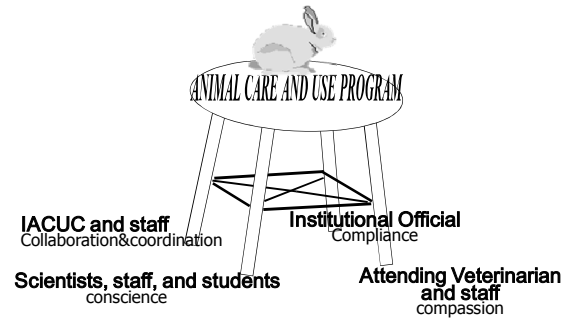


Writing an Animal Protocol

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Team Members Responsibilities



What IACUC

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

คณะกรรมการดูแล
การเลี้ยงและใช้สัตว์ทดลอง

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.

Protocol Review Consideration

Benefit **MUST** outweigh cost

- | | | |
|----------------------------|---|---------------------|
| * Human health ↑ | ↓ | * Animal use |
| * Animal health | | * Pain |
| * Advancement of knowledge | | * Distress |
| * Scientific gain | | * Number of animals |
| * Good of society | | * 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 number :		This section will be filled by the IACUC.
Received by IACUC (dd/mm/yy)		
Approved/Request Modification (dd/mm/yy)		
Resubmitted (dd/mm/yy)		
Approved/Disapproved by IACUC (dd/mm/yy)		
Approved/Disapproved by IO (dd/mm/yy)		
Expiration Date (dd/mm/yy)		

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.....

Protocol Writing

Cover Sheet

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 Cover Sheet

Principal investigator: Name.....
 Position: Department

Faculty/Institute

Tel. Fax.....

E-mail

* Animal use license no.....Expired date.....

Contact Person in Case of Emergency:

Office/Affiliation:

Phone:

E-mail:

* Animal use license no.....Expired date.....

Protocol Writing

Statistical Review: Name

_____ (Signature) _____ (Date)

Safety Review: Name

_____ (Signature) _____ (Date)

Attending Veterinarian: Name

* Animal use license no.....Expired date.....

** Veterinary practitioner license no.....Expired date.....

_____ (Signature) _____ (Date)


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

1. Non-technical summary

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

*** use common language and define all abbreviation which animal will be used and easily understood by non-scientists.***

- 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: 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 total of 61 articles were found using the above search terms. Several articles focused on novel vaccine development and one compared viremia induced by different strains of dengue-2. None of the articles looked expressly at dengue-2 and dengue-3 virus in the nonhuman primate model. Therefore, this is not a duplication of any previous research

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?

- Provide a complete description of what will be done to the animals. Succinctly outline the formal scientific plan and direction for experimentation, sequential description of procedures what will be done to the animals from obtain the animal to the end of study. A diagram or chart may be helpful to explain complex design

Protocol Writing

Experimental design/Materials and Methods

What will happen to the animals?

- Complete description of the proposed used 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 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

Experimental design/Materials and Methods

- Conclude with the study endpoint and the total number of animals per species required for this particular experiment.
- The universal study endpoint is always "euthanasia when moribund or survival to X days following exposure".
- Death as an endpoint is not acceptable.

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.

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

7. Animal model and species justification 7.1 Description of animals

Fill in table:

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

- 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

Protocol Writing

7. Animal model and species justification 7.1 Description of animals

- Weigh, especially application to primate, large rodents and rabbits
- Sex. Male or female-may break out the number for each
- Number of animals: - Required by species
 - The no. stated here must correspond exactly to that described in Experimental design.
- Special consideration: List specialized requirements for the animals
- Source/Vendor: -Provide a sources for the animals.
 - Animals will be legally obtained from suppliers.

Protocol Writing

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

Protocol Writing

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

7.2.1 Animal model and Species justification Justification of the selected species

- Do not use the statement "Rodents are used because they are less sentient or lower on the phylogenetic scale than NHPs..." This is not a justification.
- Look for how sensitive this model is to the agent in question and how closely it resembles the human condition of the disease.

