

Manual of Operations

Husain Lab

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I. New Lab Members

A. Intro into the University of Pittsburgh

Before you are able to complete any research at the University of Pittsburgh, you are required to:

- a. Set up an HSConnect account, which can be done at www.hsconnect.pitt.edu
Create a log-in using either your pitt or chp email address.
- b. Complete the following modules which can be found in HSConnect, located under “Applications” (click on [go] next to “Internet-based studies in Education and Research”). Search for the following modules under “All Modules.”
 - a. Blood borne Pathogen Training (Formerly RPF Module 9)
 - b. Research Integrity (Formerly RPF Module 1)
 - c. Conflict of Interest (Formerly RPF Module 4)
- c. Take a TB test and give the results to your HR representative

B. Intro into UPMC

- a. Within 90 days of hire you will need to complete the following clearances:
 - i. Pennsylvania Child Abuse Clearance History
 - ii. Pennsylvania State Criminal History Record
 - iii. Federal criminal background check (FBI)
 - iv. National Sex Offender Registry check
- b. Your HR representative will give you more information on these clearances

II. DLAR

A. Access to the DLAR requires:

- a. Completion of the following modules in HSConnect
 - i. Use of Laboratory Animals in Research and Education
 - ii. Small Animals Research and Training
 - iii. Additional modules may need to be completed depending on which animal model you are going to use
 - iv. New modules may be added; confirm at <http://www.iacuc.pitt.edu/training>
- b. You must be added to the protocol. This is done by going to the IACUC website and clicking on “Change of research personnel.” Forms are located here: <http://www.iacuc.pitt.edu/forms>

- c. The University of Pittsburgh Animal Exposure Surveillance Program has a Health Questionnaire that must be filled out as well. The form can be found at <http://www.ehs.pitt.edu/assets/docs/AESPenroll.pdf>. If you have additional enrollment questions please contact Dr. Yolanda Lang of Employee Health Services at 412-647-3407. Once you have completed the Animal Exposure Surveillance Program Health Questionnaire, email or fax the completed form to Dr. Lang at langyc@upmc.edu or 412-647-1993.
 - d. A copy of your most recent TB test must be given to the DLAR. TB tests need to be updated every 6 months. It is best to give a copy to both your HR rep and the DLAR.
 - e. A tour of the DLAR must be scheduled. To schedule a tour of the facility please contact the DLAR Facility Supervisor, Lacey Mock, at lam159@pitt.edu or at 412-692-9496
- B. Reserving a room for procedures can be done from the DLAR homepage (only if you have access to the DLAR). Click “room scheduler” on the side of the page: www.dlar.pitt.edu
- C. Reserving the Anastasia Unit can be done by sending an email to either of the vet techs, Kara Kracinousky at kbk15@pitt.edu or Jillian Jeffers jsj19@pitt.edu
- D. Ordering:
- a. All ordering of animals for research can be done at the DLAR website: www.dlar.pitt.edu. This website is linked to your HSConnect account. For troubleshooting, call the help desk at the University of Pittsburgh at 412-624-4357
 - b. On the left hand side of the website, click on “Animal Ordering (Non-NHP)”
 - c. Cost tips: Charles River and Jackson Laboratory seem to have the best prices and Charles River gives a 20% discount to University Pittsburgh employees
 - d. Try to order as many animals as possible in one shipment as shipping is expensive
 - e. After the animal is delivered, it should acclimate for two days before any procedures are done. The animal can, however, be sacrificed on the day of delivery.
 - f. A good reference on how the strains of mice are written can be found [here](#)
- E. Breeding strategies
- a. Ask for the breeder feed by marking the red card with special instructions. Breeder feed comes at no charge and does not have to be added to the protocol. Breeder feed is slightly higher in fat and is also an alfalfa free diet.

