

**Handbook for Investigators Using
Laboratory Animals
University of Nevada, Reno**

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Regulation of Research at the University of Nevada, Reno

Research utilizing animals is regulated by numerous national, state, and local regulations and policies as well as university policies to ensure optimal husbandry of animals and animal welfare. Regulations and ethical concerns place scientifically legitimate humane limitations on research procedures in animals.

Animal Welfare Act

The main federal law that regulates use of research animals is the **Animal Welfare Act (AWA) of 1966** (and later amendments, the most recent of which is the Farm “Bill” of 2008). This law covers all warm-blooded vertebrates except agricultural animals used in agricultural research, birds, and rats of the genus *Rattus* and mice of the genus *Mus* bred for research. Rats and mice comprise the vast majority of research animals so the concern some express that the AWA does not fully regulate animal research arises from this fact. The AWA is enforced by the **USDA (Department of Agriculture) Animal Care Division**. The AWA mandates an **Institutional Animal Care and Use Committee (IACUC)** oversee animal research and welfare. The AWA also mandates the university have an Institutional Officer (IO), legally responsible for animal research compliance at the university. At UNR the IO is the Vice President for Research. Institutions must file annual reports to the USDA describing institutional animal use and also requires prompt reporting of significant problems.

The USDA conducts unannounced inspections at least annually. They also make follow-up visits to assure any problems are been corrected. The inspector observes animal conditions and spot-checks animal proposals. S/He also reviews the minutes of the Animal Care and Use Committee and animal records.

Public Health Service Policy

The **Public Health Service (PHS)** also publishes federal policies regarding care of animals used in research. PHS policy is overseen by the **Office of Laboratory Animal Welfare, or OLAW**, at the **National Institutes of Health (NIH)**. OLAW administers the Public Health Service “Policy on Humane Use of Laboratory Animals” and the **Guide for the Care and Use of Laboratory Animals (“the Guide”)**. OLAW covers all live vertebrate animal use in biomedical teaching, research, or testing. Biomedical research and teaching includes most non-agricultural (food and fiber) research and teaching uses of animals. It also includes any education of veterinarians or veterinary technicians, regardless of whether standard agricultural practices are taught or studied. The Guide has more specific requirements for animal care than does the AWA and also describes institutional responsibilities and requirement for an IACUC. The Guide was made into law by the NIH Reauthorization Act of 1985. Thus the Guide has the force of law for any institution which receives federal funding for animal research.

Agricultural Research and Teaching

The **Federation of Animal Science Societies** also publishes “**The Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching**” (**the Ag Guide**). The Ag Guide outlines husbandry and institutional requirements when animals are used in any teaching or research projects. The Ag Guide requirements are generally similar to that of the NIH Guide, including the requirement that the IACUC assess all proposed research and teaching use of animals BEFORE the use begins. Most agencies that fund agricultural research including the USDA and Hatch funds require IACUC oversight, compliance with the Ag Guide and, when applicable, the NIH Guide.

Hatch Act funds are to support agricultural research. Use of Hatch funds requires IACUC approval for animal use.

Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)

AAALAC is a non-governmental agency that assures quality animal care is. Participation in AAALAC is voluntary but is widely considered to indicate a program meets basic federal standards. AAALAC performs site visits every three years, inspecting all facilities and animal research related administrative procedures. AAALAC uses the Guide, the Ag Guide, and the AWA as inspection standards. AAALAC interacts with the institutional official, the Animal Care and Use Committee, and the laboratory animal veterinary staff and the animal care staff. NIH automatically accepts AAALAC accreditation (UNR is AAALAC accredited) as a standard of quality animal care. AAALAC may suspend accreditation or place an institution on probation for lapses in animal care or program deficiencies. In addition to covering vertebrates AAALAC also covers invertebrate animals such as crabs, octopi, etc.

Controlled Substances Act

The US Drug Enforcement Agency oversees compliance with the controlled substances act. Some people confuse prescription drugs with controlled substances. Many items which are available only from a licensed professional are prescription items but are not

federally controlled. Antibiotics, for example, are usually prescription but not controlled. Controlled substances include many pain relieving agents (analgesics) and anesthetics that are derived from morphine or barbiturates. Controlled substances are assigned to one of several “schedules” (I-V) depending on the abuse potential. Use of controlled substances requires a DEA registration. If you use small quantities of controlled substances you may be able to use the DLAM registration and have the agents “prescribed” to your study. Investigators using larger quantities of controlled substances or schedule I and II agents will be required to attain their own DEA registration. Controlled substance use must be precisely logged, with information documenting receipt of each shipment, the lot number of the shipments, which animals received the substances and the date and quantity of the use of all the contents of a vial. Records must precisely account for all the controlled substance attained. Failure to accurately document use of all substances received can result in federal criminal penalties. If you attain your controlled substances from OLAM you will be required to return an accurate and complete log sheet before obtaining more substance. Controlled substance agents must be stored behind 2 locks to which only authorized individuals have access.

Wildlife and CITES Laws

Guidelines for work with many wildlife species have been established by respective wildlife and biological societies. Adherence to these published guidelines is required for publishing in most wildlife journals. These guidelines are also used by the IACUC when reviewing wildlife protocols. Societies that have published guidelines include the American Ornithologists Union, the American Society of Ichthyologists and Herpetologists, the American Society of Mammalogy, and the Wildlife Society.

Many national, state, and local regulations govern use and possession of wildlife and wildlife tissues. The IACUC and investigator must assure compliance with all applicable regulations when using wildlife for research or education purposes. Many of these laws regulate use of animals out of concern for wildlife populations and conservation. Others focus on protection of human health. Laws such as the Lacey Act create strict penalties for infringing on the existence of protected species.

International laws also specify certain species which may not be moved across international boundaries without permits. The most exhaustive law is the Convention on International Trade in Endangered Species (CITES). Violating CITES regulations constitutes a federal offense and may carry international penalties as well. Investigators at many universities have faced charges for seemingly minor infractions such as accepting slides of orangutan blood for pathology analysis, shipping pet-store acquired chameleons internationally for collaborative studies, or bringing feathers from migratory birds from Mexico.

Good Laboratory Practice Acts

Good Laboratory Practice Acts (GLP) are often referred to as a single legislation. In reality there are multiple GLPs; the primary one of concern to biomedical research is that

administered by the US Food and Drug Administration. Most academic research is not conducted under the auspices of the GLPs but work performed for pharmaceutical companies or that will be used to apply for drug or device patents may require compliance with GLP regulations.

State and Local Regulations

UNR must also comply with state and local regulations such as the veterinary practice act. Some aspects of the veterinary practice act do not apply to institutionally owned animals. However, much of the practice act still governs how we may work with animals and how veterinary care is provided. Attaining pharmaceutical agents for studies or treatment of animals may be covered by the practice act.

Other state and local regulations and laws covering laboratory animals are designed to protect the interests of animal and ensure the public that animals in research are used humanely. Working under an approved IACUC protocol removes much of the potential onus of some but not all of these regulations. The OLAM has copies of all legislation and regulations, and invites enquiries.

Occupational Safety and Health Administration (OSHA)

Research conducted at UNR must comply with OSHA standards. Research facilities must also comply with these standards. The IACUC (see the following section) in conjunction with the UNR ES&H department, ensures this compliance through regular inspections.