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

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

Animal Care Consideration

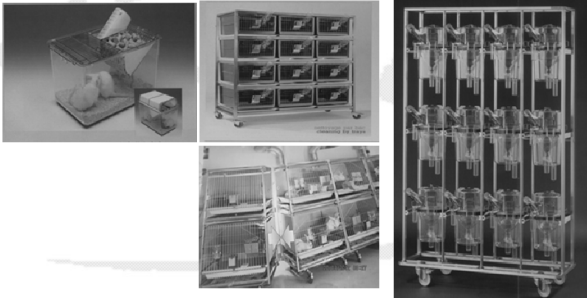
- 8.3 Caging:
- Solid bottom, open top
 - Suspended cages, wire bottom
 - Individual ventilated cage (IVC)
 - Other, please specify
 - Static filtered top cages
 - Metabolic cages
- 8.4 Cage size: W x L x H, (inch).....
- 8.5 Caging materials
- Plastic
 - Stainless steel
 - Other, please specify

8.6 Number of animals per cage.....

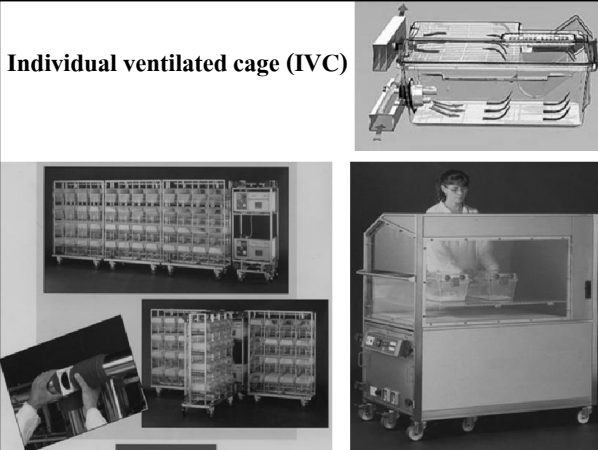
HOUSING

Caging type:

- Shoe box cages
- Open cage
- Filter top cage
- Hanging cage
- Metabolic cage



Individual ventilated cage (IVC)



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

Protocol Writing

Animal Care Consideration

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

Protocol Writing

Animal Care Consideration

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, everyday(s)

Protocol Writing

Animal Care Consideration

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? *(This is allowed for conducting experiment (s) only not for housing. In addition, the holding period must be less than 12 hours.)*
 No Yes

If **yes**, please provide information below:

- Where the experiment is expected to be conducted? Please provide the building name and room number.

- Please provide the animal experimental procedures in detail.

- Estimated total time period that live animals will be kept in the laboratory is.....hours
- How will the animal sample or carcass be disposed?

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

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

10. Animal welfare

10.1 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 to be examined. Therefore it is necessary to perform these studies with (this agent) in an animal model.

Protocol Writing

Reduction

minimum group size as needed to obtain statistically significant data.

- 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

Keep the balance

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

Protocol Writing

Refinement

changing experiments or procedures to reduce pain or distress

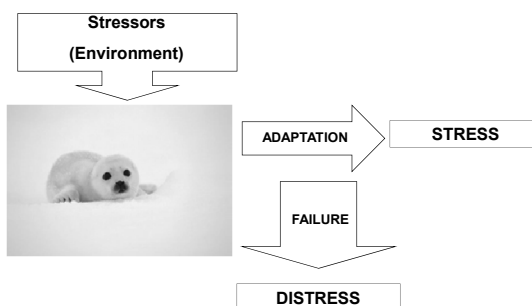
- Pilot studies
- Adjust techniques
- Skilled technicians/ Well trained personnel
- Environment enrichment
- Anesthesia/Analgesia
- Close observation/ Monitor clinical signs & Pathology
- Early endpoint

