Writing an Animal Protocol

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IACUC and staff
Collaboration & coordination
Institutional Official
Compliance

Scientists, staff, and students
Conscience

Attending Veterinarian and staff
Compassion

Team Members
Responsibilities

Protocol Review Systems
- Full Committee Review
- Designated Committee Member Review
- Pre-review or multilevel reviews
  - Mixing
    * Quorum
    * Discussion
    * Voting

Good protocol review relies on thorough, well-written documents.

What IACUC

- Institute
- Animal
- Care and
  Ethical Committee
- Use
- Committee

Protocol Review Consideration

Benefit MUST outweigh cost

* Human health
* Animal health
* Advancement of knowledge
* Scientific gain
* Good of society

* Animal use
* Pain
* Distress
* Number of animals
* Species

Harm-Benefit Analysis

Protocol Review

- Scientifically sound
  - Objective / hypothesis
  - Statistical consultation
  - Study design
- Animals
  - Appropriate species
  - Appropriate number
  - Appropriate housing/care
- Personnel appropriately trained to perform animal manipulations
The Research Proposal

- Before a research program can begin, the scientist must write a detailed outline of the proposed research.
- The document, referred to as a research proposal, explains the specific aims and expected results of the research and describes what methods will be used to accomplish these aims.

The Animal Use Protocol

- If the scientist plans to use animals as part of the research, he or she must explain in a separate written document, called an animal use protocol,
  Why animals are needed to accomplish the aims,
  What procedures will be performed on the animals and
  How the animals will be housed and cared for throughout the project.

Protocol Approval

- Protocol approval before any use of animals begins.
- Animal protocol – Follow standard form:
  "MU Animal Care and Use Protocol"

Protocol Review Objectives

- Ensures that the animal research is being performed in an ethical manner.
- Ensures that the animal research is performed according to the highest standard.
- Ensures that animals are not subjected to unnecessary pain and distress.

Protocol Review

PI vs IACUC

Protocol Amendments

- Significant change
  - Objective
  - Species or number of animals
  - Degree of invasiveness
  - Switch from nonsurvival to survival surgery
  - Anesthetic or analgesic agents
  - Methods of euthanasia

Protocol Writing

Cover Sheet

Protocol Title:
(Thai)…………………………………………………………………………..
(English)………………………………………………………………………

If this protocol is a part of the Main Project, please provide the Main Project Title:
(Thai)…………………………………………………………………………..
(English)………………………………………………………………………

Funding Source(s): ………………………………………………………
Grant has been: ☐ Submitted
☐ Approved. If approved, duration of approval………
Anticipated Protocol Period: From………………To……………………….

Type of Animal Protocol

[ ] Research: In the Field of
[ ] Testing/Monitoring (please specify)
[ ] Teaching: Course Title/Level
[ ] Biological Production: (please specify)
[ ] Animal Breeding (please specify)
[ ] Other (please specify)
### Protocol Writing

#### Protocol Title

This is an animal protocol, therefore it must have animal listed in the title

***Effect of Antigen A on Vaccine Efficacy***

***Effect of Antigen A on Vaccine Efficacy in Mice***

### Protocol Writing

#### Non-technical summary

***Example:*** Malaria is the most important parasitic disease worldwide. Severe anemia is often the cause of morbidity and mortality related to malaria. The study of a XXX in a severe malaria mouse model may lead to improved therapy for patients with severe malaria anemia (SMA)

### Protocol Writing

#### Non-technical summary

***Example:*** In people dengue causes flu-like symptoms, joint pain, fever, rash, and rarely, upon subsequent infections, generalized bleeding. A preventive vaccine is required. Candidate vaccines must first be tested for protective efficacy in animal models including non-human primates such as rhesus monkeys. Such testing requires that vaccinated animals be challenged with live dengue viruses. Although dengue infection in the rhesus is asymptomatic the challenge viruses can be detected in the blood as a viremia, the presence of which is used as a surrogate for disease. This protocol is designed to test three candidate dengue challenge virus strains for their ability to reproducibly induce viremia in non-immune rhesus macaques, so that these challenge viruses can be used in a subsequent vaccine efficacy study.
Protocol Writing

2. Rationale and literature review (Background)

Provide a brief description of the project expressing its significance and needs for undertaking the study.

