by administering warmed intravenous

solutions. Hot water bottles and radiant heat may also be used if the animal is protected from burn injury

4. The anesthetist and another member of the investigative team must remain with the animal until extubation. Following extubation, the animal must be attended continuously by the anesthetist and/or another member of the investigative team until it can maintain sternal recumbancy (quadrupeds) or sit upright (nonhuman primates), is hemodynamically stable, and exhibits good respiratory exchange. Documentation of complete recovery from anesthesia should be made in the Individual Animal Medical Record (IAMR). Alternatively, recovery can be recorded in the anesthetic record or the operative note. For additional information and guidelines concerning post-procedural care, please refer to the Policy on Post-Operative and Post-Procedural Care .

5. Appropriate analgesia must be provided as described in the IACUC-approved protocol, and should be determined by the nature of the procedure and in consultation with the facility veterinarian. The American College of Laboratory Animal Medicine (ACLAM) recommends that analgesics be administered pre-procedurally to provide maximal pain relief, unless contra-indicated for the particular study. Additional information is available on the CCM website (https://mghresearch.partners.org/veterinary-information/) or by contacting the facility veterinarian (https://mghresearch.partners.org/about-ccm/).

6. All treatments must be noted in the Individual Animal Medical Record (IAMR).

7. If the approved pain management regimen is determined to be inadequate, the attending veterinarian (AV) or their designee must be contacted for consultation. .

8. Unrelieved pain and/or distress must always be scientifically justified in the protocol and approved by the IACUC.

INVESTIGATOR RESPONSIBILITIES: RODENTS

1. Anesthetic administration and monitoring of anesthetic depth must be followed as described in the IACUC-approved protocol. While dedicated personnel and documentation of anesthetic monitoring are not required for rodents, a member of the investigative team must monitor the animal continuously while it is under anesthesia. During the recovery period, the animal must be attended continuously until it is ambulatory, hemodynamically stable, and exhibits good respiratory exchange. The animal's body temperature should be supported periprocedurally by applying external heat sources, such as a water-circulating heating pad. The anesthetic event must be recorded on the Rodent Record Card (for mice and rats bred for research) or in the IAMR (for rodents other than mice and rats bred for research).

2. The use of bell jars or open-drop exposure for rodent anesthesia is prohibited unless scientifically justified in the protocol. If approved, the bell jar must be contained in an

appropriate chemical fume hood and have the means to separate the animal from contact with the anesthetic agent.

3. Appropriate analgesia must be provided as described in the IACUC-approved protocol. The American College of Laboratory Animal Medicine (ACLAM) recommends that analgesics be administered pre-procedurally to provide maximal pain relief, unless contra-indicated for the particular study. All analgesic administration must be recorded on the Rodent Record Card (for mice and rats bred for research) or in the IAMR (for rodents other than mice and rats bred for research). Additional information is available on the CCM website (https://mghresearch.partners.org/veterinary-information/) or by contacting the facility veterinarian (https://mghresearch.partners.org/about-ccm/).

4. Unrelieved pain and/or distress must always be scientifically justified and approved by the IACUC.

INVESTIGATOR RESPONSIBILITIES: NON- MAMMALS (fish, frogs, reptiles, birds)

Anesthesia and analgesia must be provided as described in the IACUC-approved protocol. All administration of anesthesia and analgesia must be documented on the "Rodent Record Card" or equivalent, in the laboratory notebook, or other method.

CCM RESPONSIBILITIES

The Director, CCM, and their designees are responsible for providing guidance and consultation to investigators on the appropriate use of anesthetics and analgesics in order to minimize pain and distress.

USE OF ETHER ANESTHESIA

The MGH IACUC does not permit the general use of ether for animal anesthesia due to the dangers of storage and handling of this volatile agent and the inability to deliver it in a safe and controlled manner to the animals. Any exceptions to this policy must present strong scientific justification for the use of ether anesthesia in an IACUC protocol. Acquisition, storage, and use of ether must conform to the policies and procedures for hazardous agents outlined by the MGH Safety Office.

RELATED POLICIES

Policy on Animal Observation and Record Keeping
Policy on Post-Operative and Post-Procedural Care
Policy on Surgery and Other Experimental Procedures

REFERENCES

American College of Laboratory Animal Medicine. ACLAM Position Statement on Pain and Distress in Research Animals. 2016.

U.S. Department of Agriculture. Animal Welfare Act and Regulations (Animal Care Blue Book). Code of Federal Regulations (CFR), Title 9, Chapter 1, Subchapter A, Parts 1-4. 2020.

U.S. Department of Health and Human Services. Public Health Service Policy on Humane Care and Use of Laboratory Animals. Office of Laboratory Animal Welfare, National Institutes of Health: Bethesda, MD, 2015.

v1.1, 20 September 2006
v1.2, 4 October 2011
v1.3, 29 April 2016
v1.4, 17 May 2017
v1.5, 16 August 2017
v1.6, 16 June 2021

MASSACHUSETTS GENERAL HOSPITAL
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON USE OF ADJUVANTS IN RESEARCH ANIMALS

GENERAL POLICY

This policy describes the acceptable use of adjuvants in research animals housed within MGH facilities. Adjuvants are vehicles employed to enhance the immune response of specific immunogens, which are rarely sufficient to induce a satisfactory antigenic response alone. They work through a number of mechanisms serving as antigen-depot-forming substances, as delivery vehicles or inert carriers, as immunostimulators or modifiers, or any combination of these. In addition, the source of the antigen preparation must be considered. Many immunogens are identified and isolated from polyacrylamide gels, which alone is inflammatory and has adjuvant properties. Ideally, the immunogen should be eluted from the gel before immunization. If this is not possible, the gel should be trimmed so that the least amount of gel is administered. Lastly, preparation of antigens for injection in aqueous solution should be performed aseptically such that contaminants (i.e. unwanted toxins, pyrogens, unintended bacteria and other pathogens) are eliminated and the pH of the injection solution adjusted to within physiological limits.

Adjuvant Selection

Many adjuvants can cause moderate to severe inflammatory responses at the site of administration. The Principal Investigator should first consider whether an adjuvant is actually required to induce the desired immune response. Highly aggregated antigens are likely to induce the appropriate immune response without the aid of an adjuvant. Soluble, relatively pure small molecule antigen preparations are less likely to induce an adequate response alone thereby justifying the need for adjuvant-mediated enhancement. Adjuvant type and administration site should be selected based on three criteria: 1) adequate antigenic response; 2) least amount of inflammatory response at the administration site; 3) best site for minimally affecting the normal posture and movement of the animal.

Non-inflammatory adjuvants such as aluminum compounds and subcutaneous implanted chambers should be considered first since they cause less inflammation. Well-developed alternative adjuvants commonly used in immunology studies include the RIBI adjuvant system (oil-in-water emulsion), Titermax (copolymer water-in-oil emulsion), and Montanide ISA Adjuvant (oil/surfactant-based). Water-in-oil emulsions such as Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA) will induce more severe inflammation, should be used only when alternatives do not elicit an acceptable immune response, and require a documented literature search for alternatives in the protocol.

Administration Considerations

In addition to selection of the appropriate adjuvant, the administration site must be considered so that exposure to the least amount of pain/distress is possible. Injection sites should be aseptically prepared (clipped of hair, surgically scrubbed and allowed to dry) prior to antigen-adjuvant administration. Compounds can be administered intravenously (preferably small particulate antigens), subcutaneously (preferred for CFA), or intradermally; intramuscular injection is discouraged. Intraperitoneal immunization is permissible only in mice as a single immunization and scientifically justified if CFA is used. Footpad injections using water-in-oil adjuvants (CFA and/or IFA) are particularly painful/distressful and will only be approved when it is documented that there are no other acceptable methods. Because antigens injected into the footpad are processed by the popliteal lymph node, injections made at the tail base or in the area of the popliteal node are a more humane alternative. Aerosol, oral, and intranasal routes are also utilized when an IgA response is desired.

When administering compounds subcutaneously or intradermally, injecting small volumes into multiple injection sites is more beneficial than injection of larger volumes in fewer sites, from both a humane and scientific perspective. Care should be taken that there is adequate separation between injection sites to avoid the coalescing of inflammatory lesions that could lead to tissue sloughing or abscess formation. Booster immunizations may be given to maintain adequate antibody levels long-term. Frequency of booster immunization should be based upon the time required for the animal to process the immunogen and should be given when the titer has peaked and started to decrease. Booster injections containing antigens such as bacteria, virions or cells may be given intravenously as long as they are not likely to cause anaphylaxis; booster injections involving CFA must be scientifically justified. Soluble antigens with a higher risk of causing anaphylaxis should be administered subcutaneously. Booster injections should be given at sites different from the primary immunizations whenever possible. Animals should not be boosted if adverse reactions were noted during a prior immunization.

Monitoring Requirements

Animals used in antibody production need to be monitored closely after administration of primary and booster adjuvant-antigen injections. Anaphylaxis is a common adverse effect that will determine whether further injections should be performed. Animals should be monitored immediately after injection, one hour later and then 2-3 times a day for the first two days post-injection. Daily observations are necessary to ensure that the approved protocol is followed and to ensure the welfare of the animals. In many cases it is appropriate to administer analgesics to animals in immunization studies. These should be given as stated in the approved protocol. It is generally recommended that analgesics are administered prophylatically to minimize the pain/distress. If animals exhibit pain/distress during the study (e.g., not ambulating normally, not eating, depressed activity, self-trauma), then additional analgesics should be provided or the animal should be euthanized. Consultation with veterinarians from the Center for Comparative Medicine (CCM) is recommended.

Post-procedural monitoring and treatments must be documented on the Individual Animal Medical Record, the Rodent Record Card, laboratory notebook or other method in accordance with the Policy on Animal Observation and Record Keeping.

Blood collection for antibody harvest is a critical aspect of the immunization process. The goal of collection is to obtain a suitable volume of undamaged blood while minimizing adverse physiologic effects on the animal. Please refer to the IACUC Policy on Blood Collection for more information.

If investigators are unfamiliar with any of these techniques, they must contact CCM to schedule training or to request veterinary technical services to perform these procedures.

Related Policies:
Policy on Animal Observation and Record Keeping

[Most recently revised October, 2016]

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

POLICY ON BLOOD COLLECTION

GENERAL POLICY

The volume of blood removed, and the time course over which that blood is removed, are critical factors in determining acceptable blood collection regimens that minimize the effect of blood loss on the animal.

The volume of blood that can be collected without serious side effects is determined by the animal's total blood volume. Most healthy animals can tolerate losing up to15% of their total blood volume over a two-week period. Collection of volumes within this range along with good hemostasis will minimize hypovolemia and allow the animal to regenerate adequate numbers of erythrocytes before the next collection.

If the amount taken exceeds 15% of the animal's total blood volume, additional justification is required. This may include a review of the literature documenting that adverse effects were not found when greater volumes were collected in this specific species and strain, under the same experimental conditions. Alternatively, a scientific justification may require higher volumes but include the provision of additional veterinary care, including close monitoring for signs of pain/distress, hematocrit (packed cell volume) measurements, and specific supportive care (e.g. provision of fluid replacement and/or nutritional support). A CCM veterinarian should be consulted for specific recommendations.

Additional factors to consider in minimizing effects of blood loss include age, health status, genetic traits, and nutritional restrictions (e.g., low iron diet) or other components of the protocol (e.g., irradiation or pharmacological treatment) that may retard hematopoiesis. Such factors may require that less blood is taken at longer intervals than stated above or that parenteral fluids may be indicated immediately following blood collection to minimize the consequences of blood collection on the animal's welfare.

Blood Collection Volume Calculation

The easiest way to determine the total blood volume for an animal is to follow the general principle that total blood volume is equivalent to 6% of the animal's body weight.

TOTAL BLOOD VOLUME (mls) = BODY WEIGHT (grams) X 0.06

For example, if 10 blood collection timepoints in a 300-gram rat were needed over a 24-hour period with 250 µL of blood to be taken per timepoint, the equation would be:

10 X 250 µL = 2500 µL or 2.5 ml blood needed

300 gms X 0.06 = 18 mL total blood volume

2.5/18 X 100 = 13.9% of total blood volume (acceptable amount)

For larger species, the same formula can be provided as:

TOTAL BLOOD VOLUME (liters) = BODY WEIGHT (kilograms) X 0.06

Blood Collection Sites

Recommendations for species specific blood collection sites are located on the IACUC website (https://mghresearch.partners.org/iacuc-policies/).

Depending on the frequency of blood collection, species, volume and duration, additional methods such as surgical cannulation may be an option for blood sampling. Investigators should consult with a CCM veterinarian for additional guidance and training.

v1.1, December 2004
v1.2, December 2005
v1.3, 17 September 2014
v1.4, 19 April 2017
v1.5, 16 December 2020

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON EUTHANASIA

GENERAL POLICY

This policy provides guidance, and the most commonly used and approved methods, for performing euthanasia of laboratory animals used in research at Massachusetts General Hospital (MGH).

Euthanasia is the act of inducing humane death in an animal by a method that induces rapid loss of consciousness and death with a minimum of pain, discomfort or distress. All euthanasia procedures performed within MGH animal facilities must be consistent with the recommendations outlined in the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition.

Euthanasia is performed as required by protocol study endpoints, to relieve pain/distress and suffering from experimental manipulations or spontaneous conditions, and as appropriate in other situations deemed necessary by a CCM veterinarian. Animals that are experiencing undue pain/distress must be euthanized humanely unless these conditions are required for scientific objectives that have been documented and justified in a study protocol and approved by the IACUC.

It is important for researchers to delineate criteria for euthanasia in the IACUC protocol, including measurable physiological parameters and observable signs indicative of pain and distress. Euthanasia methods must be indicated, including the trained staff who will be responsible for observations and euthanasia procedures. CCM veterinary services should be consulted if needed to demonstrate and/or discuss these techniques.

Distress vocalizations, fearful behavior, and release of pheromones by a frightened animal can all cause anxiety and apprehension in other animals. Therefore, in general, animals should not be euthanized in the animal housing room/area to minimize stress on the remaining animals. Exceptions may occur given scientific justification (e.g., to prevent spread of infectious disease), for emergency euthanasia when an animal cannot be readily moved, or situations that do not induce stress in the remaining animals (e.g., complete microisolator caging systems for housing rodents and thus will not be exposed to the effects mentioned above).

Any animal found to be “near death” (moribund) must be immediately addressed. Rodents found moribund by CCM staff will be euthanized in accordance with CCM SOPs and the Rodent Health Concern Booklet. For species other than rodents, when an animal is found moribund, attempts will be made to contact the Principal Investigator (PI) or specified contact person for that study. If the PI or contact person is unavailable, an agreement cannot be reached, or a delay will result in further distress to the animal, the CCM veterinarian has full authority by the IACUC and the Attending Veterinarian (AV) to euthanize the animal. No animal should ever be left in a moribund state.

Regardless of the technique used, all animals must be evaluated following euthanasia to confirm that they are dead. This includes observing or palpating the absence of a heartbeat and respiration. For rodents, an approved secondary physical method such as bilateral thoracotomy, cervical dislocation, exsanguination, or decapitation can also be used to confirm death. Animals may have absence of respiration but still have a heartbeat and recover fully. For large animals, adjunct vital signs used to assess death include fixed

and dilated pupils. Confirmation of death is required because most euthanasia agents may also induce deep anesthesia that the animal can recover from if not administered properly. Failure to ensure death of animals after euthanasia procedures (e.g., failed euthanasia with CO_2) is a noncompliant event that must be reported to the regulatory agencies overseeing laboratory animal research.

All personnel performing animal euthanasia must be trained, knowledgeable, and proficient in the chosen techniques and verification of death. PIs are responsible for ensuring that their staff are trained in the relevant euthanasia method. Trained research staff are encouraged to euthanize their own animals whenever possible. CCM staff can euthanize rodents for research staff as a service.

If there are any questions about euthanasia or if proper equipment/drugs cannot be found, the CCM facility veterinarian or the On-Call veterinarian (if after hours, weekends or holidays) should be contacted immediately.

Carcasses must be disposed of immediately after euthanasia has been confirmed. Carcasses should be placed in a carcass bag, then should be placed in walk-in coolers, refrigerators, or chest freezers specified for this purpose in CCM animal facilities or designated area in the laboratory.

EUTHANASIA METHODOLOGY GUIDELINES:

(For doses, refer to the species-specific Insight form or consult with your facility veterinarian.)

