Anseth Lab Orientation Checklist

In order for the lab to operate in the most efficient and safe manner possible, it is imperative that all lab members are familiar with lab policies and procedures. This checklist offers a description of essential guidelines for working in the Anseth Group.

Safety

Lab Attire – Safety glasses, long pants and closed-toed shoes must be worn at all times in the chemistry areas. Safety glasses, long pants and closed-toed shoes must be worn while actively experimenting in biology areas.

Lab Etiquette – Absolutely no food or drink including chewing gum is allowed in chemistry areas. Food or drink may only be consumed at your personal desk space in the biology areas. In the desk space there should be no experimental equipment including chemical reagents, gloves and lab coats.

Undergraduate Researchers – Undergraduate researchers are welcome to use lab and desk space during normal business hours. Undergraduates should not be working in laboratories after hours without supervision. Mentors should be present when undergraduates are actively experimenting in the lab or should designate a contact person for the times when the mentor is not available.

Material Safety Data Sheets (MSDS) – Detailed information about the reactivity, dangers, and handling of each chemical in the lab is located in alphabetical order in the MSDS Notebooks, which can be found on the shelves across from the ordering computer in A351. If you bring a new chemical into the lab, you must add the appropriate MSDS sheet to the notebook, if one is not already present. **Please read MSDS sheets to determine which chemicals, if any, can be used openly on your bench in the biology area.**

Showers and eyewash stations – 3 showers and 3 eyewash stations are located in the lab. Be sure to familiarize yourself with their locations (A351, A391, A390).

Fire extinguishers – 3 fire extinguishers are located in the lab. Be sure to familiarize yourself with their locations (A351, A390, hallway outside A370).

Fire Alarms – The nearest fire alarm is located outside of the lab in the hallway across by the stairs.

First Aid Kits – There are 3 first aid kits located in A351, A391 and A390. If you have a minor cut, please use these kits. If the injury is more severe (more than a band-aid), seek medical attention at the nearest medical facility and report the incident to Dr. Anseth, IBC and EH&S.

Spill Kits – Whenever a volatile solvent is spilled, the area should be cleared and the spill should be soaked up with vermiculite (brown flaky stuff) stored in large cardboard box in A390A and in spill kits located throughout the lab. Once solvent is soaked up by vermiculite, the solvent-containing vermiculite should be put in a sealable container, labeled properly and sent away as solid waste.

Emergency Action Plan (EAP) – Refer to the established EAP. A quick reference is posted outside in the hallway.

Contact Numbers – Important phone numbers are posted on the lab doors.

EH&S Training – All members of the lab must complete the EH&S safety training prior to working in the lab. Contact EH&S to schedule training (http://www.colorado.edu/ehs/training/index.html). Training must be updated yearly.

Biosafety Training - All lab members that do cell culture or use the tissue culture room must complete biosafety training (http://www.colorado.edu/ehs/training/biosafety.html). The following modules must be completed by all members: biosafety training, bloodborne pathogen training, biosafety cabinet training, and shipping biological materials training. If you do any type of cloning or work with plasmids/vectors or recombinatnt DNA, you must also complete the recombinant DNA training module. Training must be updated yearly. Contact the Bioside Coordinator for more details.

Tissue Culture Training – All lab members that use the tissue culture lab must be trained by the tissue culture coordinators before using the tissue culture room.

Lab Supplies

Glassware – There are several cabinets of assorted glassware located in A390. The user should clean dirty glassware. At the minimum, all glassware must be rinsed with acetone and washed with soap and water. After washing with soap and water follow the instructions for more thorough cleaning using the base and acid baths.

General–use disposable supplies – Stocks of tips, tubes, pipettes, gloves, and other disposable lab supplies are maintained for general use. These can generally be found on the shelves and in drawers around the lab.

Overstock boxes – most of these lab supplies have full cases that serve as overstock and are located on the top shelves above their lab storage location. When breaking into an overstock box, **unpack the whole box** so that it will be clear to the person that is doing the ordering that the overstock has been used.

