Common Techniques When Using Mice in Cancer Research Part II

NCI Mouse 101

Dr. John Dennis
Laboratory Animal Sciences Program
November 2009

SAIC From Science to Solutions
SAIC-Frederick, Inc.
A subsidiary of Science Applications International Corporation

Expectation for All Investigators

- Before collection of research data, ensure competency and proficiency in all technical procedures.
- Improperly performed techniques can result in harm to animals and compromised research results.
  - ***" An animal doesn't need to be moribund to be scientifically invalid."***
- Training resources AND technical resources are abundant within the NCI, NCI-Frederick and elsewhere on the NIH Campus

Availability of Training .....

- *Facility Veterinarians, Technicians, Supervisors, and Managers*
- *NCI/NIH Collaborators*

Location	*Vet Associate*
Bldg 37	Gail McMullen
CRC Vivarium	Danielle O'Mard
NCI-Frederick	Julie Bullock

Manager of Technical Operations

- NCI-Frederick: Mark Shrader
- NCI-Bethesda: Larry Cook

  - Short-term or long term technical support
  - Advance notice requested

Blood collection

- How much blood can I take from a mouse without endangering its life?

- Answer: The amount depends on the size of the mouse, the frequency and interval of collections and whether or not replacement fluids are given.

Blood Collection

- Rapid removal of 20-30% : SHOCK
- 30-40% removal : Over 50% Die
- >40% blood volume removed : DEATH

- For most species, 10% of total blood volume (no fluid replacement) may be removed at one time every 2-4 weeks. (Blood volume = 55-70 ml/kg). With Fluid Replacement, up to 15% can be removed at once.

Blood Collection (cont)

- Detailed guidelines for intervals and volumes in NIH ARAC Document,

  Guidelines for Survival Bleeding of Mice and Rats

  http://oacu.od.nih.gov/ARAC/Bleeding.pdf

For a 25 g mouse the recommended withdrawal volumes are 0.13 ml weekly or 0.18 ml q 2 weeks or 0.26 ml monthly