- b. Ask the tech to autoclave some of the crinkled paper. The mice like the change in the texture.
 - c. Huts help; either the plastic or the cardboard will work.
- F. Animal care
- a. For any questions about care, please ask any of the DLAR staff
 - b. For any questions about health, please ask the vet techs
 - c. Cuts, wounds, etc. should be addressed with the vet techs
 - d. If you notice the mice or rats are developing bald spots, this is called barring (over grooming). To end this behavior, place a hut in the cage or another sensory object.
 - e. Rats have a set number of sensory items in their cages. One rat should have three objects, while two rats should have two objects each, etc.
 - f. It is discouraged to house animals alone as they are social creatures. If you must house them separately, they should be housed with two sensory objects in their box and a sticker on the cage signifying the reason for solitary confinement.
 - g. Weaning of new pup must be done 21 to 28 days after birth. There is a sheet on the back of the door that has to be filled out stating that the mice were weaned, fed, and have water. You must also write down the number of mice weaned and number of new boxes added. A “Recently Weaned” card should also be added to the box.
 - h. Always check to see that the box you are taking a mouse from has the least amount of mice possible in it. If you can finish a box, please do so.
 - i. If you do empty a box, please mark it down. There is a clipboard on the rack where clean cages are kept in which you can write how many boxes were used or added.
 - j. Pink slips are Health Care Reports. These are warnings, not fines, but should not be accrued. Examples of warnings include: a double litter, a death, overcrowding in a box, or deteriorating animal health.
- G. Jackson Labs
- a. Mouse Colony Software-tool to help keep track of your colony
 - i. <http://research.jax.org/data/index.html> (you will need someone from IT to assist with the download)
 - ii. If you need to add a new strain, you must get in contact with the Jackson Lab to have it installed

III. Lab meeting

- a. Lab meeting are held every Monday at 8:30am in room 7109
- b. Each lab member is to put together a presentation of their work from the previous week and goals for the upcoming week

- c. PowerPoint presentations are preferred, but verbal presentations are acceptable
- d. All PowerPoint presentations are to be saved into the lab R-Drive

IV. Resources

- a. IT Help Desk: 412-692-9000 (know the device ID number found on either the top right hand corner of the desktop or the computer tower before calling)
 - i. Gary Kopcho – IT for Rangos: gary.kopcho@chp.edu 412-692-5600
- b. Histology Core Department - Basement, B209 CSB
 - i. Lori Schmitt – Manager of Core: Lori.Schmitt@chp.edu
 - ii. If you need any histology work done, make sure you have the correct form filled out
- c. Building Manager 3rd Fl. Rangos:
 - i. Shawn Ward: shawn.ward@chp.edu 412-692-6360
 - ii. Call if you need lab equipment moved or fixed
 - iii. Shawn also has a storage space of old equipment if you are looking for a replacement or need to borrow equipment
- d. Admin for Diane Cline, 3rd Fl. Rangos:
 - i. Linda Cherok: linda.cherok@chp.edu 412-692-6438
 - ii. Email Linda for laptop and/or projector reservations
 - iii. If you would like a locker, please contact Linda and give her a few possible locker numbers
- e. Core Imaging Contact - 8th Fl. Rangos: Dr. Kimmi Tobito: kit3@pitt.edu. 724-816-0463
- f. Bioluminescence Imaging Software Contact, 6th Fl Bay 17: Carl Egman: Carl.Engman@chp.edu
- g. Admin. 7th Fl. Rangos: Coleen McGill: Coleen.McGill@chp.edu 412-692-5412
 - i. Email Coleen to reserve a room
- h. Pitt HR 1st Fl. AOB: Jeanne Johnson: johnsonj@upmc.edu
- i. Employee Health - 1st Fl. AOB (TB tests can be given here for UPMC employees; if you are a Pitt Employee, you will have to go to the UPMC Health Building in Oakland)
- j. Clinical Director of the DLAR: Dr. Joseph Newsome: jnewsome@pitt.edu 412-648-8950 (Pitt), 412-864-7558 (Hillman), 412-692-9499 (Rangos)