- All acronyms must be spelled out first.
- Any statement of discovered fact should be referenced.
- Long, moderate or short to highly technical, aimed at the scientific audience.
- Bring the reader to a jumping off point — The next step
- Scientific reference to genus and species of agent or animal should be italicized

Protocol Writing

3. Literature search for Duplication

To be performed to prevent unnecessary duplication of previous experiments.

- Literature Source(s) Search: BIOSIS, BRD, PubMed
- Date of Search: Perform no earlier than six months prior to the IACUC meeting. (dd/m/yy)
- Period of Search:
  a. BIOSIS: 1926 to present
  b. BRD: 1998 to 2015
  c. PubMed: 1950 to present
- Key Words of Search: hit, animals species used, agent type.
- Result of Search:………………………………………………

Protocol Writing

Literature search for Duplication

Result of Search

Duplication of research was determined by the PI is not sufficient.

The number of articles found and why they are not duplicative.

Provide a narrative description of the results of the literature search.

Show that "There are not unnecessary duplicating previous experiments."

It is up to the IACUC to see that adequate information is provided.

Protocol Writing

Literature search for Duplication

Result of Search

A review of the literature showed publications for Plasmodium berghei and severe malaria related publications in mice. There were __ publications for XXX application in human anemia, in swine, dogs, cats and sheep. There was no publication studying XXX in P. berghei mouse severe malaria anemia.

Protocol Writing

4. Objectives/Hypothesis

State the objective of this protocol to be accepted or rejected.

- non-technical terms —
- Type a full sentence:
  "The objective of this protocol is to determine or develop........"
- More than one objective is acceptable.
  The objective is to:
  1) determine........
  2) test the vaccine........etc
- Number the objectives for clarity.
Protocol Writing

5. Experimental design/Materials and Methods

- What will happen to the animals?
- Complete description of the proposed use of animals, all necessary information needs to be included here.
- Clearly describe the numbers of animals and their distribution
- Identify all groups in the design
  * Include control and experimental groups
  * Number of animals per group
  * Number of iterations of testing/sampling/injections
- Outline the formal scientific plan and direction for experimentation
- Describe the experimental design of sequential studies if more than one experiment will be performed.
- Flow charts, time lines and tables are very useful

With Humane Standards

Protocol Writing

Experimental design/Materials and Methods

- If study utilizes several species, please identify experimental procedures for each species separately
- Describe any non-surgical manipulations:
  Injections, scans (x-rays), sample collection, Route, volumes, frequency included
- Outline All Surgical manipulations
  * Describe surgical manipulations including site preparation, surgical approach, and unique techniques for each surgical procedure.
  * Provide information on Pre- and post operative care
  * Anesthesia and Analgesia
  * Intra-operative monitoring
  * Wound Closure and suture removal

With Humane Standards

Protocol Writing

6. Data Analysis and statistical method

- List the statistical test(s) planned or describe the strategy intended to evaluate the data.
- Describe the statistical methodology used to determine group size and total number of animals.
- A power based assessment of the sample size may be included.
- If an experiment must be repeated in order to increase the numbers or power needed for the study include a short statement justifying the repetition.

Example: the data will be analyzed using SPSS 12.0 for Window and StatXact-7. Antibody will be log-transformed before testing the difference. T cell responses will be analyzed by using the kruskal-Wallis test and the Mann-Whitney rank sum test (non-normally distributed data) with Bonferroni correction for multiple comparison. P-value < 0.05 will be considered statistically significant.
7. Animal model and species justification

7.1 Description of animals

Fill in table:

- **Common name**
- **Genus and Species**
- **Strain/Stock**: If inbred or specialized animals are required, use proper terminology (See the AV for assistance)
- **Age**, application for some species like rodents
- **Weight**
- **Sex**
- **Number**

<table>
<thead>
<tr>
<th>Common name</th>
<th>Genus and Species</th>
<th>Strain/Stock</th>
<th>Age</th>
<th>Weight</th>
<th>Sex</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Rattus norvegicus</td>
<td>Wistar</td>
<td>8 wk</td>
<td>250-300 g</td>
<td>Male</td>
<td>40</td>
</tr>
<tr>
<td>Mouse</td>
<td>Mus musculus</td>
<td>ICR</td>
<td>4 wk</td>
<td>20-22 g</td>
<td>Female</td>
<td>80</td>
</tr>
<tr>
<td>Mouse</td>
<td>Mus musculus</td>
<td>Balb/c</td>
<td>3 wk</td>
<td></td>
<td>Female</td>
<td>20</td>
</tr>
</tbody>
</table>

