OLAC Newsletter

Office of Laboratory Animal Care

Volume 5, Issue 1

May 2013

Upcoming AAALAC Site Visit

Patricia Coan, DVM, PhD, DACLAM

AAALAC International is a private, nonprofit organization that promotes the humane treatment of animals in science through voluntary accreditation and assessment programs. AAALAC stands for the "Association for Assessment and Accreditation of Laboratory Animal Care." AAALAC endorses the use of animals to advance medicine and science when there are no non-animal alternatives, and when it is done in an ethical and humane way. Having AAALAC International accreditation demonstrates that an institution is serious about maintaining high standards for animal care and use and is committed to animal welfare

"More than 890 companies, universities, hospitals, government agencies and other research institutions in 37 countries have earned AAALAC accreditation, demonstrating their commitment to responsible animal care and use. These institutions volunteer to participate in AAALAC's program, in addition to complying with the local, state and federal laws that regulate animal research".

UTKA's animal care and use program has a long history of AAALAC accreditation. The UTMCK became accredited in 1968. The UTCVM and WLS facility both became AAALAC accredited in the early 1980's. In 2000, the entire UTKA animal care and use program became accredited as one unit, including the Agricultural campus and the Research and Education Centers located around the state. We have been one fully AAALAC accredited animal care and use program for 13 years now.

The accreditation process includes the submission of an Animal Care and Use Program Description and a site visit once every three years. This year's document was over 500 pages long with over 30 housing and specialized use areas on campus. The AAALAC site visit will happen the week of July 29, 2013. There will be four AAALAC site visitors touring every area where vertebrate animals are used for research, teaching, or testing. They will compare the Program Description to what they see onsite. They will visit all surgery and housing areas, including laboratory areas that use vertebrate animals. They will ensure that our institution adheres to both the 8th Edition of the Guide for the Care and Use of Laboratory Animals (Guide), NRC 2011; and the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Ag Guide), FASS 2010. The site visitors will look at the appropriate components and cleanliness of the area. They will ask questions about the program to the veterinarians, researchers, students and technicians. They will thoroughly evaluate every aspect of our animal care and use program. OLAC is currently conducting mock site visits in the dedicated animal facilities. If you would like a mock site visit to help prepare for the upcoming AAALAC site visit, please call us at 974-5634 and we will be glad to schedule one with you.

References:
http://www.aaalac.org/about/index.cfm

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OLAC Office
2431 Joe Johnson Drive
336 Ellington Plant Science
Knoxville, TN 37996
Phone: 865-974-5634
Fax: 865-974-5649
OLAC is published by the University of Tennessee Office of Laboratory Animal Care.
UT is an EEO/AA/Title VI/Title IX/ Section 504/ADA/ADEA institution.
Publication #: E18-9909-002-08

Does the Glove Fit?

A Guide to the Selection and Use of Disposable Gloves

Brian Ranger, MS, SM(NRCM), CBSP & Susan Fiscor, CIH, CSP, CHMM

Disposable gloves are a commonly used type of personal protective equipment. They provide a quick, easy-to-use, and relatively inexpensive barrier to grease, grime, and potentially hazardous materials (biological, chemical and radiological). However, it is important to understand that there isn't a single type or brand of glove that is guaranteed to cover all tasks. Disposable gloves have many limitations depending on the base material, thickness (gauge), elasticity, manufacturer, performance testing stringency, and types of (hazardous) materials that may be encountered or handled.

Table 1 summarizes the types, advantages and limitations of disposable gloves used for most research, teaching, and clinical applications at the University of Tennessee, Knoxville-area campuses.