University Policy

All units at UNR are registered as a single research entity with the USDA and under a single assurance statement with the Public Health Service. As such, the Animal Welfare Act, Guide for the Care and Use of Laboratory Animals, and the Ag Guide apply to all applicable species and studies.

Institutional Animal Care and Use Committee (IACUC) Policies and Procedures

The IACUC, mandated by both the USDA and the NIH, is comprised of many scientific and non-scientific members. Members are chosen to assure representation of all the administrative units that utilize animals for research and to ensure the non-university community is represented as well.

The IACUC is required to conduct inspections of animal facilities and review the research oversight program at least twice a year. All findings of these reports must be sent to the IO. The USDA routinely requests these inspection reports when it conducts inspections.

All animal research, teaching and exhibition must be approved by the IACUC before it occurs. Investigators submit a protocol form to the committee BEFORE beginning any use of animals or ordering animals. The IACUC meets monthly to review animal use protocols. Protocols specify which personnel can work on the project, outline personnel credentials to do the work, and contain thorough details of planned animal activities. Protocols can be valid for a maximum of three years. Principal investigators (PIs) must also file annual reports updating the committee on research projects. Failure to submit annual reports by the anniversary date of protocol approval or renew by the third anniversary will result in protocol expiration. All animal work under these expired protocols must stop until the PI completes the required paperwork and receives approval of the annual reports or renewal protocol. Most protocols are reviewed by a convened quorum of the IACUC. Protocols may require modification to attain approval but almost all protocols that have satisfied central committee concerns pass by unanimous vote. Votes against a protocol have to be recorded in minutes. Votes against a protocol do not constitute a federally defined “minority opinion.” Minority opinions refer to dissenting views about facility inspections, training or animal care program. Such opinions have to be reported to federal agencies but “nay” votes regarding individual protocols do not require reporting.

Minor amendments to approved research/teaching protocols may be requested by the researcher. They are reviewed by the committee veterinarian or another appropriate committee member and, when approved, added to the protocol file. Major amendments require full committee review. Major amendments are defined as those which increase animal numbers (in most cases by more than 10%) or add significant potential for increased pain or distress to animals. PHS has specific guidelines regarding changes which should be considered major.

Completing IACUC Protocols

Every procedure performed with animals must be approved by the IACUC before it occurs. As such, your protocols and amendments must cover everything you do with animals. It is strongly recommended that PIs maintain copies of their protocols in their laboratories and that laboratory staff be well versed in the protocols. Inspectors and site visitors may query lab staff about which protocols they work under and whether they

know if their procedures are approved. More detail about IACUC protocol composition may be found in the OLAM document “Guide to Completing an IACUC protocol.”

The committee is most concerned with animal pain and distress (see Categories below) and ensuring the use of animals is justified for the scientific objectives. Although committees formerly focused primarily on pain levels, they are also charged to pay heed to the more nebulous level of distress. In general, pain or distress does not include momentary and mild pain, such as drawing small quantities of blood. Although it can be difficult to know if an animal is experiencing pain or distress, if the procedure would normally cause pain or distress in a human it must be considered painful/distressful in animals. Pain and distress must be justified and alleviated in research. Pain treatment must be compatible with standards of veterinary and human medical practice. For instance, appropriate analgesics are usually required for 48-72 hours after major surgery, in compliance with current veterinary and laboratory animal practice. Multiple survival surgeries are forbidden without adequate scientific justification, and animals may not be transferred to another project with survival surgery if they have already experienced major surgery except if that surgery was for a documented veterinary treatment.

IACUC Protocol Review

Protocols must be submitted via eSirius (<http://esirius.unr.edu/esirius/>) to the OLAW at least 10 days prior to the next meeting. Meetings currently occur on the third Tuesday of each month. The electronic protocol is disseminated to appropriate IACUC members for suggested changes. If the PI can make these changes prior to the meeting it increases likelihood the protocol will successfully pass review. Once considered at the meeting the IACUC can approve a protocol, require changes to secure approval, table the protocol for future consideration or disapprove a protocol, suggesting the work is not likely to be approved at UNR. Disapprovals are unusual but many protocols will require changes to secure final approval.

The committee consists of people in many different disciplines, and they cannot be expected to be experts in all areas of research at the university. The committee is also not expected to perform significant scientific review on protocols that are otherwise peer reviewed (NIH study section, etc.). However, this statement can cause confusion. As per federal policies, the IACUC must ask questions related to the science if those questions are necessary to assure proposed procedures are adequately justified. NIH makes it clear that while the primary focus of study sections is scientific review they rely upon IACUCs for judging the relevance and ethics of proposed animal use. Thus at times there are overlaps between scientific and welfare review. NIH’s specific guidance on this issue is quoted below:

Although not intended to conduct peer review of research proposals, the IACUC is expected to include consideration of the [U.S. Government Principles](#) in its review of protocols. Principle II calls for an evaluation of the relevance of a procedure to human or animal health, the advancement of knowledge, or the good of society. Other PHS Policy review criteria refer to sound research design, rationale for involving animals, and scientifically valuable research. Presumably a study that could not meet these basic criteria is inherently unnecessary and wasteful and, therefore, not justifiable.

The primary focus of the SRG is scientific merit and the primary focus of the IACUC is animal welfare. The two bodies have differing constitutions, mandates and functions. However, since it is not entirely possible to separate scientific value from animal welfare some overlap is inevitable. SRGs may raise concerns about animal welfare and IACUCs may question the scientific rationale or necessity for a procedure

Although many qualified scientists and lay individuals serve on the committee, it is likely the committee is not expert in your area of study. Therefore, the lay summary of the project is critical. The use of flow diagrams to explain the logic of a procedure is also highly recommended and appendices supporting assertions in the proposal may be beneficial.

Amendments and Annual Reports

If you need to change any procedures you perform with animals or add personnel to the protocol you will need to file an amendment before changing procedures or having new people work with animals or their unfixed tissues. You must also submit annual reports by the anniversary date of your protocol to prevent your protocol from expiring. Amendments and annual reports are also submitted using the eSirius system. Animal orders are also placed by investigators using the eSirius system.

Whistleblower Protection

In accordance with federal law, any individual may express concerns regarding the care and use of animals at UNR. Any concerns with animal research or teaching may be brought up by anyone to any IACUC member, the veterinary staff, or the IO. If desired, the report may be anonymous. However, full investigation of animal use concerns may be difficult or impossible if anonymity is an absolute requirement. Federal laws and university policy prevent negative sequelae to individuals who express concern regarding animal care and use.

Research Involving Hazardous Agents

Hazardous agents are used in a variety of studies. The primary class of hazards used are primarily radiation hazards, biosafety (biological hazards), toxic chemicals, and recombinant DNA. If another committee oversees use of specific agents, the IACUC will have to have notice of approval from that committee.

Radioactive Materials

The Radiation Safety Committee (RSC) oversees the purchase, use, and disposal of radioactive materials. Use of radiologic agents in animal studies will require RSC approval in addition to IACUC approval prior to their use. In addition, specific requirements for carcass removal will have to be met. Check with OLAM personnel during planning of these studies to ensure they can properly dispose of affected carcasses.

Use of external irradiation does not create tissue residue concerns. Using instruments such as cesium irradiators is highly regulated and will require specific approval from the radiation safety office.

