Contents

IACUC Forms

Use of animal cell lines and tissues

The form "Application to use animal tissue" has been modified to streamline the processing of using commercial or established cell lines.

The modification is in response to the frequently asked question and feedback on the need to submit an application for the use of animal cell lines and tissues.

Obviously, if the animal cell lines and tissues are used on (administered to) animals, such as cancer cell lines to induce tumours in animals, or if animals are specifically sacrificed to obtain the required cells and tissues, an application using the form "Application to use animals for research" has to be submitted for IACUC review.

In the case where animal cell lines and tissues are solely used for *in vitro* work, without the PI having to specifically sacrifice animals to obtain the cells or tissues, an application using the "Application to use animal tissue" form has to be submitted to IACUC to document such use. Although their use is not regulated by the Animals and Birds Act which defines animal as 'any live vertebrates', the tissue form is necessary to address concerns that the use may involve ethical, occupational health and safety issues, e.g. tissues carrying pathogens, tissues obtained from another protocol, etc.

However, the concerns are ameliorated when commercial or established cell lines are used, and the project has the risk assessment approved by OSHE.
(Note: submission of a risk assessment for all research projects is required by OSHE).

Modifications to form :
The tissue form is thus modified to include 2 parts; part 1 for documentation of the use of commercial and established cell lines in *in vitro* work ONLY, and part 2 for documentation of use of other animal tissues.

Part 1 does not require IACUC review and approval. It is only for documentation purposes.

Part 2 requires IACUC review and approval as per normal.

What to do:

For the use of commercial or established animal cell lines in *in vitro* work ONLY :
Complete General Information and **Part 1** of the tissue form for documentation if you are using

1. **Commercial Cell lines** purchased from well established dealers/vendors (e.g. ATCC).
2. **Established cell lines** supplied by collaborators from AAALAC accredited laboratories or other well established research institutes / laboratories. Correspondence (email) indicating the agreement of your collaborator to provide the cell lines should be submitted as supporting document

For the use of other animal tissues
Complete General Information and **Part 2** of the tissue form for IAICUC review and approval if you are using primary cells, tissues, blood, fluids and cadavers.

Back to Contents

IACUC / Protocol Matters

Annual protocol review / renewal of protocol

By Dr Balaraman Deivakumar, IACUC office

After granting an approval, the IACUC is required under the law to "conduct continual reviews of activities involving the use of any animal for scientific purpose, including any approved project of long duration and any long-term continual use of any individual animal for such purpose, at appropriate intervals but at least once a year".

This explains the reminders for an annual review submission to principal investigators (PIs) a month before the anniversary of the protocol approval date.

In fact, depending on how the Federal and State regulations are interpreted, many IACUCs in the US require PIs to submit an Annual Protocol Review for protocol renewal before they can continue to use the animals.

If we adopt such a practice, about half of the projects due for annual reviews each month would have been suspended temporarily. Why is this?

One month before the due date (i.e., 1st, 2nd or 3rd year of protocol approval), a first reminder to submit the Annual Protocol Review (APR) form will be sent to the PI. Reminders will be sent monthly until the APR form is received. The following are the average percentages of ARP submission after each reminder:

1st reminder – 56%
2nd reminder - 24%
3rd reminder - 14%
Final reminder - 6%

The statistics indicate that 44% of the protocols were not 'renewed' on time and animal work should have been suspended. It may be further interpreted that 44% of the PIs are not taking the annual review of protocol seriously. This assumption is supported by the following common discrepancies / inaccuracies in the information provided in the ARP form.

	Information provided by PI in the APF form	**Records from IACUC office**
1.	no change in the protocol	amendments were made to the approved protocol.
2.	yet to start the animal work; no animal work or no *in vivo* work was done in the past year	animals have been purchased from CARE .
3.	project already completed	final report not submitted (soon after completion) until IACUC office sends reminder of annual review.
4.	protocol does not involve breeding of animals	protocol involves breeding of animals
5	APR of an expired protocol submitted	no amendment submitted for extension of the project.

IACUC seeks PIs' understanding and co-operation in this matter. If issues of non-cooperation and non-compliance continue, IACUC would have to impose work suspension.

We would like PIs to note the following:

1. The 1st reminder will now be sent 2 months ahead of the due date.

2. Under the NACLAR Guidelines on Responsibilities of Investigators (chapter 8), a copy of which is included in the course materials in RCULA training, "investigator must inform the IACUC in writing when each Project is completed or discontinued; and the outcome of the Project".

