



ANIMAL TRACKS



A newsletter for the Duke research community

January 2007

<http://www.duhs.duke.edu>

Monitoring General Anesthesia

Monitoring an anesthetized patient requires knowledge of normal physiologic parameters of respiration and circulation, as well as skill in interpreting variations in these parameters. Experience with the species, the procedure being performed, and the equipment are vital to success. The anesthetist must keep in mind that each animal is different, and that evaluation of physiologic signs must involve ALL signs, not any individual one. An anesthetic log should be maintained for each patient as a recording of vital parameters every 10-15 minutes while the animal is anesthetized. This is helpful to see how the patient responded to the anesthesia, but also a regulatory requirement. For rodents, this log may be the laboratory notebook. For rabbits, dogs, or other animals the log should be a medical record.

The following parameters are the most useful in assessing the well being of an anesthetized patient. Most of these can be monitored by some form of equipment, in fact most monitoring devices are able to assess multiple parameters at once.

Pulse: Taking the pulse on most species is done by palpating the femoral artery on the medial surface of the hind leg. In addition, the mandibular artery on the lower jaw and the digital artery between the toes can be palpated on larger animals such as dogs, sheep, and pigs. In small rodents the heart is palpated directly over the chest wall. Experience will aid the technician in learning to evaluate the strength and character of the pulse as a means of assessing the response of an animal's cardiovascular system. A monitoring device can be used to continually evaluate an animal's pulse while it is anesthetized.

Respiratory Pattern: Adequate monitoring of the respiratory pattern includes observing the depth and character of the animal's respiration. Depth is self-

explanatory, that is, how much is the animal expanding and contracting its thorax during a breath. Character involves two parameters: the first is rate, or how many times per minute the animal is breathing and the second is called area of initiation. Area of initiation means the spot on the animal's body that moves first when it starts to breathe. In a normal, awake animal, the first spot that moves is the center of the thorax (thoracic respiration). As the animal's anesthetic state becomes deeper, the area of initiation moves caudally on the body, first to the end of the rib cage (thoracic-abdominal respiration), and then to the abdominal muscles (abdominal respiration). Shallow, thoracic respirations are generally characteristic of the lighter stages of anesthesia, with deeper, abdominal movements characteristic of deep anesthesia. Surgical anesthesia is characterized by a pattern of regular breathing initiating in the thoracic-abdominal area. Animals that are attached to an anesthetic machine can be monitored by observing the valves and the rebreathing bag, as well the animal's thorax. Monitoring respiration in a mouse requires very careful observation, since respiratory rates in excess of 200 per minute can occur. Some circumstances require an animal to be placed on a ventilator, in which the respiratory rate of the animal is controlled by the machine and can be adjusted as necessary to maintain an appropriate anesthetic plane of the animal during surgery.

Blood Pressure: Continuous blood pressure monitoring provides additional information on cardiac output. The common non-invasive method that is used to monitor blood pressure is by means of ultrasonic Doppler flow detectors. This device usually employs a cuff that is placed around the animal's tail or leg. The cuff is then electrically coupled to a monitoring device such as a display screen. Some procedures require invasive blood pressure monitoring, in which case an arterial line is surgically placed (usually in the femoral artery since it is most easily accessible) for this purpose.



Body Temperature: Surgical anesthesia paralyzes the heat regulatory center of the brain, resulting in a temperature drop in the patient (hypothermia). The lower the temperature, the lower the patient's ability to metabolize drugs. A lowered body temperature, therefore, slows the animal's recovery from anesthesia. The animal's heat loss can be minimized during anesthesia by placing it on a

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Whistleblower Policy

Some of you have requested copies of the Duke Whistleblower policy so here is a copy for your convenience. Simply print this page, cut out, and post in your laboratory or animal use area.

QUESTIONS, CONCERNS, OR COMPLAINTS ABOUT THE CARE, USE AND/OR WELFARE OF RESEARCH ANIMALS?

