# Getting Started with agar

You may have heard or read about using agar plates in many online communities or books. It has become a popular tool in mushroom growing. Growing mushrooms from scratch requires being able to store and properly propagate mushroom cultures on nutrient rich agar.

***The objectives are:***

- Start with a sterile piece of mushroom tissue and transfer it onto agar
- Start from a spore syringe and grow out the best genetics
- Create multiple copies of the culture
- Avoid contamination of the culture
- Transfer clean mycelium to liquid culture for inoculating multiple spawn bags or jars.

**Properly transferring cultures allows you to "grow out" mushroom different species for the purpose of making grain spawn. It also allows for the ability to "clean" a culture that has been contaminated.**

**Things You'll Need**

- Sterilized Nutrient Rich Agar Plates
- A sterile disposable scalpel or a sharp knife
- An alcohol lamp or a steady flame source for sterilizing the blade
- A Laminar Flow Hood (Recommended) or a quality Still Air Box
- Parafilm or masking tape
- Spore Syringe, Spore Swab, Spore Print, Fresh mushroom tissue, or Liquid Culture

**1. Clean Up**

Start by cleaning the outside of the dishes / slants with rubbing alcohol. Ensure that your hands, scalpel and all other tools are clean. Wear nitrile gloves and a surgical mask to prevent contaminants landing on the fresh plate from your hands or breath.

**2. Set Up the Dishes**

Remove the Parafilm or tape. Set the dishes side by side in front of the flow hood. It may be easier to set the new plate on the opposite side of your working hand.

**3. Flame Sterilize Scalpel**

Start by flaming the scalpel blade until it is glowing red hot. This needs to be done between each and every transfer, so have a constant flame going on your work bench. This can be achieved most easily with an alcohol lamp, but you can also use a shot glass that is ¾ full with rubbing alcohol. Just ensure that the shot glass cannot tip over while it is burning! You can also purchase boxes of disposable sterilized disposable scalpels for cheap on Amazon.

**4. Cool the Blade**

Once the blade is red hot, cool it off rapidly by dipping into the agar on the receiving dish. Gently lift the lid off the dish keeping your hand on the back half of the lid, downstream from the rest of the plate. Try not to contact the very edge of your plate with your hands. Handle it minimally, and never remove the lid from the stream of laminar flow.

With the lid removed, quickly dip the blade into the agar near the edge of the dish. You will hear an audible sizzle. Quickly replace the lid back onto the receiving dish.

**5. Decide what to inoculate the plates with:**

- Spore Syringe: Lift the lid, squirt 1cc of solution and make an X with the liquid, close the lid and tape shut using Parafilm, Micropore tape or masking tape.

-Fresh Mushroom: Pick a fresh full size mushroom. Pull apart the mushroom from the bottom stem and tear apart in the flow hood or still air box. With sterile scalpel cut a small 1-2mm piece of inside tissue where the stem and cap meet and drop on the agar. Close and tape up.

-Spore Print: Using a sterile scalpel, scrape a small amount of spores around the entire dish. Make sure the spread out evenly. Close lid and seal with tape.

-Liquid Culture: Using the same technique as the spore syringe, make an X around the plate with the liquid culture. Make sure to shake well before using!

**6. Incubate the Agar plates at 77-83 degrees**

You will want to find a warm clean place to incubate the dishes for 5-7 days. You can grow them at lower temps but its best to strive for 80 degrees for optimal results

**7. Transfer Mycelium from growing Agar dish to new plate**

Open the lid in the culture-containing dish. Hold it steady while you cut a small piece of agar (containing strong mycelium growth) out of the dish. A **1 cm x 1 cm piece will usually suffice**. Once the piece is cut, stab it with your scalpel, and quickly transfer it over to another receiving dish, placing it at the center. Ensure that the culture piece stays in the laminar flow, preferably upstream of the plates.

***The reason we transfer is to make sure there are no contaminates and also ensure we are transferring a healthy colony of mycelium. You can repeat this step as many times as needed until you have a healthy growing contaminate free sample growing on your dish.***

**8A. Transfer to grain spawn**

You can transfer your healthy mycelium to grain spawn by simply cutting a 1cm x 1cm piece out and dropping it in the grain jar or bag. Make sure you do this in a flow hood or still air box. Work as fast as possible to avoid contamination.

**8B. Transfer to Liquid Culture Jar**

Transferring to liquid culture jar allows you to inoculate hundreds of grain bags or jars. Simply cut the same 1cm x 1cm piece from the plate and open the lid of the liquid culture and drop in and mix. Let the culture grow for 5-7 days or until a healthy cloud of mycelium is formed.