Biological Agents

Use of biological agents in animals will require approval by the Institutional Biosafety Committee (IBC) in addition to IACUC approval. For biologic agents the risk is defined in “Biosafety in Microbiological and Biomedical Laboratories” (BMBL) CDC/NIH. 5th edition, available online at: <http://www.cdc.gov/OD/ohs/biosfty/bmb15/bmb15toc.htm>.

Some biological agents may not pose a known risk but also are not tested to define lack of risk. For example, many human cell lines maintained by the American Type Culture Collection (ATCC) are Biosafety Level 2. These cell lines are not known to be a specific hazard but are not tested and known to be free of infectious agents such as tuberculosis, HIV, CMV, etc.

While experimental inoculation of animals with infectious agents is described in detail in the BMBL, a less obvious risk involves natural pathogens of the host animal or pathogens that may be introduced in tumors or other biological materials. Well known natural pathogens which may pose risk include Cercopithecine herpesvirus 1 (B virus) in simian primates and “Q” fever (*Coxiella burnetii*) in sheep. While no nonhuman primates are housed at UNR, some labs work with their unfixed tissues. The unfixed tissues pose risk. Similarly, sheep should almost always be considered a Q fever risk, particularly if the sheep are pregnant or lambing.

Toxic Chemicals

Toxin use in animals may pose exposure hazard through contamination of bedding or aerosols. It is important to identify these risks so steps can be taken to minimize exposure to animal care staff and investigative staff. ES&H oversees use of toxins at UNR and may need to oversee disposal of bedding and carcasses following dosing with some toxins.

Recombinant DNA

Use of recombinant DNA (rDNA) must comply with the NIH Guidelines for Research Involving Recombinant DNA molecules, Federal Register May 7, 1986. All *in vivo* work with rDNA must be approved by the IBC in addition to the IACUC before work begins. Appropriate containment and disposal of genetically altered animals will be required to ensure the safety of animals and humans.

Stem Cells

Injection of stem cells into animals is a common research technique used in species from mice to farm animals. While this is a powerful research technique, the source of cells used raises various issues which must be considered. For example, using mouse stem cells in other mice requires the assurance the mouse stem cells are free of pathogens otherwise excluded from the facility. This may require polymerase chain reaction (PCR) or mouse antibody production (MAP) testing prior to allowing the stem cell use. Use of human stem cells often requires documentation of Institutional Review Board and Institutional Biosafety Committee approval prior to IACUC approval. Human stem cells may require animals be housed under BSL-2 conditions. Although Nevada does not yet

have regulations governing stem cell propagation in animals, several states prohibit breeding of animals reconstituted with human stem cells.

Animal Welfare Considerations

Animal Pain and Distress Categories

The USDA pain/distress levels differ slightly from categories previously used at UNR. The following briefly outlines the classifications:

B: breeding of animals (e.g. colony to generate animals for research use)

C: only momentary pain or distress (e.g. most injections, blood collection, ear tagging, genotyping young mice, brief restraint, euthanasia & tissue harvest)

D: more than momentary pain or distress relieved by anesthesia or analgesia (e.g. survival or non-survival surgery, genotyping older mice, restraint with anesthesia)

E: more than momentary pain or distress which for scientific reasons cannot be relieved by analgesics or anesthetics (prolonged restraint without complete training, no post-operative analgesia, etc.).

Committees cannot approve category E studies without sufficient scientific justification for withholding analgesics or anesthetics. Examples that may be approved include: Infectious disease studies where analgesia has been demonstrated to alter data; analgesics affect the target organ (e.g. GI motility decreased by opioids); etc.

The committee must also consider that stress caused by unrelieved pain may also affect experimental objectives.

Pain/distress categories are discussed more fully in the “Guide to Filling Out an IACUC Protocol” available from the OLAM.

Blood Collection and Substance Administration

Investigators frequently ask how much blood can be collected humanely from animals or what volume of substances may be administered. The circulating blood volume of most non obese animals is approximately 5-7% of total weight or 55-70 ml/kg body weight. As a rough estimate, up to 10% of the circulating blood volume can be taken on a single occasion from normal, healthy animals with this repeated every 4 weeks. An excellent guide used by the IACUC to determine other blood collection and substance administration standards is entitled “A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes” by Diehl, et. al. available online at:

<http://www.fcv.unl.edu.ar/bioterio/guias/diehl2001.pdf>

Investigator Training

Federal law requires the IACUC assure all faculty, staff and students who use animals in research, teaching, demonstration, or testing are appropriately trained. The depth and duration of training needed depend upon the particular project. Basic research animal welfare compliance training is attained online through the CITI website. Contact OLAM for directions regarding completion of this training. This training must be completed by all who will participate in an IACUC protocol before they begin work and once every

three years thereafter. Study specific training such as mouse handling, surgery, etc., is available on a one-on-one basis and is arranged directly through OLAM. Investigators may be required by the IACUC to secure approval of certain techniques from OLAM staff if there is any concern that their training is not current.

Specific topics on which all who work with animals or their unfixed tissues will be trained include:

- Federal regulations and institutional policies for the care and use of animals used in research and education, including essential IACUC functions
- Information regarding basic husbandry and use needs of relevant species
- Instruction regarding humane and correct methods of experimentation, handling, restraint, pre- and post-procedural care of animals, euthanasia, and sample collection
- Alternatives to use of animals and procedures which minimize animal pain or distress
- Available services (Medline, etc.) that provide information regarding appropriate methods of animal care and use, alternatives to the use of live animals, and avoiding unnecessary duplication of animal research
- Proper use of analgesia, tranquilizers, or anesthesia relevant to studies and the species used
- Methods whereby deficiencies in animal care and treatment or protocol compliance are reported (see whistleblower discussion below)
- Occupational health concerns including zoonotic diseases
- And if you perform surgery, guidelines for aseptic surgery

In addition, the IACUC is responsible to ensure that all scientists, research technicians, animal technicians, and other personnel involved in animal care, treatment, and use are qualified to perform their duties. The IACUC must ensure training is provided to individuals who are not yet proficient in these duties

Occupational Health and Safety Program

The IACUC occupational health and safety program is designed to comply with the NIH standards outlined in “Occupational Health and Safety in the Care and Use of Research Animals,” available online at: <http://books.nap.edu/openbook.php?isbn=0309052998>. Individuals who work with animals or their unfixed tissues must be informed of the risks that such work may pose. Many potential hazards are discussed in the online CITI training. Others are briefly discussed in Appendix 1 of this handbook, Zoonoses in Animal Research. Specific training may also be offered for certain studies or species. In addition, all who will work with animals or their unfixed tissues must complete the Occupational Health Questionnaire. While all must complete the form, you may decline further participation by signing the declination page in the form. No one will be allowed access to animals or their unfixed tissues until they have completed this form. When the form is completed, place it in a sealed envelope and write your name on the outside of the envelope. Return the envelope to the OLAM. The OLAM administrative assistant will record your name indicating you have participated in the program. The remainder of the program is contracted through UNR Family Medicine. No one in OLAM sees your

information other than your name on the envelope, rather OLAM forwards the sealed envelopes to Family Medicine nurses and physicians. You may be contacted by Family Medicine and invited to discuss means of minimizing your risks if they deem you may have significant risks. In addition you may contact Family Medicine personnel to schedule such a discussion. If you are interested in contacting them for a discussion ask the OLAM for current contact information.

