

Inoculation Techniques Video Transcript

In this video I'm going to demonstrate various inoculation techniques and I'm off camera because I want you to see what I'm doing in front. You don't need to look at my lovely face right now. So, what we're going to do is inoculate a grain spawn jar from liquid culture and inoculate a plate from liquid culture. This is a standard malt agar plate you can also use PDA which stands for potato dextrose agar fungi love both of them. Note that I have labeled my plate prior to this procedure. You want to label everything that you do with the variety and the date the procedure was performed. And when I have finished inoculating, I will actually store it with the agar side up so the label is easy to read. That's why we label it on that side. Storing it agar side up makes the plates easier to handle and less prone to contamination.

I am very very emphatic when I say contamination is bad. So the first thing we're going to do is clean off everything with an alcohol wipe. Going to clean off the top of the specialized mycology lid which as you can see, this is a filter because fungi are obligate aerobes, and they need the air as much as we do. This is a self-healing port. This one is made of a rubber gasket. You can make your own using a synthetic batting such as a pillow batting for the filter and a sealant for the self-healing port. But I found these are wonderful to work with. Absolutely wonderful. They cut my contamination rate down quite a bit as compared to the lids that I made.

This is a standard 10 milliliter syringe for your liquid culture. They can be obtained from a number of reputable sellers. The Stamets book has all kinds of them. One thing that we did when I was in mycology class was do a quick sterilization. This is one procedure you can perform outside of your still airbox because of this lid. So you wipe with alcohol the needle and then you flame the needle with a standard lighter until the needle is red hot. (My lighters have been doing a lot of this lately, so it's already turned up.) It is red hot. Insert the needle into the self-healing port, depress the syringe, and don't be afraid to give it a heavy inoculation. It will help your jar colonize faster. Give the jar a good shake. This is why we don't fill them up completely. So we can give them this good shake. That heavy sterilization is good, and the heavy inoculation will help the jar colonize a lot more quickly and become the dominant organism in the jar.

Yes, we do sterilize these in our pressure canner as given in the instructions: 15 pounds pressure for about an hour and a half for the jars. But things still happen. Okay, things still happen, and that's not a good thing. So we've done the jar, we take our alcohol wipe and, you can get away with doing this outside of you're still airbox if you are very, very careful. Okay, we again wipe the needle and flame the needle until it is red hot. That will kill anything on the

needle and even if it's fresh out of the sterilized wrapping, I do this. Open it very very carefully and no more than you need to get that in there. Depress the syringe and just roll the liquid inside around on the plate that will be enough to inoculate your plate Okay, And that is how we inoculate plates outside of the still air box. In a moment, I will set up and show you how we do these things inside of a steel airbox.

In this part of the video, I'm going to demonstrate inoculation procedures that we do inside of the steel airbox. So the first thing I'm going to demonstrate is how to properly clean the still air box. Because everything that we are going to use needs to go inside of this box. Bleach: go around the box with your bleach solution and give it a nice wipe all the way around. You want to do everything that's going into the box. This is not an absolutely perfect thing, but it is the best we are going to be able to do. I'm going to demonstrate inoculating from plates into grain spawn and from green spawn to grain spawn. I have a slight problem with my shiitake plate, it has gained an infection. That is a green mold - that is bad. So one thing I can try to do is subculture away from the mold into another plate. And that's what I'm going to try and do to save my plate.

We clean everything that is going into the still airbox and I'm going to use a different grain culture for this. And I will in fact use an alcohol wipe and do it again when I get in there. We clean EVERYTHING that is going in there. The next thing we do is clean the inside of the box with the same bleach solution. Spritz down the gloves, the sides the top, wipe them off. Otherwise, you'll have bleach dripping all over everything. You don't quite want that. But you do want as clean as possible. Close up the box and spritz a little bleach inside to clean the air as much as possible.

Now working inside of a still air box is kind of a pain, no doubt about it. But as I've mentioned in the lessons, it is a lot less painful than throwing things out. So put our hands inside of the gloves that we cleaned inside of the steel airbox. The first thing I'm going to do is try to subculture the shiitake plate. Okay, we open things up and I use my butter knife and I choose a portion well away from the contamination as much as I possibly can. That's not easy because this plate is pretty heavily contaminated. But I do the best I can and just flop it on the plate. Be sure of which lid you are putting back on your plate because the lid from the contaminated plate is likely contaminated as well. So that matters. Okay. Now we'll just toss the old shitake plate in fact.

When it comes to inoculating from a green spawn jar getting into that alcohol wipe is not the easiest thing. And yeah, I'm probably paranoid, but having tossed things due to contamination is a natural paranoia, is a natural side effect. We want to clean again, and clean, and clean the lids basically. We've already spritzed the jars themselves. Now you open up the lid and you open up

the other lid. You can use the butter knife, and I prefer a butter knife to a pocketknife because there are fewer surfaces that can be contaminated. Okay. Loosen up a little of the spawn and toss it in just like that.

Now, if this were a sawdust block, which I show in another video, I would inoculate more heavily but this is a small jar of leftover green. You'll want to close everything right back up before you do anything else, because spillage is also not a very fun deal. And again, there's no point in working inside of the still air box if you open up before everything is sealed. Okay, so that is how to inoculate a plate from another plate and how to inoculate a grain spawn jar from another grain spawn jar. If you want to inoculate your grain spawn jar from a plate you would do much the same thing. You would just cut a piece from the agar and toss them into the jar. Very, very easy to do. Okay, so those are inoculation techniques that may not be familiar to everyone. I learned many of them when I was in college. Thank you and Happy Shrooming!