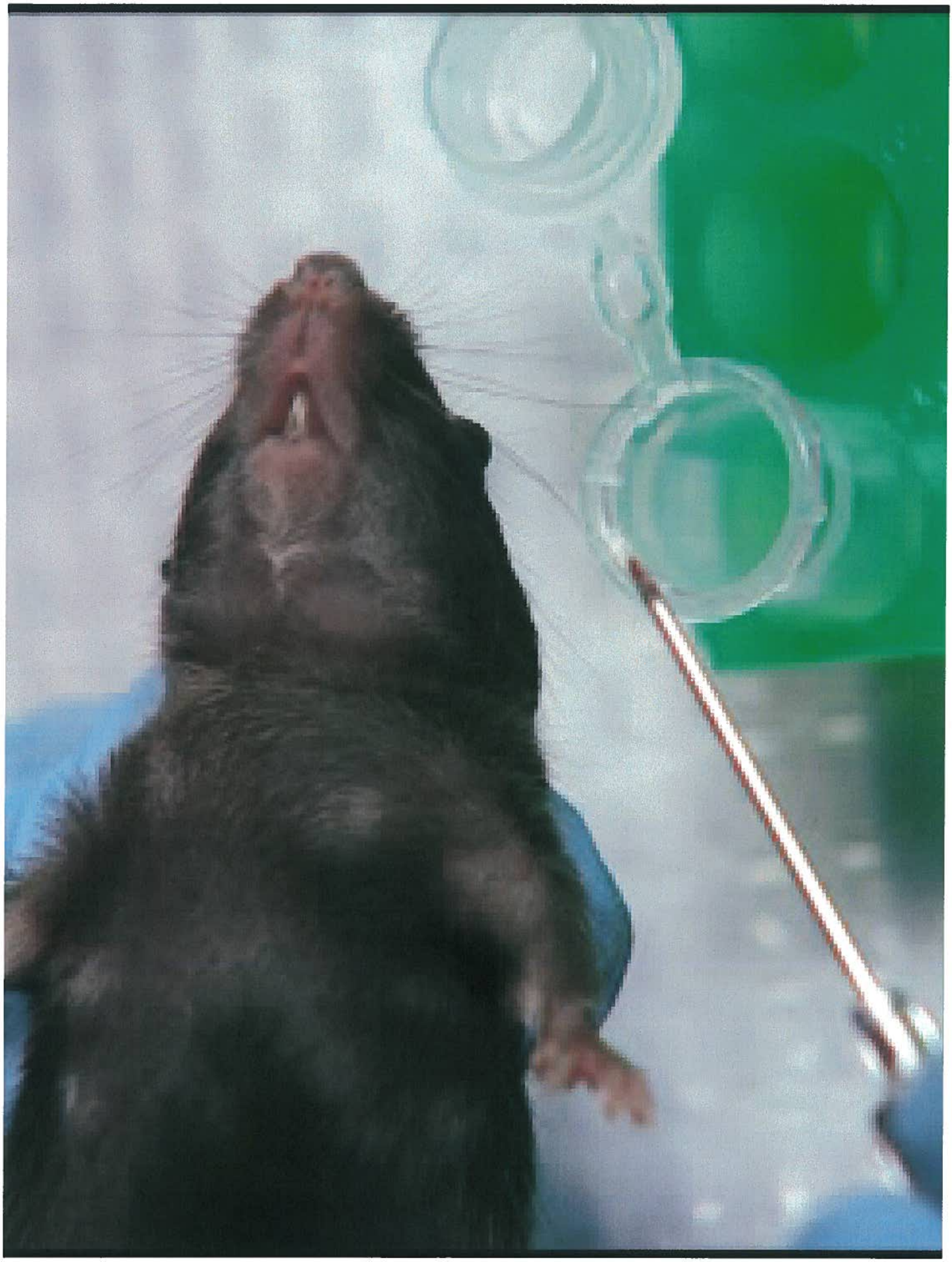
Blood collection

- Survival awake
- Each method has pros/cons
  - Mandibular/facial artery 20 ga or lancet
  - Tail nick (ventral tail artery) (25 ga)
  - Saphenous vein 25 ga
  - Jugular vein 25-27 ga

    Rapid, minimal risk to animal, anesthesia not required. High throughput limited by restraint requirements. Repeat sampling is possible if vessel is pierced, rather than severed. Ensure that bleeding stops.
  - Tail catheter (lateral tail vein) requires anesthesia

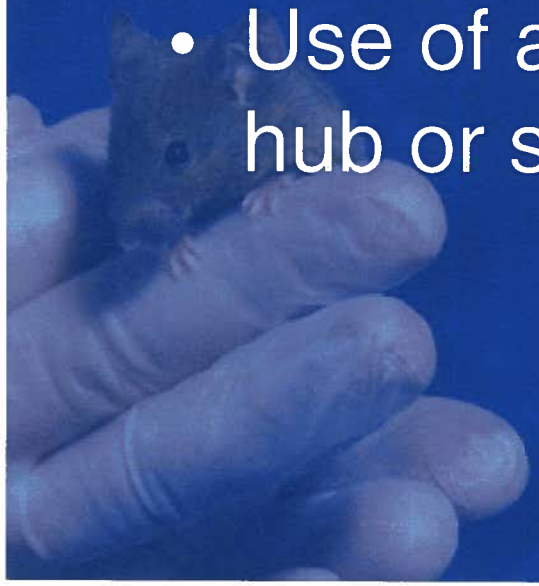
Blood Collection

- Laceration or amputation procedures yield samples that are contaminated with tissue products.
- The degree of contamination increases with the degree of tissue trauma
- Pure blood samples typically require placing a needle into a blood vessel or catheterization
- Different collection sites may result in different cell counts
- The person doing the procedure is the most important factor for success / proficiency and consistency in results



Blood collection

- The vein or vessel should be clearly visible or else known by location in conjunction with landmarks
- Dilation of vein w/ heat or vasodilators
- Pluck or shave hair – saphenous vein
- Use of anti-coagulants in the needle hub or storage device may be useful



Blood collection: retro-orbital

- Acceptable on awake animals only when scientifically justified and performed by a skilled operator
- Topical or general anesthesia recommended
- Mouse has a *large* retro-orbital venous sinus
- Accessed from medial or lateral canthus
- Microhematocrit tubes 75-200 μl
- Do not touch or damage the eye. There is a risk of injury to the mouse eye and a risk of pain/distress to the animal
- Allow 2 weeks for recovery
- Alternative methods are available, making this method uncommon and frequently discouraged. Not permitted at some institutions
- High throughput is possible by a skilled handler

Blood collection in conjunction with euthanasia

- Anesthesia or CO_2 narcosis required prior to exsanguination
  - Retro-orbital/mandibular
  - Cardiac puncture - percutaneous
  - Posterior vena cava – rapidly open the abdomen (pure blood)
  - Axillary vessels

Normal blood parameters

- Vary by strain and age
- Jax Phenome database is great resource
- Flow cytometry or diagnostic lab staff can help interpret
- Facility veterinarian or veterinary pathologist may also be of assistance

Urine collection

- Rapid restraint free-catch
- Metabolic cages
- Cystocentesis usually at necropsy

Other sample collections

- CSF (8-25 μl from anesthetized mouse)
- Milk (mouse milking machines)
- Bile (chronic collection surgery prep)
- BAL (bronchiolar-alveolar lavage)
- Bone marrow
  - Aspirates from anesthetized mice
  - Flush from excised long bones
- Peritoneal fluid
  - Prime peritoneal cavity w/ 3% thioglycollate
  - Terminal or awake collection by saline lavage (6-8 ml warmed, aseptic)