Certain vaccines may be strongly recommended and may be a requisite for specific types of work. For example, tetanus is required at least once every 10 years for all who work with animals or their unfixed tissues. Annual TB testing is required. Rabies vaccines may be required for work with wild carnivores or bats.

Animal Allergies

Animal allergies are becoming one of the major career limiting developments for those who work with animals (especially rodents). Individuals who become allergic to laboratory animals must have access to medical consultation through the occupational health program. The university must work with medical staff and the affected individual to minimize exposure to allergens in the animal facility, such as proper PPE, use of ventilated equipment, and reduced exposure. Appropriate use of hoods, properly balanced animal facility HVAC systems, ventilated cage systems and appropriate transportation of animals helps minimize allergic risk. Dirty cages should only be emptied of their soiled contents in the designated HEPA filtered dump stations.

Wherever possible, personnel in animal facilities should not be wearing street clothes. If street clothes are worn they should be covered lab coats or coveralls. This and additional PPE minimizes continued exposure to allergens or spread of allergens to other areas. HEPA filtered respirators or positive air pressurized respirators (PAPRs) are effective in removing allergens and may allow some individuals to continue animal related work. Surgical or dust masks do not provide allergy protection. Personnel with a history of airway disease (e.g. asthma, emphysema) must consult a physician before working with animals or using fitted masks.

Zoonotic Diseases

Zoonotic diseases are those which spread from animals to humans (anthroponoses are those spread from humans to animals). There are few zoonoses of concern from clean, vendor supplied mice and rats. However, experimental manipulations can create zoonotic risks from these species. Other species carry several organisms that can cause disease in humans. Risk of contracting a zoonosis is reasonably high when working with animals. Some of these zoonotic diseases are mild and you may not realize you have contracted them. Others are untreatable and potentially fatal. A list of many of the major zoonoses which may be seen in laboratory animals is included in Appendix 2

Reporting Bites and Injuries

All bites and animal related injuries (needle sticks, cutaneous exposures, etc.) must be reported to appropriate supervisors immediately. In most cases bites and injuries are

workman's comp issues and will be handled as per university policies. OLAM management must also be notified of all bites or animal related injuries within 24 hours.

UNR Animal Facilities

UNR has approximately 20,000 sq. ft. of animal housing, which is coordinated through the Office of Laboratory Animal Medicine at three primary locations (Medical School, south campus, Main Station Field Lab). OLAM accommodates studies in the several facilities as space is available. The more lead time an investigator can give to OLAM, the less chance that the start of the project may be delayed because of lack of research space.

The Central Animal Facility

The central animal facility in the Nellor and Anderson buildings at UNR house animals which are maintained free of pathogens. This is particularly true of laboratory mice, rats and guinea pigs. Therefore, visitors (including investigators) are required to follow procedures to help maintain the health of all animals, not only their own. Persons conducting research at the UNR must be instructed on entrance policies before they enter and get a keycard for the building. All personnel must wear facility apparel (personal protective equipment or PPE), which can be a lab coat to cover street clothes, or surgical scrubs. Facility footwear or shoe covers over closed toed shoes are required inside the animal facility. Shorts and open toed shoes are generally not allowed when working in animal areas. This PPE is to protect both personnel and the animal colony. For barrier areas, other dedicated clothing will be required, and depending on whether the desire is to keep diseases out or in (or both), showers may be required. Each investigator will meet with LAR personnel about his or her project before it commences to discuss requirements.

Animal holding areas outside the central facility may have differing PPE requirements as posted at or near entrances to the rooms.

Access to Laboratory Animal Housing Areas

Access to the central animal facility is controlled by key cards and (after hours) a unique personal identification number (PIN). The OLAM personnel will order a key card and, if after hours access is granted, assign a PIN to the facility upon the completion of each person's Key Request Form. To have access to the animal facility a person must be listed on an IACUC protocol or an amendment and have completed relevant training. Key cards are to be used only by the individual to whom they are issued. Key cards are the property of UNR OLAM and must be returned to OLAM when no longer required. Sharing key cards or PIN numbers is strictly prohibited and may be grounds for loss of access privileges. Individual key cards must be used when entering the facility, piggy-backing on other's key cards is prohibited. Other animal facility entrances may be controlled by hard keys. Investigators are expected to follow the same standards when granting access to hard keys (only individuals listed on a protocol and having appropriate training, IACUC members, or the veterinary or OLAM staff are provided access). The DLAM and veterinary staff must have keys to all animal housing areas to allow for emergency access.

The front doors to the OLAM office are open from 8:00 am to 5:00 pm Monday-Friday, except university holidays. Perimeter doors to the central animal facility have key card readers on the doors. The key card suffices for entry during the office hours listed above. After hours access also requires use of your PIN. The animal facility is always locked, so must be entered with a key card. Propping open animal facility doors is not allowed, in part for security and in part as it will affect the building's air balance, which sequentially eliminates differential room air pressures, an important component of biosecurity and biosafety (see below for more about biosecurity and biosafety). Propping open doors may be grounds for loss of access privileges.

Husbandry Restrictions and Considerations

For most animals in research, husbandry standards should be carefully standardized. This allows you to compare data attained this year with data you attained last year, in another facility, or compare your data to published data. Criteria such as housing density, temperatures, noise levels, air quality, feed, water quality, bedding, light/dark cycles, etc, can have profound effects on research animals. This is particularly true of rodents as rodents are very sensitive to their environment. That said, most species are more attuned to their environment than are modern techno-sensory overloaded humans so noises or diet alterations that would be trivial to us have marked effects on research.

As such, we work hard to preserve housing condition standards. Routine access to animal rooms during dark cycles is strongly discouraged and requires IACUC approved scientific justification. Whenever possible access to rodent rooms should be restricted to people that routinely interact with the animals. Even mice demonstrate different behavior when unknown individuals enter rooms vs. staff with whom they are familiar. Noise levels should be carefully controlled, precluding personal audio devices or cell phone use in animal rooms.

Animal Procurement

Upon approval of an IACUC protocol an investigator may order animals. In most cases animals are ordered through eSirius, the software program used by OLAM and the IACUC. Once the order is reviewed by OLAM and confirmed by the vendor, a confirmation will be sent via email to the investigator.

Agricultural animals for biomedical studies must be attained from approved vendors. Agricultural animals for agricultural use may be purchased from private sources but should be free of diseases outlined in the agricultural animal quarantine SOP. Animals that cannot be tested free of these diseases are tested during quarantine as described in the SOP.

Wildlife species may be procured for use on campus as allowed by federal law and as stipulated on the investigator's permits and IACUC protocol.

Animals from Non-Standard Vendors

Most animals used at UNR are from approved vendors known to be free of specific diseases. Investigators may need to acquire animals from another institution or entity

other than an approved vendor. OLAM has an obligation to facilitate research but must also protect animals already in the institution. As such, investigators must contact OLAM when planning import of animals from non-approved vendors. Generally, OLAM will contact the source institution for colony health records. Depending upon the health status of the originating colony OLAM may disallow importation or provide for quarantine to ensure imported animals are free of specific pathogens before use.