Ordering general-use disposable supplies – In order to avoid duplication of orders, these general lab supplies are maintained by only one person in the lab. If you notice that one of these stocks is getting low, let the orderer know. This is particularly true if you break into the last overstock box of an item.

Chemicals – Chemicals are stored in several locations, depending on their storage conditions and usage:

A390 Chemicals Cabinet – General-use chemicals, stored in alphabetical order by name. Typically only solid chemicals are stored here.

A390 Chemicals Refrigerator – General-use chemicals that require storage at $4^{o}C$, stored in alphabetical order by name.

A390 and A351 Flammables Cabinets – General-use flammable liquids (organic solvents, alcohols, etc.)

A390 Acid Cabinets – General-use acids are stored in the cabinet.

A390 Base Cabinet – General-use bases are stored in the cabinet.

A390 PEGs – General-use PEGs are located on the shelves next to the general chemicals cabinet.

Equipment reagents – Specific chemicals are required for the operation of certain instruments in the lab. Often, these stocks are kept in the area where they are used. Do not use these stocks for other purposes unless cleared by the person who is responsible for maintaining the instrument.

Personal chemicals – Each researcher in the lab may have reagents that they have ordered specifically for their project. These chemicals are generally stored at that person's bench or in their designated storage areas in the lab refrigerators and freezers. Before using these personal chemicals stocks, it is imperative that permission is granted from the lab member to whom it belongs.

Ordering chemicals: If you empty a general-use chemical it is your responsibility to make sure that a replacement has been ordered. If it has not been ordered (or is not already in stock), put the chemical in the ordering log. If you notice that a general-use chemical is getting low, please put it in the ordering log so that it will arrive before the old one runs out.

Waste Disposal

Glass Containers – Anything that has the potential of being sharp, but is not a syringe, needle, or knife should be disposed of in the glass waste boxes. These regular sharps include non-biologically contaminated:

1. *pipette tips*
2. *plastic pipettes*
3. *glass pipettes*
4. *broken glass*
5. *microscope slides and coverslips*
6. *Eppendorf tubes*
7. *Conical tubes*

Note: Pipette tips can be collected in a secondary puncture resistant container. When full please seal the container closed and label as non-hazardous waste.

If a glass box is full, use the cardboard flap to close the opening of the lid and secure the lid onto the box with tape. Secured boxes are to be thrown away in a dumpster.

Sharps Waste Containers –The following items are NOT considered "regular" sharps and must be disposed of by placing them in specific, puncture-proof waste containers (large red plastic containers labeled "Regulated Medical Waste"):

1. *Needles*
2. *Syringes*
3. *Razor blades*
4. *Scalpels*
5. *Scalpel blades*

Syringes are considered "sharps" because of the public opinion that they are biohazards. Therefore, they cannot be sent to the landfills and must be handled as hazardous waste.

Sharps that are used for biological applications must also be autoclaved. Biohazardous sharps containers are located under the hoods in each tissue culture room.

Paper/Cardboard – Bins are located around the lab for recyclable paper.

Regular trash – Anything that is neither recyclable nor potentially hazardous (package wrapping, tip containers, non-contaminated paper towels etc.).

Hazardous Waste - Hazardous waste is unwanted or discarded hazardous materials that may harm the health or well-being of people or the environment. The basic waste types are:

Biohazardous
Chemical
Radioactive

Whenever possible keep these types of waste separate from each other. Disposal of mixtures of these is difficult and expensive. Also, to help minimize hazardous waste

generation – be sure to keep your hazardous wastes separated from normal nonhazardous wastes as much as possible.

Biohazardous waste – Biohazardous waste is biological, infectious, and some noninfectious waste. Biological waste includes cultures, plates, media, and other materials that contain or come in contact with living cells, body fluids, viruses, clinical materials, and other microorganisms. All materials involved in cell culture (bacterial or mammalian) such as media bottles, pipette tips used for tissue culture, agar plates, etc. are considered biohazardous waste. Basically, if there is a chance that something will be able to grow on it, it needs to be rendered not biohazardous via autoclaving so it must be disposed of in biohazardous waste containers.