- **Common name**
- **Use scientific name “Genus and Species”**
- **Strain/Stock**: If inbred or specialized animals are required, use proper terminology (See the AV for assistance)
- **Age**, application for some species like rodents

7.2 Scientific justification for animal species and number requested.

*How will the proposed use of animals benefit human or animal health*

7.2.1 Animal model and Species justification

**Animal model**

- **Alternatives are considered.**
- **If animals are indeed necessary, use the least sentient (“aware”) species capable of providing the needed data.**
- **Provide rationale for using animal models versus non-animal models**

**Justification of the selected species**

- **Presence of a large body of scientific knowledge validates use of particular species or animal model.**
- **The presence of previous work as an animal model of human diseases in the literature.**
- **Characteristics of the species make it uniquely suited for the study.**

7.2.1 Animal model and Species justification

**Justification of the selected species (con’t)**

- **Describe the characteristic of the animal that make appropriate for the study.**
- **Look for how sensitive this model is to the agent.**
- **Good representative of the human disease; physiological and/or morphological.**
- **Used in the previous protocol and need to complete.**
- **Disease – cause, currently therapy, how proposed animal experiments might better.**
- **Explain the medical terms.**

**Protocol Writing**

**Justification for animal species**

- **Use the least sentient species**
  - Apes (chimps, orangutans, gorillas)
  - Monkeys (baboons, rhesus monkeys, marmosets)
  - Large animals (dogs, cats, pigs, goats)
  - Rabbits
  - Rodents (guinea-pigs, hamsters, rats, mice)
  - Non-mammalian vertebrates (poultry, amphibians, reptiles, fish)
  - Invertebrates (crustaceans, slugs)
  - Smaller life forms (insects, arachnids, worms)
  - Single cell organisms (yeast, bacteria etc.)
Protocol Writing

Justification for animal species

EXAMPLE: The rat is most appropriate animal for numerous reasons. First, to link symptom severity to neuronal activity we must be able to make neural recordings during awake and behaving conditions. The rat is the most often used animal under these circumstances. Second, of the two widely used animal models of Parkinson’s Disease, rat experiments are the more humane.

Protocol Writing

7.2.2 Number of animals required

Determine the right number of animals for obtaining valid results

- Justify group size or experimental repetitions:
  - match exactly those described in experimental design.
  - Consider power analysis and statistics
  - consult a biostatistician
- be include animals for controls or technique development
- For new surgical or other techniques
  - studies on cadavers from other approved protocols
- Pilot experiments to demonstrate feasibility or provide a justification for proceeding with subsequent studies.
- ***If personnel training is part of the study, include sufficient animals to ensure adequate training

Protocol Writing

8. Animal Care Consideration

Determine whether the living conditions are appropriate for the animals

8.1 Study location: (Study room where the animals will be housed)

-----------(Laboratory Animal Unit - MU Testing Center)

8.2 Housing System:

- Conventional
- Strict hygienic conventional
- Clean conventional
- Barrier maintained
- Laminar flow
- Isolator maintained
- Other, please specify .............................................

Conventional Animal

Microbial burden is unknown and uncontrolled, and housing is generally in open rooms that have unrestricted access.

Monitor or Clean Conventional Animal

Free of major pathogens, demonstrated by sequential monitoring. Housed in a low security barrier and to be

Specific Pathogen Free Animal (SPF)

Free of specified list of pathogens, but still have normal flora.

Germfree Animal

Free of associated forms of life

Protocol Writing

8.3 Caging:

- Solid bottom, open top
- Static filtered top cages
- Suspended cages, wire bottom
- Metabolic cages
- Individual ventilated cage (IVC)
- Other, please specify .............................................

8.4 Cage size: W x L x H, (inch).............................................

8.5 Caging materials

- Plastic
- Stainless steel
- Other, please specify .............................................