Blood vessel cannulation

- May be necessary for timed repeat blood sampling of awake mice or injections
- Carotid artery or jugular vein
- Requires micro-surgery skills
- Individual housing /protective jacket recommended
- Daily flushing
- Tethering is also possible
- Mice may be instrumented by vendor

Necropsy/Tissue Collection

- Necropsy- critically important for characterizing genetically engineered mice (GEM)
- Should be performed on clinically normal GEM and when such mice show clinical problems
- Mouse pathology services are available at NCI-Frederick and NIH-Bethesda
- Virtual mouse necropsy http://www.geocities.com/virtualbiology/

Necropsy (continued)

- Supported by other clinical diagnostics, cultures, when appropriate
- Recognition of normal vs. abnormal (may be subtle) – facility veterinarian
- Refrigeration, preservation, fixation
- Histopathological evaluation by experienced mouse pathologist can make a huge difference

Fasting / Withholding Food

- Not required prior to blood collection or anesthesia
- Gastric emptying – 4 hours in the mouse ; entire GI tract requires 3 days!
- Temporary suspension of food or water provision requires ACUC approval and alerting the animal care staff
- Special cage signage

Anesthesia and Surgery

- ***"There are no safe anesthetic drugs, only safe anesthetists."***

- ***"Getting to survival is not enough."***

Anesthesia and Analgesia

- Restraint vs. Minor vs. Major surgery
- Pre-emptive analgesia is probably beneficial for major surgery.
- Prevent hypothermia – the # 1 cause of rodent anesthetic complications
- Protect corneas during anesthesia
- Determine which method (gas or injection) is most advantageous
  - In general, properly delivered gas anesthesia is safest for the animal

Ketamine and ket combinations

- Ketamine produces immobility, analgesia, and amnesia in most species
- Alone, it is not satisfactory for surgery and should be combined with a tranquilizer or other drugs to produce balanced anesthesia
- Useful starting dose for mice is 100 mg/kg ketamine + 10 mg/kg xylazine
- Mixtures should be diluted 1:5 or 1:10 in saline and used within one week
- Ketamine is Controlled Substance and must be procured through the DVR Pharmacy in Bldg 14, in keeping with NIH Policy

Avertin (2,2,2-tribromoethanol)

- Not recommended anymore for survival procedures. Refer to ACUC policy.
- Peritoneal inflammation is common
- Subject to rapid photo-degradation, so prepare fresh just prior to use.
- When used a second time in the same animal, deaths have been reported.
- Advantage is this is not a Controlled Substance
- 240-375 mg/kg

Gas Anesthesia : Isoflurane

- Need precision vaporizor to ensure proper delivery rate
- Need for scavenging for personnel safety
- Safest for animals when properly delivered
- Rapid recovery can be a problem if residual pain is not covered by an analgesic

Monitoring the Depth of Anesthesia

- Response to painful stimuli
- Respiratory rate
- Alterations in perfusion, heart rate, temperature, or ventilation physiology
- Supplementary monitoring devices EKG, pulse oximetry, capnograph, are available and useful when animals cannot be seen directly (inside imaging device)

Analgesia

- *We are not that good at recognizing pain in animals; therefore, analgesics should be used routinely.*
  - Local analgesia- bupivicaine
  - Opioid analgesia – buprenorphine
  - NSAIDs – Carprofen, Meloxicam
  - Provision recommendations guided by chart based on anticipated degree of pain
  - Research effects of unrelieved pain may outweigh temporary side effects of analgesics

Mouse intubation

- 20-24 ga catheter w/ stylet- trimmed down to extend only to mid-sternum
- Injectible anes. supplemented by gas
- Magnification and fiberoptic supplemental light and specialized restraint are useful to visualize larnyx
- Required for open chest procedures, in conjunction with mechanical ventilation
- Verify proper placement and secure in place

Surgical techniques

- Choice of good-quality surgical instruments
- Sterilization of instruments prior to first surgery of the day (250 F for 15 min or 270 F for 5 minutes)
  - Alternatives to steam: dry heat. Ethylene oxide, gluteraldehyde, chlorine dioxide
- Between animals: wipe with sterile saline and place tips in glass bead sterilizer

Surgery – The 4 A's

- Anesthesia-Analgesia
- Asepsis
  - The critically important factor to speed recovery and minimize research complications
  - Improves with practice and consciousness
- Atraumatic tissue handling
  - Efficient, rapid, less tissue destruction, less bleeding, and less pain
  - Some people do this more naturally and skillfully than others

Preparing for Performing Surgery

- Research the techniques
- Talk to others, esp. the lab animal veterinarian
- Gain competency through practice
  - "success comes from experience, and experience often comes through previous failures…" Therefore: practice on cull animals or non-important or deceased animals.