APR forms can be used as a final report to IACUC, but it has to be submitted soon after the project is completed or discontinued.

Back to Contents

Compliance audit on animal use –how did we fare?

By Dr Mark Vinson Vallarta, IACUC office

The IACUC audit team began unannounced visits to the laboratories in Feb 07. Generally, IACUC is satisfied with the outcome of the audits so far but non-compliance issues were detected in some.

2006 Compliance Audit Statistics

Total number of compliance audits conducted	10
Total number of protocols audited	47
Total number of laboratories (of 11 departments) visited	16

Some deficiencies/deviations (from the approved protocols) detected :

1. Unavailability and/or inaccuracy of records of the following:
   - breeding
   - daily health monitoring
   - post operative monitoring
2. Inappropriate method of euthanasia
3. Lack of proficiency in handling animals
4. Discrepancies in the protocol (which necessitates the submission of an amendment application to IACUC)
5. Undeclared housing location
6. Personnel at work who were not listed in approved protocol
7. Change in endpoints
8. Inappropriate or inadequate personal protective equipment
9. Inadequate animal care and post-operative care; infrequent monitoring
10. Inappropriate methods used in the transportation of animals
11. Inadequate housekeeping
12. Inappropriate method of animal identification
13. Continued use of expired drugs
14. Humane endpoints – personnel do not know when early euthanasia should be performed for animals experiencing unrelieved pain and distress.
15. Copies of the approved protocol are not available
16. Completion of project un-reported

IACUC would like to remind PIs of the seriousness of non-compliance in animal protocols. It may result in project suspension and closure of the laboratory. In fact, two PIs had been censured for serious non-compliance or persistence in non-compliance. They have subsequently rectified the situation.

To minimise inadvertent non-compliance issues, IACUC would like to advise that team members should be familiar with the approved protocol and copies should be made available and accessible to facilitate reference.

Back to Contents

Protocol processing time

We are pleased to advise that IACUC office has managed to maintain the processing time of approved protocols to an average of 4 to 6 weeks.

We will continue to ensure that this continues to be on track.

IACUC alternates

To address the problem of a meeting quorum, IACUC has at its meeting on 3 Oct 06, proposed alternates for members in case they are unable to attend a scheduled monthly meeting.

The introduction of alternate members has enabled us to meet quorum and hold meetings as scheduled, thereby avoiding delays in approving animal protocols due to cancellation of non-quorate meetings.

Back to Contents

Animal Care Matters

Seeing **red** – Animal disease detection

(Source: Dr Leslie Retnam, LAC)

Sentinel animals in LAC's housing facilities are regularly sent for serology multiplex tests for the presence of a number of pathogens. When the sentinel serology indicates animals are positive for any of the pathogens, at least one animal from each cage of the positive racks in the affected animal rooms will be tested by LAC for follow-up serology. Principal investigators (PIs) will be notified of the follow-up serology results when they become available.

In the case where follow-up serology results are positive, you (users of the animal facilities) should be aware of the following:

1. The rooms are now considered "dirty" and will be marked with a **red** health status sheet on the door. The positive rack(s) will also be marked. Work must be conducted by working from "clean" to "dirty" animals. When working in the "dirty" rooms, animals on the positive rack must be handled last.

2. Animals in the positive cages identified on follow-up serology may be euthanized within the animal facility. These animals are **not to be moved out** of their rooms.

3. You should contact the Lab Officers of the facilities **before euthanizing *any* animals** housed on positive racks in the rooms. LAC will euthanize these animals for you at no charge.

4. If you have animals in the affected room(s) which are **immuno-compromised**, you should contact the LAC veterinarian (Dr Leslie Retnam via ahurl@nus.edu.sg or tel ext 3051) immediately.

5. If you have moved **ANY** animals from these rooms into another room, onto another rack, to another investigator, and/or facility, you should immediately contact the LAC veterinarian (Dr Leslie Retnam's contact details earlier given) as these animals pose a possible health threat to the animals of other investigators within the facility. Moved animals must be tested.

6. Individuals with dirty or suspect (yellow) rooms are expected to restrict animal-related activities to their animal room, as use of shared procedure rooms can advance pathogen spread within the facility.

7. Special or additional measures and precautions may be required when accessing the affected rooms. The protective mechanism will differ depending on the disease and the mode of transmission. For instance, if the affected rooms are positive for Tyzzer's, GDVII and Parvo, the precautions are :

- When entering a 'dirty' room, individuals must don an **additional** pair of gloves over their regular barrier apparel.
- When leaving the 'dirty' room, individuals must remove the additional gloves and dispose of them as they leaving the 'dirty' room, in the 'dirty' room. **Do not** wear these gloves into the hallway, as they may be contaminated.

