Writing an Animal Protocol

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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.

What ?????

- FTM
- Animal
- Care and Use Committee

Protocol Review

It is up to the IACUC to decide what type of review system will work best for their institute

* Full Committee Review
* Designated Committee Member Review
* Pre-review or multilevel review
* Mixing

The PI may present an interactive, oral presentation of the protocol.

Protocol Review

Benefit MUST outweigh cost
* Human health
* Animal health
* Advancement of knowledge
* Scientific gain
* Good of society
* Animal use
* Pain
* Distress
* Discomfort
* Species

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
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 Approval

- Protocol approval before any use of animals begins
- Animal protocol – Follow standard form:
  “FTM Animal Care and Use Protocol”

Protocol Review

- 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 Topics

- Non-technical summary
- Rationale and literature review
- Literature search for duplication & alternative
- Objective(s)
- Experimental design
- Data analysis and statistical method
- Animal model and species justification
- Animal care
- Animal welfare
- Surgery
  - Blood or body fluid withdrawal/tissue collection/injections, tail clip, gavaging

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.***

Non-technical summary

- Brief narrative description
- Describe simply the reason for the study, which animals will be used, and why.
- State what will be done with animals: Provide brief description of the experimental design
- Tie scope of work into possible human or animal health benefit
Protocol Writing
Non-technical summary
Example:
This protocol describes a procedure in which ICR outbred mice are used as a source of tissue fluid for chigger (larval) mites in order to develop into the next stage of its life cycle to maintain our chigger mite colonies. The chigger colonies are required for a variety of studies on the transmission of Orientia tsutsugamushi, the causative agent of scrub typhus.

Protocol Writing
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
Literature search for Duplication
To be performed to prevent unnecessary duplication of previous experiments.
- Literature Source(s) Search: Generally performed by Librarian
- Date of Search: Perform no earlier than six months prior to the IACUC meeting.
- Period of Search: a. BIOSIS: 1926 to present
  b. BRD: 1998 to 2013
  c. PubMed: 1950 to present
- Key Words of Search: hit, animals species used, agent type.
- Result of Search:………………………………………………

Protocol Writing
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

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 description of 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 experiment design of sequential studies
- Flow charts, time lines and tables are very useful

With Humane Standards

Protocol Writing

Data Analysis

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

Protocol Writing

Data Analysis

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

Protocol Writing

Data Analysis

Animals: Description of animals

Fill in table:
- Common name
- Use scientific name “Genus and Species
- Strain/Stock
- Age, application for some species like rodents
- Weigh, especially application to primate, large rodents and rabbits
- Sex, Male or female may break out the number for each
- Number, remember to calculate total of animals
- Source
- Special consideration
Protocol Writing

Justification for the Use of Animals

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

• Describe the characteristic of the animal that make appropriate for the study.
• Good representative of the human disease; physiological and/or morphological.
• Disease – cause, currently therapy, how proposed animal experiments might better
• Explain the medical terms
• There is a wide body of knowledge regarding the model.
• Used in the previous protocol and need to complete.

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 the number of animals

Determine the right number of animals for obtaining valid results

• Justify group size or experimental repetitions:
  - match exactly those described in experimental design.
  - use statistical analysis
  - 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.

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 (MPTP-monkey and 6-OHDA rat), rat experiments are the more humane.

Protocol Writing

Justification for the number of animals

Example: We used the JMP statistical package to perform power analysis to arrive at the proposed group size. Using parameters of alpha = 0.05; power = 80%; effect size = 50%; and standard deviation = 30% of the mean, we arrived at a group size of 7 for a comparison of two groups. A group size of 10 was chosen because it provides an 80% chance of observing a difference of 50% in the histologic score at a level of significance of 0.05
The formula for the sample size required at each time point is:

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Justification for the number of animals
Determine the right number of animals for obtaining valid results

Example: The statistical analysis we use to measure significance are Students t-test and repeated measures. Sample size for each study is carefully monitored to assure no excess studies are performed. Using an alpha probability of 0.05; a power of 0.8 and an effect size of 1.2 X SD, the sample size to detect a statistical difference would be 15

Protocol Writing
Animal Care Consideration
Determine whether the living conditions are appropriate for the animals

Routine housing and care conditions.
- study room
- cage flooring
- floor space
- group housing
- cage changing
- environment
- feeding
- watering

Specialized living conditions
- care for surgically altered animals
- special light cycle
- special diets
- using metabolic cage

Protocol Writing
Animal Care Consideration
Determine whether the living conditions are appropriate for the animals

Examples of non-standard...
- High/Low salt diet; high/low fat diet, etc
- Drugs / Compounds (Sprite, alcohol) added to drinking water
- Single housing, metabolic caging

For each non-standard situation...
- Include description of what is added
- Provide reason
- Identify an individual responsible for overseeing the process
- Develop lab book
- Label appropriately

Protocol Writing
Veterinary Care Provision

- 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
Consideration Animal Alternative
"The 3 Rs"
Replacement, Reduction and Refinement.

Replacement with non-animal techniques
- organ, tissue or cell culture > incubators for cell lines
- immunologic bench assays > bioassays involving animals
- computer simulations > model the pharmacokinetics
- invitro techniques > growing monoclonal antibodies
- Use of membrane feeding

Protocol Writing
The “3 Rs"
Replacement, Reduction and Refinement.