Adult Animals

A. Carbon Dioxide (CO_2) Asphyxiation using Compressed Gas
   1. **Dry ice is not an acceptable source of CO_2**
   2. Carbon dioxide must be supplied in a precisely regulated manner and in a purified form without contaminants or adulterants, typically from a commercially supplied cylinder or tank.
   3. CO_2 euthanasia stations and portable units are located in the CCM animal facilities with specific instructions posted in these areas
   4. Prefilled chambers are unacceptable
   5. CO_2 delivery must be maintained for at least one minute after respiration ceases
      a. Rodents (mice, rats, hamsters, guinea pigs, gerbils), avian
         i. Use CO_2 flow rate that displaces 30-70% cage volume/minute.
         ii. Euthanasia in the home cage is urged whenever possible to minimize stress (e.g., when euthanizing some but not all mice in a cage, keep the mice to be euthanized in the home cage and move the remaining mice to a clean cage).
         iii. Rodents from different cages should not be combined.
         iv. Rodent cages must not be overcrowded (follow the IACUC Policy on Rodent Breeding and Cage Density)
      b. Rabbits < 2 kg
         i. Use a CO_2 flow rate that displaces 50-60% cage volume/minute
         ii. Sedation is strongly recommended prior to exposure to CO_2

B. Barbiturates/Sodium Pentobarbital/Pentobarbital Combinations (e.g., Euthasol)

1. Categorized as DEA Schedule II and/or III Controlled Substances and must be stored in a double-locked cabinet.
2. Dosages should be 2-3 times the appropriate anesthetic dose
3. Routes of administration should ensure rapid circulation
    a. Intraperitoneal: mice, rats, hamsters, guinea pigs. May administer IP in rabbits, sedation/anesthesia recommended.
    b. Intravenous: rabbits, dogs, swine, small ruminants, nonhuman primates, avian
    c. Intracoelomic: amphibians
    d. Intracardiac (under general anesthesia only): rabbits, dogs, swine, small ruminants, nonhuman primates, avian, ferrets

C. Dissociative Agent Combinations
1. Overdose used in rodents, rabbits
2. Ketamine must be used in combination with an alpha-adrenergic agonist (e.g., ketamine/xylazine anesthetic overdose)
3. Routes of administration:
    a. Intraperitoneal: mice, rats, hamsters, rabbits, guinea pigs
    b. Intravenous: rabbits

D. Inhalant Anesthetic (such as isoflurane)
1. Use of precision vaporizer is required. Bell jars are prohibited.
2. Adequate ventilation and scavenging must be provided to ensure personnel safety
3. May be used as overdose in rodents and avian species or as general anesthetic followed by non-survival surgery or secondary physical method (e.g., exsanguination) in rodents, rabbits, nonhuman primates, small ruminants, swine, dogs

E. Cervical Dislocation
1. Can be used as a secondary physical method to ensure death in anesthetized rodents < 200 grams and small birds.

2. To use as primary euthanasia means in non-anesthetized animals (rodents < 200 grams, small birds):
    a. Must be scientifically justified in IACUC protocol
    b. Personnel should be trained on anesthetized and/or dead animals
    c. Personnel must demonstrate technical proficiency
    d. Those responsible for the use of this method must ensure that personnel performing cervical dislocation have been properly trained and consistently apply it humanely and effectively.

F. Decapitation with Guillotine or Scissors
1. Can be used as a secondary physical method to ensure death in anesthetized rodents and small birds.
2. To use as primary euthanasia means in non-anesthetized animals (rodents, small birds):
    a. Must be scientifically justified in IACUC protocol
    b. Personnel should be trained on anesthetized and/or dead animals

c. Personnel must demonstrate technical proficiency to whom?
d. Those responsible for the use of this method must ensure that personnel performing decapitation have been properly trained and consistently apply it humanely and effectively.
e. Guillotine or decapitation scissors must be in good condition, kept clean and with sharp blades. Guillotines should be maintained as recommended by the manufacturer.
f. Use of restraint methods, such as decapicones, is recommended for either guillotine or scissors methods.

G. Adjunctive Methods (Used Only in Anesthetized Animals)
1. It is unacceptable to perform these methods in a conscious animal
2. Animal must demonstrate Stage III anesthesia (surgical plane of anesthesia defined as loss of consciousness, loss of reflex muscle response, and loss of response to noxious stimuli) before performing.
3. Rodents, rabbits, dogs, swine, small ruminants, nonhuman primates
   a. Exsanguination:
      i. Collection of large volumes of blood (≥ 50% of total blood volume)
   b. Potassium Chloride:
      i. Intravenous or intracardiac route of administration to induce cardiac arrest
   c. Other Methods: Chemical perfusion, bilateral thoracotomy (pneumothorax), removal of vital organs (non-survival surgery)

H. Tricaine methanesulfonate (MS 222)
1. Used in amphibians and fish
2. Appropriate safety precautions should be taken when working with powder or concentrate
3. A 10 gram/liter stock solution can be made and sodium bicarbonate added to saturation, resulting in a pH between 7.0 and 7.5 for the solution
4. Stock solution must be protected from light and refrigerated or frozen if possible. Labelling and storage should be performed in accordance with the IACUC Labeling Guidelines and Recommended Expiration Dates for Research Drugs and Agents
   a. Amphibian dose: 5 grams/liter.
   b. Fish dose: 0.5 grams/liter
      Note: For zebrafish, 30 minutes exposure time following loss of rhythmic opercular movement is required.

Fetuses (mice and rats):

Up to and including 14 days of gestation:	Because the neural development is minimal, pain perception in the fetus is considered unlikely. Euthanasia of the mother or removal of the fetus should ensure rapid death due to loss of blood supply and non-viability at this stage of development.
15 days - birth:	Possibility of pain perception documented in fetuses of this age; perinates are not sensitive to inhalants therefore chemical anesthesia (administered i.p.) is recommended. Decapitation, cervical dislocation, or rapid freezing are acceptable but may be aesthetically unpleasant. For fetuses that require chemical fixation, anesthesia such as hypothermia or a deep anesthesia of the mother with pentobarbital (which crosses the placenta) should be done prior to immersion or perfusion.

Neonates (mice and rats):

Birth – 10 days old (mice): Birth – 7 days old (rats)	Acceptable methods include IP pentobarbital or dissociative anesthetic overdose, decapitation, or cervical dislocation. Neonates can be exposed to inhalant gases or CO_2, but exposure times are extremely lengthy (e.g., up to 50 minutes in CO_2 for mice) and a secondary physical method must be used to assure death after no response is found to painful stimuli. For neonates requiring chemical fixation, anesthesia is required and after loss to painful stimuli determined, then neonates may be chemically fixed or perfused. Neonates $\leq$ 6 days old may be anesthetized using hypothermia (gradual cooling to 4 °C), followed by a secondary physical method after loss of movement.
Older than 10 days (mice): Older than 7 days (rats):	Follow guidelines for adults.

REFERENCES

American Veterinary Medical Association Panel on Euthanasia. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. American Veterinary Medical Association: Schaumburg, IL, 2020.

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National Research Council of the National Academies. Recognition and Alleviation of Pain in Laboratory Animals. *National Academies Press: Washington, D.C., 2009.*

National Research Council of the National Academies. Recognition and Alleviation of Distress in Laboratory Animals. *National Academies Press: Washington, D.C., 2008.*

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v2.1, July 2013
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v2.2, 20 September 2020
v2.3, 15 September 2021
v2.4, 16 February 2022

Policy on Controlled Food and Fluid Intake in Laboratory Animals

General Policy

Some research projects require the regulation of food or water to achieve the desired experimental results. The objective of any study using controlled access should use the minimum regulation necessary to achieve the scientific objective while maintaining animal well-being. The restriction must be scientifically justified and approved by the IACUC. Experimental procedures utilizing food or water restriction must include a program for monitoring of physiologic and behavioral indexes, including criteria (such as weight loss or state of hydration) for temporary or permanent removal of an animal from food or fluid control.

This policy outlines how animals housed at MGH on controlled food or fluid should be managed and monitored. It outlines specific guidelines that when followed, should facilitate the protocol review and approval. Any exceptions to this policy must be appropriately justified.

Definitions

Food or Fluid Controlled: An animal that receives anything other than *ad libitum* access to food and fluid are considered controlled.

Scheduled Access: Animals are permitted access to food or fluid sources at regular intervals so that those animals may consume as much as desired.

Restriction: The total volume of food or fluid consumed is strictly controlled and monitored.

Fasting: Withholding of food for a defined period prior to induction of anesthesia or sedation. The length of this period will vary by species. Unless otherwise described and justified, water is always provided.

Standard pre-anesthetic fasting does not need to be specifically described on a controlled food and fluid intake form. For those species capable of vomiting, such fasting is accounted for on the specific procedure form (usually 12-18 hours for monogastric animals and 12-24 hours for ruminants). Note that some species such as rodents (mice and rats) and rabbits must *not* be fasted unless it is specifically described and justified in the protocol.

Protocol Requirements

All protocols that utilize controlled food or fluid access are required to have a "Controlled Food or Fluid Intake" form. All aspects of the control must be described on this form. This includes but is not limited to the following:

- All variations to the standard provisions of *ad libitum* food and water must be described in the protocol and be scientifically justified.
- Alternatives that would facilitate the desired outcome, such as positive reward systems, *without* the need for food/fluid restrictions should be discussed. Explanations as to why such positive reward systems cannot be used must be provided.
- The least restriction that will achieve the scientific objective should be used.
- Include a detailed description of the times at which animals will have access to food and fluid and at what amounts (e.g., if there are "working" and "non-working" periods). This may range from hours to chronic scheduled restriction. The maximum period of restriction must clearly be stated.
- Detail the monitoring schedule – how often animals are checked. This includes the frequency with which animals are weighed.
- The specific criteria that result in cessation of controlled food/fluid access and a return to *ad libitum* access must be provided.
- In the event of termination of controlled access, any additional supportive care (e.g., intravenous or subcutaneous fluid support, provision of hydrogels) that are given must be described as well.
- The inclusion of citations/references is strongly encouraged in order to support the planned controlled intake program.
- Controlled access to food and fluid may impact the USDA pain and distress categorization. Investigators are encouraged to review the IACUC guidelines for assigning categories and consult with their facility vet or members of the IACUC to aid in this aspect of protocol development.

Other General Considerations:

- Sections of the protocol where amounts of fluid access are described should be provided in terms of mL/kg/day or % reduction compared to baseline.
- Efforts should be made to match the eating and drinking schedule of animals with species-specific eating and drinking habits (e.g., rodents preferentially eat and drink at night).
- Baseline intake of either food or fluid should be obtained whenever possible prior to initiating controlled access. This is due to normal variations based upon age, gender, health status, stain, etc.
- Investigators must ensure foods and fluids managed by the lab are not expired and that any effective component in the fluid/food is clearly described in the animal medical record or the rodent record card.

- Similar to fluid restriction, controlled food intake may be based upon weight of a complete diet or total energy consumption.

Types of Controlled Food or Fluid Access

Scheduled Access

Scheduled access to food or fluid involves the withholding of food and/or fluid for specific short periods of time. Outside of these periods, animals may consume as much food or fluid as they wish. The brief period of withholding is used when animals need to perform specific tasks during a set period of the day. Given animals are permitted *ad libitum* access to food and fluid outside of the brief scheduled period, animals should not lose weight as compared to age-matched controls.

Restricted Food or Fluid Access

Animals receiving less than *ad libitum* food and fluids are considered food or fluid "restricted." Contrary to animals under scheduled access, animals that are restricted may be expected to lose some amount of weight.

The criteria outlined above in terms of the content of the protocol, schedule of control and monitoring, etc. must be detailed. In addition, there are other general considerations that should be included in the protocol:

- Animals on food or fluid restriction must be monitored daily and all observations must be documented in such a way that CCM staff may access the records at any time. Investigators must coordinate with the appropriate CCM facility manager and veterinarian to agree upon how animals on restriction will be identified in the facilities and how documentation will be provided. This method must be described in the protocol.
- Animals undergoing restriction must be readily identifiable (e.g., rodent special husbandry cards)
- Chronic restriction should not be abrupt; animals should be acclimated to restriction. Investigators should plan to progressively reduce available food or water over a period time (typically seven to fourteen days) until the desired degree of restriction is attained.
- In general, animals should receive a nutritionally adequate diet. However, if animals are to have restricted access to specific nutrients in the food that would normally be present in a complete diet, justification for the restriction and a description of any anticipated detrimental effects must be provided.
- Research staff are responsible for monitoring animals under food/fluid restriction or regulation studies. They must be trained and competent to evaluate the animal clinical condition.

There are many clinical criteria that should be used as a basis for evaluating if alterations in schedule or volume of food/fluid access, or even removal from restriction, is necessary. The following is a list of criteria that is common to all species while on restriction (species-specific distinctions are described later in this document):

- Anorexia

- Body condition score
- Behavioral abnormalities such as increased aggression and/or depression
- Clinical indicators of dehydration such as delayed skin turgidity, sunken eyes, and dry/tacky mucus membranes
- Scant or excessively dry feces
- Oliguria/anuria (reduced or lack of urine production)

Species-Specific Considerations: Mice and Rats

General Considerations

- It is essential to obtain baseline weights within 48 hours prior to initiating any controlled intake. If practicable, baseline daily water intake amounts may be obtained.
- Weight checks should occur regularly, generally not less than twice weekly for animals that are restricted. The frequency, however, is dependent upon the specific nature of the project and more frequent checks may be required.
- The standard endpoint for body condition score is 2 out of 5. If a lower BCS is required, it must be scientifically justified.
- Young or growing animals (<14 weeks) are especially sensitive to fluid restriction and malnutrition. Health and minimum growth requirements must be met.
- In general, fluid volumes below 40 mL/kg/day/mouse or 50% of the normal intake for rodents are not recommended and the protocol must provide specific justification that less restriction is not scientifically acceptable.
- In general, once a rodent attains 15% weight loss since initiation of controlled access compared to age- and gender-matched controls (or compared to vendor growth charts), that animal should be provided *ad libitum* access.
    - These animals should then be weighed daily and may not undergo additional restriction until they have achieved 95% of their weight prior to initiation of restriction or 95% of the weight of age-matched controls
    - If additional supportive care other than return to *ad libitum* access (e.g., administration of subcutaneous fluids) is to be provided, those details should be included in the protocol.
    - Any animals that continue to lose weight despite being provided *ad libitum* food and water must be euthanized.

Anesthetic and Surgical Procedures

If rodents are to undergo surgery prior to starting any controlled food/fluid access, then those animals must meet *both* of the following criteria before food/fluid limitations begin:

1. At least one week has passed since the procedure and animals have received food and water ad libitum during this time.
2. The animal has regained any weight loss that may have resulted from the procedure

If a protocol stipulates that animals that are already currently on controlled food/fluid access require an anesthetic procedure, then those animals must be offered *ad libitum* access to food and fluid for 24 hours prior to anesthesia and 48 hours after. If that anesthesia involves a surgical procedure, then the aforementioned two criteria must be met before animals may resume controlled food/fluid access. Short non-surgical procedures do not require ad lib access to water. Exceptions to this must be justified.

Species-Specific Considerations: Non-human Primates (NHPs)

Prior to Restriction

- Baseline serum chemistry, behavioral parameters, body weight and body condition must be collected and documented within a 3-month period prior to initiation of fluid control.
- Body weights of young growing monkeys need to be monitored every 6 months until mature.
- For fluid-restricted animals, baseline water consumption must be determined. This may be defined by one of two methods. The preferred method involves recording the daily amount of fluid that the animal consumes on an ad libitum basis for two weeks. The second method involves calculating the normal daily fluid consumption using the formula 40 mL fluid/kg body weight.
- In general, fluid volumes below 40 mL/kg body weight/day are not recommended and must have documented scientific justification that higher volumes are not acceptable
- Water content for dietary supplementations such as fruit should not be subtracted from the ration of fluids to be given.
- The investigator must arrange for a veterinarian to perform a complete physical examination

During Restriction

The investigator should be prepared to consider and address a range of behavioral, environmental, or equipment-related variables that might hinder training or disrupt performance. Personnel newly involved in fluid restricted studies should be particularly sensitive to the fact that problems in training or maintaining a fluid-motivated animal often do not mean that the restriction is not strict enough and other types of variables should be considered first. Fluid regulation in NHPs will often result in reduced appetite for dry diets (e.g., biscuits), therefore fluids should be provided during mealtimes to encourage

consumption of food, thereby reducing body weight loss.

The following parameters are intended to assist the veterinarians in assessing the hydration status of animals and must be documented in the animal's medical record.

Working/Training Day Assessments

- Volume of fluids administered during working/testing
- Any additional supplemental fluids provided
- Behavior/ Performance on study
- Physical examination (i.e., skin turgidity, condition of the eyes, etc.)
- Amount of feed consumed
- Amount and character of feces

Other Regular Assessments

- Body weight (weekly)
- Urine specific gravity, ketone levels (obtained by free catch weekly)
- Serum chemistry and CBC including total protein, BUN, and creatinine (monthly)

Criteria for Termination of Restriction

A CCM Veterinarian will evaluate all animals on fluid control at least twice monthly to assure that the practices in place meet the expected outcomes approved by the IACUC. If abnormal findings are present, the CCM Veterinarian will meet with the Principal Investigator and designated Contact Person to discuss the animal's current clinical condition and working performance. Alterations in the working/training and restriction schedule should be considered to assure that the animal may remain a part of the study.

If changes in the working/training schedule do not address the abnormal health conditions of the animal, the animal must be removed from fluid control until other causes have been identified or until the animal reaches normal physiological status. In addition to the general criteria that apply to all animals described above, species-specific criteria that may be used in determining if alterations in schedule or volume are necessary include, but are not limited to:

- Weight loss of 10% in an adult animal (15% weight loss should lead to removal from restriction)
- Any weight loss in a juvenile animal expected to continue to maintain or add body weight because of natural growth phase
- Abnormal serum chemistry and/or CBC results (greater than 15% difference from normal)
- Ketonuria
- Urine specific gravity of greater than 1.035
- Veterinarian discretion

When fluid restriction is initially lifted, measured amounts of fluids should be offered to assure that the animal does not drink too rapidly and bloat. These amounts should be offered on at least 2 occasions separated by at least 3 hours for the first day followed by ad libitum fluids. These animals may also require more than the amount of normal daily fluid consumption as they replenish from restriction.