Any individual who has concerns related to the use of animals in biomedical research at Duke University is encouraged to voice those concerns. Please contact the Duke Animal Welfare Hotline (919.684.3535) or Email the Duke IACUC at IACUC@duke.edu

Duke University will not tolerate any mis-use or neglect of animals nor will the institution accept reprisal against an individual who has come forward with concerns or allegations of wrong-doing involving the care and use of animals. Such reprisal is prohibited by federal law (USDA Regulations & the 9th Code of Federal Regulations). Individuals who feel that action has been taken against them because they reported an apparent violation of animal care and use requirements, should present their case to the Chair of the IACUC, the Director of the Division of Laboratory Animal Resources, or the Director of the Office of Animal Welfare Assurance.

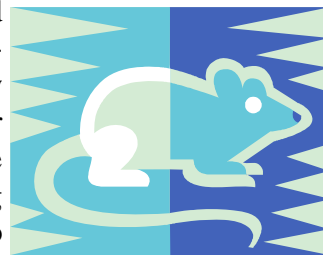
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Did You Know?

When tail clipping mice: Only the minimum amount of tissue necessary for analysis should be taken – less than 5 mm of the tail tip. Tail clipping is not considered a surgical procedure. Because it is not a surgical procedure, alcohol can be used to wipe down the tail. Even so, all instruments must be clean and free of visible debris and can be wiped down with alcohol. Ideal methods for cleaning instruments include exposure to autoclave, glass bead sterilizer, or chemical disinfectants. Neonatal mice should be hand held (generally not in a restrainer). Place mice on paper pad and with gentle pressure, restrain the mouse and extend the tail. After removing tissue a piece of gauze should be applied to the distal portion of the tail with pressure to ensure hemostasis. If done before a mouse is weaned at 21 days, anesthesia is not necessary. After 21 days of age (generally this is the date of weaning), anesthesia and analgesia are required. If anesthesia cannot be provided to a post-21 day animal, scientific justification is required by the IACUC specifying why anesthesia and/or analgesia cannot be used, and why the procedure cannot be done before 21 days.



Toe clipping in mice: Toe clipping for identification purposes alone is not acceptable without clear and prevailing scientific justification. Toe clipping MUST be performed prior to 12 days. Only the minimum amount of tissue necessary should be taken. Tail clipping is not considered a surgical procedure. Because it is not a surgical procedure, alcohol can be used to wipe down the tail. Even so, all instruments must be clean and free of visible debris and can be wiped down with alcohol. Ideal methods for cleaning instruments include exposure to autoclave, glass bead sterilizer, or chemical disinfectants. Neonatal mice should be hand held (generally not in a restrainer). Place mice on paper pad and with gentle pressure, restrain the mouse and extend the tail. After removing tissue a piece of gauze should be applied with pressure to ensure hemostasis. Anesthesia for toe clipping is recommended. Topical anesthetics with epinephrine may be used effectively. Alternative identification methods are preferred and include: a) Micro-chip; b) Indelible markers; c) Ear tags; d) Tattooing

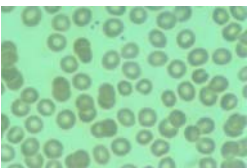
Q & A

Whose interests does the Duke IACUC represent?

The Animal Welfare Act charges the members of the IACUC with representing “society’s concerns regarding the welfare of animal subjects.” In addition, the non-affiliated (outside) member is specifically charged with representing general community interests in the proper care and treatment of animals. The PHS Policy references the IACUC as “an agent of the institution” that will “oversee the institution’s animal program, facilities, and procedures”. All of this is regulatory language to simply say that the Duke IACUC is expected to oversee and evaluate all aspects of the institution’s program for animal care and use. The IACUC is the single body that ensures Duke is compliant with the federal laws found in the Animal Welfare Act, the federal policies defined in the PHS Policy, and the recommendations listed in ‘The Guide.’ The membership of the Committee consists of scientist, veterinarians, non-scientific members and representatives of the local community. The Committee is required to assure animal welfare and well-being, appropriate use of animals, and humane care and use of animals. The IACUC serves as the local oversight arm of federal agencies and accrediting bodies, such as APHIS/AC, NIH/OLAW, and AAALAC.

Must the IACUC review and approve protocols using tissues, cells, or biological fluids acquired from sources other than the IACUC’s institution?