Animals from unapproved vendors are quarantined after arrival. The length of the quarantine varies with the health status of the originating colony. Animals are released from quarantine once sentinel animals confirm lack of specific pathogens. Quarantine lasts 5-12 weeks depending on the status of the originating colony. Under some circumstances investigators may receive approval to manipulate animals during quarantine (i.e., breeding, etc.) but they must adhere to biosecurity measures. Quarantined animals have a higher *per diem* rate than regular rodents because of the containment procedures needed. Occasionally investigators are approved for acute studies in which animals are not quarantined but are used in terminal experiments outside the facility immediately upon arrival.

Animal Care Services

OLAM provides full care to most laboratory animals on campus. Rates for daily care (per diem rates) are determined per animal or per cage, and depend on what type of housing is utilized. Call the OLAM for current per diem rates. Per diems include setting up, cleaning, feeding and maintenance of animals on a daily basis. They also include veterinary care and, in the case of mice and rats, a sentinel program (below). Some requests, such as use of disposable cages may incur additional expense. OLAM staff are trained to carry out animal care to avoid spread of disease throughout an animal facility. Some of the precautions caretakers take include hand washing on leaving each animal room, room-specific lab coats or other PPE, changing of gloves between populations of animals and showering before or after entering a room.

In general animals may not be housed outside central animal facilities. Federal policies provide strict guidance regarding animal housing areas. These facilities must be separate from personnel areas, air must be 100% fresh and exhausted, surfaces must be water resistant and easily sanitized, etc. These policies are to ensure uniform environments for the animals which reduces scientific variability and minimize the risk of non-trained individuals from contacting animals or waste products (dander, etc.). The IACUC may approve terminal use of animals in investigator laboratories but it is critical that animals taken to laboratories not remain in these areas overnight as this constitutes housing of animals. In addition investigators and their staff are required to ensure that all animal use areas are approved by the IACUC before they are used.

Animal housing and use areas fall under different regulations and policies than do other university areas. For example, eating and drinking is prohibited in all animal areas. Open toed shoes and shorts will usually be prohibited in animal areas. PPE such as laboratory coats and gloves may be required in all animal areas.

Cages and pens must also allow animals to remain dry, clean, and exhibit as many species specific behaviors as possible. Cage density is explicitly outlined in both the AWA and Guide. Investigators will be asked to reduce populations in overcrowded cages. If the situation is not corrected or if overcrowding is causing behavioral or welfare concerns the OLAM staff may immediately correct overcrowded cages and notify the investigator.

Investigators frequently need to split cages or otherwise manipulate housing. This is generally acceptable as long as investigators ensure new cages or enclosures have adequate clean bedding, food and water. Cage cards or animal identification must also be clear on any reconstituted cages.

Animal Identification

The AWA and Guide require appropriate identification of all animals. Appropriate methods of identification vary by species but include cage cards, collars, bands, ear notches, ear tags, microchips, tattoos or brands. The AWA requires that certain species be identified by tattoo or tag. If USDA tags are used the tags must be removed and retained by the facility for at least one year after the animal's death.

For most species used in biomedical research cage cards are required as per the Guide to have specific information including the investigator's name, protocol number, species, sex, date of birth or approximate age, date of acquisition, breed or type, distinctive markings and any identification information. OLAM provides cage cards that contain the required information as well as a bar code which allows OLAM to assist the investigator ensure animal numbers are consistent with that approved in the IACUC protocol. Investigators are welcome to use additional cards with study specific information as long as the OLAM cage card remains on the cage and the bar code remains visible.

Management of Animal Health and Welfare

Under some circumstances the DLAM or other OLAM personnel may authorize treatment or euthanasia of animals based on veterinary and welfare concerns. When possible OLAM staff attempt to contact investigators or their designees before performing such tasks. When investigators cannot be contacted or if investigators or their staff do not attend to concerns in a timely manner the OLAM staff will be authorized to address the welfare or veterinary issues. In some cases performing such tasks may incur expenses in addition to standard per diem rates, consistent with rates charged for special services.

Maintenance of Breeding Rodents

No more than **one litter** should be present in a standard shoebox-sized cage, in the company of no more than one or two adults. If harem breeding is used, any apparently pregnant female must be removed and housed singly prior to giving birth. In the case of "accidental" births in cages with more than two adults, all adults other than the dam should be removed as soon as possible. In some cases the male may stay with the parturient dam if post-parturient breeding is required. Exceptions to this policy based on scientific need must be approved by the IACUC prior to harem housing nursing females.

Mouse litters must be weaned promptly by 21 days of age unless the IACUC has approved an exemption to this requirement prior to extending the nursing period. Most exemptions are for transgenic or knockout mice that require a longer nursing period but it is rarely justified to nurse longer than 28 days. If you are approved for an extended nursing period, ensure you do not breed on the post-partum estrus as this will result in two different aged litters nursing simultaneously. Upon weaning, separate mice by sex and house them at no more than five per cage (or 11 in a large cage).

Investigators are expected to control cage overcrowding and wean animals at the proper time unless there is a service request for the OLAM staff to do this. If investigators have crowded cages or cages requiring weaning, they are notified by email and the cages are marked. If investigators fail to thin cage populations or wean within 24 hours of notification, animal care staff will thin cages or wean animals. There may be a charge for this service. The IACUC will be notified of investigators that repeatedly fail to properly maintain breeding colonies.

Maintaining a breeding colony also requires the investigator carefully monitor offspring and unexpected mortality. The eSirius cage card system is designed to facilitate animal census management. Please ensure you accurately report weaned animals through this system.

Social Housing and Cage Density

Many species used in animal research are social. As per the Guide, single housing of social species causes distress and should not be routine. Single housing of social species may be a scientific necessity or may be dictated by veterinary concerns. Scientific needs for single housing may occur when hormonal effects alter data, surgical sites are likely to be foci of aggression, etc. Housing animals singly due to scientific needs must be approved by the IACUC prior to initiation of the social housing. Veterinary issues which may dictate social housing include individual aggression, timid animals, separation of peri-parturient females prior to giving birth, or medical conditions. Veterinary separation of animals is coordinated between the investigator and veterinary staff. This does not require specific IACUC action but records should be kept to document medical justification for single housing. Whenever possible, singly housed social animals should be in visual, olfactory, and auditory contact with conspecifics. Singly housed social animals should be provided added enrichment unless scientific necessity precludes this.

In general the number of animals allowed per cage is based upon a mathematical formula printed in the Guide. In some cases animals may require more space than the minimum listed in the Guide. For example, many strains of mice may be housed at 5 per cage based on criteria in the Guide. In the case of male mice, five per cage may be too many, with fighting or excessive ammonia levels dictating a lower housing density. If fighting lesions are seen or waste products accumulate before scheduled cage changes may occur, animals must be separated. Severe injuries may require euthanasia.

Animal Transportation

Transportation of animals is highly regulated. Reasons for these standards include both protection of animals and protection of people. Animals should not be moved through public areas unless absolutely necessary. When animals are moved through public areas they must be in containment caging and covered unless otherwise approved by the IACUC. When trans-facility transit is required, animals must only be moved in approved vehicles. This protects animals from environmental extremes and potential contact with non-pathogen free animals. It also protects humans who may inadvertently be exposed to allergens or pathogens carried by the animals. While air systems in animal facilities are designed to minimize risk to non-animal users, serious human illness has been reported several times when animals were inappropriately moved through public areas, including elevators that lacked appropriate filtered ventilation.