Anesthetic & Surgical Procedures

Animals scheduled for any type of procedure requiring anesthesia to a surgical plane must be offered ad libitum fluids for 24 hours prior to the anesthesia and at least 48 hours after. If the procedure is surgical, animals must be offered ad libitum fluid 48 hours prior and 2 weeks following surgery. Short procedures requiring chemical sedation (i.e., ketamine sedation) do not require ad lib access to water.

Other Species

If species not specifically discussed in this document are to undergo controlled food or fluid intake, the investigator should contact their facility veterinarian to discuss protocol development.

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1. Association of Primate Veterinarians. Guidelines for Fluid Regulation of Nonhuman Primates in Biomedical Research. *J Am Assoc Lab Anim Sci*. **2022**, 61(5), 397-402.
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Effective Date

V1.0, 1 December 2023

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON MONOCLONAL ANTIBODY PRODUCTION

GENERAL POLICY

The mouse ascites method of monoclonal antibody (MAb) production causes significant discomfort, distress, or pain to an animal. Practical *in vitro* methods exist that can replace the ascites method for many experimental applications without compromising the aims of a study. The IACUC critically evaluates the proposed use of the mouse ascites method as described in the **Adjuvants Use, Ascites/Antibody Production** form in each protocol. Prior to approval of protocols that include the ascites method, the IACUC must determine that (i) the proposed use is scientifically justified, (ii) methods that avoid or minimize discomfort, distress, and pain (including *in vitro* methods) have been considered, and (iii) the latter have been found unsuitable for scientific reasons (see Appendix 1 for examples). Justification based on financial costs or on the length of time required are not acceptable. The Principal Investigator (PI) is responsible for documenting in the protocol that alternative *in vitro* methods for production of the monoclonal antibodies of interest have been attempted and found to be unsuitable.

PROCEDURES

When the mouse ascites method is used for monoclonal antibody production, efforts to minimize pain or distress (i.e. frequent observation, limiting the number of abdominal taps, clinical criteria for euthanasia if signs of distress appear) must be described in the protocol.

The following guidelines must be followed:

- The first step in producing monoclonal antibodies *in vivo* is to prime the mice with an agent that facilitates implantation of the hybridoma cells to be injected and the subsequent development of ascites. The volume of primer used should be the minimum required to stimulate the necessary reaction. Recommended agents and volumes are: Pristane, 0.1-0.2 ml injected intraperitoneally, two weeks prior to inoculation of hybridoma cells, or Incomplete Freunds Adjuvant (IFA), 0.1 ml injected intraperitoneally, 24-48 hours prior to inoculation.

- Prior to use, hybridomas must be tested and demonstrated free of viruses and *Mycoplasma spp*. that could contaminate the animal colony and introduce unwanted variables. Hybridoma cell suspensions should be prepared aseptically.

- Following injection of hybridoma cells, animals must be monitored twice daily (including weekends and holidays) by the PI or his/her designee for signs of abdominal distension (e.g., abdomen becomes tight, body weight has increased 20% from baseline, activity impairment) at which time the abdominal pressure must be relieved by aseptic paracentesis (i.e., abdominal tap) to withdraw ascitic fluid and relieve intra-abdominal pressure.

- Once ascites develops, animals must be monitored daily, at minimum, for signs of distress. Signs of distress include, but are not limited to, rough hair coat, hunched posture, decreased activity, rapid shallow breathing, pallor, and decreased appetite or water consumption. Animals should be monitored for changes in body weight. Rapid weight loss may be a sign of distress; the weight measured after tap should be compared to animal's pre-inoculation weight as the pre-tap weight will be artificially elevated due to fluid retention.

- Animals may be tapped a maximum of three times, the third tap following euthanasia. Post-tap monitoring is critical to avoiding hypovolemic shock. Warm saline or lactated Ringer's solution may be administered subcutaneously at the time of the tap to avoid shock. If multiple taps are used, the animal should be tapped at intervals not to exceed 48 hours in duration in order to avoid excessive abdominal distension. The number of taps and interval between taps must be specified in the protocol.

Deviations from this policy must be scientifically justified and approved by the IACUC.

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Stills, H.F., Jr. Antigens, Antibodies, and Blood Collection. In *The IACUC Handbook*, 3rd edition; Silverman, J., Suckow, M.A., Murthy, S., Eds. CRC Press: Boca Raton, FL, 2014; pp 447-459.

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v1.3, 17 February 2021

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON ANIMAL OBSERVATION AND RECORD KEEPING

GENERAL POLICY

The well-being of research animals depends upon careful and frequent observation and monitoring of these animals by investigators and research staff, as well as by the veterinary staff. Every IACUC-approved protocol must include a plan for observation and monitoring of animals for signs of pain or distress based upon the research procedures performed on the animals and the possible effect of the procedures on animal health and well-being. Observation and monitoring is done by both the investigator/research staff and the veterinary staff, as dictated and described by both IACUC policies and by the specific research protocol.

The Animal Welfare Act Regulations (AWAR) and PHS policy require proper documentation of animal care and use to assess compliance with research protocols and clinical care procedures. This policy outlines the expectations with regards to appropriate and necessary record-keeping for research animals at MGH.

DEFINITIONS

USDA-covered species, non-rodents: Animal species that are taxonomically defined as lagamorphs or higher and covered by the Animal Welfare Act Regulations (AWAR), e.g., rabbit, cat, dog, ferret, sheep, goat, swine, non-human primate.

USDA-covered species, rodents: Animals that are taxonomically defined as rodents and covered by the AWAR, e.g., guinea pigs, hamsters, gerbils, ground squirrels, and mice/rats except for mice (*Mus*) and rats (*Rattus*) bred for research.

Non USDA-covered rodents: Mice (*Mus*) and rats (*Rattus*) bred for research.

Non-mammal: Vertebrate animals that are not classified as mammals. Animals included in this category are fish, amphibians, reptiles, and birds.

USDA-COVERED SPECIES

Individual Animal Medical Records (IAMRs) are required for all USDA-covered species at MGH. The IAMR is expected to contain documentation of all observations, treatments, and procedures for these species.

Individual Animal Medical Record (IAMR)

The IAMR serves as:

- A permanent record of animal health and research results;
- One means of communication among the research team, and between the research team and veterinary staff;
- Documentation of adherence to the protocol and standards of veterinary medical care; and documentation of compliance with regulations and standards required by the Animal Welfare Act and the Public Health Service Policy.

The IAMR must contain at minimum:

- Basic demographic information (species, strain, stock or breed, source of the animal, animal identifier, protocol number, responsible investigator, contact info, and pertinent dates)
- Clinical information: detailed clinical and diagnostic information (e.g., lab results, x-rays, pathology reports)
- Dates of inoculations, routine physical examinations, quarantine clearance
- For survival and non-survival surgeries, complete surgical records to include animal history, IACUC protocol number, preoperative preparations, surgical reports, anesthetic monitoring records, intraoperative monitoring notes, postoperative care and recovery notes.
- Any animal manipulation, conscious or anesthetized, including imaging or behavioral training sessions, obtaining physiologic parameters (e.g., blood, urine, or spinal fluid collection), breeding notations, or any other non-surgical procedures.
- Any agents, whether research or treatment related, administered to the animal, including the agent name, dose, and route of administration.
- Behavior treatment progress and status
- Documentation of veterinary/clinical exemptions to the Policy on Environmental Enrichment, Social Housing, and Exercise of Laboratory Animals. The veterinarian will record the basis for the exemption, the period of single housing and frequency of reevaluation, unless it is a permanent condition.
- Final disposition of the animal or euthanasia method (include agent, dose and route) and necropsy report, if applicable

The IAMR is created and maintained by the facility where the animals are housed. In order to facilitate compliance with required medical record elements, a series of forms has been created. Form templates may be found at on the CCM intranet site (CCM - Forms and Documents); hard copies are available in the CCM facilities. Investigators are highly encouraged to use these templates and/or forms or confirm their own system includes the required components. IAMRs must remain near the animal and be available for review. Investigators must return complete

IAMRs to the facility manager within two weeks after the end of study. IAMRs must be retained as described below in **Best Practices and Record Retention**.

Research Staff Responsibilities with Regard to IAMR

Investigators and research technicians should document in the IAMR the animal's general health status, as well as any treatment, medication, or procedures performed. If an animal is having an unanticipated health problem or if an animal is in unrelieved pain or distress, this must be communicated to the veterinary staff and this communication should be documented in the IAMR. Entries should be legible, and include the date, time, and initials of the person making the entry.

Veterinary Staff Responsibilities with Regard to IAMR

The documentation of routine veterinary care for healthy animals is governed by the IACUC Policy on Adequate Veterinary Care. For animals under treatment or observation for specific concerns/conditions, veterinary staff should document in the IAMR all observations, examinations, veterinary interventions, and response to such treatments. Each entry should include a future care and observation plan until the issue at hand is resolved. Communications to the PI regarding any of the above should also be documented. If an animal is having an unanticipated health problem or if an animal is in unrelieved pain or distress, this should be communicated to the research staff and this communication should be documented in the IAMR. CCM veterinary staff documents in the IAMR the general health status as well as any treatment, medications, procedures performed, and the resolution of any clinical problems whenever they observe or perform procedures on animals. The veterinarian will also record the basis for the exemption, the period of single housing and frequency of reevaluation, unless it is a permanent condition. Entries should be legible, and include the date, time, and initials of the person making the entry.

NON USDA-COVERED SPECIES

An IAMR is not required for non-USDA-covered vertebrate species. However, documentation of observations, treatments, and procedures is still required for these species, in accordance with the approved IACUC protocol.

Mice (genus *Mus*) and Rats (genus *Rattus*) bred for research.

Rodent Record Cards are to be used for mice and rats as part of the record-keeping requirements, outlined below, and may be supplemented by other sources including laboratory notebooks. Rodent Record Cards, cage cards, notebooks and other documents must be available for audit by the IACUC, and must have sufficient detail such that compliance with IACUC policy and approved protocols can be confirmed. These records must also be easily accessible to veterinary staff. The Rodent Record Card is available from CCM staff and in rodent procedure rooms. Instructions for using the card are posted in rodent procedure rooms and are available on the IACUC Web site (Rodent Record Card Instructions). Additional information and record templates are also available on the IACUC website (Rodent Record Card).

Anesthesia record-keeping requirements.

Anesthetic events must be documented on the Rodent Record Card, as outlined in the instructions for using the record card (date, agent, initials). Additional information that is not required on the Rodent Record Card must be recorded elsewhere and may be captured on the Rodent Record Card, in the laboratory notebook, or using another method (e.g., dose and route).

Adequacy and depth of anesthesia must be confirmed before initiating a procedure, and animals must be monitored for anesthetic depth every 15 minutes. There is no requirement to document that anesthetic depth was checked. Instead, the attestation on the Rodent Record Card, that monitoring will be conducted in accordance with the approved protocol, must be checked, and the animal must be monitored as outlined in the protocol and per this policy.

Procedural and surgical record-keeping requirements.

Any procedure performed that has a procedure form or surgery form in the approved IACUC protocol must be documented on the Rodent Record Card. For all survival and non-survival surgical procedures, the record card must include the IACUC protocol number, and the name and date of surgical procedure. Exceptions are genotyping and identification procedures.

All agents administered to the animal(s) must be documented on the Rodent Record Card.

The information recorded on the Rodent Record Card must be consistent with the instructions for the use of the card. A group of animals in one cage may be documented on a single Rodent Record Card.

Post-operative and post-procedure record-keeping requirements.

At a minimum, all treatments administered to the animal(s), including analgesics and anesthetics, must be documented on the Rodent Record Card.

An attestation must be provided on the cage card that animals will be monitored in accordance with the approved protocol. Monitoring checks may be documented on the Rodent Record Card, in the laboratory notebook, or using another method.

Observations about the animal(s) and any additional information such as animal weights, body condition scores, tumor sizes, laboratory test results (e.g., CBC), radiographs and other imaging information, histopathology/necropsy records, etc., may be documented on the Rodent Record Card, in the laboratory notebook, or using another method. Information about the animal must be recorded in accordance with the approved protocol and IACUC policy.

A group of animals in one cage may be documented on a single Rodent Record Card.

Non-mammals (fish, frogs, reptiles, birds bred for research)

For non-mammals, a Rodent Record Card or equivalent, or the laboratory notebook may be used

to document all experimental manipulations of the animals. Records should also include all observations, treatments, laboratory test results (e.g., CBC), radiographs and other imaging information, and histopathology/necropsy records.

Records must be easily accessible to veterinary staff and must be available for audit by the IACUC; records must have sufficient detail such that compliance with IACUC policy and approved protocols can be confirmed.

BEST PRACTICES AND RECORD RETENTION

All entries in IAMRs, on Rodent Record Cards, in laboratory notebooks, etc., should be made proximally to the time of the treatment or observation (that is, write it down as it happens). The records must be sufficiently detailed so that compliance with IACUC policies and approved IACUC applications can be audited easily. All records must be readily available for inspection by CCM staff, Office of Animal Welfare Assurance staff, IACUC members, MGH officials, and outside regulatory agencies.

IAMRs, Rodent Record Cards, and other records are considered to be research materials that must be retained according to the MGB Guidelines on Retention of Research Data, Materials, and Records.

- IAMRs must be kept while the animal is living and will be retained by CCM for seven years after the end of a research project/activity or as required by MGB guidelines, whichever is longer. Principal Investigators are responsible for retaining IAMRs for the same period in non-CCM housing areas.
- Rodent Record Cards and other records must be retained while the animal(s) is living. If the Rodent Record Card is the sole research record, it must be retained for seven years after the end of a research project/activity or as required by MGB guidelines, whichever is longer. If the information from the Rodent Record Cards was copied into a laboratory notebook, the notebook must be retained in accordance with the MGB guidelines.

RELATED POLICIES

Policy on Adequate Veterinary Care
Policy on Anesthesia and Analgesia
Policy on Food and Fluid Restriction in Laboratory Animals
Policy on Post-Operative and Post-Procedural-Care

REFERENCES

American College of Laboratory Animal Medicine (ACLAM). Position Statements: Medical Records for Animals Used in Research, Teaching, and Testing. September 30, 2020.

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v1.1, 20 February 2008
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INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON POST-OPERATIVE AND POST-PROCEDURAL CARE

GENERAL POLICY

Adequate and appropriate post-operative/post-procedural care must be provided to all experimental animals used in protocols involving major and minor surgical procedures as well as non surgical procedures at MGH. Provision of post-procedural care is the responsibility of the Principal Investigator (PI) through his or her designated personnel listed in the approved protocol. The Director, Center for Comparative Medicine (CCM) and his or her designees are responsible for providing consultation and guidance on post-operative care to ensure that proper procedures and practices are followed to minimize pain and distress. CCM staff may provide emergency veterinary care when the Attending Veterinarian (AV) or his or her designee determines that immediate intervention is needed to save the animal's life or relieve pain, and neither the PI nor his or her research staff members are available or cannot be contacted within a reasonable time (determined by the nature of the veterinary emergency).

The above stated interventions by CCM are not intended to compromise research results; however, unless specifically described in the protocol and approved by the IACUC, unrelieved pain associated with surgical procedures will not be permitted. The same assigned responsibilities and actions also apply to potentially painful procedures in non-surgical protocols.

DEFINITIONS

USDA-covered species, non-rodents: Animal species that are taxonomically defined as lagamorphs or higher and covered by the Animal Welfare Act Regulations (AWAR), e.g., rabbit, cat, dog, ferret, sheep, goat, swine, non-human primate.

USDA-covered species, rodents: Animals that are taxonomically defined as rodents and covered by the AWAR, e.g., guinea pigs, hamsters, gerbils, ground squirrels, and mice/rats except for mice (*Mus*) and rats (*Rattus*) bred for research.

Non USDA-covered rodents: Mice (*Mus*) and rats (*Rattus*) bred for research.

Non-mammal: Vertebrate animals that are not classified as mammals. Animals included in this category are fish, amphibians, reptiles, and birds.