8.6 Number of animals per cage..............................
Protocol Writing

Animal Care Consideration

8.7 Environmental requirements:

- Temperature: ..........................................................
- Humidity: ..........................................................
- Light: 
  - Standard fluorescent
  - Other, please specify .....................................
- Light cycle: 
  - Standard 12:12 (light:dark)
  - Other, please specify .....................................

8.8 Food:

- Type of food: 
  - Standard diet
  - Other, please specify .................................
- Feeding schedule: 
  - Routine feeding (Ad libitum)
  - Other, please specify .................................

8.9 Water:

- Type of water: 
  - Hyperchlorinated ....... ppm.
  - RO water .............
  - Other, please specify .................................
- Provision of water: 
  - Routine feeding (Ad libitum)
  - Other, please specify .................................

8.10 Bedding or litters:

- No
- Yes, please specify  
  - Sterile
  - Non-sterile
- Type of bedding or litters: 
  - Wood shaving
  - Sawdust
  - Paper
  - Other, please specify .................................
- Schedule of bedding changing: 
  - Weekly
  - At specified interval, every .... day(s)

8.11 Environmental Enrichment: It is MU policy to provide environmental enrichment through nesting material for all laboratory animals.

- Acceptable
- Not acceptable. Please justify.
Protocol Writing
Animal Care Consideration
8.12 Is this project intended to conduct the animal experiment in other building? [ ] No [ ] Yes
If yes, please provide information below:
1. Where the experiment is expected to be conducted? Please provide the building name and room number.
2. Please provide the animal experimental procedures in detail.
3. Estimated total time period that live animals will be kept in the laboratory is........... hours
4. How will the animal sample or carcass be disposed?

Protocol Writing
Veterinary Care Provision
9. Veterinary medical care: (Describe the routine veterinary care. List the criteria used for health evaluation while the animals are on study).

- Routine
  - daily observation or more frequently and by whom
  - indicate what will happen if the animal becomes ill during the study and requires supportive therapy
  - if the animal will be euthanized and by whom
  - justification for not providing supportive care for clinically ill animals
- Emergency
  - during recovery after surgical
  - fighting
  - etc.

Protocol Writing
Animal welfare
10. Does the proposed research duplicate any previous work?
   [ ] Yes [ ] No
   If yes, explain why it is scientifically necessary to duplicate the experiment.

10.2 considered each of the following alternatives (the 3Rs)
   10.2.1 Replacement………………………………………..
   10.2.2 Reduction………………………................................
   10.2.3 Refinement……………………………………………..

Protocol Writing
Animal welfare
Consideration Animal Alternative
The “3 Rs” Replacement, Reduction and Refinement.

Replacement with non-animal techniques
- Not common but should be considered
- In vitro methods: organ, organ, tissue or cell culture
- Immunologic bench assays
- Computer simulations
- Invertebrate animal (fruit fly)
- Use of membrane feeding

Protocol Writing
Animal welfare
Consideration Animal Alternative
The “3 Rs”-Replacement
Non-animal alternatives considered:
Address what non-animal alternatives were considered, and why PI can’t use them.
Ex: To date there are no non-animal models for testing the efficacy of a potential (agent) vaccine. Only in living, whole animals can the immune system be examined. Therefore it is necessary to perform these studies with (this agent) in an animal model.
Protocol Writing
Reduction
- Careful experimental design
- Avoid redundancy
- Use the correct model
- Statistically well planned - minimum group size as needed to obtain statistically significant data
- Shared control groups/ share tissue
- Use newer instrument that improves precision
- Preliminary screening in non-animal systems
- Minimize loss thru well trained staff and good animal care

Double the procedures on the same animals may not justify the reduction of the animal number.

Protocol Writing
Refinement
- Pilot studies
- Adjust techniques
- Skilled technicians/ Well trained personnel
- Environment enrichment
- Anesthesia/ Analgesia
- Close observation/ Monitor clinical signs & Pathology
- Early endpoint

Protocol Writing
Refinement: changing experiments or procedures to reduce pain or distress
Ex. Animal handling and techniques will be performed by experienced animal technicians and result in minimal discomfort. Mice are conditioned for 3 to 5 days before the beginning of study and they are trained to be familiar with manual handling. Mice are observed twice daily or more often during the day when necessary for early endpoint clinical signs.