You should consult the LAC veterinarian on the special or additional measures and precautions to be taken when your rooms are affected.

Back to Contents

Animal Use Matters

Alternatives to retro-orbital blood collection

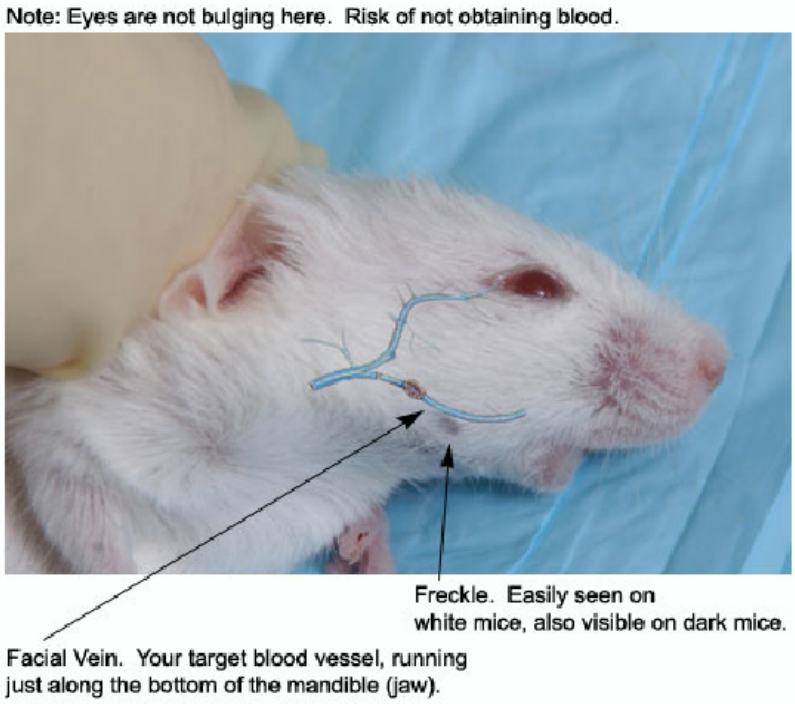
(Source: Dr Leslie Retnam, LAC)

We continue to receive protocols describing retro-orbital route as the method of routine/serial blood sample collection. It is a technique that can have severe consequences for the animals and, therefore, the IACUC does not recommend retro-orbital bleeding for non-terminal procedures, other than exceptional and justifiable circumstances, under which the procedure must be performed with anaesthesia and by competent persons and, repeated sampling from the same site (same side of the eye) should not be performed at intervals shorter than 4 weeks. It is, however, acceptable as a terminal procedure under anaesthesia.

Retro-orbital method can easily be replaced by the following techniques:

1. Facial vein (limited to adult mice):

This is a relatively new technique and repeated sampling is possible by using alternate sides of the face in the area of the mandible. The sample may be a mixture of venous and arterial blood. The method is said to require less training than tail, retro-orbital sampling to reliably withdraw a reasonable quantity of blood. It may be performed on conscious animals that are properly restrained to allow proper site alignment and venous compression for good blood flow (see Figure below). A minimal amount of equipment is required and can be performed relatively rapidly. 20G or smaller size needles should be used to prevent excessive bleeding.



More information on this technique may be found at the web site:
http://www.ahc.umn.edu/rar/facial_vein.html

For video clips, see the following commercial video at Medipoint:
(Windows Media) http://www.medipoint.com/html/directions_for_use1.html
(Quicktime) http://www.medipoint.com/html/directions_for_use2.html

2. Saphenous vein:

A technique for obtaining blood from the saphenous vein of mice and other small animals has been described and is rapidly becoming the technique of choice for many investigators.

This procedure does not require that the mouse be anesthetized to collect a blood sample, and is much less invasive than retro-orbital method. A tube can be used to restrain the mouse, and the hind leg is extended by applying gentle downward pressure just above the knee. The hair over the tarsal area is shaved with clippers followed by a number 11 scalpel blade, and the vein is pricked with a needle (25 gauge is usually adequate). Blood can be collected in a microcapillary tube.

Smearing a small amount of silicone grease over the area to be punctured helps to prevent the blood from coming into contact with the skin and minimizes blood clotting.