Protocol Writing

Refinement: *changing experiments or procedures to reduce pain or distress*

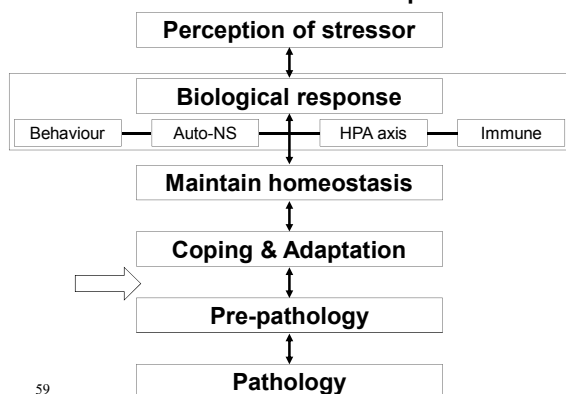
Ex. Animal handling and techniques will be performed by experienced animal technicians and result in minimal discomfort. Mice are conditioned for 3 to 5 days before the beginning of study and they are trained to be familiar with manual handling. Mice are observed twice daily or more often during the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

Pain and Distress



58

Model of Stress Response



59

Protocol Writing

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?

 2) Who will monitor the clinical condition of the animals?

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

Protocol Writing

Pain and Distress assessment

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
- Failure to groom, causing and unkempt appearance
- 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 normality

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

(year of citations b. MEDLINE : 1998 to 2013

cover by database) 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
 ECG 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 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

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

Protocol Writing

Surgery

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.

11.6 Describe long-term care of chronic survival procedure.

Protocol Writing

Surgery

11.7 Multiple survival surgery procedures: Multiple major operative procedures on the same animal must be adequately justified for scientific reasons by the principal investigator in writing.

11.7.1 Procedure:

11.7.2 Scientific justification:

11.7.3 Who will be the responsible for post-surgical care and treatment?

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

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)

	Anatomic location	Needle size/ catheter size and length	Biopsy size	Volume collected (ml)	Volume administered (ml)	Frequency (times per day)
Blood withdrawal
Body Fluid withdrawal
Tissue collection
Injection/ infusion
Tail clip
Gavaging
Other

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

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.

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

Species	Oral	Subcutaneous	Intramuscular	Intraperitoneal	Intravenous
Mouse (20 g.)	0.5 ml	Scruff, 0.5 ml/site 25 G	Quadriceps/posterior thigh, 0.01 ml/site, 25G	1.0 ml, 25G	Lateral tail vein, 0.2 ml, 25G
Rat (200-250g.)	2.0 ml	Scruff, back, 0.5ml/site <23G	Quadriceps/posterior thigh, 0.1 ml/site, <23G	5.0 ml, <23G	Lateral tail vein, Sublingual vein, penile vein
Guinea pig (300-400g.)	5.0 ml	Scruff, back, 1.0 ml/site <21G	Quadriceps/posterior thigh, 0.1 ml/site, <21G	10-15 ml, <21 G	Ear vein, saphenous vein, dorsal penile vein, 0.5 ml, <23G
Rabbit (2-2.5 kg.)	7.5 ml	Scruff, flank, 1.5 ml/site <21G	Quadriceps/posterior thigh, lumbar muscles, 0.2ml/site<21G	20ml, <21G	Marginal ear vein, 10 ml, (slowly), <21G

Practicable volumes of blood to take from various animals.

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

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

Protocol Writing

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.

Protocol Writing

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.

	Amount restricted/added	Duration	Compound supplemented	Compound deleted	Frequency
Food restriction
Fluid restriction
Nutrient alterations

Protocol Writing

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.

Protocol Writing

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.
- * For subcutaneous tumors the maximum allowable size is 20 mm in diameter for a mouse or 40 mm diameter for a rat.
- * The site of tumor implantation should be chosen to minimize damage to adjacent normal structures.
- * Sites involving the special senses should be avoided.

Protocol Writing

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) :deal 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

Protocol Writing

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)

Protocol Writing

17. Euthanasia / Disposition of animals

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):
(the point at which pain or distress in an experimental animals is prevented, terminated, or relieved).

Yes No
 Early endpoint criteria used are

Protocol Writing

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

Protocol Writing

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.

Protocol Writing

19. Biohazard/safety:
 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

Name	Responsibilities	Description of relevant experience or training

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

Personnel

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

Include **EVERYONE** involved in the research.



1. How they were trained?
2. Who trained them.
3. How much experience they have had performing

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.

