Basic Lab Etiquette for Working in the Potter Valley Tribe Mushroom Lab

New to the lab? Welcome! Every lab is different, but when it comes to working in a mushroom lab there are some basic ideas and techniques to keep in mind to set yourself up for success. Here we are going to do our best to **avoid biological contamination**, namely other fungi (molds) and bacteria. This stuff is everywhere, so we have to consider everything that hasn't been sterilized and sealed to be contaminated. The name of the game is to *reduce* contamination risk whenever possible - you're never going to completely eliminate it, but following these tips will help.

Remember: you are entering the lab "dirty". Try to wear clean clothes and have showered recently. Leave your overcoat and hat outside of the lab. Once you enter the lab, **rub your hands with alcohol**. You'll want to make every effort to **be aware of everything you touch** - always sanitize your hands before touching something sterile, or after touching something dirty.

Keep a clean workspace! This means free of clutter, dirt, debris… and spray it down with alcohol if you can. Sweep out any dirt you track in, dispose of any spill grains or sawdust, and remove the trash daily.

This lab has a **flow hood**, which is used to generate a "clean air field" for you to work in. The further you get from the flow hood, the more likely you are to encounter contamination. If you stand in front of the flow hood or place items in front of the flow hood, you may be blowing contaminants towards your nice clean substrate. **Always stand behind your work**, keep the most sensitive items closest to the flow hood, and leave substrate or cultures open to the air for as little time as possible. If you've just sterilized your scalpel, but then waved it around outside of the clean air space, it's not clean anymore! Do not touch the flow hood filter or you risk puncturing it. It's a good idea to turn the flow hood on at least 10 minutes prior to use.

This is a **shared space**. Please label things (including what it is, your name, & the date), make note of problems, and sign in & out of the lab book. Keep it clean, respect the space. Thank you!

How to Make Agar Plates for Mushroom Culturing

Once you've collected a mushroom you're interested in culturing or growing, you'll need to prepare some agar plates. The purpose of this process is to create *nutrient dense* and *sterile* substrate for the mushroom tissue to begin growing out as mycelium again. You're going to take a chunk out of a mushroom and ask it to keep growing, so you'll have to provide it with *easily accessible nutrients* and *no competition*. We recommend working with a pre-mixed enriched malt extract agar (anti-biotic free), but you can experiment with your own amendments to the agar. Here we will provide a recipe for 15-20 plates, but feel free to apply this ratio to any number of plates you'd like to pour.

Supplies:

- ☐ Digital kitchen scale
- ☐ Spoon
- ☐ Enriched malt extract agar (agar media)
- ☐ Easy pour bowl or measuring cup (750mL+ capacity)
- ☐ Distilled water, or tap is OK
- ☐ 750 mL glass bottle with modified lid for gas exchange
- ☐ Tin foil
- ☐ Pressure cooker
- ☐ Petri dishes
- ☐ Flow Hood
- ☐ Isopropyl alcohol
- ☐ Paper towels
- ☐ Parafilm

Instructions:

1. Measure out 25 g of agar media with digital scale.
2. Mix into 500mL of warm water in an easy pour measuring cup, ensuring all the agar has dissolved.
3. Pour into glass bottle, screw lid on, cover cap with tin foil, and place into pressure cooker in a secure position so that it will not fall over.
4. Pressure cook for 45 minutes at 15 PSI: Once pressure has reached 15 PSI, adjust heat to maintain this pressure. Stay within earshot of pressure cooker to ensure the pressure regulator is rattling. Turn off heat when done. Do not let rise above 15 PSI.
5. Wait for the pressure to drop to 0 PSI (DO NOT OPEN UNTIL PRESSURE HAS DROPPED TO 0), and remove bottle when cool enough to handle, but still warm enough to remain a liquid. (The agar will solidify when cool, but you want this to happen when it's in the petri dish, *not* in the bottle)
6. In front of the flow hood, wipe down the outside of the petri dish sleeve with alcohol and a paper towel. Rub alcohol on your hands and allow to air dry. Now, it is very important to be mindful of proper lab technique and possible sources of contamination.
7. Stack the petri dishes (right side up - the larger lids should be on top of the smaller bases) in front of the flow hood in manageable size stacks.
8. In front of the flow hood, remove the tin foil and cap from the glass agar bottle.
9. Moving swiftly but carefully, start pouring your plates! Pour from the bottom of the stack to the top, keeping each plate open for as little time as possible.
10. Leave the flow hood on and allow plates to cool for up to an hour before using or wrapping with parafilm for storage.

Mushroom Tissue Culturing

You've got your mushroom, you've poured your agar plates, and you're ready to try growing out something you've collected. Tissue culturing is when you take a small chunk of the mushroom tissue and place it onto an agar plate to grow out new mycelium. This can then be used to inoculate grain bags and begin growing out more mushrooms. You can also store your petri dishes longer term in the fridge to develop a bit of a "library", or do bench scale experiments to try introducing different toxins or food sources to the fungi. In any case, tissue culturing is a fundamental part of mushroom cultivation. You'll likely be working with saprotrophic mushrooms, but you're welcome to experiment. Oysters are a great one to learn this skill.