Replacement
- Careful consideration of necessity
- 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

Reduction
- Preliminary screening in non-animal systems
- Well trained staff and good animal care
Protocol Writing
The “3 Rs”
Replacement, Reduction and Refinement.
Refinement: changing experiments or procedures to reduce pain or distress
- Pilot experiment
- Adjust techniques
- Skilled technicians
- Training
- Anesthesia/Analgesia
- Close observation
- Early endpoint

Protocol Writing
Literature Search for Alternative
• Literature Source(s) Search: Generally performed by Librarian
  - Date of Search: Perform no earlier than six months prior to the IACUC meeting.
  - Period of Search:
    a. BIOSIS: 1926 to present
    b. BRD: 1998 to 2013
    c. PubMed: 1950 to present
• Key Words of Search: hit, animals species used, agent type.
• Result of Search: .................................................................

Protocol Writing
Result of Search
Provide a narrative description of the results of the literature search.
Show that “There are no applicable non-animal alternative”.
It is up to the IACUC to see that adequate information is provided.

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 XXX
The effects of XX on………. Cannot be evaluated in vitro. Currently, there are no in vitro methods that can provide accurate estimates of XX for……

Pain and Distress
Stressors
(Environement)
ADAPTATION STRESS
FAILURE
DISTRESS
Pain and Distress assessment
No pain: routine procedures e.g. injection, deep palpation, blood collection from vein, observation studies of animal behavior, tissue collection after euthanasia
Alleviated pain: appropriate anesthetics or analgesics will be administered to avoid or alleviate pain e.g. surgery, tattooing, small tumor removal
Unalleviated pain: animals are subjected to painful procedures without the use of anesthetics, analgesics, or tranquilizers e.g. lethal dose studies, pain studies
Protocol Writing

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

Anesthesia and Analgesia

- Pre-anesthetic fasting
- Duration of anesthesia
- Agents, dose, routes, and site
- Indicate needle size
- Methods of monitoring and care during anesthetic recovery
- Analgesic (pre-emptive and post procedure provision)

Who will be administering the analgesics, anesthetics during the study?

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
- instruments and work area should be cleaned

Major surgery: - Surgery that penetrates and expose 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
Protocol Writing

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

Post-Operative Procedures

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

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

Substance Administration Volumes Considered Good Practice

Route and Volumes (ml/Kg or *ml/site)

<table>
<thead>
<tr>
<th>Species</th>
<th>Oral</th>
<th>SC</th>
<th>IP</th>
<th>IM</th>
<th>IV</th>
<th>IV Slow</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP</td>
<td>Max</td>
<td>GP</td>
<td>Max</td>
<td>GP</td>
<td>Max</td>
<td>Max</td>
</tr>
<tr>
<td>Mouse</td>
<td>10</td>
<td>50</td>
<td>10</td>
<td>40</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>Rat</td>
<td>10</td>
<td>40</td>
<td>5</td>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Rabbit</td>
<td>10</td>
<td>15</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>20</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>78</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>

GP= Good practice dosages, Max= Maximum dose values
Protocol Writing

Prolong Restraint

Prolong restraint should be avoided unless it is essential for achieving research objectives

- 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

Restrictions of Food and Water

- Water restriction is defined as water deprivation for longer than 12 hours.
- Food restriction is defined as food deprivation for longer than 24 hours for simple stomach animals, or longer than 48 hours for ruminants.
- 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).
- 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.

Restrictions of Food and Water

- The reason for restricting food or water
- The period and frequency of food or water deprivation
- The procedure for monitoring the animals
- In prolong deprivation, be included
- Physiological or behavioral parameters use in monitoring the animals.
- Criteria for temporary or permanent removal of an animal from the study.
- Body weight should be recorded at least weekly

Euthanasia and Disposition of Animals

Gentle or Easy death

To kill an animal with the minimum of physical and mental suffering
- painless
- rapid and complete
- peaceful
  - safe for the operator, observer
  - simple to perform

Provide both a Primary Method and a Secondary (adjunct) Method to ensure death of the animal

2013 Report of the AVMA Panel on Euthanasia

Euthanasia and Disposition of Animals

AVMA Euthanasia Guidelines

- Acceptable methods
  - Barbiturate overdose
  - CO₂ cylinder
- Conditionally Acceptable
  - requires IACUC approval of scientific justification
  - cervical dislocation, decapitation
- Unacceptable methods
  - Chloral hydrate, chloroform, Cyanide

Euthanasia and Disposition of Animals

- State the method of euthanasia (according to SOP).
- Giving needle size and syringe size range, if applicable.
- State how death will be ensure when euthanizing rodents with CO₂.
- State who will perform the euthanasia DVM personnel, PI and staff (by name)

AVMA Panel on Euthanasia
**Protocol Writing**

**Experimental and Humane Endpoint**

Experimental Endpoint: when the scientific aims and objectives have been reached. Should be humane and scientifically sound.

Humane Endpoint: the point at which pain or distress in an experimental animal is prevented, terminated, or relieved. Should be defined when possible before the start of the study. Determination of the humane endpoints should involve the PI, the veterinarian and the IACUC.

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**Humane Endpoint Criteria**
The criteria used to intervene in research studies to prevent unnecessary pain and distress.

- 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.

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**BIOHAZARD/SAFETY**

- Potential occupational health and safety issues?
- Any hazardous agents?
- How they will be handled?

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**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.

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**Personnel Qualification and Training**

<table>
<thead>
<tr>
<th>Name</th>
<th>Qualification and Training</th>
<th>Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>BSc, Trained... on...</td>
<td>Animal handling, IV Injections, Euthanasia.</td>
</tr>
<tr>
<td>B</td>
<td>PhD, Trained... on...</td>
<td>Catheter Placement, Splenectomy.</td>
</tr>
</tbody>
</table>

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