V. Experiments

- a. Experimental write-ups
 - i. All experiments are to be written after they are conducted
 - ii. All of the experimental write ups should be saved in the R-Drive in personal folders
 - iii. A copy of the write up should also be handed to Dr. Husain
- b. Saving data
 - i. All data must be saved by the year_month_day_initials and summary of experiment. Example: 2013_09_aio_CnA beta mice gene typing
- c. Protocols
 - i. Protocols should be saved in your personal folder in the R drive

VI. Surgery

- a. If you are going to be conducting surgery, please read the following:
 - i. AALAS Learning Library – Animal Care and Use in Research and Education
<https://www.aalaslearninglibrary.org/demo/course2.asp?strKeyID=2B340697-3402-4476-9EC5-182627579679-0&Library=10&Track=8&Series=1243&Course=2451&Lesson=26659>
 - ii. Techniques in Aseptic Rodents Surgery
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2587003/>
 - iii. Principles of Rodent Surgery for the New Surgeon
<http://www.jove.com/video/2586/principles-of-rodent-surgery-for-the-new-surgeon>

VII. Journal club

- a. Journal Club is on Thursday mornings at 9 am on the 6th Floor Rangos, Conference Room 6109
- b. Journal Club is a time when we can come together as a lab to keep up to date with current research. Every week, one person will present one article in detail. We will rotate through lab members; a calendar should already be set up.
- c. The presenter should send out their chosen paper a few days before Journal Club. The PMC number should also be recorded.

VIII. EH&S

- a. Hazardous Waste Disposal
 - i. Hazardous waste is put in a vault every other Tuesday in the basement of Rangos
 - ii. Green Hazardous Waste stickers should be filled out for all material that is being stored until it is disposed
 - iii. The Disposal Form needs to be faxed 3 days prior to the date of disposal
- b. Eye Wash
 - i. The Eye Wash stations needs to be tested every week for 15 minutes
 - ii. Upon completion of testing, the form should be filled out and saved for inspections
- c. EH&S has mandatory training sessions that are held in Oakland (102 Benedum Hall) at 9 am. You need to have a Pitt ID to attend. It covers the following topics:
 - i. Bloodborne Pathogens
 - ii. Chemical Hygiene/Formaldehyde
 - iii. Dangerous Goods Shipping
- d. Yearly Inspections are conducted and should be properly prepared for

IX. Purchasing

- a. If you are going to be purchasing anything for the lab, you are required to certain courses. For scheduling contact Cathy Lewis at clewis@cfo.pitt.edu
- b. Panther Buy is used for ordering from the selected Pitt-approved vendors
- c. Prisim is used for ordering from the selected Pitt-approved vendors
- d. P-Cards are lab credit cards used for purchases that are not from the approved Pitt vendors. They are primarily used for conferences, travel, etc.

X. Best practices

- a. All reagents must be dated and initialed
- b. Before a reagent run out, please write its name on the white board
- c. Never continuously thaw and refreeze reagents such as TLCS or Cerulean as they will breakdown, making them less potent
- d. Always ask before using someone's personal items, including tools and solutions. This avoids contamination, as well as having things misplaced
- e. Be careful when using Excel templates, as problems may result when copying and pasting formulas

XI. Animal Protocols

- a. Adding Modifications can be found at the ICAUC website under the forms tab: <http://www.iacuc.pitt.edu/forms>
- b. Adding Lab Members to the protocol can be done by going to the ICAUC website. A Pitt ID number is required:
<http://www.iacuc.pitt.edu/protocol/a>
- c. If you have any questions when doing a Modification please refer to the “*Guide for the Care and Use of Laboratory Animals (Guide)*” NRC 2011, as a basis for evaluation. This will go over in detail what ICAUC is looking for and help with the overall process.