According to NIH/OLAW policy, the outside source (vendor) providing the tissues, cells, or fluids must have a NIH/OLAW Assurance if live animals are being used to produce these products specifically for the institution (or researchers), versus a standard “off-the-shelf” product the company sells commercially. So, if you or anyone else could simply buy it out of the catalog, then it does not require a protocol. If the vendor is producing this tissue, cell, or biological fluid specifically for you, then the vendor must be PHS Assured.



Does the purchase of antibodies from commercial sources require IACUC approval?

According to NIH/OLAW policy, IACUC approval is not required if the standard reagent antibodies (e.g., mouse-antihuman) are produced by a commercial supplier using their own resources and offering them for general sale, for example, through a catalog, the institution may consider the antibodies to be “off-the-shelf” reagents and the supplier is not required to file an Assurance with OLAW. If, on the other hand, a supplier or contractor produces custom antibodies using antigen(s)



provided by or at the request of a principal investigator, the antibodies are considered “customized” and the vendor or subcontractor must file an Assurance with OLAW. Usually it is known in advance that someone intends to perform

this kind of work under a PHS grant. In such cases, the researcher must mark the PHS Grant Application (PHS Form 398) “yes” for vertebrate animal involvement and include the appropriate Animal Welfare Assurance number(s), verification of project-specific date of IACUC protocol review, and the identification of all project performance sites. All animal-related activities supported by the PHS must be conducted at Assured institutions and must be reviewed and approved by an IACUC.

According to USDA Policy #10, if that commercial company uses rabbits (or any other regulated species) to produce antibodies, it must be registered as a research facility and have its own IACUC review relevant procedures. The Duke IACUC will require the commercial company’s USDA registration number in this case.

For commonly used, well-established clinical procedures, such as taking a blood sample from a rabbit’s ear vein or blood from a mouse tail, why does the IACUC request details of how the procedure itself will be performed?

The IACUC must make an informed decision, and the information concerning preparation of the skin, desired volume of blood to be drawn, and the frequency of draws, and needle size is all information that assist the IACUC in clearly understanding the proposed activity. It would not generally be necessary to ask for the angle of needle insertion or the depth of the needle insertion.

Upcoming Events

January 4	SC meeting
January 8	New protocol deadline
January 8	SC deadline
January 18	SC meeting
January 22	SC deadline
January 25	IACUC meeting
February 1	SC meeting
February 5	New protocol deadline
February 5	SC deadline

SC= Significant change

Anesthetic monitoring from page 1

material which insulates it from the surgery table and placing it on a warming device such as a hot water blanket. External devices such as heat lamps should be avoided due to the potential for thermal burns on the patient. It is good practice to provide an area where the animal can escape the warming device if it chooses, such as placing the water blanket under half of the rodent cage. The animal's body temperature should be monitored frequently during anesthesia and recovery.

Mucous Membrane Color: If a normal animal is receiving enough oxygen during anesthesia, its mucous membranes remain pink. This can be evaluated by observing the conjunctiva of the eye, the lips, the gingiva (gums), and in albino animals, the ears and pads of the feet. A bluish discoloration (cyanosis) indicates that the animal is not getting sufficient oxygen. A pulse-oximeter measures oxygen saturation of the tissues from a sensor that is placed on the animal's body (usually on the tongue of larger animals) to allow this to be continuously monitored.

Capillary Refill Time: Capillary refill time is the time it takes the capillaries to refill with blood (return of pink color) following the release of physical pressure that keeps them empty. For example, pressing a finger against the animal's gum, releasing it, and noting how long it takes for the pink color to return to the gum gives an indication of cardiac output. Normal capillary refill time for most laboratory animals is less than two seconds. A longer refill time suggests depression of cardiac output. This parameter should be checked frequently on normal, unanesthetized animals in order to acquire the judgment necessary to evaluate an anesthetized patient.

Eyes: The size of the pupil of the eye is a poor indicator of anesthetic depth, especially when atropine has been given as a preanesthetic. The eye is more often used to evaluate the palpebral reflex, which can be observed by a light touch to the medial canthus (corner) of the eye, or just to the eyelashes. A lightly anesthetized animal will blink when touched. Animals in surgical anesthesia do not have a palpebral reflex. The corneal reflex, which involves very carefully touching the cornea with a small piece of cotton, persists after the palpebral reflex is lost. This reflex may be present in surgical anesthesia and thus is not a reliable indicator of the animal's depth of anesthesia. The use of either the corneal or palpebral reflexes for small mammals is not a good idea – these reflexes are not consistent in the small mammal species.