When the blood has been collected, gentle pressure applied with a piece of gauze should be used to effect hemostasis. Pictures demonstrating this technique may be found at the web site: http://www.uib.no/vivariet/mou_blood/Blood_coll_mice_.html

Back to Contents

An update on the use of Buprenorphine

(Source: from the "Vet Regulatory News" June 2007).

In Aug 06, AVA received notification from MOH that Buprenorphine or B has been classified as a Class A Controlled Drug under the First Schedule of the Misuse of Drugs Act w.e.f. 14 Aug 06. The sole supplier, Schering-Plough can no longer import B which effectively bans the use of B for vet use.

MOH received an appeal from a vet for MOH to allow the use of B as an analgesic for veterinary practice. On 16 Mar 07, AVA sought feedback from practicing vets on MOH's position and requested vets to indicate if they are required to use B in their course of work. AVA has sent the consolidated feedback and appeal for MOH to re-consider permitting vets to use B as an effective analgesia for alleviation of pain in animals.

In May 2007, MOH replied to indicate that AVA should discuss the matter with the Central Narcotics Bureau (CNB) as the use of B falls under the Misuse of Drugs Act.

The AVA is currently in talks with CNB and would update the veterinary community accordingly.

Back to Contents

The 3-staR PIs

By Dr Balaraman Deivakumar, IACUC office

It is gratifying that some researchers are taking the 3Rs principles of animal care and use seriously. They have **R**eplaced animals, **R**educed animal numbers or **R**efined procedures to animals, during the course of their project.

The IACUC is pleased to list these researchers below in appreciation of their efforts :

Name of the PI/ Department	Replacement / Reduction / Refinement
Prof Ariff Bongso O & G	**Refinement**: Use of finer gauge hypodermic needles for administration of substances.
Dr Steve Cheung Nam Sang Biochemistry	**Refinement:** Euthanasia by CO_2 instead of cervical dislocation.
Prof Chou Loke Ming DBS	**Reduction:** Animal sampling indicated small size animals were not suitable. The experimental group for small size animals was eliminated.
Dr Davide Lomanto Surgery	**Replacement:** Live animals were replaced with virtual surgical simulators. Multiple procedures performed on deeply anaesthetized animal before euthanasia, and tissue sharing with other researchers/specialists.
Prof Ding Jeak Ling DBS	**Replacement:** Use of cell lines instead of live animals to test bioactive peptides.
A/P Feng Si-Shen ChBE	**Refinement**: Improved injection rate of infusion.
Dr Gan Yunn Hwen Biochemistry	**Refinement:** Instead of infecting the animals, macrophages are isolated and infected; thereby eliminating potential pain and distress due to infection.
A/P Go Mei Lin Pharmacy	**Replacement:** Use of cell lines instead of live animals to evaluate the neuroprotective effects of synthesized compounds on cells challenged with the neurotoxic agent.
Prof Barry Halliwell Biochemistry	**Reduction**: Reduce the number of doses in experiment.
A/P M Prakash Hande Physiology	**Replacement:** Collect mouse fibroblasts from others (tissue sharing) instead of procuring animals.
Prof R Manjunatha Kini DBS	**Refinement:** Euthanasia by CO_2 instead of cervical dislocation.
Dr Koh Hwee Ling Pharmacy	**Refinement:** Purchased rabbit blood from LAC, hence minimizing pain and distress to the animals and allowing more experiments to be carried out.
Prof Li Fong Yau Sam Chemistry	**Reduction:** Reduced number of animals using laser induced fluorescence system.
A/P Eugene K W Sim Surgery	**Reduction:** Conduct pilot study to establish group sample size.
A/P Wilder-Smith E P Medicine	**Refinement**: Refined procedure to reduce number of tests.
Dr Zhu Yi-Zhun Pharmacology	**Refinement**: Rats are kept warm in between the water maze tests.

Back to Contents

Occupational & Environmental Health & Safety

Making the tree shrew facility a safe and healthier place (for personnel and animals)

By Mr G Saravanan, OSHE.

A room at AHU has been renovated for animal work involving tree shrews. OSHE conducted a walkthrough inspection before it was commissioned, and the following changes were made by AHU staff to ensure a healthy work environment. .

Improved ventilation

The fans (Fig.1) were configured to give a negative pressure potential in the room, as with any other BSL-2 facility.

Mosquito netting was also installed to prevent mosquito vectors from entering and spreading any potential pathogens that may be present in the animals.