Table 1. Summary of commonly-used fluid-resistant disposable gloves

Glove Material	Advantages	Limitations
Latex	Elastic with good tensile strength Durable while performing routine, low-impact tasks Tear, abrasion and puncture resistant[1] under routine, low-impact conditions Good tactile sensitivity Comfortable with low modulus (resistance to hand movement) Good for biological and water-based materials	Limited chemical protection; can be degraded by oils and many organic solvents Deteriorate with long-term exposure to oxygen, ozone, and UV light Can induce or exacerbate latex allergies (leading to anaphylaxis in some cases) Difficult to detect small punctures in glove matrix-may lead to accidental exposures
Nitrile	Resistant to a wide range of chemicals including oils, alcohols, aldehydes, and some acids/bases Durable while performing routine, low-impact tasks Tear, abrasion and puncture resistant under routine, low-impact conditions Clear indication of punctures or small breaks Comfortable Good alternative for those with latex allergies Good for biological materials	Can have a high modulus/ stiffness leading to hand fatigue Deteriorate with long-term exposure to oxygen, ozone, and UV light Tactile sensitivity is not as good as for latex gloves, and may be inadequate/poor with thicker gauges
Neoprene	Resistant to a wide range of chemicals including oils, alcohols, aldehydes, peroxides and some acids/bases Durable while performing routine, low-impact tasks Tear, abrasion and puncture resistant under routine, low-impact conditions	Poor for aromatic or halogenated hydrocarbons Can have a high modulus/ stiffness leading to hand fatigue Deteriorate with long-term exposure to oxygen, ozone, and UV light Tactile sensitivity is not as good as for latex gloves, and may be inadequate/poor with thicker gauges
Vinyl (Polyvinyl Chloride)	Resistant to oils, fats, peroxides, and some acids/bases Not as prone to deterioration via oxygen/ozone exposure Abrasion resistant	Poor durability Poor elasticity and tensile strength; gloves readily tear or rupture Poor resistance to many chemicals including alcohols, aldehydes, and many organic solvents Not form-fitting, increasing risk for exposure to hazards Not adequate for handling infectious materials

[1]Puncture resistance does not include protection from needles, sharp devices, or anything else that focuses pressure across a very small area.

In addition to glove selection, proper use is essential for the gloves to successfully protect against contamination. Key points are:

1. Prior to putting on gloves, dry your hands thoroughly. Wet hands increase friction when trying to don gloves, making breaks and tears likely. Likewise, it is important to trim/file fingernails and remove any jewelry with jagged points or edges.
2. Avoid using lotions and waterless hand sanitizers just prior to putting on gloves as oils/alcohols may jeopardize glove integrity and reduce the breakthrough time, especially when wearing latex gloves.
3. Avoid powdered gloves. Powder can defeat the intent of glove barrier protection by functioning as a vehicle for the transport of infectious microorganisms and interfering with the local resistance to infection in wounds where powder is deposited. Glove powder may also cause dermatitis with cracks and open lesions on the hands. This break of the natural skin barrier

Does the Glove Fit?

continued

may enhance microbial access into the body. Powder can also absorb and aerosolize disinfectants, drugs and other chemicals with which the powdered glove comes in contact.

4. Make sure gloves are form-fitting. Loose, baggy gloves may cause work errors or lead to a spill or leak. Gloves should not be so tight that they jeopardize circulation, dexterity, or glove integrity.
5. In some cases, double gloving should be considered. Layering gloves provides additional protection against infectious agents and some chemicals, and may prevent animal bites/scratches from breaking the skin. Additionally, this allows grossly contaminated outer gloves to be quickly removed and replaced. However, dexterity and tactile sensitivity may be greatly reduced when double gloving.
6. As gloves are donned, check them thoroughly for holes, tears, or other manufacturing defects that would make them unusable.
7. Do NOT wash gloves with alcohols or detergents prior to handling hazardous materials. This practice weakens the glove integrity and does not sterilize the outer surfaces. If procedures require sterile gloves, then they should be purchased as such.
8. Do NOT touch common contact surfaces such as phones, doorknobs, keyboards, water fountains, etc. with gloved hands. Even if you are certain that they are not contaminated with hazardous material, those observing the practice are not.

9. Change gloves immediately if they come in direct contact with concentrated chemicals. Disposable gloves provide an initial barrier, but many chemicals degrade or permeate the gloves (sometimes quickly). Act fast; don't wait until the end of the procedure!
10. Gloves contaminated with infectious or radiological material are to be changed immediately to minimize the spread of contamination and risk of personal exposures.
11. Change gloves if they show signs of degradation or fatigue. Signs of degradation include:
    a. Hardening or becoming brittle;
    b. Loss of strength;
    c. Softening (may see extending of fingertips);
    d. Loss of tear resistance;
    e. Tackiness (stickiness);
    f. Loss of elasticity;
    g. Cracking; or
    h. Change in color.
12. When removing gloves, avoid contact with exposed skin. Hands must be washed with soap/water immediately after glove removal. Contaminated gloves should be segregated into the proper hazardous materials waste stream.

Although disposable gloves cover a wide range of applications when worn and used properly, it is worth noting that they do not give adequate protection against high heat, cryogenic liquids, cutting instruments, some concentrated acids/bases or highly toxic compounds (e.g. methylmercury). Therefore, it is important to contact the UT Safety Offices (Biosafety, EH & S, or Radiation Safety) for guidance in the proper selection and use of gloves, especially for work involving highly toxic materials.