Pain and Distress
Stressors
(Environmen)
Stress
Adaptation
Failure
Distress

Model of Stress Response
Perception of stressor

Biological response

Maintain homeostasis

Coping & Adaptation

Pre-pathology

Pathology

10.3 Potential animal pain and distress assessment:
10.3.1 Please indicate pain category according to USDA Pain and Distress. (Appendix A)

1) Number of animals:
- Category C _________
- Category D _________
- Category E _________

2) Pain relief/Prevention
(Address how to minimize discomfort, pain and distress)

10.3.2 During the study:
1) How often will the clinical condition of animals be monitored?

……………………………………………………………………………………

……………………………………………………………………………………
Protocol Writing

Pain and Distress assessment

No pain (C): routine procedures e.g. injection, deep palpation, blood collection from vein, observation studies of animal behavior, tissue collection after euthanasia

Alleviated pain (D): appropriate anesthetics or analgesics will be administered to avoid or alleviate pain e.g. surgery, tattooing, small tumor removal

Unalleviated pain (E): animals are subjected to painful procedures without the use of anesthetics, analgesics, or tranquilizers e.g. lethal dose studies, pain studies

10.3.3 Are the animals expected to experience any specific study-induced or related problems (i.e. health problems, pain, distress, complications, etc.) or any health problems as a result of the phenotype of the animal?

☐ Yes  ☐ No  If yes, please answer the following questions:

1) Describe the expected problems: …………………………………………………………………………………………………………..

(Ex. Mice are challenged with malaria and will develop severe malaria anemia (SMA) condition. All animals will be observed closely and animals that meet the early endpoint criteria will be humanely euthanized.)

Protocol Writing

Pain and Distress assessment

2) What criteria(s) will be used to assess pain, distress, or discomfort?

Check all that apply:

☐ Inactivity  ☐ Loss of appetite  ☐ Restlessness

☐ Loss of weight (   ) 5% (   ) 10% (   ) 15% (   ) 20% weight loss

☐ Abnormal resting postures, somnolence or hunched posture

☐ Licking, biting, scratching, or shaking a particular area

☐ Failure to show normal patterns of inquisitiveness

☐ Guarding (protecting the painful area)

☐ Loss of mobility  ☐ Red stain around the eyes of animals

☐ Unresponsiveness  ☐ Torpor

☐ Self-mutilation  ☐ Labored breathing

☐ Other, please specify……………………………………

Protocol Writing

Pain and Distress Recognition:

Attempts to recognize pain and distress in animals rely on observing a combination of behavioral and physiological variables, and looking for deviations from normally:

Signs of Acute Pain

• Decreased appetite, anorexia

• Restlessness

• Porphyrin discharge (Red stain around the eyes of rats)

• Increased respiration

• Vocalization

• Licking, biting, scratching, or shaking a particular area

• Biting or shaking the affected body part.

Who will observe the animals? How often? Criteria?

Protocol Writing

Signs of Chronic Pain

• Loss of weight 5% 10% 15% 20% weight loss

• Reluctance to move

• Failure to groom, causing and unkempt appearance

• Abnormal resting postures, somnolence or hunched posture

• Change in fecal and urine activity

• Change in behavior

Who will observe the animals? How often? Criteria?

Protocol Writing

10.3.4 Literature Search for Alternative

to procedure that cause pain & distress

• Literature Source(s) Search: Ex: BIOSIS, MEDLINE, PubMed

• Date of Search: Perform no earlier than six months prior to the IACUC meeting.

• Period of Search: a. BIOSIS: 1926 to present

   b. MEDLINE: 1998 to 2013

   c. PubMed: 1950 to present

• Key Words of Search: hit, animals species used, agent type.

• Result of Search:………………………………………………
Protocol Writing

Literature search for Alternative

Result of Search

Provide a narrative description of the results of the literature search. Show that "There are no applicable non-animal alternative".

IACUC ensure that the PI has considered alternatives when proposing painful or distressing procedures.

Protocol Writing

Alternative

Alternatives are always considered

There are no in vitro or computer modeling alternatives for studying immune responses to and protection against dengue viruses. Animals with an intact immune system are required.

Literature review showed that no alternatives was available to replace the in vivo testing of XXX in mice malaria model.