Syringes, needles, glass pipettes and pipette tips – All go in the red biohazardous sharps containers labeled "Regulated Medical Waste".

Media bottles, plastic pipettes, plastic plates – All go in the containers lined with red biohazard bags.

If your material/waste has a biohazardous component, please contact Bioside Coordinator for proper training. You will be required to complete biosafety training through EH&S.

Chemical waste – Chemical waste is any waste that is toxic, corrosive, ignitable or otherwise listed by the Environmental Protection Agency (EPA). This includes laboratory chemicals, cleaning products, paint, copier toner, batteries, fluorescent bulbs, electronic devices, photographic and shop chemicals. If you are unsure whether the waste you generate is hazardous, please contact EH&S. Certain expired and unusable chemicals are hazardous waste and subject to regulations. Watch for expiration dates on containers of peroxide-forming chemicals including ethers.

Chemical wastes should be separated based on the type of chemical present: (1) organic solvents, (2) acids, (3) bases, and (4) organic and aqueous wastes. Here are a few general rules.

Organic solvents – Organic solvents only (no contaminants, reactants, or byproducts from reactions) should go in the waste containers labeled flammable. Document the volume of solvent added on the sheet with the container. This container should be vented (back valve should have hole in it). DO NOT ADD REACTION WASTE TO THIS CONTAINER; IT CAN REACT AND CAUSE AN EXPLOSION.

Acids – Acids should go in the appropriately labeled container. Document the grams of acid added on the sheet with the container (note calculation on how to do this on sheet). This container should be vented (back valve should have hole in it).

Bases – Bases should go in the appropriately labeled container. Document the grams of acid added on the sheet with the container (note calculation on how to do this on sheet). This container should be vented (back valve should have hole in it).

Mixtures – Reaction waste containing various solvents, reactants, catalysts, and other types of organic compounds should be collected into a personal waste container that you are responsible for maintaining this vessel, documenting the chemicals within it, and submitting a waste tag to EH&S when the container is full.

- You should separate the chemicals into different waste containers so that you do not mix molecules that can react with each other (MIXING CHEMICALS THAT ARE REACTIVE CAN LEAD TO EXPLOSIONS WITH FLYING GLASS SHARDS AND LAB FIRES!.

- Specifically any violently reactive molecules should be quenched prior to disposal; seek assistance if you do not know how to do this.

- When you are adding chemicals to a waste bottle, do the addition in the hood with the sash lowered.

- Leave the cap on the bottle lose to allow for vapor expansion with temperature changes.

- Store the bottle in an SAA when not adding chemicals to it.

- Do not fill a bottle more than 3/4ths full (head space is need for vapor expansion, otherwise bottles can explode!

Piranha – Piranha waste, a mixture of sulfuric acid and hydrogen peroxide. There is a container specifically designated for this type of waste. The container is a 4L glass bottle with a white cap. The cap used is a self-venting cap and should be completely sealed (not left lose for venting). Additional caps for this waste are kept in the secondary container next to the piranha waste.

Empty Chemical Containers – When you use the last of a chemical reagent, clean the empty container and place it on the "Out" bin next to the chemical inventory computer (A351) for disposal. Depending on the reagent, the protocol for cleaning the container will be based on the nature of the chemical. If you are not sure how to properly clean the container, ask someone who knows.

Unknown – If you are unsure of how to properly dispose of something, you must ask for help. Improper waste disposable can lead to dangerous situations, environmental contamination, large fines, etc.

Waste Generators' Responsibilities - Anyone who handles or manages hazardous waste is considered a hazardous waste generator and is subject to the federal and state Hazardous Waste regulations. As a hazardous waste generator you must:

- Receive initial hazardous waste training when beginning work with hazardous materials through EH&S (http://www.colorado.edu/ehs/training/index.html).
- Complete annual refresher training as long as you are actively working with hazardous materials with proctor or online quiz.
- Manage hazardous chemicals and waste properly to prevent pollution, minimize waste and protect human health.
- Know your lab safety precautions, and safety equipment (MSDS's, eyewashes, safety showers, personal protective equipment, etc.).