References & Resources:

FirstHand-Critical Glove Barrier Issues (2001). Kimberly-Clark Health Care Education Information: http://www.kchealthcare.com/media/66820/fhv1_critical%20glove%20barrier%20issues.pdf.

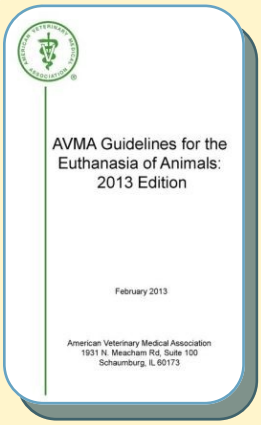
Glove Nation Chemical Resistance Chart: http://www.glovenation.com/gn_chem_chart.pdf.

UC San Diego Glove Selection Chart. UCSD Safety Program: http://blink.ucsd.edu/safety/occupational/PPE/gloves/chart.html.

2013 AVMA Guidelines on Euthanasia

Rodent Euthanasia

Joleen Adams, DVM



Our institution utilizes the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia as one of our primary guiding documents in our animal and care use program. Just recently, the updated AVMA Guidelines for Euthanasia of Animals: 2013 Edition was released. Although the document has increased from 30 pages to now roughly 100 pages, we do not anticipate major changes in current policies regarding euthanasia of our research and teaching animals. This piece will highlight some of the changes in the updated AVMA guidelines on euthanasia of rodents.

There are a few modifications in use of injectable anesthetics that can now be used in rodent euthanasia. Injectable barbiturates and their derivatives remain acceptable agents that can be used in the euthanasia of rodents. The AVMA now includes the use of a dissociative agent such as ketamine, as an acceptable method of rodent euthanasia. Dissociative agents should be used in conjunction with an alpha-2 adrenergic receptor agonist, such as xylazine or diazepam when used for euthanasia. Both injectable barbiturates (and derivatives) and use of dissociative combinations are the only euthanasia methods considered to be acceptable without conditions. All other methods discussed here have conditions that must be met to be considered acceptable.

Tribromoethanol is now considered an acceptable method of euthanasia of rodents when certain conditions involving preparation, storage and administered dosage are met. All inhaled methods of euthanasia such as isoflurane overdose and CO_2 asphyxiation are now considered acceptable with conditions. Utilizing CO_2 in the euthanasia of animals has generated debate as to whether it is distressful to the animal, as the gas will form a weak acid (carbonic acid) that can be irritating to mucosa membranes. For CO_2 to be acceptable and not cause distress to the animal, several conditions have to be met, many of which this institution already has in place. Prefilling the chamber with CO_2 is considered absolutely unacceptable now by the AVMA. It is stated in our rodent euthanasia standard operating procedures that the chambers are not to be prefilled. However, it could be argued that after euthanizing several animals in the same chamber with CO_2, as CO_2 is heavier than air, the chamber does become "prefilled". Having CO_2 euthanasia equipment that allows the animal to be euthanized in its home cage would prevent the inadvertent prefilling from occurring. The AVMA also now encourages CO_2 euthanasia to occur in the animal's home cage in order to mitigate the stress of being moved to an unfamiliar environment. Another requirement for CO_2 euthanasia to be acceptable is the use of equipment in the gas delivery process that can ensure sufficient gas displacement needed for the size of euthanasia chamber. It still remains imperative to ensure death prior to removal of the animal from the chamber. Adjunctive methods that can be utilized to ensure death (ex. cervical dislocation, decapitation) remain the same, however thoracotomy is now qualified as being bilateral.

Use of inhalant anesthetics, such as Isoflurane is still acceptable, however with conditions for euthanasia of animals weighing less than 7 kg. In animals that have not demonstrated distress, administration of high concentration of the gas is preferred. However, in those species that show aversion to high concentrations, a gradual fill method is the preference. Interestingly, the Guidelines do not specify preference for euthanasia in a home cage environment when inhalant anesthetics are utilized.

Some of the changes now in the AVMA Guidelines will afford more flexibility in euthanization of rodents as some injectable anesthetics that before were not listed as acceptable are now considered humane methods of euthanizing rodents. Currently our SOP for CO_2 euthanasia of rodents is consistent with AVMA Guidelines, and we are exploring options that can be utilized to euthanize rodents in their home cage when CO_2 overdose is used.