PROCEDURES

USDA-covered Non Rodents Species

After surgery is completed:

1. Should be transferred to a designated recovery room or their pens/cages and monitored continuously with particular attention to adequate respiration and perfusion. A method of documentaion is to be kept with the animal throughout the entire post-operative period, defined as the time until the animal can maintain sternal recumbancy (quadrupeds) or sit upright (nonhuman primates), is hemodynamically stable, and shows good respiratory exchange. Documentation of complete recovery from anesthesia can be made on the anesthetic record, the operative note, or in the Individual Animal Medical Record (IAMR). Prior to exhibiting that level of recovery, an animal should be turned from one side to its other no less frequently than every thirty minutes.
2. Once the animal is deemed stable and able to swallow, the endotracheal tube (if applicable) is removed. If the animal is under the effect of an anesthetic or paralytic that suppresses the gag reflex, even greater attention may be necessary to avoid removing the endotracheal tube prematurely so aspiration of vomitus does not occur.
3. During recovery, the animal's body temperature should be supported by applying external heat sources such as commercially available warming devices and/or administering warmed intravenous solutions. Hot water bottles and radiant heat may also be used if the animal is protected from burn injury.
4. Post-procedural analgesics will be administered as stated in the approved protocol. If an animal continues to display obvious pain or discomfort, the AV or his or her designee will determine if additional or different analgesic therapy is indicated, after consultation with the PI or their designee.
5. The PI and AV, or their designees, should be notified immediately if post-procedural complications are noted; these may include, but are not limited to, bleeding, difficulty breathing, seizures, vomiting, and the incision site dehiscence. During evenings, weekends, and holidays, the on-call veterinarian must be contacted. Contact information is posted in each facility.
6. Post-operative and post-procedural monitoring should continue as described in the IACUC-approved protocol. However, more frequent monitoring may be required for animals deemed physiologically unstable or not fully recuperated from the procedure. Skin sutures and wound clips must be removed in a timely fashion (usually 10-14 days after surgery). All observations, procedures, and treatments must be noted in the Individual Animal Medical Record (see IACUC Policy on Animal Observation and Record Keeping).

USDA-covered Rodent Species

After surgery is completed:

1. Should be transferred to a clean cage and monitored continuously until the animal is ambulatory, hemodynamically stable, and shows good respiratory exchange.

The animal's body temperature should be supported by applying external heat sources, such as a water-circulating heating pad. Animals should not be returned to the housing area until they are completely recovered; moving around the cage with activity similar to what they showed prior to the procedure.

2. Post-procedural analgesics will be administered as stated in the approved protocol. If an animal continues to display obvious pain or discomfort, the AV or their designee should be contacted and will determine if additional or different analgesic therapy is indicated, after consultation with the PI or their designee.
3. Post-operative and post-procedural monitoring should continue as listed in the IACUC-approved protocol; however, more frequent monitoring may be required for animals deemed physiologically unstable or not fully recuperated from the procedure. Skin sutures and wound clips must be removed in a timely fashion (usually 10-14 days after surgery). All observations, procedures, and treatments must be noted in the IAMR

Non USDA-covered Rodents and Non-mammals

After surgery is completed:

1. Should be transferred to a clean cage/tank and monitored continuously until the animal is ambulatory, hemodynamically stable, and shows good respiratory exchange. For non-mammals, animals should be monitored continuously until alert and responsive. Animals should not be returned to the housing area until they are completely recovered; moving around the cage with activity similar to what they showed prior to the procedure.
2. Post-procedural analgesics will be administered as stated in the approved protocol. If an animal continues to display obvious pain or discomfort, the AV or their designee should be contacted and will determine if additional or different analgesic therapy is indicated, after consultation with the PI or his or her designee
3. Post-operative and post-procedural monitoring should continue as listed in the IACUC-approved protocol. However, more frequent monitoring may be required for animals deemed physiologically unstable or not fully recuperated from the procedure. Skin sutures and wound clips must be removed in a timely fashion (usually 10-14 days after surgery). All applicable procedures and treatments must be documented, including all anesthtetic and analgesic episodes, on the Rodent Record Card; observations must be documented on the Rodent Record Card, in the laboratory notebook, or using another method. For non-mammals, the Rodent Record Card, laboratory notebook or other method may be used to document observations, procedures and treatments.

RELATED POLICIES

Policy on Anesthesia and Analgesia
Policy on Animal Observation and Record Keeping

v1.1, 16 February 2005
v1.2, 20 September 2011
v1.3, 19 October 2011
v1.4, 29 April 2016
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INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON RODENT BREEDING AND CAGE DENSITY

GENERAL POLICY

The goal of this policy is to define appropriate cage density for laboratory rats and mice, including animals that are used for breeding. The primary objectives of cage management are to ensure a healthy environment for the animals housed therein and to assure compliance with state and federal regulations as well as current accreditation standards. Rodent welfare can be impacted by higher than acceptable cage densities because they can affect the cleanliness of the cage and the frequency of required cage changes.

Additionally, excessive cage densities may adversely affect breeding colonies. All of these situations can cause unnecessary stress for the animals and may also compromise intended research objectives. All personnel involved with animal research are responsible for ensuring that the cage environment is appropriately maintained.

While the Center for Comparative Medicine (CCM) staff provides basic husbandry to most rodents housed at MGH, it remains the responsibility of the Principal Investigator (PI) to maintain his/her animals in compliance with Institutional Animal Care and Use Committee (IACUC) policies and approved protocols. Rodents are housed in accordance with guidelines provided in the *Guide for the Care and Use of Laboratory Animals*. Those guidelines are described below for housing mice and rats, respectively. Genetically modified animals, that have a new phenotype which may express an adverse health condition, may require increased monitoring or husbandry practices and notification to the IACUC. If an investigator is found to have overcrowded cages resulting from any of the causes described below, the IACUC may withhold approval to breed or use animals until appropriate oversight procedures have been established.

CAGE CAPACITY GUIDELINES

Adult Mice and Rats

Adult mice are defined as any sized mouse that is weaned and at least 28 days of age. Adult rats are defined as any sized rat that is weaned and at least 21 days of age. The IACUC has adopted the minimum space dimensions listed in the *Guide* (p. 57) for the standard shoebox mouse cage (6.5" x 10.5"), as listed in Table 1, and the standard shoebox rat cage (17" x 8.5"), as listed in Table 2.

Table 1. Mouse Housing Guidelines*

Weight of Largest Mouse	Maximum Number of Adult Mice
Less than 25 grams	5
25 grams or more	4

Table 2. Rat Housing Guidelines*

Weight of Largest Rat	Maximum Number of Adult Rats
Less than 300 grams	4
300 to 400 grams	3
400 to 500 grams	2
More than 500 grams	1

** To address the impact of cage density on adequate veterinary care, CCM Veterinary Staff may require lower number of animals to ensure the welfare of the mice involved. Examples include diabetic animals, obesity models, aging models, chronic fighting, and animals needing to be isolated for veterinary purposes.*

For any cage of adult mice or rats found outside of the parameters described in Tables 1 and 2, CCM staff will notify the PI or his/her designee on the need to reduce animal density. Any cages remaining unchanged after three consecutive calendar days following notification will be separated immediately by CCM staff to bring the cage within compliance, and fees may be incurred by the investigator. Potential CCM interventions to possible overcrowding scenarios are described in Table 5.

Breeding Colony Management

1. Breeding Pairs:

For any approved protocol that involves breeding, it is the responsibility of the PI to assure the breeding colony is properly maintained. This includes the selection of breeding pairs, pairing for mating, separation of breeders after mating, and weaning of offspring.

The PI is also responsible for assuring animals are weaned at appropriate times. The latest acceptable weaning dates are outlined in Tables 3 and 4. It is not necessary to wait until the last day for weaning. Mice and rats can be successfully weaned as early as Day 18 if the pups satisfy the criteria described below.

Table 3. Latest Acceptable Mouse Weaning Day

Mice in the Cage	Latest Weaning Day
Dam with litter, +/- sire	Day 28

Table 4. Latest Acceptable Rat Weaning Day

Rats in the Cage	Latest Weaning Day
Dam with litter	Day 21
Dam and sire with litter*	Day 21

**For rats, dam and sire with a litter is only allowed with special larger caging that is only available in some CCM facilities. Please contact your facility manger regarding availability.*

2. Criteria for Weaning

For weaning at any age, pups must satisfy the following requirements:

- Erupted front teeth: the teeth must be present to allow the animal to eat solid food
- Sufficient size: the animal must be of a sufficient size to be able to reach the food and water supply. General minimum size guidelines are as follows, but may vary depending upon the strain:
    - Mice: 2.5” in length and 8-12 g
    - Rats: 4” in length and 25-30 g

In the case of strains with litters that are too immature to wean by Day 21 (rats) or Day 28 (mice), delayed weaning must be approved by the IACUC for the protocol covering the strain in question.

3. Breeding Trios and Harem Breeding Cages

Breeding cages containing a male and multiple females are referred to as “trios” (1 male and 2 females) or “harems” (1 male and up to 3 females). If utilizing a breeding strategy involving multiple females in the same cage, it is the responsibility of the PI to assure that females are separated as they become pregnant (detectible visually by embryonic day 18) to ensure there can only be one litter born to a cage.

For rats, trio or harem breeding is only allowed with special larger caging that is only available in some CCM facilities. Please contact your Facility Manager regarding availability.

4. CCM Interventions for Overcrowded Cages

Table 5. Possible CCM interventions for overcrowded cages

Possible Separation Scenario	**CCM Action**
Dam with 1 litter (with or without sire)	Notify PI or designee 3 days in advance of weaning date; wean and separate by sex if animals reach weaning date and are not yet weaned
Dam with 1 litter + multiple other animals	Separate other animals if not corrected within 24 hours after discovery (unless approved in protocol)
Dam with older litter and new litter	Separate older litter by sex if not corrected by 1:00 PM on the calendar day after discovery
2 dams plus 2 litters	Separate 1 dam and 1 litter if not corrected by 1:00 PM on the calendar day after discovery (unless approved in protocol)
More than 5 adult (weaned) mice in cage	Separate immediately
Exceeding maximum number of adult animals	Notify PI or designee; separate if not corrected within 3 consecutive calendar days

Possible Separation Scenario	CCM Action
Special health-related conditions	Notify PI or designee; separate if not corrected by the time required

DOCUMENTING AND TRACKING RODENT BREEDING

- The use of a breeding colony management system is integral to the reduction of animals unnecessarily produced in the research environment and preservation of genetically modified strains. The *Guide* (p. 76) states "Care should be taken to preserve such resources through standard genetic management procedures, including maintenance of detailed pedigree records and genetic monitoring to verity the presence and zygosity of transgenes and other genetic modifications."
- The use of a breeding colony management system is required for all rodent breeding.
- Rodent breeding colony management systems should:
    - Document and track current and past breeding pairs
    - Document and track individual offspring produced
    - Include sufficient information to calculate breeding performance indicators
- See Appendix 1 for the minimum data required to be tracked in the selected colony management system
- The number of animals produced by breeding, including breeders, animals used for experimental purposes and animals euthanized without being used (e.g., incorrect genotype), together with the number of animals purchased should not exceed the number of animals approved by the IACUC. Tracking approximate numbers of animals produced by breeding is required to avoid overproduction relative to the numbers approved on the protocol.

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v1.1, 15 June 2005
v1.2, 20 September 2006
v1.3, 23 October 2013
v1.4, 18 June 2014
v1.5, 15 September 2021
v1.6, 17 August 2022

Appendix 1 – Required data for colony management system

Current and Past Breeding Cages:

1. Room/rack/cage location/identifiers
2. Strain being generated if different from breeding pair
3. Animal identifiers
4. Dates of birth (DOB) of breeders
5. Strain/genotype
6. Date of pairing/mating
7. Estimated # of pups born and DOB for all litters produced
8. Date of tissue collection for genotyping if applicable
9. Weaning date & number of pups weaned for all litters produced
10. Date of disposition (removal of breeding program by transfer to studies, retirement, euthanasia, death)

Current and Past Progeny (Replacement Breeders):

1. Room/rack/cage location identifiers
2. Animal identifiers
3. Date of Birth (DOB) of breeders
4. Strain/genotype(s)
5. Date of tissue collection for genotyping (post weaning, if applicable)
6. Weaning date
7. Date of disposition (removal of breeding program by transfer to studies, retirement, euthanasia, death)

Optional helpful information for breeding management

Primer sequence and protocol or qPCR
Date strain last “managed” or evaluated for performance
Mating goals
Source of strain (commercially available, collaborator)
Breeding performance indicators

- Average litter size
- Average number weaned/litter
- Pups weaned per female per week
- Age in mating or weeks in breeding

MASSACHUSETTS GENERAL HOSPITAL (MGH)
INSTITUTIONAL ANIMAL CARE AND USER COMMITTEE (IACUC)

POLICY ON SPONTANEOUS AND INDUCED TUMOR PRODUCTION IN RODENTS

GENERAL POLICY

Special consideration is required for humane endpoints based on known tumor biology for studies that employ tumor models, according to the *Guide for the Care and Use of Laboratory Animals* and the "Guidelines for the Welfare and Use of Animals in Cancer Research" adopted by AAAALAC International,.[1,2] Predictable indications of pain, distress or significant deviation from normal behavior should be considered.

INDUCTION SITE CONSIDERATIONS

The method and the site for implantation of transplantable or induced solid tumors** requires considerable care to minimize trauma to the host animal. Sites should be chosen that minimize damage to adjacent normal structures and will not interfere with normal body functions such as ambulation, eating, drinking, defecation, and urination. Implantation of tumors in the muscle, footpad, tail, brain and eye are discouraged and will require scientific justification (note: metastatic tumor cells may be administered by IV injection into the tail).

For spontaneous and transplanted tumors, important features include growth rate, invasion, distension, ulceration, metastases, and production of cachectic factors. All of these factors can differ with tumor type and may impact both the frequency at which the animals must be observed and the duration of the study.

In the case of leukemias, internal, disseminated, metastatic or other occult tumors, determination of the tumor burden may be difficult. The development and/or use of appropriate biochemical and pathological laboratory methods to determine the onset of these tumors may be required. Careful monitoring of the animal's overall clinical condition is necessary in these situations.

The technical staff must be aware of the parameters of the study, such as tumor growth potential, whether a tumor is likely to become ulcerated and/or appropriate biochemical or pathological endpoints. The Principal Investigator (PI) must clearly define study parameters and endpoints in their IACUC Approved Protocol.

Particular care should be taken with monitoring the development of spontaneous tumors in all transgenic animals and especially those strains that are known to be cancer laden. Careful and regular clinical examination should be carried out to allow for the detection of both predicted and unexpected sites of tumor development.

***Contamination of tumor cell lines with human and/or rodent viruses and other microorganisms may compromise experimental results, as well as cause an outbreak of disease among laboratory animals. Screening of cell lines for rodent viruses and mycoplasmas is required.*

Studies involving the use of primary human tumors or tissues with uncharacterized blood-borne pathogens must first be reviewed and approved by the Partners Biosafety Committee (PIBC).

MONITORING GUIDELINES

The IACUC emphasizes the need for frequent monitoring during tumor development to allow for appropriate intervention before significant deterioration of animal health or death occurs. Effective monitoring systems and endpoints should include defined limits on the tumor burden and severity of tumor-associated disease. The use of altered physiological, biochemical, and other biomarkers are encouraged as potentially additive objective and reproducible endpoints than clinical signs.

All tumor-bearing animals must be directly observed at an established frequency to assess their physical condition, tumor growth, and /or metastasis in accordance with your protocol. The monitoring plan should be based on known information about growth characteristics and biology of the proposed tumor model and onset and nature of any adverse effects on the animals. The IACUC requires that tumor-bearing animals be observed 1-2 times weekly, but more frequent monitoring may be required depending on tumor size, growth rate and associated disease. If information is unknown regarding the proposed tumor model, frequent observations should be planned until tumor growth and animal welfare impact is characterized.

Records must be kept with all pertinent information in the Individual Animal Medical Record, Rodent Record Card, laboratory notebook or equivalent, in accordance with the IACUC Policy on Animal Observation and Record Keeping. Information that should be documented includes protocol number, time and frequency of monitoring, the name (initials) of the person monitoring the animal, identification of the animal, animal weight, type of clinical signs, tumor size (if appropriate), and any treatments given to the animal, in accordance with the approved IACUC protocol. Records maintained by the laboratory personnel should be available to the veterinary staff and/or the IACUC upon request.

- The maximum size for tumors (i.e. size in any one dimension) is 20 mm for a mouse or 40 mm for a rat. In cases of multiple tumors, the maximum size for any one tumor is 10 mm for mice and 20 mm for rats. The size of superficial tumors should be assessed using calipers. Scientific justification to exceed this size restriction must be approved by the IACUC.

- Clinical observations and/or palpation will be necessary to monitor for deterioration of clinical condition. Special examination techniques may be required for specific sites (e.g. respiratory rate for lung involvement, neurological disturbance for brain neoplasms, and blood cell counts for leukemias).

  Adverse clinical signs which may be associated with tumor progression:

Decreased food/water intake	Loss of body condition^{+}
Lethargic/depressed activity	Restlessness
Vocalization	Respiratory difficulty
Cranial deformity/neurological	Perianal soiling

signs	
Rough haircoat	Hunched posture
Skin pathology	Restricted mobility
Jaw deformity/malocclusion	Changes in feces/urine

[+] *See Appendix A for a Body Condition Scoring System for Mice*

- Measurement of body weight changes (both positive and negative changes compared to controls) can be used to assess tumor burden. Baseline body weights must be recorded for each animal at the start of the study and periodically through completion of the study. The period should be stated in the IACUC approved protocol. Considering both weight loss and weight gain from growth, tumor burden should not exceed 10% of the animal's normal body weight. Weight loss should not exceed 20% of the animal's body weight at the start of the experiment. For younger animals (depending on species and strain), failure to maintain weight gain to within 15% of untreated control animals should be considered as an indication of significant healthdeterioration.