Supplies:

- ☐ Fresh mushroom
- ☐ Flow hood
- ☐ Scalpel
- ☐ Bacti-cinerator
- ☐ 70% isopropyl alcohol
- ☐ Sharpie
- ☐ Agar plates (see "How to make Agar Plates…")
- ☐ Parafilm
- ☐ Paper towels

Instructions:

1. Do your best to remove any debris from your mushroom before bringing it into the lab.
2. In front of the flow hood, rub hands with isopropyl alcohol and clean workspace with alcohol.
3. Place petri dish in front of the flow hood and remove parafilm, without removing lid.
4. Rub hands with alcohol again.
5. Tear the mushroom in half, exposing inner tissue. Set aside one half (in front of the flow hood and with the clean side facing up).
6. Use the bacti-cinerator to sterilize your scalpel.
7. Carefully cut out a small chunk of the interior tissue, about ⅛ to ¼ inch wide, keeping the piece on the tip of the scalpel.
8. Swiftly remove the lid of the petri dish, and gently place the tissue onto the center of the petri dish, closing lid immediately.
9. Seal with fresh parafilm and lab the dish.
10. Wait 1 to 3 weeks for the mycelium to grow. Check back frequently for signs of contamination.
11. Once colonization is complete, you may store your dish in the fridge or use to inoculate grains.

*If contamination occurs, you can either toss your plate or try to isolate away a section of "clean" culture. You may use a similar process as described above to remove a piece of the new tissue from the plate and place onto a clean plate.

Techniques for Reducing Substrate Contamination

There are many different ways to grow mushrooms, and there are many ways to reduce chances of contamination. Here, we'll go over some basic techniques for sterilizing your growing mediums and when to use them. This is meant to provide an overview of techniques to help you find the right one for your project. You can research more about these methods online. We recommend using learn.freshcap.com/growing for great step-by-step instructions.

You don't always need to pressure cook your substrate before inoculating, but sometimes it's essential. Consider the sensitivity of the situation and weigh it against the capacity and tediousness of the possible sterilization techniques. For example, when preparing a very nutrient dense substrate, like agar, your mushrooms aren't the only things that would grow quickly. It is the perfect micro-climate for all sorts of microbiology. Spores and mushroom tissue are the most likely things you'll grow out on the agar, both of which have a higher risk of contamination. You'll need to pressure cook your agar and start with sterile petri dishes. See "How to Make Agar Plates" for more info. The pressure cooker is only 23 qt so it will work to sterilize agar and up to 6 bags of 4lbs of grain for spawn. But while it might be ideal to completely sterilize all your substrate, you likely don't have the ability to pressure cook a pile of wood chips for growing in your garden. In order to reduce the contamination for dozens of pounds of substrate you can pasteurize instead. These are often less intensive methods and are detailed below.

Sterilization

Pressure Cooking

If you need to sterilize your substrate or materials, this is the way to go. Use this for components which will be used to expand out into more substrate and very nutrient dense mediums. Agar, grain, and sawdust can be pressure cooked at 15 PSI- the timing depends on what you're cooking. Be sure to put enough water in for the length of time you plan to cook.

Autoclave

Usually used in bigger labs - don't worry about this one right now!

Pasteurization

Cold Water Pasteurization

Hydrated Lime

This technique works great for pasteurizing straw. Straw is a bit more prone to contamination than wood chips. The idea behind this method is to soak the straw in water, using *hydrated lime* to raise the pH of the water. The anoxic environment and the high pH will kill off a lot of mold and bacteria, giving the mushrooms an upper hand at colonizing the substrate. You can soak anywhere from 12 hours to 4 days. A lime soak is generally used when preparing substrate

to grow oyster mushrooms, as they can tolerate a higher pH substrate than most other fungi.

Cold Soak

This technique works well for wood chips. This method is similar to the hydrated lime soak, except without the lime! Just soak your chips in water (completed submerged) for 12 hours to 4 days. We recommend a longer soak than the lime soak, utilizing the anoxic environment to kill off contaminants.

Hot Water Pasteurization

Pillow Case Tek

This method is useful for straw or wood chips, but is generally used for straw. A pillow case is used to contain the substrate in boiling water, serving almost like a giant tea bag (except you want to keep the straw, not the water). You can use a thermometer to bring the substrate up to temperature, boiling for 1.5-2 hours.

Hot Water Bath

A hot water bath is generally preferable over a cold soak if you can swing it. This method works well for wood chips primarily. Boiling water is poured over your substrate in a tub and allowed to soak for 2-12 hours.

Heat Pasteurization

Oven

This is a less common method, but will do in a pinch for coco coir, vermiculite, peat moss, or other casing layer type substrates. The substrate is mixed just above field capacity (dripping wet), and baked slow and low in a turkey tin covered with tin foil in the oven.

Steam

This method is very popular for pasteurizing large batches of sawdust. This can be done in a 55-gallon drum over propane, in a plastic tub hooked up to a steamer, or even in something as big as a shipping container attached to a boiler! The sawdust substrate is prepared and then packed into specialty filter-patch plastic bags before being cooked. A thermometer is used to ensure the internal temperature has reached & sustained 140℉ for a minimum of 2 hours.

Nothing!

Working with fresh substrate

Are you working with wood chips or logs? Best to get it fresh (within a few weeks for chips, within 2 months for logs). This is great for anything you're growing outdoors. A higher inoculation rate will do well here to ensure your mushrooms have a good chance of outcompeting wild molds and bacterias. You do want your substrate to have some moisture content, so if you find they've dried out prior to inoculation, you can do an overnight soak.