Spotlight on Models in Animal Research

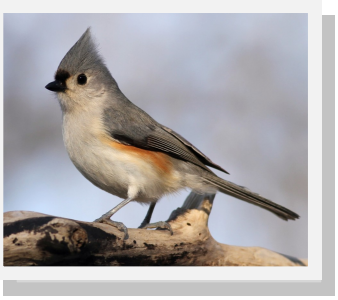
Todd M. Freeburg, PhD

Todd M. Freeberg , PhD

Todd Freeberg is an Associate Professor in the Department of Psychology, where he is Director of the Experimental Psychology Program, and is an Adjunct Member of the Department of Ecology & Evolutionary Biology. He received his BS in the Department of Ecology, Ethology, and Evolution from the University of Illinois at Urbana Champaign, and his PhD in the Department of Biology at Indiana University - Bloomington. He did postdoctoral work at Purdue University in the Departments of Audiology & Speech Sciences and Biological Sciences before joining the faculty of the University of Tennessee in 2002.

Students and I aim to understand links between the social environments of animals and the communicative signals they use. We are particularly interested in the question of communicative complexity. Why is there such incredible diversity in signal complexity? What factors drive the evolution of complex signals? A major hypothesis to explain communicative complexity in animals is the Social Complexity Hypothesis, which posits that species or populations forming complex social groups require more complex signaling systems than species or populations that form simpler groups. More complex social groups might relate to greater numbers of individuals or social roles, or greater diversity in social relationships among group members, in comparison to simpler groups. Our work has attempted to address this and related hypotheses to explain communicative complexity, with a focus on calls of Carolina chickadees, *Poecile carolinensis*, and tufted titmice, *Baeolophus bicolor*, as a model system.

Both species possess the chick-a-dee call system, which is argued to be one of the more structurally complex signaling systems outside of human language. One reason for this complexity is that the call system is open-ended. Given the rules that govern how notes are composed into calls, a potentially limitless number of unique calls can be produced by an individual chickadee or titmouse. Why many chickadee and titmouse species have such a complex call system is not yet known. We are testing the possibility that a driver of this vocal complexity is the complex social structure of these birds. Unlike most bird species, chickadees and titmice form relatively stable social groups in the overwintering months. We are using combinations of naturalistic observation studies and experiments in field settings, as well as captive aviary studies, to try to determine what features of the social structures of these birds influence variation in the calls they produce.



We know from several field experiments conducted by our lab, as well as several other labs, that variation in chick-a-dee calls of these two species (and related species) is not random. Birds produce distinct call variants (that is, they vary the composition of notes in their calls) to different environmental contexts, such as the presence food, novel stimuli, predators, and whether those predators are perched or flying. Furthermore, some call variants seem to relate to behavioral tendencies. As one example, chickadees in flight produce distinct call variants compared to chickadees that are perched. Finally, recent work suggests that the 'personality' of the individual chickadee or titmouse might help explain some of its call variation – bold or aggressive birds produce distinct call variants compared to shy or submissive birds.

The idea that social complexity may influence vocal complexity is one of the major recent arguments for language origins in humans. There are increasing calls to widen our approach to questions of language origin and the evolution of complex communication, to include a broader range of taxa with diverse social and communicative behavior, and we believe our system provides a powerful approach to these difficult questions. We additionally hope to uncover ways in which variation in calling behavior might influence the social structure of birds communicating with one another over time – how vocal complexity might also drive social complexity.

Maintaining a Colony of Laboratory Mice

Chris Carter, BS, LVT, LATg

It is a common practice for investigators to breed mice as part of their research objective. The usual reasons for doing so are to maintain a strain, produce animals for experiments, or to produce a new strain. The goal when managing any mouse colony should be to maintain adequate numbers of animals in as little shelf space as possible, while adhering to the university's policies regarding health and well-being of the mice. Doing so will reduce waste of valuable resources associated with costs, time, and animals.

To properly manage a mouse colony, an understanding of the fundamentals of mouse reproductive biology is essential. Generally, laboratory mice become sexually mature between five and eight weeks of age. Mice are prolific and can produce back to back litters if bred during the postpartum estrus which occurs 14-24 hours after parturition. They can breed for about 7-8 months, producing four of more litters. Litter size can vary from 3 to 12 or more pups per litter and is highly depended on strain. Weaning age depends on weanling size and maturity, although most strains are weaned when they are 21 days old.

The size of the colony of mice that needs to be maintained will largely depend on the investigator's experimental needs and the breeding characteristics of a given strain. The example outlined in table 1 on the next page gives a straightforward step-by-step approach for determining mouse colony size.