The effects of XX on Physiologic parameter cannot be evaluated in vitro. Currently, there are no in vitro methods that can provide accurate estimates of physiologic effects of XX candidates for efficacy evaluation.

Protocol Writing

10.4 Anesthesia

☐ Yes  ☐ No

If yes, please answer the following questions:

1) Pre-anesthetic preparation:  
2) Type of anesthesia used:  
3) Dose:  
4) Route of administration:  
5) Frequency of anesthesia:  
6) Length of anesthesia:  
7) Who is responsible for maintaining anesthesia?:  

Protocol Writing

Anesthesia (con’t)

8) If inhalation anesthetics are used, describe the system for scavenging waste anesthetics gas.  

9) What criteria(s) will be used to assess level of anesthesia? Check all that apply:

☐ Respiration rate  ☐ Body temperature  ☐ Heart rate  
☐ EKG  ☐ Toe pinch  ☐ Tail pinch  
☐ Corneal reflex  ☐ Pedal reflex  ☐ Muscular relaxation  
☐ Color of mucous membrane  
☐ Other (pulse oximeter, respirometer) please list

10) How animals are kept warm?  

Protocol Writing

10.5 Analgesics and/or tranquilizers:

☐ Yes  ☐ No

If yes, please specify

1) Type of analgesics used  
2) Dose  
3) Route of administration  

10.6 Describe post-anesthetic treatment or intervention:  

Protocol Writing

Surgery

Survival surgery: the animal regains consciousness after anesthesia:

- aseptic techniques
- properly prepare the incision site
- clip the hair and disinfect the skin

Non-survival surgery: the animal is euthanized while under anesthesia and does not regain consciousness:

- minimum the surgeon should wear gloves
- clip the surgical site
- instruments and work area should be cleaned
Protocol Writing

Surgery

Major surgery:
- Surgery that penetrates and exposes a body cavity, such as the chest or abdomen after anesthesia.
- Surgery that produces substantial physical or physiological impairment.

Minor surgery: the less invasive surgery.

Multiple major survival surgeries:
- Scientific justification
- Conservation justification
- Medical justification

Facilities for Aseptic Surgery

Major survival operative procedures on non-rodent species:
- Conducted only in dedicated facilities intended for that purpose and under aseptic conditions.
- Separate areas for surgical support, animal preparation, surgeon preparation, operating room, and animal recovery.

Non-major operative procedures and all rodents surgery:
- Do not require a dedicated facility but must be using aseptic technique.

Protocol Writing

11. Surgery

[ ] Yes [ ] No

If yes, please answer the following:

11.1 Surgical procedure is:
1) [ ] Non-survival [ ] Survival
2) [ ] Major [ ] Minor
3) [ ] One time [ ] Multiple

11.2 Location: Give the location/room number for the proposed surgical procedure.

11.3 Surgeon/qualification: Indicate who will perform the surgery, and his/her qualifications, training, or experience in the proposed procedure.

11.4 Procedure: Describe in detail the surgical procedure.

11.5 Pre- and post-operative provision:
- Detail the provision for both pre- and post-operative care, including provisions for post-surgical observation.
- Describe long-term care of chronic survival procedure.

11.6 Post-operative procedures:
- Post operative care
  - By whom? How frequency?
  - Weekends, holidays, after hours care and monitoring
  - How will pain and distress be evaluated?
  - Analgesic name, dose, frequency, route

Ex. The anesthetized mice are observed until they completely recover from anesthesia and resume normal activity. Care will be provided during anesthesia and recovery to maintain body temperature by using either a water-circulating heating pad. Animals will be placed on lateral recumbency. They will be monitored for stable respiration and they will be turned on the alternate side every 3 to 5 minutes.
Animal Manipulations

12. Blood or body fluid withdrawal/tissue collection/injections, tail clip, gavaging: (Describe in detail method(s), needle sizes, volume(s) collected or administered, and frequency of collection or injections)

<table>
<thead>
<tr>
<th>Anatomic location</th>
<th>Needle size</th>
<th>Volume collected (ml)</th>
<th>Volume administered (ml)</th>
<th>Frequency (times per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood withdrawal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Fluid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue collection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tail clip</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gavaging</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total blood volume ........ ml in total .......... study days or ........ months