The Sentinel Program

Rodents and rabbits may harbor pathogens that can have negative effects on other animals in the colony. For this reason all mice and rats on campus have “sentinel” animals of the same species which live with them, in the same room or rack. Sentinels are naïve disease-free animals ordered directly from approved vendors. Samples of litter from all colony cages are introduced to the sentinel cage during cage changing. Almost all pathogens of concern in a rodent colony are spread by the fecal-oral route so this method provides reasonable assurance of detecting pathogens. Some agents such as mouse parvovirus are not as easily spread by the fecal-oral route so may be more challenging to detect on a regular basis. Additional tests may be required for accurate detection of these agents. Sentinel animals are bled on a regular basis and the blood tested for antibodies for important murine diseases. Sentinel animals also are tested for pathogenic internal parasites. Approximately quarterly sentinels rotated and tested by a contract laboratory.

Most pathogens require approximately 3 weeks for detection by have sentinel animals. Costs for the sentinel program are included in the per diem rate charged for colony animals. Animals subject to quarantine are not released from quarantine until one or occasionally two rounds of sentinels are confirmed free of antibodies to pathogens and free of internal parasites.

Since wild rodents may infest areas outside the central housing areas, rodents are not allowed to return to the animal facility once they have been removed. Exceptions may be granted but only after the animals are re-quarantined.

These principles also apply to the use of live tissue (e.g., tumors, etc) to be implanted into laboratory animals at UNR. Tissues may contain live pathogens which may result in serious disease outbreaks in research animals or contagion risk to humans who work with the animals. As seen recently in other institutions, it is also possible to alter non-pathogenic organisms into serious pathogenic organisms through use of sera-enriched tissue culture broth. It is important to know the status of the animals, humans, or sera for tissues and cultures used in research animals. Diagnostic testing or housing animals at an increased biosafety level may be required for those receiving implants or sera.

While most attention regarding quarantine is given to mice and rats procedures are in place to ensure all animals which enter UNR do not pose a risk to other animals or humans. Quarantine policies must be strictly adhered to by OLAM and the university community.

Morbidity Reports

Morbidity Reports are generally filled out by animal caretakers as they encounter illness or injury during animal care, but reports may be made by anyone. These reports need to be complete regarding animal identification and location. A Morbidity Report will document every incident; a copy of the report goes to the veterinary staff and copies remain on the cage. Investigators are welcome to one of the copies on the cage. In general the veterinary staff will confer with investigative staff or the investigative staff can take care of animals before the veterinary staff becomes involved in cases. Whenever possible, investigators are contacted by the OLAM veterinary staff before initiating treatment. If the investigator cannot be contacted, treatment or euthanasia will be administered if it is essential to the animal's health or welfare. In almost all cases animals in per diem supported housing receive veterinary and diagnostic support without any additional charge. Exceptions to this include experimentally induced disease or diagnostics required for the experimental protocol.

Mortality Reports

Like Morbidity Reports, Mortality Reports will be filled out by animal caretakers when animals are found dead. In some cases investigators inform OLAM they do not wish for necropsies following unexpected deaths but in general those that die unexpectedly are necropsied. If an animal is found dead, it is bagged with identification and placed in the OLAM cooler. The Mortality Report, containing all pertinent information, is then passed to the veterinary staff. Animals may have only a gross necropsy or may have tissues submitted for analysis to assist in determining cause of death.

OLAM Technical Services

Work Orders

Some research services, special feed, transportation of animals, unusual housing and supplies can usually be accommodated by LAR with advance notice. Requests must generally be in writing to insure accurate communication and delivery of goods/services. The forms are available at the OLAM, and may be delivered personally or faxed (784-4201).

Veterinary Care

The University Veterinarian is charged with the oversight of all areas of animal use at the university. He reports directly to the IO and has access to all animal areas in the university. He has the authority to suspend research studies or teaching use for animal welfare or regulatory reasons. Dr. Hasenau can be reached at the OLAM office, 784-4874.

Veterinary care for laboratory or teaching animals is available at all times. The on-call veterinarian and contact numbers are posted in throughout animal care and use areas. Otherwise, illness or injury is reported on Morbidity Reports. Any investigators that employ their own veterinarians must maintain health records for the animals near the animals and accessible to the OLAM veterinarians and USDA or other inspectors. Inspecting entities need to see animal records in the animal rooms or an adjacent animal room. Any treatment, whether experimental or therapeutic, needs to be in the individual or group record.

Surgical Facilities

The OLAM maintains a surgical suite and a procedure area, including a prep and a recovery area for investigator use. Investigators are encouraged to use this for surgeries and other procedures unless they have IACUC-approved areas to perform work. To reserve a surgical room or procedure room please speak with the OLAM administrative assistant. Federal and local laws require a designated surgical area for survival surgery. The exception is that surgery on laboratory-raised mice and rats may be done in a designated CLEAN area within a laboratory. Surgery is not to be performed in animal housing areas. ALL locations for survival surgery must be approved by the IACUC and inspected at least semiannually. If you wish to set up a designated rodent surgery area in a laboratory it is advisable to seek input from OLAM veterinary staff before beginning work.

Investigator Work Areas

Several procedure rooms are available within the central animal facility. These rooms contain cabinets, exam tables, countertop space and sinks. A euthanasia area and necropsy room is also available for small animal necropsies. Please contact OLAM to schedule use of these rooms.

Special Procedures

Special procedures can be performed by OLAM technical staff to assist research protocols. Commonly performed procedures include blood collection, injection and gavage of rodents, tattooing, ear tagging, ear punching of many species, and weaning. Management of rodent breeding colonies by OLAM may provide an alternative to an investigator performing these routine duties. Administration of anesthesia, post-operative care and administration of analgesics or other drugs can be arranged. The veterinary staff is available for surgical procedures.

An hourly technical or veterinary service fee or a prearranged set charge is assessed for these procedures; please call to inquire about these charges. OLAM will train research staff in proper methods of euthanasia and other animal techniques. OLAM may have excess rodents which can be utilized for instruction. Please contact OLAM if you wish to arrange a training session.

Euthanasia

OLAM provides euthanasia, by request, for animals under OLAM care. A completed work order form or request for euthanasia form is used to arrange euthanasia. These are

available from the OLAM and may be returned in person or faxed. The AVMA Guidelines on Euthanasia is available online at: http://www.avma.org/issues/animal_welfare/euthanasia.pdf. Euthanasia methods for research animals must conform to techniques published in this guide.

Carcass Disposal

OLAM will accept small experimental animals for disposal. Carcasses that may be biohazardous must be double bagged in red, strong non-leaking plastic bags legibly identified with the investigator's name and biohazards. Carcasses are stored in the OLAM cooler in the Anderson building.

Miscellaneous Policies

Food and Drink in Animal Use and Housing Areas

Food and drink, applying cosmetics or inserting contact lenses, and use of tobacco products are not allowed in animal use or housing areas. Keep in mind if you have an area of your laboratory approved for animal use it must comply with this policy.

Animal Health Records

Records must be kept regarding animal health. The animal care staff maintains daily observation records for most animals. In a few select laboratories or housing areas the investigator may be responsible for weekend or holiday observations. Please ensure observations are made and documented every day in these situations.