PROTOCOL EXPERIMENTAL DESIGN CONSIDERATIONS

Endpoints must be established to minimize the potential for pain and/or distress. The investigator must have a plan for pre-emptive euthanasia based on clearly defined endpoints in the protocol**.** Investigators should describe in their research protocols the behavioral observations and clinical indicators of pain and distress that will be used as criteria for euthanasia. ***NOTE: The "intentional death" end point will not be allowed unless it is scientifically justified to the IACUC. Animals expected to become moribund should be euthanized prior to reaching this state****.*

- Particular attention must be paid to the body system and/or organ system (e.g., skin, peritoneum, spleen, lymph node, etc.) most likely to be affected by the tumor type. The site for injection should be carefully chosen to permit room for tumor growth and to avoid unnecessary distress. Subcutaneous or intradermal growth on the back or flank is considered to cause the leastdistress.

- Some tumors may result in ascites, leading to severe abdominal distention. Distention interferes with a number of physiological systems including but not limited to the respiratory and gastrointestinal systems. Ascitic tumorsproducing large volumes of fluid can rapidly deplete the animal of essential nutrients such as protein and hasten cachexia.

- Tissue necrosis or ulceration of the skin overlying the developing tumor may occur. Ulceration or necrotic tissue may result in a continuous loss of body fluids and/or infection, and should require euthanasia of the affected animal unless approved by the IACUC. When it is necessary to maintain an animal with an ulcerated tumor, the status of the ulcer and the animal's overall condition mustbe assessed in consultation with the veterinary staff.

- Animals with tumors that impact mobility and/or interfere with the animal's ability to acquire food or water, may require supportive care oreuthanasia.

HUMANE ENDPOINTS

All animal experiments must provide for a humane endpoint. As a general guideline, animals used in experimental procedures involving tumor development will be considered for euthanasia if the following conditions occur:

- Tumors exceed maximum allowable size unless approved by the IACUC. (The maximum size for tumors is 20 mm for a mouse or 40 mm for a rat. In cases of multiple tumors, the maximum size for any one tumor is 10 mm for mice and 20 mm for rats).
- Tumor size or metastatic growth interferes with normal behavior and condition of the animal (e.g. ambulation, eating, drinking, grooming) or causes pain or distress due to its location.
- Weight loss exceeding 20% of the body weight of a conspecific normal animal (taking into account the tumor mass).
- Body Condition Score (BC) = 1
- Tumor becomes ulcerated (break in overlying skin), infected, or necrotic.
- Palpation of tumor elicits a pain response.
- Self-induced trauma associated with tumor location.
- Animal appears weak with "hunched posture", is unresponsive, or moribund
- Animal becomes anorectic.
- Animal appears dehydrated.
- Animal shows respiratory difficulty.
- Ascites production due to tumor progression and which results in an increase in body weight of 20% due to ascitic fluid.

Note: Following veterinary assessment of animals exhibiting the above adverse clinical signs, euthanasia may be performed at the discretion of a CCM veterinarian.

Related Policies:

Policy on Animal Observation and Record Keeping

[Most recently revised October 2016]

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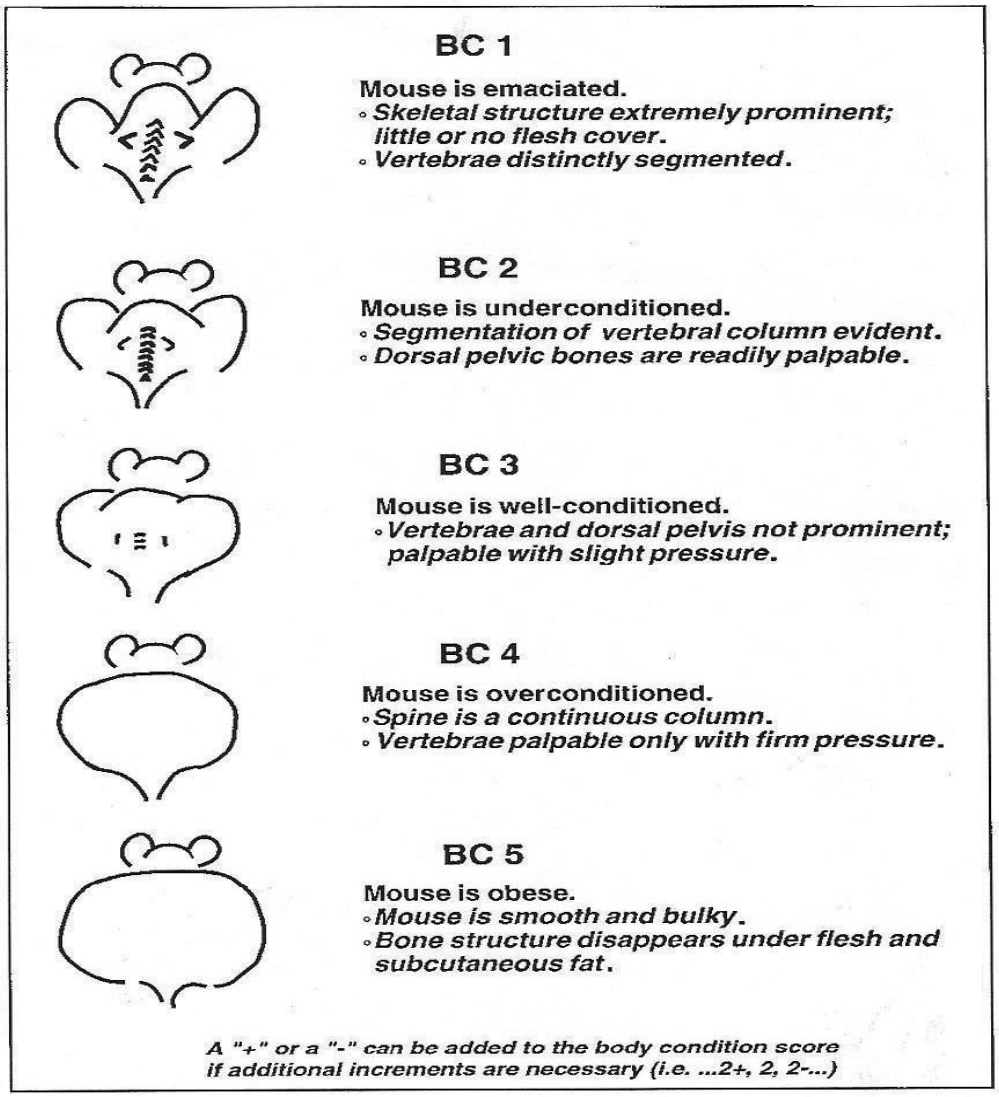
APPENDIX A

Body Condition Scoring System for Mice



Ullman-Cullere MH, Foltz CJ. Body condition scoring: a rapid and accurate method for assessing health status in mice. *Lab Anim Sci.* 1999 Jun;49(3):319-23.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON SURGERY AND OTHER EXPERIMENTAL PROCEDURES

GENERAL POLICY

Any person participating in a research activity involving vertebrate animals must be listed in the approved protocol, and must be qualified to perform the intended procedures on that particular species. Investigators may be required to provide proof of proficiency or credentials to perform a particular procedure or work with a particular animal species (see Peri-Operative/ Peri-Procedural Support below). Experience and training may be obtained from the Principal Investigator (PI), through the Center for Comparative Medicine (CCM), from relevant outside training opportunities, or other experienced third-party whose proficiency on the procedure is documented on their own protocol or equivalent.

DEFINITIONS

Non-surgical procedure: Manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition, including injection, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, behavior training and testing, euthanasia, etc. Non-surgical procedures may or may not require the use of a sedative or anesthetic, and may or may not require the use of analgesics.

Surgical Procedure (Surgery): Usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition. Surgery usually requires anesthesia and analgesia, and is further sub-classified as major or minor, and survival or non-survival (see below).

Survival Surgery: Animal regains consciousness, for any period of time, after anesthesia.

Non-survival Surgery (Acute Surgery, Terminal Surgery): Euthanasia is performed while the animal is under general anesthesia. The animal never awakens or regains consciousness.

Major Surgery: Any surgical intervention that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic function, or involves extensive tissue dissection or transection.

Examples of **major surgery** include:

- Surgical access of a body cavity (i.e. cranial, thoracic, abdominal, pelvic, ocular or orbital, skeletal, joint)
- Injury or repair of a tendon or ligament

- Bisection (partial or complete) of muscle or tendon
- Amputation of a limb
- Nephrectomy or nephrotomy
- Enucleation
- Open surgical biopsy of a major organ
- Surgical implantation of indwelling medical or monitoring devices
- Orthopedic procedures involving a surgical approach
- Neutering of male animals

Minor Surgery: Any surgical intervention that does not penetrate a body cavity and causes little or no physical or physiologic impairment.

Examples of **minor surgery** include:

- Percutaneous or cut-down approach to a superficial artery or vein for catheterization or other purposes
- Tissue biopsy not involving surgical exposure of a body cavity
- Skin and subcutaneous implants
- Head post implants
- Removal of small digits or tail amputation in small animals
- Endoscopy, colonoscopy, tracheoscopy, and laparoscopy (in which only a small penetrating incision is made in the skin)
- Surgical repair of a superficial injury

USDA-covered species, non-rodents: Animal species that are taxonomically defined as lagamorphs or higher, e.g., rabbit, cat, dog, ferret, sheep, goat, swine, non-human primate.

USDA-covered species, rodents: Animals that are taxonomically defined as rodents and covered by the Animal Welfare Act Regulations (AWAR), e.g., guinea pigs, hamsters, gerbils, ground squirrels, and mice/rats except for mice (*Mus*) and rats (*Rattus*) bred for research.

Non USDA-covered rodents: Mice (*Mus*) and rats (*Rattus*) bred for research.

Non-mammal: Vertebrate animals that are not classified as mammals. Animals included in this category are fish, amphibians, reptiles, and birds.

Aseptic Technique: A technique used for surgery or other procedures that is designed to maintain an object or anatomic area in a condition as free as possible from all microorganisms and infection. Aseptic technique is used whenever body tissues must be penetrated and the animal is intended to recover from anesthesia. It is designed to protect the animal as well as the person performing the procedure and may require the use of a dedicated room or area. Aseptic technique is further defined below as “strict” or “modified”, depending on the type of procedure performed and the animal classification.

Strict Aseptic Technique includes:

- A dedicated, clean, and uncluttered work area
- A pre-sanitized work area
- Pre-surgical or pre-procedural preparation of skin surfaces (i.e., hair clipped, skin shaved if applicable, disinfectant soap scrub)
- Surgery/procedure preparation of the skin site (iodophor and/or alcohol scrub)
- Sterile draping of the surgery/ procedure skin site
- Use of sterile instruments and supplies
- Surgeons' preparation, including removal of jewelry from hands and wrists and 5 minutes of thorough scrubbing of fingers to elbows using an appropriate disinfectant
- Wearing of surgical clothing (scrub suit, cap/bonnet, mask, shoe covers, sterile gown, sterile gloves)

Modified Aseptic Technique: Depending on the type of surgery or procedure and classification of animal, some of the requirements of strict aseptic technique (e.g., the need for a dedicated work area, requirement for sterile instruments and supplies, wearing of surgical clothing, pre-surgery or pre-procedure preparation of the animal subject or surgeon, and sterile draping of the surgery/procedure site) may be waived as described in the IACUC-approved protocol.

NONSURGICAL PROCEDURE AREAS ("Procedure Areas")

The location in which a nonsurgical procedure is to be performed must be specifically identified in the protocol. If procedure locations change, the protocol must be updated to capture the new locations. Procedure locations not previously approved by the IACUC must be reviewed by the Office of Animal Welfare Assurance, the Center for Comparative Medicine, Environmental Health & Safety (including Biosafety, if required), and the Research Space Management Group. All items identified during the review must be addressed before the area may be approved for use.

When procedures cannot be performed in a designated animal treatment or procedure room within an animal facility, the following additional requirements must be adhered to:

- The procedure must be performed in an area separate from other activities
- Activity in the area must be limited to the procedure conducted
- The area must be kept neat and uncluttered, and easily cleaned and sanitized
- Personnel access to the area should be limited
- Modified aseptic technique should be used

USDA-covered Species, Non-rodents

Routine non-surgical and euthanasia procedures must be performed in IACUC-approved locations.

Non-survival experimental procedures in large animals, including euthanasia, must not be performed in animal housing rooms.

All Rodents

Whenever possible, routine procedures using animals must be performed in designated hoods in animal housing rooms, or in dedicated procedure spaces within the CCM animal facilities, or other IACUC-approved specialized locations (e.g., irradiators, imaging suites). If the procedures are performed in locations outside of centralized animal facilities, rodents should be returned to specified holding rooms instead of to their original housing rooms in order to prevent spread of infectious agents potentially encountered while outside the vivarium.

If it is not possible to perform procedures within these dedicated, specialized rooms, procedures may be conducted in an Investigator-owned closed-door room, with the appropriate air flow. In circumstances where a dedicated room is not available and procedures must be conducted in shared laboratory spaces, procedures should be conducted within a laminar flow hood or bench-top plexiglass containment device unless there is a justification for working outside the flow hood or containment device. In such instances, work with animals should be conducted as close to the room exhaust as possible and signage must be posted alerting laboratory personnel that animal research is on-going. Every effort must be made to limit allergen release into shared work environments.

Non-survival procedures on all rodents (mice, rats, hamsters, and guinea pigs) including euthanasia, should not be performed in animal housing rooms except as required in BL-2 rooms or rooms under quarantine, but may be performed either in dedicated procedure rooms in animal facilities or in other IACUC-approved locations as described above.

SURGERY AREAS

The location in which a surgical procedure is to be performed must be specifically identified in the protocol. If surgery areas change, the protocol must be updated to capture the new locations. Animal surgery areas not previously approved by the IACUC must be reviewed by the Office of Animal Welfare Assurance, the Center for Comparative Medicine, Environmental Health & Safety (including Biosafety, if required), and the Research Space Management Group. All items identified during the review must be addressed before the location may be approved for use.

The surgery area requirements are determined by animal classification and type of surgery, as follows:

Major Survival Surgery, USDA-Covered Mammalian Species, Non-rodent

Operations must be performed in a dedicated surgery facility using strict aseptic technique. IACUC-approved surgery facilities comprise:

- Surgical support areas
- Animal preparation areas
- Surgeon preparation areas
- Surgical supply areas
- Operating room(s)
- Post-operative recovery/ care animal areas
- Access to the surgery area is limited.

Minor Surgery, USDA-Covered Mammalian Species, Non-rodent

Operations do not require the use of dedicated surgery facilities, but strict aseptic technique must be followed. The surgery area requirements are:

- Separate, easily sanitized, and uncluttered area (a separate room is not required)
- Activity in the area must be limited to the procedure conducted
- Access to the room must be limited

Non-survival Surgery, USDA-Covered Mammalian Species, Non-rodent

Operations may not require the use of dedicated surgery facilities or strict aseptic technique; however, consultation with CCM veterinarians is recommended, as the requirements listed below may be modified based on the duration and/or type of non-survival surgery.

The surgery area requirements are:

- A separate, easily sanitized, and uncluttered area (a separate room is not required)
- Activity in the area must be limited to the procedure conducted

- Access to the room must be limited

Modified aseptic technique must be followed:

- Sterile supplies are optional
- Drapes are optional
- Instruments must be clean and sanitized.

Survival Surgery, All Rodents and Non-mammals

Operations do not require the use of dedicated surgery facilities. The surgery area requirements are:

- A separate, easily sanitized, and uncluttered area (a separate room is not required)
- Activity in the area must be limited to the procedure conducted
- Access to the room should be limited

Modified aseptic technique must be followed, including:

- Sterile supplies
- Dedicated laboratory clothing (e.g., lab coat, surgery scrubs, clean surgical gown); sterile clothing is optional
- Face mask and sterile gloves
- Maintenance of a sterile field
- Sterile drapes (optional, but recommended)

Non-survival Surgery, All Rodents and Non-mammals

Operations do not require the use of dedicated surgery facilities. The surgery area requirements are:

- A separate, easily sanitized, and uncluttered area (a separate room is not required)
- Activity in the area must be limited to the procedure conducted
- Access to the room must be limited

Modified aseptic technique must be followed:

- Sterile supplies (optional)
- Dedicated laboratory clothing
- Drapes (optional, but recommended)

PERI-OPERATIVE / PERI-PROCEDURAL SUPPORT

The PI is responsible for the provision of pre-/intra-/post-operative or procedural animal care as described in the IACUC-approved protocol. Please refer to the IACUC Policy on Anesthesia and Analgesia for monitoring animals peri-operatively / peri-procedurally.

Persons providing perioperative support may be asked to demonstrate proficiency to IACUC-designated personnel before they are approved to participate in this role unsupervised. Evaluations will include, but may not be limited to, the following:

- Calculating and administering appropriate pre-anesthetic drugs
- Calculating and inducing anesthesia
- Endotracheal intubation and securing the animal to the procedural surface
- Preparing the surgical site per the needs of the protocol
- Assessing depth of anesthesia, documenting vital signs
- Calculating and administering anesthesia-reversing drugs, if included in the protocol
- Providing supportive post-op care in accordance with IACUC policy, including proper documentation

The Director, Center for Comparative Medicine (CCM) and their designees are responsible for providing guidance and consultation to ensure that proper procedures and practices are followed to minimize pain and distress. CCM staff may provide emergency veterinary care when the Attending Veterinarian or his or her designee determines that immediate intervention is needed to save the life of the animal or relieve pain, and neither the PI nor his or her research staff members are available or cannot be contacted within a reasonable time (determined by the nature of the veterinary emergency).