Table: Site of injection, maximum normally accepted volume and needle size

<table>
<thead>
<tr>
<th>Species</th>
<th>Site of injection</th>
<th>Maximum normally accepted volume (ml)</th>
<th>Needle size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse (20 g.)</td>
<td>Scruff, back</td>
<td>5 ml</td>
<td>21G</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intramuscular</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intraperitoneal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intravenous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat (250-300 g.)</td>
<td>Scruff, back</td>
<td>5 ml</td>
<td>23G</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intramuscular</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intraperitoneal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intravenous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea pig (300-400 g.)</td>
<td>Scruff, back</td>
<td>5 ml</td>
<td>21G</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intramuscular</td>
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<td></td>
<td>Intraperitoneal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intravenous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit (2-3.5 kg.)</td>
<td>Scruff, flank</td>
<td>10 ml</td>
<td>21G</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intramuscular</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intraperitoneal</td>
<td></td>
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</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Practicable volumes of blood to take from various animals.

<table>
<thead>
<tr>
<th>Blood volume (ml)</th>
<th>MOUSE</th>
<th>RAT</th>
<th>GUINEA PIG</th>
<th>RABBIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total blood</td>
<td>80</td>
<td>50</td>
<td>75</td>
<td>70</td>
</tr>
<tr>
<td>Volume (ml/kg.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Available volume</td>
<td>25</td>
<td>20</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>When bled out (ml/kg.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum safe</td>
<td>7</td>
<td>5</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Volume at one bleeding (ml/kg.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Protocol Writing

13. Restraint with mechanical devices:

☐ Yes ☐ No

If yes, describe device, duration of restraint, frequency of observation, conditioning procedures and steps to assure comfort and well-being.

If prolonged restraint is used, must provide justification.

- Restraint devices are not normal methods.
- Restraint devices should not be used for convenience.
- Period of restraint should be minimum.
- Training the animals
- Provide veterinary care

Animal Manipulations

Nonsurgical methods

Injections, administration

- List all types of injection, administration and the procedure
- Provide detail on needle and syringe size, routes, frequency and volumes.

Samples collection

- All blood, urine, faces, tissue etc sampling from living animals should be annotated.
- For blood, list the volume to be taken, the frequency and how.

Physical Restraint

Physical Restraint: usually brief periods

Prolong restraint:
- should be avoided unless it is essential for achieving research objectives
- Should be suitable in size, design, and operation in minimize discomfort, pain, distress and the potential injury.

Procedures:
- Animal can be trained through the use of positive reinforcement techniques.

Guidelines:

- Animals that do not adapt to necessary restraint systems should be removed from the study.
14. Project involving food and water deprivation, or dietary manipulation:

- Yes
- No

If yes, describe methodology. State objective criteria used to assess physical condition and pain, discomfort, stress, and distress during the course of study. Include clinical signs or manifestations expected from the procedure. What criteria will be used to determine a humane endpoint before severe morbidity and death?

- Individual animal’s weight is monitored every ……… days.
- Individual animal’s weight is not monitored.

<table>
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</tr>
<tr>
<td>- Water restriction is defined as water deprivation for longer than 12 hours.</td>
</tr>
<tr>
<td>- Food restriction is defined as food deprivation for longer than 24 hours for simple stomach animals, or longer than 48 hours for ruminants.</td>
</tr>
<tr>
<td>- Restriction for research purposes needs to be scientifically justified and a program established to monitor physiologic or behavioral parameters, including criteria for removal of the animal from the experiment (such as weight loss or hydration state).</td>
</tr>
<tr>
<td>- Precautions that should be used in cases of fluid restriction to avoid dehydration include daily recording of fluid intake and recording of body weight at least three times per week - or more often for smaller animals, such as rodents.</td>
</tr>
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15. Tumor and disease models, toxicity testing:

- Yes
- No

If yes, describe methodology used for tumor/disease and/or toxicity testing. State objective criteria used to assess physical condition and pain, discomfort, stress, and distress during the course of study. Including clinical signs or manifestations expected from the procedure. What criteria will be used to determine a humane endpoint before severe morbidity and death?

- The visible size of the tumor is only one of the criteria used for determination of humane endpoint.
- The site of tumor implantation should be chosen to minimize damage to adjacent normal structures.
- Sites involving the special senses should be avoided.