You must also maintain records of scientific manipulations which may affect animal health such as surgery, administration of substances, etc. For rodents it may be permissible to maintain group records; for most USDA regulated species individual records must be maintained in a manner that complies with the state veterinary practice act. Health records may be maintained either by OLAM staff or by the PI. If maintained by the PI they must be available for examination by the IACUC, veterinary staff, auditors or inspectors.

Pharmaceutical Grade Reagents

Federal policies require that investigators use pharmaceutical grade reagents when such reagents are available. Use of non-pharmaceutical grade reagents requires review and approval by the IACUC before they are used. Approval must be based upon evidence that available veterinary or human pharmaceutical agents are insufficient for the scientific objectives.

Expired materials

Use of expired medical materials including pharmaceutical agents, fluids, or sutures is not allowed by federal and state policies and regulations. An exception may be granted for use in non-survival surgical procedures where the agent's expiration date will not compromise animal welfare or science. For example, some sutures may be acceptable for use in non-survival procedures. Expired materials must be separated from non-expired materials and clearly marked "not for survival procedures."

The IACUC may disallow expired materials from use in any animate procedure. Expired anesthetics and analgesics are not acceptable for any procedures.

Photographing animals or animal facilities

The capturing of images (including photographs, video recordings, movies, or other images of any kind in any media) is prohibited in all research animal facilities.

Exceptions require approval of the DLAM prior to capturing images. This policy is designed to protect the integrity and confidentiality of university research and promote a safe and respectful workplace.

Radios in Animal Use Areas

No radios are allowed in the animal rooms, surgery rooms, procedure rooms or laboratories where live animals are present. This includes personal radios or musical devices. Any exceptions must be approved by the attending veterinarian and IACUC and used as “white noise” to background noise stress on specific animals.

Appendix 1

Zoonoses of Research Animals

"Domestic" or Laboratory Rodents/Rabbits: Most rodents and rabbits in research are specific pathogen free (SPF), so the major zoonotic risk is allergies. Rats are one of the most allergenic animals. Many people also develop allergies to rabbits. In the worst cases, allergies and asthma may be life threatening. The most allergenic proteins are probably lipocalins in the urine, saliva, and possibly hair.

Wild Rodents/Rabbits: These animals may carry many diseases, including:

Hantavirus (especially in deer mice)--Most recent cases in the Four Corners area were pulmonary and fatal. The disease is usually spread via infected deer mouse feces. This disease is unlikely in a research facility but is a concern if for those performing field research.

Dermatomycosis (ringworm)--These organisms cause scaly skin lesions which are easily treated.

***Staphylococcus* and *Streptococcus* spp.**--These bacteria are usually spread by bites. SPF rodents may have these bacteria.

Rat-bite fever (*Streptobacillus moniliformis*)--This flu-like disease spread by bite wounds. It is usually not serious.

Lymphocytic Choriomeningitis Virus--This flu-like disease, may progress to serious or fatal encephalitis.

Plague (*Yersinia pestis*)--Spread by the rat flea, this flu-like disease most commonly associated with prairie dogs. May progress to fatal septicemia

Tularemia (*Francisella tularensis*)--A non-specific flu-like disease with an incubation period of 1-10 days, tularemia often progressed to fatal septicemia. Spread is by aerosol, ingestion, direct contact, or by arthropods (ticks). This is commonly seen in when animals are necropsied by ungloved people. Typical lesions are seen in infected animals are white necrotic spots on the liver, occasionally spleen or lymph nodes.

Amphibians, Reptiles, and Fish: Zoonotic diseases of amphibians, reptiles, and fish are common; wear gloves if possible and wash hands after handling animals. Common zoonotic diseases include:

Salmonellosis--This bacterium is very common amongst these animals and causes severe gastroenteritis (vomiting, diarrhea), occasionally septicemia with disseminated abscesses and, rarely, death. Many animals are asymptomatic carriers, up to 90% or more of various populations shed *Salmonella* in feces. Attempts to eliminate *Salmonella* w/antibiotics are generally unsuccessful, leading to increased resistance. Animals can be infected before or at birth through contact with other animals or by eating feces (typical for hatchlings).

Yersinia--enterocolitica is the most common amphibians, reptiles, and fish. Symptoms are similar to *Salmonella*

Campylobacter and other enterics (Klebsiella, Aeromonas, Citrobacter, Serratia, etc.)--These are common in most amphibian, reptile, and fish species; symptoms are similar to Salmonella.

Q-fever--This disease has been spread by pythons. It is described under "Sheep."

Erysipelothrix rhusiopathiae--Crocodilians are known to carry this bacterium which may cause septicemia in people.

Mycobacteriosis--Several amphibian and reptile species carry various *Mycobacteria* (including tuberculosis and mariner's itch). Human infections are contracted through scratches, bites, or curing cage cleaning

Fungal infections seen include Aygomycosis, Aspergillus, etc.

Equine encephalitis-- Reptiles and amphibians may be viremic but it is not known whether they are a source for zoonotic infection

Worms--Worms are common in reptiles and amphibians. Reptile pentastomids are the most serious. Pentastomid eggs can contaminate cages and are spread during cleaning. In humans worms may encyst in lymph nodes, liver, lungs, etc., then calcify, inducing inflammation.

Ticks--Reptile ticks are not zoonotic but can spread equine encephalitis virus, etc.

Birds: Zoonotic diseases of birds include the following:

Psittacosis--Caused by *Chlamydia* (*Chlamydia psittaci*), psittacosis is usually contracted from inhaled organisms liberated from psittacine (parrots, etc.), duck, and turkey feces. Strains from other bird species appear to be less virulent for humans but cases from pigeons have been reported. Flu-like illness in humans including shortness of breath which can progress to debilitation, pneumonia, meningitis, kidney disease, and liver lesions if untreated.

Salmonella spp.--Birds commonly carry this bacterium but human infection from birds is rare. Signs in people are as described above.

Listeria--Avian sources of this bacterium reportedly cause human conjunctivitis.

Yersinia pseudotuberculosis--Birds are a major reservoir' the bacterium is more common in Europe than in the US. Symptoms include severe intestinal inflammation, headaches, anorexia, vomiting, arthritis, skin sores, and occasionally death. Treatment is challenging.

Campylobacter spp.--The zoonotic potential of avian *Campylobacter* has not been fully investigated. These bacteria may cause diarrhea in children.

E. coli--Severe forms are rarely spread from birds but can be found in feces.

Tularemia--as described below. Avian transmission is unlikely but birds carry the bacterium.

Tuberculosis--Many birds carry *M. avium intracellulare*. Infection has become common in immunosuppressed humans, especially HIV patients.

Viruses--birds may act as reservoirs for Equine Encephalitis, etc.

Giardia--birds may serve as a reservoir of *Giardia spp.*

Cats and Dogs: Zoonotic diseases of cats and dogs include the following:

Dermatophytosis (ringworm)--Mild skin infection is caused by these fungi.

Staphylococcus and Streptococcus spp., Capnophagia spp., etc. are often spread by bites. They cause mild to moderate subcutaneous infections.

Cat-scratch fever (*Bartonella henselii*)--This organism usually causes self-limiting lymphadenopathy.