RELATED POLICES

Policy on Adequate Veterinary Care
Policy on Anesthesia and Analgesia
Policy on Animal Observation and Record Keeping
Policy on Post-Operative and Post-Procedural Care

REFERENCES

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v1.1, 22 June 2006
v1.2, 1 June 2016
v1.3, 19 July 2017
v1.4, 16 June 2021

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON TOE-CLIPPING OF MICE

Overview/Purpose

This IACUC policy describes when mouse toe clipping is permitted, and the standard procedures required for performing this technique. The policy is intended for use by mouse users only when individual mouse pup identification or genotyping is required prior to an age when other methods (e.g., ear punch, tail biopsy, tattoo, ear tag, or microchip) are appropriate. Toe-clipping should not be used when less painful methods can be performed.

Requirements

1. Toe clipping is a potentially painful procedure depending on the age in which it is performed and its use in animal studies is discouraged.

2. Toe clipping should be performed only on neonatal mice 7 days of age or younger.

3. Toe clipping as a method of identification or genotyping should be used only when no other individual identification method is feasible or when there is a scientific need to obtain genotyping results at an early age, before tail snipping or ear notching are viable options.

4. The investigator must justify use of this method in the animal care and use protocol.

    a. Breeding form. The Breeding form must include an explanation why identification or genotyping of individual neonatal animals is necessary within the context of the experiment and why the use of other methods of identification or genotyping are not possible. The explanation should describe the scientific need to genotype neonates rather than waiting until the animal is large enough to utilize less invasive methods such as ear notching or tail biopsy. Examples include studies involving mutant strains exhibiting a phenotype at a young age that must be genotyped and identified prior to tissue harvest or administration of experimental substances before ear notching or tail biopsy can be performed

    b. Procedure form. A Procedure form that includes a detailed description of the toe clipping procedure must be added to the protocol; a Procedure Bank form is available for importing into the protocol (Toe Clip for Mice). All toe-clipping must be approved by the IACUC.

5. Toe clipping should be performed only by trained personnel using sanitized, sharp scissors of suitable size, in a manner that instantly removes the last end joint of the toe with minimal trauma. Under all circumstances aseptic practices should be followed. Scissors must be sanitized between mice. The IACUC also recommends that the scissors should be frequently sharpened or replaced.

6. Only the end joint of the toe should be removed per diagram below:

Figure 1. Proper technique for toe clipping requires removal of the entire most distal toe bone (3rd phalanx) and nail bed, which often requires removal of a small portion of the 2nd phalanx (adapted from Dahlberg, et al., 2013).

7. No more than 2 toes per foot may have their end joints removed. Do not cut thumbs on the forefoot.

8. Toe-clipped mice should be observed for at least 5 minutes to ensure that bleeding is controlled. Operators should be prepared to apply pressure with sterile gauze if bleeding continues.

9. Mice that have had toes clipped should be observed closely to confirm the absence of infection.

10. If toe-clipping is approved on the IACUC protocol, the toe tissue, when possible, should be used as a source of DNA for genotyping instead of collecting additional tissue samples.

11. If tissue samples alone are needed, the investigator should consider ear punching or other alternative methods.

12. If identification alone is needed, the investigator should consider tattoo application, non-toxic permanent marker, ear notching, or other alternative methods.

13. The IACUC will monitor implementation of this policy and will modify it based upon its experience, as well as emerging material trends or policy.

14. Investigators who need to perform toe clipping other than as described above must provide further scientific justification as to why they cannot comply with the policy. Additional requirements, such as anesthesia and analgesia, will likely be required when toe clipping is performed other than as described in this policy.

REFERENCES

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v1.1, March 2017
v1.2, 19 August 2020

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
POLICY ON ANIMAL ACQUISITION

GENERAL POLICY

All incoming animals purchased or imported into Massachusetts General Hospital must be acquired lawfully on to an approved IACUC protocol. MGH maintains strict health requirements for the animals entering the facilities to minimize the risk of pathogens that can adversely alter scientific objectives or pose a potential harm to people or other research animals.

DEFINITIONS

Animal orders: The Center for Comparative Medicine maintains a list of key approved animal vendors that provide current, complete and reliable health status for direct entry into animal facilities. These requests are termed "animal orders."

Animal imports: All animals coming into any MGH animal facility from a non-approved vendor or collaborator (universities, hospitals, etc.) must have prior approval from a CCM Veterinarian before they can be received. These requests are termed "animal imports."

Information on animal acquisition processes can be found on the Animal Order & Transfer page of the CCM website.

PROCUREMENT AND HEALTH ASSESSMENT

All animal procurement must be managed by CCM, with the exception of zebrafish housed in IACUC-approved satellite facilities. Vendors are reviewed and approved by the veterinary staff based on use, numbers and vendor program specifications. Participation in the CCM Approved Vendor Program is based on an appreciation and alignment of the vendor and MGH husbandry and veterinary practices such that further evaluation in a formal quarantine program is not warranted. All animals originate from lawful sources and their procurement, transportation and receipt must be compliant with federal, state and local animal welfare regulations. Animals received through the approved vendor route may go directly into the animal facility for acclimation. Procurement requests from non-approved vendors (including universities, hospitals, etc.) must have prior approval from a CCM Veterinarian before they can be received. Isolation and quarantine for further evaluation of health may be required. Assessment and preventive medicine programs such as vaccinations, ecto- and endoparasite treatments and other disease control measures are incorporated according to current veterinary practices appropriate for each species. Overall, the health status of all animals used in the MGH animal care and use program is assessed based on species, strain/lines, source and experimental aim to protect general institutional animal and human health. Facility design, operational standard operating procedures and staff training is in place to ensure that this is sustainable. See Animal Health & Treatment on the CCM website.

[Most recently revised September 15, 2021]

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON ADEQUATE VETERINARY CARE

Adapted from ACLAM Position Statement on Adequate Veterinary Care

GENERAL POLICY

The Attending Veterinarian (AV) must be appointed by the President of MGH and reports directly to the Institutional Official (IO). The AV is responsible for assuring that adequate veterinary care is provided to all vertebrate animals maintained within MGH and MGH-affiliated research facilities and may also serve as the Director of the Center for Comparative Medicine (CCM). He or she must be knowledgeable of all current and planned use of animals thorughout the institution. A veterinarian, typically the AV, will serve as a member of the IACUC. No more than 3 individuals from CCM will serve as voting members of the IACUC. The AV has full authority to execute the duties inherent to assure the adequacy of veterinary care and to oversee other aspects of animal care and use to ensure that the program meets applicable standards. The AV and designees (CCM veterinary staff) must have the authority to initiate treatment immediately, if indicated.

The AV should be board certified by the American College of Laboratory Animal Medicine (ACLAM). The continuing education of all veterinarians associated with the program is an essential component of maintaining competence.

Led by the AV, all veterinarians involved in clinical care should foster and support enhancement of the program through the identification and adoption of techniques, procedures and policies that improve laboratory animal health and well-being. When possible, the veterinarian should discuss health concerns with the research contact to determine a course of action consistent with experimental goals. If the research contact is not available, or if an agreement cannot be reached, the veterinarian has full authority by the IACUC and the IO to act to protect the health and well-being of the animal or colony. All veterinarians must ensure that their professional judgements are not influenced by institutitonal interest to the detriment of the animals under their care.

DISEASE DETECTION AND SURVEILLANCE, PREVENTION, DIAGNOSIS, TREATMENT AND RESOLUTION

Procurement and Health Assessment

All incoming animals purchased or imported into Massachusetts General Hospital must be acquired lawfully on to an approved IACUC protocol. MGH maintains strict health requirements for the animals entering the facilities to minimize the risk of pathogens that can adversely alter scientific objectives or pose a potential harm to people or other research animals. Please see the IACUC Policy on Animal Acquisition.

Daily Animal Obserations and Veterinary Care:

All animals must be evaluated daily for abnormalities and proper treatments or actions should be taken based on what is approved in the protocol or when unexpected research outcomes are observed. Within CCM-managed facilities, research animal specialists (RAS) are trained to identify and assess the severity of abnormal clinical signs for all of the species housed. In satellite facilities, this is done by either CCM or trained lab staff depending on whether there is a contractual agreement with CCM for full husbandry and veterinary care.

Clinical rounds that assess and review animals with open health concerns are generally performed weekly, however, the frequency may be changed at the discretion of the veterinarian. The AV is responsible for assuring that communication between animal care staff and the facility veterinarians are effective and timely. This must exist for both CCM facilities as well as satellite housing facilities.

CCM veterinarians share on-call duty to provide coverage during evening, weekend, and holiday hours throughout the year. One On-Call mobile phone is rotated between them and this number must be posted throughout all animal facilities in case of an emergency. MGH Police & Security and MGH Buildings & Grounds should also have the On-Call mobile number and should contact it for any animal or animal facility-related concerns that they observe. All staff members performing weekend care must contact the On-Call veterinarian if there are any clinical cases that need immediate assessment.

Disease Surveillance

Disease surveillance for all animal facilities must be managed by CCM VS. This includes routine monitoring of colony animals for the presence of parasitic, bacterial, and viral agents that may cause overt or inapparent disease. Any positive screening results may be confirmed by secondary method and depending on the agent appropriate containment, treatment or depopulation steps will be taken by CCM VS. Additionally, cells, tissues, fluids, and transplantable tumors that are to be used in animals must be screened by the researcher. These results are reviewed as part of the IACUC protocol review process for infectious agents that may cause disease in animals or compromise research data. Details of the

testing panels, vendors for biologic screening, and other applicable information may be found on the Animal Health & Treatment page of the CCM website under Cell Lines.

Diagnostics

CCM provides diagnostic laboratory services either through internal clinical pathology resources or through outsourced services such as anatomic pathology with reputable diagnostic laboratory organizations. Laboratory diagnostic services include histopathology, microbiology, clinical pathology, serology, and parasitology as well as other routine or specialized laboratory procedures, as needed. Services can be found on the Research Support Services page of the CCM website under Clinical Pathology Laboratory. CCM Veterinarians or the CCM Clinical Pathology laboratory management is also available to help researchers identify diagnostic resources when needed.

HANDLING AND RESTRAINT; ANESTHETICS, ANALGESICS AND TRANQUILIZER DRUGS; AND METHODS OF EUTHANASIA

CCM veterinarians should be available to provide consultations regarding anesthesia, analgesia, surgery, assessment of pain/distress, and euthanasia in general and with respect to specific research procedures. Detailed policies or guidelines for these program components are provided on the IACUC website (IACUC Policies & Guidance). CCM VS has developed template procedural descriptions that are available in Insight; these procedure forms can be views on the IACUC website (Standard Procedure Forms). Available templates include examples of proper handling and restraint, appropriate peri-operative requirements, analgesia, anesthesia, tranquilization, and euthanasia. IACUC members should utilize these procedural templates and guidelines when performing their review as well.

SURGICAL AND POSTSURGICAL CARE

CCM veterinarians who serve on the IACUC review and approve preoperative, surgical, and postoperative procedures as a part of the Full Committee (FCR) or Designated Member (DMR) review process. The AV or designee also serve in the monitoring and provision of recommendations concerning preoperative procedures, surgical techniques, the qualifications of institutional staff to perform surgery and postoperative care. The PI must confirm that personnel are

adequately trained and competent to perform the procedures. In addition, the AV or designee works in consultation with Research Space Management Group and the IACUC to ensure designated surgical facilities are appropriate for the intended surgical procedures.

ANIMAL WELL-BEING

Adequate veterinary care must include responsibility for the promotion and monitoring of an animal's well-being before, during and after experimentation or testing. CCM VS oversees the environmental enrichment program that covers all animals to support both their physical and psychological well-being in terms of environmental comfort, freedom from pain and distress and appropriate social interactions, both with conspecifics and with humans. Policies and guidelines that address social housing and environmental enrichment can be found in the IACUC Policy on Environmental Enrichment, Social Housing, and Exercise of Laboratory Animals.

APPROPRIATE USE OF ANIMALS IN RESEARCH AND TESTING

CCM Veterinarians should advise on the design and performance of experiments using animals as related to model selection, collection and analysis of samples and data from animals, and methods and techniques proposed or in use.

RELATED CONCERNS

Other areas of professional concern and responsibility by the AV and designees include the following:

1. Participating in the development and administration of training for institutional staff in the care and use of laboratory animals.
2. Assisting institutional health officials to establish and monitor an occupational health program for all animal care workers and others who have substantial animal contact.
3. Monitoring for zoonotic diseases such as leptospirosis, toxoplasmosis, rabies, Q-fever, B-virus infection, hantavirus infection, and lymphocytic choriomeningitis.
4. Advising on and monitoring of standards of hygiene among institutional staff involved with research animal care and use.

RELATED POLICIES

Policy on Environmental Enrichment, Social Housing, and Exercise of Laboratory Animals
Policy on Animal Acquisition

REFERENCES:

AAALACi. Position Statement: The Attending Veterinarian and Veterinary Care. May 2020.

American College of Laboratory Medicine. ACLAM Position Statement on Adequate Veterinary Care *Journal of the American Association for Laboratory Animal Science*. 2016, 55(6), 826-828.

National Research Council of the National Academies. *Guide for the Care and Use of Laboratory Animals,* 8th edition. National Academies Press: Washington, D.C., 2011.

U.S. Department of Agriculture. *Animal Welfare Act and Regulations* (Animal Care Blue Book). Code of Federal Regulations (CFR), Title 9, Chapter 1, Subchapter A, Parts 1-4. 2020.

U.S. Department of Health and Human Services. *Public Health Service Policy on Humane Care and Use of Laboratory Animals*. Office of Laboratory Animal Welfare, National Institutes of Health: Bethesda, MD, 2015.

v1.1, December 2004
v1.2, December 2005
v1.3, October 2011
v2.1, 18 September 2017
v2.2, 15 September 2021

MASSACHUSETTS GENERAL HOSPITAL
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY: LAB ANIMAL VIDEOS

GENERAL POLICY

Videos depicting laboratory animals (alive or dead) at Massachusetts General Hospital(MGH) or Shriners Hospitals for Children (SHC) shall not be uploaded to the internet, disseminated, or otherwise made available without prior IACUC approval. This includes, but is not limited to, animals (or animal parts) used in research, testing, and education.

Version: August 18, 2010

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON QUARANTINE AND ACCLIMATION OF RESEARCH ANIMALS

GENERAL POLICY

The *Guide for the Care and Use of Laboratory Animals,* 8th edition, defines **quarantine** as the separation of newly received animals from those already in the facility, in a way that prevents potential spread of contaminants, until the health and possibly the microbial status of the newly received animals have been determined. Regardless of whether the animals are quarantined, newly received animals should be given an **acclimation period** for physiologic, behavioral, and nutritional normalization. During this acclimation period, no experimentation may be conducted (including withdrawal of food, blood draws for experimental purposes, or use of restraint devices). Habituation to routine husbandry procedures and positive human interaction is encouraged during this time. The length of time for acclimation will depend on the type and duration of animal transportation, the species, and the intended use of the animals. .

REQUIREMENTS

Quarantine

Rodents, rabbits, livestock, and dogs coming from CCM approved vendors do not undergo quarantine.

Rodents coming from non-approved vendors must go through a period of quarantine.

Frogs, nonhuman primates and other species from non-approved vendors undergo a period of quarantine as determined by the Center of Comparative Medicine (CCM) veterinarians to eliminate risk of potential infectious pathogens.

Quarantine of any animal or room or species may be necessary by a CCM veterinarian when a suspected or confirmed disease is discovered. This will be done in consultation with the Principal Investigator(s).

Acclimation

Table 1. Acclimation periods for commonly used species*

Species*	Acclimation for survival surgery	Acclimation for non-surgical procedures requiring anesthesia	Acclimation for procedures not requiring anesthesia	Acclimation for non-survival surgery/procedures
Rodents **Amphibians** **Reptiles**	24 hours	24 hours	No acclimation required	No acclimation required

Livestock **Dogs** **Squirrels**	24 hours	24 hours	24 hours	No acclimation required
Rabbits	5 days	5 days	24 hours	No acclimation required
Nonhuman primates after quarantine and transferring between MGH facilities	1 week	24 hours	No acclimation required	No acclimation required
Nonhuman primates from Biomere	1 week	1 week	1 week	No acclimation required

*If you are working with a species not listed in the table, please contact your facility veterinarian for recommended the acclimation period.

The IACUC may grant exceptions to this policy on a case-by-case basis, following careful consideration to the potential impacts to the animal(s).