Reflexes: The palpebral and corneal reflexes have already been discussed. Another useful test of anesthetic depth is the pedal reflex, sometimes called the toe pinch reflex. This response can be elicited by pinching an animal's toe or the webbing between two toes. Pulling the foot away or flexing the muscles of the leg is sufficient to

indicate a response. Like the palpebral reflex, the pedal reflex is normally not present in the level of surgical anesthesia. The laryngeal reflex, also called the cough reflex, occurs if the area close to the animal's larynx is touched. This reflex is not usually present during surgical anesthesia (except with ketamine), and it is used as an indication that it is time to remove the endotracheal tube of an intubated animal that is recovering from anesthesia.

Eradication of Cross-Contaminated Cell Lines

Roland M. Nardone, Ph.D. states in a white paper report available on the web site of the Office of Research Integrity, Department of Health and Human Services (web address of the article is: <http://ori.hhs.gov/education/CellContamination.shtml>): "Extensive cross-contamination of human and animal cell cultures with a variety of human and animal cell lines is a long-standing problem." The article notes that up to 20% of the cultures of major repositories may be cross-contaminated. Dr. Nardone worries that as "continuous cell lines increase in use and as immortalization of cell lines becomes more common the problem will become exacerbated unless strong, realistic measures are taken to correct the situation."

Dr. Noland's 'white paper' outlines methodology that could rapidly diminish the problem. Certain of his recommendations could significantly impact the research process at Duke. Dr. Noland recommends that "... cell line authentication as a condition for receipt of grant funds from major agencies (NIH, NSF, HHMI, ACS, etc.), authentication as a condition for publication of cell culture-based research in leading journals, and focused education opportunities for technicians and scientists regarding prevention and detection of cross-contamination" be considered.

The report encourages continued discussion and sharing of options and optimum strategies that could be engaged to discourage the further impact of the present situation. This report is a good review of causes, prevention and detection of cross-contamination methodologies. Duke researcher using cell lines in their research may wish to enact some or all of the recommendations in this report. At present there are no institutional positions concerning all of the recommendations listed, however, the Duke animal program does require assessment of safety from adventitious viruses for cells or fluids injected into research animals. The Duke DLAR can assist researchers with evaluating new or putative test agents of biological origin. Certain strategies may be internal to Duke (e.g. MAP or RAP tests) while others may be external to Duke (e.g. independent testing houses).





OAWA's Brown Bag Seminar

Thursday, January 18th, 2007

Noon – 1 p.m.

Bryan Research Building: Room 103

Dr. Ron Banks

Director of the Office of Animal Welfare Assurance will be presenting:

“Oh-No! The IACUC is Coming!”

The Duke Office of Animal Welfare Assurance will provide a practical discussion of how to effectively (and painlessly) prepare for an IACUC Semi-Annual Review of your laboratory or procedure area. Several common scenarios will be used as discussion points to clearly identify what the IACUC Site Visitors are looking for. Presenters will share preferred responses to the most common questions asked during a Semi-Annual Site Visit.

The Semi-Annual IACUC visits are an on-going part of the institution's program for animal care oversight. Often, these visits appear to bring angst and anxiety to the laboratory members, but understanding the process and how to prepare for a Semi-Annual Visit will ease the distress. This session is designed specifically for laboratory managers, but is useful for all laboratory members.

The presentation will be on **Thursday, January 18th, 2007 from noon to 1 p.m.**

The session will be held in room **103 of the Bryan Research Building**, located at 421 Research Drive, on Duke University's West Campus.

Attendees are encouraged to bring a lunch.

OAWA will provide drinks and desserts.

Please plan on arriving prior to noon in order to get refreshments, sign in, and be seated.

For those who will be coming from off campus, driving directions and parking information can be found at the following link: <http://neuro.duke.edu/Links/map.htm>