Equipment Usage

The equipment in this lab is extremely expensive and must be used with care. Misuse can result in costly repairs that hinder the success of the lab and its members. Additionally, improper use of the equipment can result in dangerous situations. When in doubt about how to use any piece of equipment in this lab, you must ask for help.

Equipment Protocols – Extensive protocols have been written for each of the major instruments in this lab. These protocols should be used to train new users and as a readily-available reference. Written protocols DO NOT substitute for hands-on training obtained from the person(s) responsible for the equipment.

Sign-up sheets – Many of the instruments are in high demand and an effort must be made to accommodate multiple users. Sign-up sheets are posted by each of these machines. Use them to block out times that you know you will use them. Be considerate. That is, don't sign up for the entire day when you only need it for a few hours. Also, update the list if your plans change and you know that the machine will be free during a time that you had signed-up to use it.

Undergraduates (UGs) – Due to the nature of specific equipment in the lab, undergraduates are not permitted to use the following instruments without direct supervision: confocal and new time-capture microscopes, Laser Capture Microdissector (LCM), peptide synthesizer (SPPS), HPLC, and GPC. For all other equipment, it will be the mentor's responsibility to properly train and oversee UG equipment usage. UG mentors will be held responsible for improper use of lab equipment by their UGs.

Ordering

Ordering is done by placing the item to be ordered into the "Anseth Ordering Log" spreadsheet that is located on the computer in A351. Only graduate students and post-docs are authorized to place orders.

Chemicals – Please write out the full chemical name and include the lab storage location. Upon arrival, the chemical must be entered in the inventory and assigned a bar code. Leave all chemicals to be entered into the inventory in the "In" bin next to the computer in A351 unless they require cold storage.

Orders over $500 must be pre-approved by Kristi.

Orders must reach a $50 minimum for each vendor before Cathy can place them. If an item is less than $50, it will be ordered only when other items from the same vendor makes the total greater than $50.

Lab Resources

Chemical Inventory – The ordering/chemical inventory computer contains an inventory of all of the chemicals that are currently maintained in the lab, where they are stored and an electronic copy of the MSDS. This is a useful resource for finding reagents. Also, check this list prior to ordering reagents, as the item you want may already be stored in the lab.

Anseth Group Protocols – Useful protocols have been written for commonly used techniques and can be found on the group website: (http://www.colorado.edu/che/ansethgroup/index.html).

Molecular Cloning by Maniatis – Considered the "bible" for molecular biologists, this 3-volume set includes detailed protocols, as well as, technique theory for most biologically-based assays. These volumes can be found on the bookshelf in room A351.

Vendor Catalogues – An assortment of frequently used vendor catalogues can be found on the bookshelf in room A351.

Anseth Group Plasmid Log – A log of the DNA plasmids that are currently stored in the lab is located on the bookshelf in room A351. The log contains plasmid maps as well as the storage location. If you would like to have one of these plasmids, ask Pete about how to go about getting it.

NMR Spectra – This 3-volume set gives NMR spectra of many common compounds. These volumes can be found on the bookshelf in room A351.

By signing below, you acknowledge that you have read the information contained in this Anseth Lab Orientation Checklist and agree to abide by the policies outlined therein.

Name: _____ Signature: _____ Date: _____

By signing below, you acknowledge that you are a qualified member of the Anseth Group and that you have personally gone over the information contained in this Anseth Lab Orientation Checklist with the person named above.

Name: _____ Signature: _____ Date: _____

By signing below, you acknowledge that have completed hazardous waste generator training through EH&S.

Name: _____ Signature: _____ Date: _____

By signing below, you acknowledge that have completed biosafety training through EH&S.

Name: _____ Signature: _____ Date: _____