XII. Tips for manuscript writing

- a. Set a strict deadline for putting together parts of your paper. Put together the key references and keep in mind their value in your work, which will help you design your paper. As the first author, it is your responsibility to put together a first coherent draft.
- b. To facilitate the paper, I would suggest that you put together an outline in PowerPoint or Word according to the following suggestions. I put this together partly based on Dr. Rustgi’s video that I recommend you go through as well (<http://www-archive.infomedica.com/dvd/AGA/index.html>).
- c. Use subheadings as much as possible throughout your outline. They are like support beams that you can take off at the end if the journal doesn’t allow them.
- d. First and foremost, think of the bigger picture:
 - i. What is the main point of your work? This should be reflected in your title
 - ii. What are the key findings? (list them out and refer to them at the end of the intro, as well as the beginning and end of the discussion).

- iii. Think about the story line and your pitch. What are the strengths of your findings and what are their limitations? (note them in the Discussion section).
- e. After you organize your thoughts, don't work in a bubble. Use your team and/or oversight committee to cross-check the progress of your manuscript work as you put it together. If you hired a contractor, you wouldn't want him to build you a home only to realize that it had fatal flaws that could have been rectified or at least recognized early on.
- f. Figures
 - i. Figures should be as self-explanatory as possible
 - ii. Tell a strong story with each picture (or figure or table)
- g. The paper title is usually written in the present tense. It is best to draft it in the active voice and be as precise as possible; it must be accurate, but here you have a little bit of editorial liberty
- h. Introduction
 - i. State the subject matter and the problem upfront
 - ii. Background (literature review); what are the key points that are necessary for others to know about what lead you to doing this project?; what have others shown previously that will help build the story of what you are doing in this paper?
 - iii. State the hypothesis or, at least, the aims of the study
 - iv. Give a brief synopsis of the results, emphasizing what is novel and what the implications of the study are
- i. Materials and methods
 - i. Start with a statement about reagents if most come from a common vender. Otherwise, state the origin of reagents as you discuss them
 - ii. Take the reader step by step through your methods
 - iii. End with how you did the statistical analysis
- j. Results
 - i. The results should be a narration of the figures. Tell a logical story of the relevant facts and why you felt it was important to perform a particular experiment in a particular order. It's like a

good bedtime story: in a story book, you will often start with “Once upon a time, in a faraway land, there were three princesses...” which creates interest. Similarly, the Results section of a manuscript could read, “To optimize micro-MRI imaging of the pancreas, we compared several protocols first in whole-fixed mice...” which creates interest. What are those protocols? I feel like I really want to read more about the work.

- ii. Describe the key points of each figure in turn, and provide a logical context from which to move from one figure to the next. Here is your chance to explain something in more detail or to provide the impact of a finding. For example, “There was three-fold increase in resolution using T1-weight imaging (Fig. x).” Or “There was an 80% reduction in histological severity relative to the negative control.” Statistics should be mentioned somewhere at least once (either in the figure legend or the results).
- iii. Figure legends and results should complement one another.

k. Discussion

- i. Provide a synopsis of the key findings and emphasize what is novel.
- ii. I prefer following this up with short paragraphs on each of the key findings, with the following info: (if there was only one key finding, then just one longer paragraph will suffice)
 - 1. How does the literature compare to your key finding?
 - 2. What new insight does the key finding now add to the body of literature?
 - 3. What are the strengths and limitations of the findings?
 - 4. How might you follow up? [Careful about this part, since reviewers may ask you to do the next step]
- iii. Bring the key findings to an overriding set of conclusions.
 - 1. What new model or confirmation of an existing paradigm does your overall work provide?
 - 2. What might be the future impact and directions of your work?

iv. Conclude with the key summary points or findings.

l. References

i. Limit to 30 to 50

ii. Original publications are ideal, although sometimes a good review is appropriate

iii. Seminal or key publications

m. Abstract

i. I usually put this together last and use the key points as the basis, but some researchers prefer to write this first as a way to organize thoughts