Surgery Preparation

- Do your homework
- Make lists of supplies and what “might” be needed
  - Lavage fluid, isotonic fluids, extra instruments
- Clip or remove the hair adequately, but do not clip the animal the day before
- Commit to principles of asepsis/ draping
- Learn from previous mistakes

Surgery- What can go wrong?

- Prolonged surgical time
  - More tissue dryness, hypothermia, reduced perfusion, reduced healing, infection
- Foreign body / inflammatory reactions
  - Hair, suture material (silk)
- Anesthetic overdose/ hypothermia
- Blood loss / hypotension
- Skin tension/ self-injury / pain

Common Myth: *Rodents are more resistant to bacterial infection than other species.*

Fact: Aseptic surgical technique in rodents is expected and is a requirement in the NIH Guide fore Care and Use of Laboratory Animals

Resources for Surgical Techniques

- Training videos / CD's
- Facility Veterinarian and Training Protocol
- Routine rodent surgeries have been reduced to SOP format, available from LASP
- Laboratory Animal Science Publications
- Academy for Surgical Research

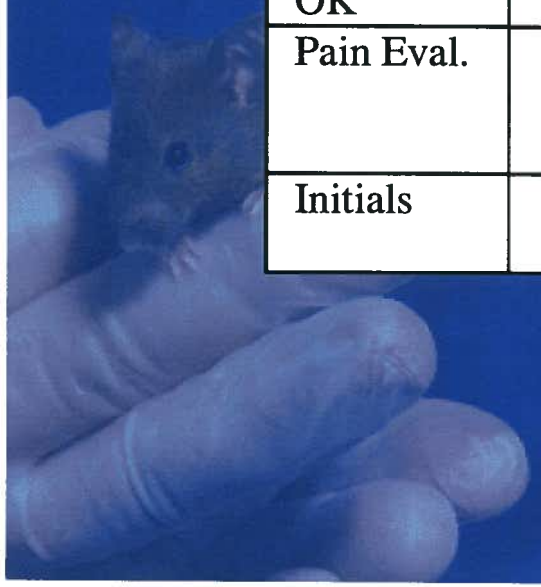
Postoperative Care

- Monitor until ambulatory or resting upright
- Mark the cage with a dated post-op tag
- Warmth
- Pain relief – give in advance or as needed
- Antibiotics – not routinely recommended
- Hypovolemia – 1 cc warm sterile saline SC
- Records – details may be on cage card
- Removal of wound clips or sutures

Postoperative Care : Record Systems
Example record for Rodent Cage Card

Surgical Procedure _____ Date _____
Surgeon _____ Number of animals _____
Anesthesia _____
Post-operative Analgesia _____
Notes / Complications _____
Post-op Monitoring

DATE							
Incision OK							
Pain Eval.							
Initials							



Daily Health Monitoring and Emergency Contact Info

- Emergency contact forms should be updated and modified prn and reviewed each year
- Any suggested medical treatments for ill mice will be discussed prior to initiation
- Animal care staff perform twice daily checks to ensure provision of food and water and looking for obvious problems; they are not responsible for post-procedure monitoring, which is typically done by the research staff.

Euthanasia techniques

- Method of euthanasia can influence immunological parameters (ex. Mitogenic lymphoproliferation) and certain gene functions
- Methods must be consistent with AVMA Guidelines (Acceptable (A) or conditionally acceptable (CA))
- Carbon dioxide / Anesthesia overdose (A)
- Cervical dislocation / decapitation when awake (CA)
- Need to be sure that the animal is dead

Humane endpoints

- Described in animal study proposal
- Defined intervention point
  - Animal to be relieved by being taken off study and/or euthanasia
- "Surrogate experimental endpoint" that predicts desired outcome
- Allows for data collection prior to onset of unnecessary pain or distress
- Should be prior to the moribund state
  - Moribund = in the process of dying
- Not just one parameter

Humane endpoints (cont)

- Examples:
  - Weight loss 15% routine, 20% approvable
  - Body condition score
  - Body temp drop of 5-6 C predicts death
  - Activity levels or neurological impairment
  - May be animal model-specific
  - Technicians can recognize progression and recommend euthanasia
- In some cases the Veterinary staff needs to perform euthanasia to protect the integrity of the animal program

Final Messages

- Problems with techniques or technical competency may never be detected unless all animal handlers are committed to excellence and are well-trained.
- Monitoring animals during procedures and post-procedure monitoring are essential .
- Repeated monitoring allows determination of progression and allows intervention before it's too late
- Be sure the animal facility technicians and veterinarian know you and how to contact to you when/if problems are found.

Questions?