References:

National Research Council of the National Academies. Guide for the Care and Use of Laboratory Animals, 8th edition*. National Academies Press: Washington, D.C., 2011.*

v1.1, 29 September 2004
v1.2, 20 February 2008
v2.1, 18 October 2017
v2.2, 17 February 2021

INSTITUTIONAL ANIMAL CARE AND USER COMMITTEE (IACUC)

POLICY ON REPORTING ANIMAL WELFARE CONCERNS

GENERAL POLICY

MGH institutional policy requires that animal care and use comply with all applicable federal, state, and local laws, regulations and policies including, but not limited to:

- Animal Welfare Act and Regulations
- Public Health Service Policy on Humane Care and Use of Laboratory Animals
- Guide for the Care and Use of Laboratory Animals, 8th edition
- ACURO Regulations, Standards, & Requirements

Principal Investigators (PIs) are responsible for ensuring that the members of their research team who are listed in approved protocols use proper procedures and techniques when performing animal experimentation. Certification of compliance with MGH policy is provided when the IACUC Protocol is signed by the PI and submitted to the IACUC for review and approval. The Attending Veterinarian, in his or her role as Director, Center for Comparative Medicine (CCM), is similarly responsible for ensuring that CCM animal care and veterinary staff use proper procedures and techniques in animal husbandry and veterinary care.

Any employee responsible for, aware of, or observing a deficiency in animal care or use, or who otherwise has concerns about care and use of animals at the institution, should report the deficiency or concern as outlined in this policy. A deficiency may include acts of perceived negligence, omission or non-compliance with approved protocols and IACUC policies, unintended accidental events with a negative impact on animal health and welfare, and intentional acts of cruelty towards laboratory animals. Retaliation against any employee reporting of a good faith issue or concern is prohibited. An employee's confidentiality will be maintained to the extent possible consistent with the need to conduct an investigation. An investigator who identifies an animal welfare-related incident, or a protocol or other non-compliance, within their own program, is also encouraged to self-report to the IACUC.

REPORTING PROCEDURES

Any employee who observes a deficiency in animal care or use should take the following steps:

- Note the date, time, building location, room number, and species of animal involved.
- Provide a complete description of the act, personnel involved, and observed circumstances. This description should contain sufficient details to allow proper assessment by the staff person receiving the report.

- Report the information either anonymously through the MGH/MGPO Compliance Helpline (617-726-1446), or by telephone, by mail, e-mail, or in person to members of the animal care and use program leadership identified in the animal welfare reporting poster (Appendix 1).

A poster with information on reporting animal welfare concerns is posted throughout all facilities and the IACUC policy on reporting is posted on the IACUC Website.

REVIEW PROCEDURES

All reports of deficiencies or concerns are reviewed by the IACUC compliance personnel for potential merit. This review may include a discussion with the reporting individual, research staff, veterinary personnel and/or a review of the approved protocol, medical records, or other documents. If no merit is found, a summary report is shared with the IACUC Compliance Subcommittee and a response is sent to the reporting individual. A summary of all reported concerns that were not investigated further due to lack of merit (based on the information provided), is provided to the IACUC at the next convened meeting.

If potential merit is found, the IACUC compliance officer convenes an investigative team to review the event. The team includes, at a minimum, the IACUC compliance officer or delegate, the facility veterinarian, and the PI or designee. Additional members will be included as appropriate, depending on the nature of the event.

The review of the event by the investigative team may include:

- Review of documentation such as the IACUC protocol, medical record, laboratory notebooks, security access logs, and/or submitted emails and reports by the research team or others.
- Interviews with pertinent personnel including the reporting individual (if not anonymous), research team members, facility staff or others.
- Review may also include a physical inspection of the animals and/or facilities involved. The facility or on-call veterinarian may take immediate action to protect animal welfare, as described in the Policy on Adequate Veterinary Care .

Violations of hospital policies outside of the IACUC's purview will be remanded to the appropriate hospital process.

The investigative findings will be presented by the IACUC compliance officer or delegate to the IACUC Compliance Subcommittee as a written report for discussion and review. The Compliance Subcommittee is comprised of the IACUC compliance officer, the IACUC Chairs, the Attending Veterinarian, the Director Animal Welfare Assurance (AWA), and a Scientist member of the IACUC, with the facility veterinarian as an ad hoc member. At a minimum, the IACUC Chair/Vice Chair or delegate or the Attending Veterinarian must be present for the subcommittee to meet. The Compliance Subcommittee may request further information or additional investigation as needed and may also make recommended revisions to the written report for clarification purposes. The subcommittee will formulate proposed, recommended corrective actions for the IACUC's review, and will

make a recommendation to the IACUC as to whether the event is reportable to the Federal agencies and AAALAC. The incident, and the recommendations from the subcommittee, will be presented to the IACUC by a member of the subcommittee at the next convened meeting. Following their review of the reported event, the IACUC may ask for additional information and/or further investigation. Once the committee agrees that they have the information needed to reach a decision, the IACUC will vote on whether the event is reportable, based on guidance and policy made available to the IACUC by the Federal and oversight agencies, and on institutional policy and procedure. The committee will also determine the appropriate corrective actions that must be put in place to prevent a recurrence of the event.

Corrective actions may include:

- Revisions to the approved protocol
- Reiteration of approved protocol practices and applicable IACUC policies
- Training/re-training of personnel
- Letter of notification/reprimand to personnel involved
- Temporary restriction on the use of animals in research by personnel involved
- Permanent prohibition of the use of animals in research by personnel involved
- Appropriate disciplinary action against personnel involved
- Remand to the appropriate MGH process if determined to be a case of misconduct in research or a serious violation of any other MGH policy
- Suspension or revocation of protocol(s).

The investigative findings and corrective action plan imposed will be reported in writing (email is acceptable) to the PI by an IACUC officer.

If there is a need for immediate action while the concern is being investigated, the investigative team, through the IACUC compliance officer, may convene a meeting of the IACUC Compliance Subcommittee to request immediate action to mitigate or correct a potential animal welfare concern. The meeting may be convened by teleconference. If a meeting cannot be convened within 24 hours, the IACUC compliance officer may notify any member of the IACUC Compliance Subcommittee to request immediate action. This action may include a request to the PI to stop the conduct of all animal research activity pertinent to the report, for whatever time is needed to complete the investigation. This action may also include the transfer of involved animals to a holding protocol or euthanasia of the animals involved. The IACUC Compliance Subcommittee member(s) may delegate this action to the IACUC compliance officer or facility veterinarian. The IACUC Compliance Subcommittee may also determine that a preliminary report to OLAW is appropriate at that time. Any such interim action will be included in the report to the IACUC Compliance Subcommittee and the IACUC.

APPEAL PROCESS

Once the IACUC has deliberated about a reported concern and decided on the corrective, and other, actions needed, any affected party may appeal such action by requesting to appear in person at the next regularly convened meeting of the IACUC, or by submitting a written request to the IACUC to reconsider their decision. A written request must be submitted to the IACUC compliance officer within 14 business days. This request will be reviewed by the convened IACUC at the next meeting.

INSTITUTIONAL REPORTING

The IACUC office will notify the PI of the committee's final determination, the corrective action plan and timeline, and whether the event was determined by the IACUC to be reportable to relevant regulatory agencies (i.e., OLAW, USDA, ACURO, funding agencies, etc.).

The outcome of reported concerns will be communicated to the concerned employee(s) when feasible, unless such concerns are reported anonymously.

The review and reporting process is summarized in Appendix 2. The Standard Operating Procedure for reporting to OLAW is captured in Appendix 3.

If, during the investigation, questions or concerns of interest to the institution and outside the direct purview of the IACUC are identified, they will be forwarded to the Institutional Official for further action. These may include human resource, research misconduct or other issues.

Reporting to OLAW

If the IACUC determines the event requires reporting to OLAW, the Director AWA submits a written report to the Director, Division of Compliance Oversight, OLAW on behalf of the Institutional Official. A verbal preliminary report will be provided to OLAW as appropriate.

Reporting to USDA

If the IACUC determines that an event involving a USDA-covered species warrants reporting to the USDA, the Attending Veterinarian with the Director AWA will make a verbal report to the USDA Veterinary Medical Officer (VMO).

Reporting to ACURO

If the convened IACUC determines that an event meets ACURO's definition of a significant deficiency, noncompliance, and/ or adverse event, the Director AWA will inform the investigator that the event must be reported to ACURO within 5 business days. In this case, either the investigator or the Director AWA will submit the report to ACURO. If the convened IACUC suspends a DoD-supported or conducted research activity, if there is a

change in the institution's AAALAC accreditation status, or if there is a socially-sensitive matter concerning, or potentially impacting, DoD-conducted or supported research activities, the Director AWA will notify ACURO within 5 business days. If USDA notifies the institution of an investigation related to a DoD conducted- or supported-study, the Director AWA will promptly notify ACURO within 5 business days of the initial notification. In any case, the Director AWA may make a preliminary inquiry regarding reporting to ACURO as needed.

Reporting to AAALAC

All reports to OLAW are forwarded to AAALAC for review. All other reporting to AAALAC follows AAALAC's reporting requirements.

RELATED POLICES

Policy on Adequate Veterinary Care

REFERENCES

National Research Council of the National Academies. Guide for the Care and Use of Laboratory Animals, 8th edition*. National Academies Press: Washington, D.C., 2011.*

U.S. Department of Agriculture. *Animal Welfare Act and Regulations* (Animal Care Blue Book). Code of Federal Regulations (CFR), Title 9, Chapter 1, Subchapter A, Parts 1-4. 2020.

U.S. Department of Defense. Institutional Notifications to the Animal Care and Use Review Office. U.S. Army Medical Research and Development Command, Animal Care and Use Review Office (ACURO): Fort Detrick, MD, 1 July 2020.

U.S. Department of Health and Human Services. NOT-OD-05-034: Guidance on Prompt Reporting to OLAW under the PHS Policy on Humane Care and Use of Laboratory Animals. Office of Laboratory Animal Welfare, National Institutes of Health: Bethesda, MD, 2005.

U.S. Department of Health and Human Services. *Public Health Service Policy on Humane Care and Use of Laboratory Animals.* Office of Laboratory Animal Welfare, National Institutes of Health: Bethesda, MD, 2015.

v1.1, 16 February 2005
v1.2, 14 December 2011
v1.3, 16 January 2013
v2.1, 20 September 2017
v2.2, 20 June 2018
v2.3, 20 February 2018
v2.4, 16 June 2021
v2.5, 20 April 2022

Appendix 1. Animal Welfare Concerns Reporting Poster

Reporting Animal Welfare Concerns

Any MGH employee observing a deficiency in animal care or use is encouraged to report it as outlined below. A deficiency may include acts of perceived negligence, omission or non-compliance with approved protocols and IACUC policies, and intentional acts of cruelty towards laboratory animals.

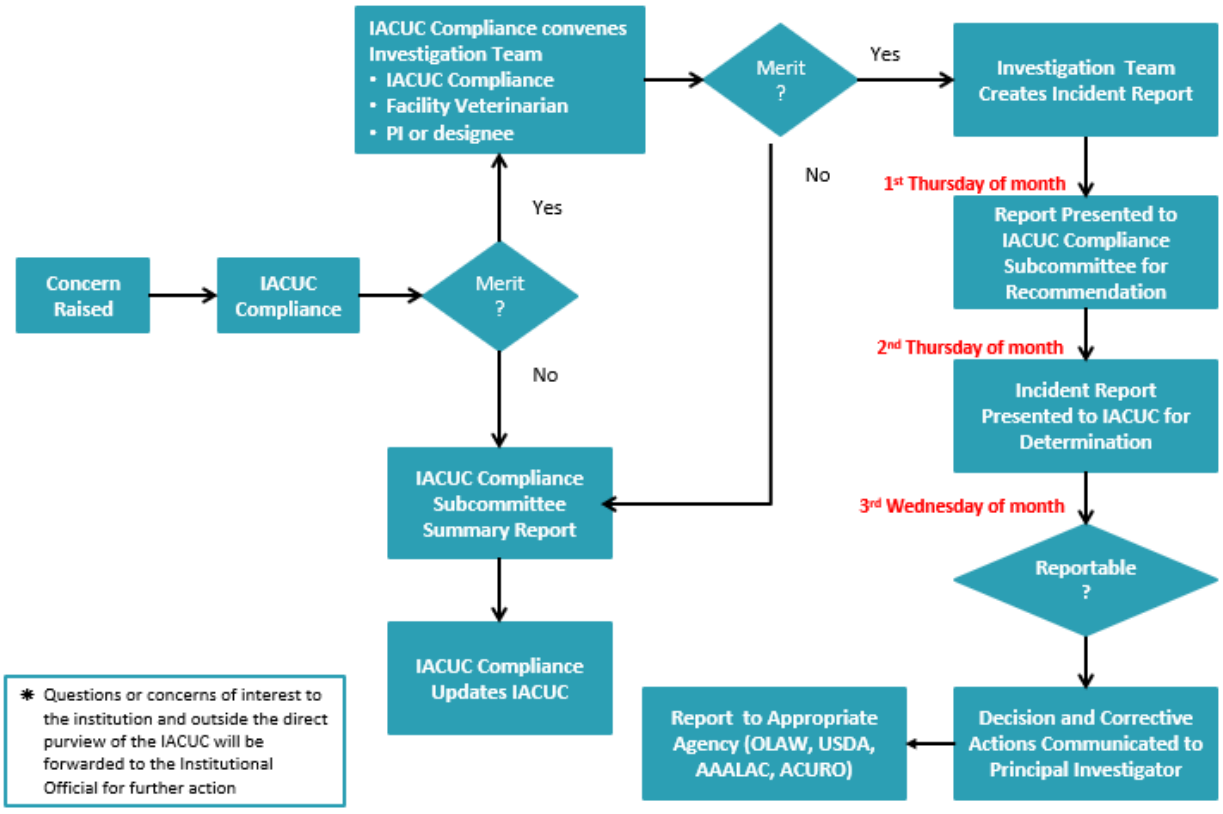
Hospital policy, as well as federal law, prohibits any discriminatory or reprisal measures being taken against any employee for reporting deficiencies in animal care or use.

MGH intends to protect, to the extent possible, the privacy of an individual who in good faith reports an apparent deficiency.

Name	Office	Phone	Email
Anne Clancy, PhD Director, Animal Welfare Assurance	CNY 149-5250	617-724-2733	aclancy1@mgh.harvard.edu
Adria Colletti, PhD IACUC Compliance Lead	CNY 149-5263	617-724-4503	acolletti@mgh.harvard.edu
Carolyn Fader, BS, CPIA IACUC Regulatory Compliance Specialist	CNY 149-5262	617-726-3495	cfader@mgh.harvard.edu
Harry Orf, PhD Institutional Official & Senior Vice President for Research	BUL-2-240E	617-724-9079	horf@mgh.hardvard.edu
Donna Matthews Jarrell, DVM Attending Veterinarian Director, Center for Comparative Medicine	CNY 149-5249	617-726-9432	djarrell@mgh.harvard.edu
James S. Allan, MD, MBA IACUC Chair	FND-7	617-724-1103	jallan@mgh.harvard.edu
MGH IACUC Compliance mailbox			IACUCCompliance@mgh.harvard.edu
MGH/MGPO Compliance Helpline **For Confidential & Anonymous Reporting**		**617-726-1446**	

For more details, refer to the IACUC Policy on Reporting Animal Welfare Concerns

Appendix 2. Animal Welfare Concern Investigation Process Flowchart



Appendix 3. SOP for reporting animal welfare concerns to OLAW

1. Director Animal Welfare Assurance will draft a notification to OLAW summarizing the event, the species involved, and the corrective actions.
2. Director Animal Welfare Assurance will email the PI, IACUC contact, and Department Administrator (DA) a copy of the draft report. The email will include the IACUC protocol number and the dates of the event. The DA will be asked to provide a list of all funds charged for animal work during the period of non-compliance, including sponsored and sundry funds. Director Research Compliance MGH, Corporate Director Research Management Post-Award and Post-Award grant administrator will also be copied on the email to the PI.
3. The relevant funds identified by the DA will be listed on the report to OLAW. Director Animal Welfare Assurance will ask Director, Finance and Administration CCM for all animal invoice information for the protocol during the period of noncompliance as a cross-check for the information provided by the DA.
4. For events where no monetary adjustment is required (e.g., adverse event), the Director, Animal Welfare Assurance will email the draft report to the PI and IACUC contact as an FYI and will copy Director Research Compliance MGH and Corporate Director Research Management Post-Award as an FYI.
5. The IACUC Director forwards the report to the Institutional Official for review and signature and forwards the signed report to OLAW on behalf of the Institutional Official. The IO, Attending Veterinarian, IACUC Chair, Director Research Compliance MGH, and Corporate Director Research Management Post Award (as required) are copied on the email to OLAW.

Post-Award grant administrator will work with the DA to remove relevant charges from sponsored funds and will work with the PI to notify sponsor/prime awardee, as required.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON CONTROLLED SUBSTANCES USED IN ANIMAL RESEARCH

GENERAL POLICY

Each investigator is responsible for purchasing, securing, documenting, and monitoring the use of controlled substances, as defined and regulated by the U.S. Drug Enforcement Agency (DEA) and the Massachusetts Department of Public Health (DPH), for use in IACUC-approved protocols. All investigators using controlled substances in their animal (non-human) research are required to follow the MGH policy on Non Human Research Controlled Substances. Detailed information can be found on the Research Compliance Use of Controlled Substances in Non-human Research page.

v1.1, February 2005
v1.2, 19 February 2014
v1.3, 19 April 2017
v1.4, 17 March 2021

MASSACHUSETTS GENERAL HOSPITAL (MGH)
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

EMERGENCIES ARISING IN ANIMAL FACILITIES

Emergencies that represent an immediate threat to personnel and laboratory animals may occur in animal facilities. A location specific Emergency Response Plan (ERP) has been generated for all approved animal housing locations and has been approved by the IACUC. Copies are also maintained on file and distributed to the research community by the MGH Research Safety Manual. This policy provides summary of those plans describing the steps to be taken if such an emergency occurs. For any emergency, the specific ERP should be referenced. The Director of CCM and the Facility Manager in whose area the emergency occurs must be notified as soon as possible and responses coordinated with other MGH departments. Each emergency and its effects must be documented by the responsible department or laboratory in a formal Emergency Incident Report (EIR), provided to the IACUC, as soon as possible after the event.