Figure 1. Fans with mosquito nets

Anteroom for changing

The initial design did not have an area demarcated for changing into the appropriate PPE for animal work. An anteroom outside the main lab was designated as a changing area. This provides the demarcation of clean and dirty areas. A fire extinguisher (Fig. 2) was also installed in this area.

Figure 2. Anteroom

An acrylic sheet (Fig. 3) placed outside the main door was intended to deflect rain. This had sharp edges and posed a physical hazard to anyone entering the room. Because of this, the sharp edges were sanded away.

Figure 3. Acrylic sheet at entrance

Lighting (Fig.4) was improved in the lab through the use of twin fluorescent tubes, alleviating eye strain and fatigue resulting from long hours in the facility.

Figure 4. Fluorescent lighting in lab

These improvements will aid in upholding safety & health standards in NUS.

What is a tree shrew? Check it out in Americazoo.com

Back to Contents

Training

Revamped RCULA training

(Source: LAC)

The RCULA course has been revamped to address participants' specific training needs and for their convenience.

1. Participants can choose to be trained in the species of interest.

2. Re-training of participants from NUS is free of charge.

3. Training will be conducted monthly, on the 3rd Thursday of the month.

D A Y O N E

General Lecture Programme

9.00 am to 12.15 pm @ CELS Auditorium

9:00 am	Registration and Introduction
9:30 am	The IACUC - Responsibilities, Functions and Guidelines on completing the IACUC application forms
10:00 am	Biomedical Research Regulations, NACLAR Guidelines and You
10:30 am	Q and A
10:45 am	Coffee Break
11:15 am	The 3R's, Ethical Responsibilities of a Researcher and the Use of Alternatives
11:45 am	Occupational Health and Safety in Biomedical Research
12:15 pm	Q and A

Rats / Mice : Species specific training including lectures which apply

2.00 pm to 6.00 pm @ AHU

Species specific training on Rats, on Mice, and on Aseptic Surgery

Includes lectures (specific to rats and mice) with information:

(i) Anaesthesia, Analgesia, Recognising Pain,

(ii) Animal Handling & Blood Collection

(iii) Intro to Surgery, Post-op Care and Euthanasia and

(iv) Animal Disease and Diagnosis and Hands-on practical (existing format using video)

D A Y T W O

Rabbits / GPigs & Pigs : Species specific training including lectures which apply

9.00am to 1.00 pm @ AHU

Species specific training on Rabbits and on Guinea pigs (lectures and hands-on)

2.00 pm to 6.00 pm @ AHU

Species specific training on Pigs (lectures only).

HOW IT WORKS

Participants will be trained in individual species.

If a participant would like training in multiple species, eg, mice, rats and rabbits, he/she would need to be present for Day One morning lectures, Day One afternoon for hands-on (for rats and mice) and then attend Day Two morning (for rabbits).

COSTS

For NUS staff and students:

Lectures	$50
Each species specific session	$50 (includes re-training sessions)

For non-NUS staff and students:

Lectures	$80 plus GST
Each species specific session	$80 plus GST

If a participant wanted training only in mice, he/she would pay $50 for lectures plus $50 for mice session.

The charge for species specific sessions includes costs for re-training should a researcher request or require that.

After attending species specific training on rats and/or mice, if researchers need to use rabbits in future, they are required to attend the next course on species specific training on rabbits. A separate charge of $50 is applicable.

Back to Contents

Newsworthy Articles

1. RIP

A special funeral and mourning ceremony was held on 28 April - for mice. Who did that and why? Read more.

It is noted that some places in the US have similar ceremonies or ways to remember the important contributions that animals make to ensure we have longer, healthier lives.

2. Use of animals for sales demonstration

Early this year, a neurosurgeon at the Cleveland Clinic (ranked one of the top 3 hospitals in the US by US News and World Report 2006) caused a furore when it was reported that he induced an aneurysm (a localized widening of an artery, vein, or the heart) in an anaesthetized dog. This was a demonstration of a medical device to sales people. The animal was subsequently euthanized.

A report by The Scientist on the investigation of the incident indicated that such animal use would unlikely be acceptable to an institution's IACUC. The report has provoked many interesting comments from its readers.

Back to Contents

IACUC Office: c/o Department of Biological Sciences, 14 Science Drive 4, Singapore 117543.
Tel: 6516 2861. Fax: 6778 6216.

IACUC Website: http://www.nus.edu.sg/iacuc/index.shtml


IACUC application forms


Application submission deadlines


IACUC meeting schedule


RCULA training course