An ongoing audit of breeding performance needs to be conducted frequently to maintain maximum productivity and quickly troubleshoot problems that arise. Optimum breeding performance can be achieved by maintaining accurate breeding records, replacing non-productive breeders, mating mice early in age, and using more experienced males when breeding. It is also wise to avoid inadvertently placing selective pressure on the mouse colony. For example, if you pick only offspring from the best breeding females to perpetuate your colony, you may select for genes that change your strain's characteristics and inadvertently develop a substrain.

Generally it is best to purchase mice directly from a university approved vendor. Although, due to unavailability of some mutant strains, it is necessary to produce mice in-house. Using these methods it can be relatively simple to realize the costs of such an endeavor in advance and subsequently maintain a colony as small and efficient as possible.

What You May Have Missed at OLAC Training

Jane Czarra, BS, LATg

One of our recent technician training sessions was presented by Dr. Prenshaw (Student Health) and Dr. Amy Knowles (OHP) on the subject of Laboratory Animal Allergies (LAA). Some interesting statistics are:

a. One third of lab animal workers have occupational allergies related to animal dander.

b. If you are going to develop these allergies, they usually appear within 1-3 years of exposure.

c. If you already have seasonal allergies, you may be more likely to develop animal allergies.

d. Smoking does not increase the risk of developing LAA but if a smoker develops animal related allergies, they are 1.5-3 times as likely to develop asthma.

e. The principle routes of exposure are inhalation and direct contact with skin and the eyes. This exposure can be minimized by utilization of PPE such as wearing N-95 respirators. Please note you should be fit tested before using N-95 respirators. Washing hands and not touching your eyes, nose, mouth, or exposed skin with contaminated gloves will also minimize exposure.

The Emergency Incident Response (EIR) Guide for Animal Facilities has been the subject of several training sessions. Brain Ranger discussed the EIR Guide in an overview session. He then followed up with practice scenarios for group discussion. Jon Phipps joined us at the facility level to practice scenarios such as spills, fire safety, natural disasters, and animal activists.

Be on the lookout for upcoming training seminars. If you have not received notification of these training seminars and would like to be notified of future training events please contact Jane Czarra at 974-5841 or jczarra@utk.edu.

Table 1. Sizing Mouse Colonies

Example: How many breeding females are needed to produce 10 female and 10 male homozygous per week using a homozygous female x homozygous male breeding scheme

Breeding Scheme	Homozygote x Homozygote
Breeding lifespan	32 weeks
Number of Litters Produced	4 litters
Litter Frequency	1 litter/8 weeks
Litter Size	6 pups
Offspring Genotypes	Homozygous only
Percent Useful Offspring	100%

1.	Number of Mice Needed	20
2.	Age requirements If must be same age, enter 1 If can have a 2-week age range (e.g., 5-6 weeks old), enter 2 If can have a 4-week age range (e.g., 5–8 weeks old), enter 4	1
3.	Frequency with which mice are needed If weekly, enter 1 If every other week, enter 2 If once a month, enter 4	1
4.	Divide Line 3 by Line 2 (round to nearest whole number)	1
5.	Sexes needed If both sexes needed, enter 1 If one sex needed, enter 2	1
6.	Breeding scheme If homozygote x homozygote, enter 1 If heterozygote x homozygote, enter 2 If heterozygote x heterozygote, enter 4	1
7.	Some surplus (insurance) mice desired If no, enter 1 If yes, enter a “fudge factor” to ensure overproduction (e.g., if 10% more mice are desired, enter 1.1)	1.1
8.	Number of mice to be produced weekly Multiply Lines 1 x 4 x 5 x 6 x 7 (round to nearest whole number)	22
9.	Average number of pups weaned per litter	6
10.	Average number of litters produced per breeder female	4
11.	Average productive female’s breeding lifespan (weeks)	32
12.	Calculate colony productivity Divide Line 10 by Line 11, multiply by Line 9 (round to nearest hundredth)	0.75
13.	Calculate number of breeding females needed Divide Line 8 by Line 12 (round to nearest whole number)	30
14.	Calculate number of replacement breeders needed per week Divide Line 13 by Line 11 (round up to nearest whole number)	1
15.	Calculate the number of additional breeders needed to provide replacement breeders Divide Line 14 by Line 12 (round up to nearest whole number)	2
16.	Total number of breeders needed Add Line 13 and Line 15	32
17.	Breeding cages Pair breeding (one breeding female per cage): – 32 cages needed Trio breeding (two breeding females per cage): –16 cages needed	
18.	Weaning cages ~11 females & ~11 males weaned per week will require ~ 6 cages (5 animals per cage separated by sex)	

References:
http://jaxmice.jax.org/manual/breeding_strategies_manual.pdf