Electrical Power Failure and Environmental Excursions

When a power outage occurs, the expected duration of the outage must be determined. If power is disrupted for only a few hours and if outside weather conditions allow, animals may be left in place while environmental conditions such as temperature and humidity are monitored on a frequent basis. Animal room doors may be propped open to improve circulation during this interruption if no hazardous materials are present in the affected rooms.

The maximum time environmental conditions may be outside acceptable ranges for animal rooms are listed below:

Species	Temperature (°F)	Humidity (%RH)	Max time allowed
Rodents	<65 or >85	<20 or >85	4 hours
Rabbits	<50 or >80	< 30 or >75	
Dogs, Livestock	<45 or >85	<30 or >75	
Nonhuman Primates	<60 or >80	<30 or >75	

If power will not likely be restored within several hours or if the room environment continues to deteriorate (e.g., too hot, too cold, too humid), B&G should be made aware of the situation in order to provide ancillary support while animals remain in the room in their cages. Such support may consist of temporary emergency power or heating, cooling, forced ventilation, humidification, dehumidification, etc., using portable equipment.

If these actions are not successful in returning the room environment into the acceptable ranges or if the power failure is expected to last more than several days, animals may be transferred to other facilities that provide an adequate environment.

CCM will consult with RSMG, B&G, and affected investigators to determine possible options or transfer scenarios.

Fire, Smoke, Flooding
Reasonable efforts should be made to protect animal rooms from these emergencies after P&S is notified and personnel are safe and protected. Such steps may consist of using fire extinguishers for small fires, making sure fire doors are closed, and diverting water from seeping into animal facilities. Animals must be secured (left in their cages) and handled as described in the applicable Emergency Response Plan until the emergency is declared under control by P&S or outside emergency responders.

Hazardous Material Exposures
If there is release of infectious, chemical, or radioactive material into the environment within or adjacent to an animal facility, P&S must be notified immediately. The response to such a release will be coordinated between P&S, B&G, and Environmental Health and Safety. Determination of appropriate action items involving laboratory animals will also include the IACUC and CCM Veterinary staff.

[Most recently revised May 25, 2014]

MASS GENERAL RESEARCH INSTITUTE
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON THE USE OF EXPIRED DRUGS AND MEDICAL MATERIALS

GENERAL POLICY

According to federal laws, regulations, and policies, the use of expired pharmaceuticals, biologics, and supplies is not consistent with acceptable veterinary practice or adequate veterinary care. Drugs administered for anesthesia, analgesia, veterinary care, or euthanasia should not be used beyond their expiration date, even if a procedure is terminal. Other expired materials must not be used unless the manufacturer verifies efficacy beyond the expiration date, or the investigator is able to document to the satisfaction of the IACUC that such use would not negatively impact animal welfare or compromise the validity of the study.

In addition, all drugs and other medical supplies used on research animals must be properly stored, and appropriately labeled.

EXPIRATION DATES

- **Unopened drugs and medical materials in the original packaging**: expiration date or use by date printed on the label or packaging by the manufacturer.
- **Opened multi-dose vials/bags of injectables**: a multi-dose vial or bag that has been opened or accessed (e.g., needle-punctured) should be dated and discarded within 28 days unless the manufacturer specifies a different (shorter or longer) date for that opened vial/bag.
- **Aliquots:** expiration date or use by date printed on the label or packaging of stock material.
- **Reconstituted solutions:** expiration date or instructions printed on the label or packaging by the manufacturer. Reconstituted solutions must be stored under the conditions described in the packaging.
- **Dilutions:** discard 28 days after preparation
- **Mixtures / formulations:**
  NOTE: Any mixture that becomes clouded or precipitates should not be used in animals
  - Discard 28 days after preparation
  - If the stability of an agent in formulation is unknown, prepare fresh prior to each use.

Aliquots, dilutions, reconstitutions, or mixtures of drugs or fluids should be prepared using sterile technique and stored under proper conditions; see Appendix 1 for recommended storage containers. These materials must be labeled as described in Labeling Requirements and Expiration Dates for Research Drugs with:

- Contents
- Concentration
- Date of reconstitution/preparation
- Expiration date
- Initials of preparer

An item is considered expired the day after the month or date indicated on the label. For example, an item with the expiration date of January 2021 on the label would be considered expired on February 1, 2021. Expired materials should either be disposed of or segregated to a location physically separated from non-expired materials. The expired materials must be clearly labeled “For Non-survival Use Only”.

SELECT USE OF EXPIRED MEDICAL MATERIALS

Expired anesthetics, analgesics, sedatives, emergency drugs, or euthanasia drugs may never be used in any live animal, regardless of whether the procedure will be terminal.

	Non-Survival Procedures	**Survival procedures**
Can be used	If the packaging has not been opened or compromised: • Parenteral fluids (e.g., saline, Ringer’s, etc.) • Surgical instruments • Suture materials • Supplies (e.g., gauze, needles, butterfly catheters, etc.) • Implantable materials or devices (e.g., cardiac catheters, etc.)	If items are re-sterilized and their function is not compromised: • Surgical instruments • Supplies • Implantable materials and devices The date of re-sterilization must be indicated on the packaging, and the pack must include the appropriate sensor strips indicating successful sterilization.
Cannot be used	Regardless of the packaging’s condition: • Physiologically active drugs • Anesthesia, analgesia, emergency drugs • Euthanasia drugs	• All drugs and parenteral fluids (e.g., saline, Ringer’s, etc.) • Functionally compromised devices (e.g., instruments, sutures, etc.).

In order to assure compliance with these regulatory requirements, the IACUC, CCM, and Environmental Health & Safety will conduct periodic audits of animal housing and use sites. Any substances and materials discovered during the course of an audit that are expired, inadequately labeled, or inappropriately stored will be discarded at that time. Failure to comply with this policy may result in suspension or revocation of animal research privileges.

REFERENCES

U.S. Department of Agriculture. *Animal Welfare Act and Regulations* (Animal Care Blue Book). Code of Federal Regulations (CFR), Title 9, Chapter 1, Subchapter A, Parts 1-4. 2020.

U.S. Department of Health and Human Services. Frequently Asked Questions, PHS Policy on Humane Care and Use of Laboratory Animals. F. Animal Use and Management, Question 5. Office of Laboratory Animal Welfare, National Institutes of Health: Bethesda, MD, January 9, 2020.

U.S. Department of Health and Human Services. Guidelines for the Select Use of Expired Medical Products . National Institutes of Health, Animal Research Advisory Committee: Bethesda, MD, September 26, 2018.

U.S. Department of Health and Human Services. Drug Packaging and Labeling Control - Expiration Dating. Code of Federal Regulations (CFR), Title 21, Volume 4, Chapter 1, Subchapter C, Part 211.137. 2020.

U.S. Department of Health and Human Services. FAQs regarding Safe Practices for Medical Injections - Questions about Multi-dose vials. Centers for Disease Control and Prevention: June 20, 2019.

v1.1, February 2005
v1.2, August 2013
v2.1, 20 September 2017
v2.2, 17 March 2021

Appendix 1. Recommended storage containers for aliquots, dilutions, or mixtures/formulations of drugs or fluids

All aliquots, dilutions, and formulations should be prepared using aseptic procedures and should be stored in sterile containers.

For drugs or agents that will be prepared and used on the same day, sterile screw cap vials or tubes like the ones pictured below may be used.

If drugs or agents will be used for more than one day, a sterile container with a rubber septum should be used. An additive free Vacutainer is an acceptable alternative.

MASSACHUSETTS GENERAL HOSPITAL
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY FOR USE OF HAZARDOUS MATERIALS

GENERAL POLICY

All animal protocols that involve the use of biohazardous agents (including recombinant DNA), hazardous chemicals, radioisotopes, or lasers must be reviewed and approved by the appropriate safety officers and/or safety committees prior to initiation of the protocol. The electronic submission system automatically notifies appropriate safety offices of submitted protocols involving any of the various safety considerations referenced above for review. Any protocol using a hazardous material will not receive IACUC approval until after approved by the appropriate safety office. Protocols involving hazardous materials are assigned an appropriate housing level (HL1, HL2) that incorporates both biosafety designation as well as chemical, and radioisotope containment. It is recommended to identify cages (either color coded cards or labels) that contain hazardous agents for easier identification.

The MGH Occupational Health service has an electronic safety report (http://safety-feedback-mgh.partners.org/RMProWeb_RM_PROD/RiskWeb3.dll) that must be completed for any animal bite, accidental skin puncture, splash to eyes or mucous membrane, splash to skin, inhalation of hazardous material, ingestion of hazardous material or other lab related accident.

Infectious Agents and Recombinant DNA

Biohazardous agents are defined as infectious agents (e.g., viral, bacterial, fungal, parasitic), toxin-producing agents, either isolated naturally or constructed by recombinant DNA technology (e.g., viral gene therapy vectors), or human material, including both primary and established human cell lines, that have the potential for causing diseases in healthy persons, animals, or plants. All animal protocols involving the use of biohazardous agents must be reviewed and approved by the Partners Institutional Biosafety Committee (PIBC), the Institutional Biosafety Committee for MGH. Principal Investigators are required to submit a separate application to PIBC for review and approval and that submission must be selected on the IACUC hazardous materials sub-form to 'link' the animal protocol to the PIBC protocol. Once PIBC has approved the protocol, the Biosafety Officer will review the animal protocol and indicate the proper handling precautions that must be taken.

The Principal Investigator on the protocol is responsible for ensuring that laboratory personnel are trained in the safe use, handling, and disposal of the agent(s) involved. The Center for Comparative Medicine (CCM) is similarly responsible for ensuring that animal husbandry and veterinary staff are trained in the same manner. Appropriate personal protective equipment (PPE) must be worn at all times when handling infectious agents, recombinant DNA, and infected animals, their tissues, and wastes.

All injury incidents and accidental exposures involving rDNA are required to be reported to the MGH Biosafety office through the MGH on-line Safety Reporting service: http://safety-feedback-mgh.partners.org/RMProWeb_RM_PROD/safety.html

Hazardous Chemicals Including Carcinogens, Mutagens and Teratogens

All animal protocols involving the use of hazardous chemicals, carcinogens, mutagens or teratogens, must be reviewed and approved by the MGH Environmental Health and Safety Office (EH&S). The Material Safety Data Sheet (MSDS) must be attached with the electronic IACUC submission and a copy kept on file in the laboratory for each toxic chemical and carcinogen used. A lab specific Chemical Hygiene Plan which includes an annually updated inventory for any chemicals to be purchased, stored or used in the lab must also be attached and available to staff.

The Principal Investigator is responsible for ensuring that laboratory personnel develop and adhere to a standard operating procedure for use of the substance, and for training laboratory personnel in the proper use and disposal of the substance. Documentation of this training must be available for review. CCM is similarly responsible for ensuring that animal husbandry and veterinary staff are trained in the same manner.

Appropriate personal protective equipment (PPE) must be worn at all times when handling hazardous chemicals, carcinogens, mutagens and teratogens, and exposed animals, their tissues, and wastes. The EH&S office (http://intranet.massgeneral.org/ehs/ehs_home.htm) also has chemical monitoring badges (isoflurane, hydrogen sulfide, chloride) that are available on request.

Radioisotopes

All animal protocols involving the use of radioisotopes must be reviewed and approved by the Radiation Safety Officer (RSO). The protocol must include an investigator who holds a radioisotopes permit for the radioactive material involved. All workers handling the radioactive material must demonstrate appropriate training and knowledge, to be assessed by the RSO. Appropriate PPE and radiation exposure badges must be worn at all times when handling radioactive material and animals, their tissues, and wastes. Radioactive materials, animal carcasses, waste and bedding, as well as other potentially contaminated materials, must be disposed of in accordance with MGH policies.

Lasers

All research proposals involving the use of lasers must be reviewed and approved by the Laser Safety Committee and EH&S. All workers using the laser or in the immediate vicinity of its use must demonstrate appropriate training and knowledge in the use of the particular laser, to be assessed by the LSC. Appropriate PPE must be worn at all times when lasers are being used.

[Most recently revised May 7, 2014]

MASSACHUSETTS GENERAL HOSPITAL

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON THE USE OF NON-PHARMACEUTICAL GRADE SUBSTANCES IN LABORATORY ANIMALS

GENERAL POLICY

Both OLAW and AAALACi provide guidance regarding the use of non-pharmaceutical grade compounds in laboratory animals. Pharmaceutical-grade substances, when available, must be used to avoid toxicity or side effects that may threaten the health and welfare of vertebrate animals and/or interfere with the interpretation of research results. However, it is frequently necessary to use non-pharmaceutical-grade substances such as investigational substances, compounded substances, and/or Schedule I controlled substances to meet scientific and research goals.

DEFINITIONS

Pharmaceutical-grade compound: A pharmaceutical-grade compound (PGC) is defined as any active or inactive drug, biologic or reagent, for which a chemical purity standard has been established by a recognized national or regional pharmacopeia (e.g., the U.S. Pharmacopeia (USP), British Pharmacopeia (BP), National Formulary (NF), European Pharmacopoeia (EP), Japanese Pharmacopeia (JP), etc.). These standards are used by manufacturers to help ensure the products are of the appropriate chemical purity and quality, in the appropriate solution or compound, to ensure stability, safety, and efficacy. The Food and Drug Administration (FDA) maintains a database listing of FDA approved commercial formulations for both FDA approved drugs (the Orange Book) and veterinary drugs (the Green Book).

The following are considered **pharmaceutical grade**:

- FDA-approved, commercially available human or veterinary formulations
- Compounded product prepared by a registered pharmacist using USP standards
- Dilutions or combinations of FDA-approved, commercially available human or veterinary formulations used to compound a needed dosage. Examples: Buprenex (buprenorphine) diluted with sodium chloride (0.9%) injection solution or Ketaset (ketamine) and xylazine diluted with sodium chloride (0.9%) injection solution for administration to rodents. *Note*: all constituents of the dosage **must** be pharmaceutical grade.

USP grade: USP grade refers to the chemical purity of a material. USP grade material is of sufficient purity to be used in the manufacture of food, drugs, or medications. The bulk drug or chemical is considered **non-pharmaceutical grade**. Example: USP grade tamoxifen purchased as a powder from Sigma.

New investigational substances: substances that are supplied by the manufacturer for testing in an experimental setting only. A new investigational substance would not have chemical purity standards established and by default is considered a **non-pharmaceutical grade** compound.

Availability: Refers to whether a product is commercially available from an active U.S. vendor.

JUSTIFICATION AND USE OF NON-PHARMACEUTICAL GRADE SUBSTANCES

The use of non-pharmaceutical grade substances (including active substance and other active or inactive constituents such as vehicles) must be described and justified in the IACUC protocol. Appropriate scientific justification for the use of non-pharmaceutical grade substances includes:

- No equivalent veterinary or human drug is available for experimental use; this includes new investigational compounds.
- Pharmaceutical grade is not available in the appropriate concentration or formulation, or the appropriate vehicle control is not available.
- Non-pharmaceutical grade is required to generate data as part of an on-going study or to generate data that are comparable to previous work without inadvertent introduction of new variables.

Cost savings alone is not considered an adequate justification for the use of non-pharmaceutical-grade substances

Agents for sedation, analgesia, anesthesia, or euthanasia should be commercially available veterinary or human preparations, unless the use of an investigational chemical or formulation is scientifically necessary, appropriately justified, and approved by the IACUC.

Non-pharmaceutical grade substances must be prepared as described in the Guidelines for Preparing Drugs and Agents for Animal Research. When formulating from bulk drug or chemical, USP grade material should be used when available. If USP grade drug or chemical cannot be obtained, material of the highest purity available, e.g., American Chemical Society (ACS) grade or reagent grade, should be used.

REFERENCES

AAALACi. Frequently Asked Questions, C. Institutional Responsibilities, 9. Non-Pharmaceutical-Grade Compounds. June 2017.

National Research Council of the National Academies. Guide for the Care and Use of Laboratory Animals, 8th edition*. National Academies Press: Washington, D.C., 2011.*

U.S. Department of Health and Human Services. Guidelines for the Use of Non-Pharmaceutical

Grade Compounds in Laboratory Animals . National Institutes of Health, Animal Research Advisory Committee: Bethesda, MD, March 4, 2016.

U.S. Department of Health and Human Services. Frequently Asked Questions, PHS Policy on Humane Care and Use of Laboratory Animals. F. Animal Use and Management, Question 4. National Institutes of Health, Office of Laboratory Animal Welfare: Bethesda, MD, November 28, 2017.

v1.1, 20 February 2019
v1.2, 21 April 2021