Rabies--As described below.

Toxoplasmosis--This is common in cats, although most people are infected from eating undercooked beef. Cats spread the organism via feces. The organism must sporulate for 48 hrs post-defecation before it is infective, so daily litter box changes prevent infection. High danger to developing fetuses means most pregnant women are instructed not to change litter boxes.

Cryptosporidiosis, *Giardia*--These enteric organisms may be zoonotic from dogs and cats, the evidence is unclear.

Nematodes (worms)--A few roundworms of dogs and cats are zoonotic. The major parasites of concern are of the genus *Ancylostoma* and *Toxocara*, causing cutaneous or visceral larval migrans, respectively (parasites migrate through subcutaneous tissues or major organs).

Allergens--Cat salivary proteins and dander are highly allergenic.

Wild Non-Rodent Mammals (skunks, bats, foxes, racoons, opossums, etc.): Zoonotic diseases of these animals include:

Rabies--This is the primary concern for most of these species, but has not been documented in North American opossums. This disease is usually spread through bite wounds but people have contracted rabies from aerosolized bat feces. Bats are the main reservoir of rabies in the western US but not the most common source of human infection. Anyone who works with wild mammals or visits bat caves must receive rabies prophylaxis (vaccination) before beginning work

Leptospirosis--the bacterium is spread primarily through urine. It can infect numerous organs in humans, causing fever, headache, rashes, and occasionally death.

Salmonellosis--As described above.

Dermatomycosis (ringworm)--As described above.

Roundworms--The racoon ascarid worm, genus *Baylisascaris* (and occasionally the skunk ascarid), can be an agent of larval migrans, preferentially migrating through Central Nervous System tissues or the eye. There is no effective treatment.

Sheep and Pigs: Zoonotic diseases of sheep and pigs include:

Dermatomycosis--As described above.

Leptospirosis--As described above.

Rabies--As described above.

Salmonellosis---As described above, but infection is relatively rare in these species.

Coxiella burnetii (Q fever)--This spirochete is a common infection in ruminants. It is considered a high risk zoonosis when working with pregnant ruminants, those giving birth, or when working with placental materials. Pregnant sheep are considered the highest risk. Although infections may be common in those who work with ruminants, serious human disease is not common. That said, some of the most serious cases have been in researchers and even lay staff in research facilities who are far removed from sheep. The disease is flu-like but can progress to serious

endocarditis, particularly in those with compromised cardiac function. Illness can be life threatening.

Orf--This poxvirus causes self-limiting skin vesicles in sheep and people.

Non-human primates: The most common zoonotic diseases of NHPs are enteric organisms which can cause serious diarrhea in humans. These commonly include: shigellosis, campylobacteriosis, yersiniosis, and entamoebiasis. These enteric organisms are shed when primates develop stress-related diarrhea. Human infections can result in serious diarrhea.

Other zoonoses of NHPs include:

Dermatomycosis--As described above.

Tuberculosis-- As described above except humans usually give this to primates rather than visa-versa.

Herpes B virus--This virus is common in macaques and is related to the human cold sore virus. More than 90% of macaques carry the virus and the virus is present in saliva, feces, and other fluids of viremic monkeys. Viremia is usually induced by stresses such as transport, anaesthesia, etc. Non-viremic animals are not contagious. Human cases are very rare (less than 70 in the 1900's) but the case fatality rate exceeds 70%. Infection is spread to humans by bites, scratches, or infected bodily fluids. Antiviral agents such as acyclovir may prevent death but must be given for the life of the patient.

Poxviruses--Several pox viruses infect macaques and are zoonotic. These organisms are rare but not usually fatal to humans.

Appendix 2

Biosecurity and Biosafety in Laboratory Animal Facilities

Modern research animals are remarkably “clean” from a pathogen perspective. This has not always been the case. In the fairly recent past rodent and rabbit colonies were colonized by numerous pathogens that are known to alter research data. This was recognized as an unacceptable research variable and by the late 1970s and early 80s the vast majority of commercially available laboratory rodents were specific pathogen-free. To maintain this status, approved vendors routinely test animals similar to what is described for the above. Vendors maintain this biosecurity using very stringent management techniques and sentinel programs similar to that described above. We work very hard to maintain biosecurity of colonies through the procedures described in this handbook. Our facilities are designed to facilitate maintenance of biosecurity in the vivarium. The flow of caging and of personnel is closely monitored for this purpose. Supply air to most facilities is HEPA filtered to exclude unwanted microbes. The New CMM building will have a dedicated barrier area to for full assurance of the biosecurity.

Steps taken to maintain biosecurity a certain status might include the following:
Hand washing on entering or exiting an animal room and donning a clean gown, foot dips or covers, and disposable gloves designated for that space only. This in a conventional animal facility might be considered the minimum acceptable PPE.

Some facilities at UNR are operated as modified barriers and full barriers will be in operation by the end of 2010. In full barriers all materials and equipment is sterilized into the facility. Personnel shower and change into clean, sterile clothing. Each animal and procedure room is sequestered from others as much as possible. Current modified barrier rooms at UNR operate with some of these procedures in place. When applying principles of biosecurity intelligently in laboratory animal facilities it is useful to address the following concerns:

- The status of the animals you want to protect
 - Are they immunocompromised and susceptible to opportunistic pathogens?
 - Do they have an flora that you would like to maintain unchanged?
- Are they extremely valuable, perhaps irreplaceable?

Personal Hygiene and PPE in Animal Facilities

- Hand washing has a dramatic effect in controlling room-to-room or cage-to-cage pathogen spread.
- Showers are generally not applicable to conventional facilities but are useful in full barriers or when working with biohazards. Showers are effective in to ensure pathogens are not inadvertently transferred.

Facility Clothing

Animal care staff usually work in scrubs because of the extended length of time they work with animals and number of different animals they encounter. Investigative and veterinary staff members may find lab coats and shoe covers suffice for protective clothing when animal contact is minimal. Lab coats and shoe covers should be removed

at the entrance to the animal room. For biohazardous or immunocompromised animals additional PPE such as back-fastening gowns, disposable coveralls head cover, mask, gloves and dedicated room footwear or shoe covers may be required..

Fomite Control

A fomite is an object carrying a potential pathogen from one place to another. Any object brought into the facility may be contaminated, including something as simple as a pen and paper, bar code reader, etc. Fomites are best controlled by ensuring that each room is fully stocked and equipment is not taken from one room to another. When equipment must travel from one room to another SOPs must be followed to minimize risk of fomite transfer. All individuals using the facility must be trained to minimize fomite transfer.

Sterilization

- Most animal facilities are supplied HEPA filtered air. This ensures that air supplies do not contaminate research animals. Propping open doors or otherwise subverting the air supply balance risks introducing unclean air.
- Food and Bedding can be sterilized by autoclaving or may be purchased after gamma irradiation by vendors. If food is to be autoclaved it must be manufactured for the purpose to allow for partial destruction of heat-labile components.
- Clothing can be autoclaved
- Equipment can in some cases with one of several gasses, depending on the nature of the equipment and what agents must be neutralized.
- Water can be sterilized or decontaminated by several methods. Autoclaving water can be tedious but is an option for certain groups of animals. Reverse osmosis systems with UV treatment systems will be utilized in the new CMM facility.