16. Behavioral studies

- Yes
- No

If yes, describe in detail types of behavioral manipulations, including placement in testing chambers or apparatus, use of aversive stimuli, duration of test periods, and frequency of test periods.

- Behavioral sciences:
  - neural (information sciences): deals with information processing of stimuli from the social environment by cognitive entities, to engage in decision making
  - social (relational sciences): deals with relationships, interaction, communication networks, associations and relational strategies or dynamics among organisms or cognitive entities in a social system

17. Euthanasia / Disposition of animals

17.1 Disposal of animals after completion of activity:

- Euthanatized
- Return to production/breeding unit/facility inventory
- Transfer to another research project:
  - Protocol No. ………… and investigator
  - ……………………………………
- Other (Please describe)
  - ……………………………………

17.2 Euthanasia method

- Anesthetic overdose, please list
  - Drugs used for euthanasia …………………
  - Dose……………………………………
  - Route of administration …………………
- Cervical dislocation Decapitation
- CO₂ Chamber
- Other (Please describe)
  - ……………………………………
Protocol Writing

Necropsy: If animals are to be necropsy:
[ ] Yes  [ ] No

- Location: ____________________________
- Who will do it, and what is their experience in the technique to be used? ____________________________
- Personnel protective equipment (PPE) ____________________________

Animal tissue and carcasses disposal: Please describe method used to dispose animal tissue and carcasses.

Protocol Writing

18. Study endpoint: (State the project study endpoint for the animals. Indicate whether recovery, euthanasia, or death is/are expected; specific plan for determining when the animal experimentation phase will be stopped).

(when the scientific aims and objectives have been reached: should be humane and scientifically sound)

Early endpoint is used (the animals are humanely euthanized prior to the expected terminate study day):

[ ] Yes  [ ] No

Early endpoint criteria used are ____________________________

Humane Endpoint Criteria

The criteria used to intervene in research studies to prevent unnecessary pain and distress for early removal from study...

- A limit on weight loss 20-25 %
- Extend anorexia over 3 days
- Sudden pain or distress that cannot be controlled with analgesics, sedatives or tranquilizers
- Severe medical conditions that cannot be controlled with appropriate therapy
- Maximum tumor volumes or tumor weight

Death or moribundity as an endpoint is used.

[ ] Yes  [ ] No

Animal Study Proposals that include moribundity as an endpoint or that includes animal procedures that have the potential to cause adverse sequellae should address the following:

- Criteria that establish when the endpoint has been reached.

- A plan for monitoring the animals both before and after a change in any of the above aspects, providing care if appropriate, and increasing the level of monitoring must be described.

- Identification of personnel responsible for evaluation, record keeping, notification of the investigator and/or veterinarian and persons responsible for euthanasia must be described.

Protocol Writing

19. Biohazard/safety:

- Infectious agent(s) is/are used: specify ____________________________
- Biohazardous chemical or carcinogen or radioactive material is/are used specify ____________________________
- Recombination agent(s) is/are used: specify ____________________________
- None

19.1 Provide a list of any potential biohazards associated with this protocol. Specify biosafety level. □ ABSL 1  □ ABSL 2  □ ABSL 3  □ ABSL 4

19.2 Explain any safety precaution or program designed to protect personnel from biohazard and any surveillance procedure in place to monitor potential exposure. ____________________________

19.3 Explain how the waste is decontaminated and disposed. ____________________________

19.4 List primary safety equipment and personnel protective equipment requirements.

19.5 List procedures if any accident, injury or illness occurs.

19.6 List specific treatment provision for accidental exposure.

19.7 List relevant occupational medical health provision.
20. Qualification of personnel:
List all individuals who will be involved in this protocol. If personnel do not have experience in working with animals, state how they will be trained.

<table>
<thead>
<tr>
<th>Name</th>
<th>Responsibilities</th>
<th>Description of relevant experience or training</th>
</tr>
</thead>
</table>

IACUC Ensure that personnel are qualified to perform the proposed procedures on animals

Personnel
Show adequate record of experience and training the personnel have in the procedures

Personnel Training
The use of animals must be covered under a protocol or an SOP

Training
➢ To understand the potential hazards.
   • From animals
   • The use of specific agents
➢ Safety practices to minimize the risk of exposure.
➢ The Available Health Care Service.