STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PLANT

TITLE: Seed flask autoclave sterilization SOP

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APPROVALS: **Process Change Committee** **DATE:**
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A. Scope

This procedure describes the method to prepare a flask for seed propagation using an autoclave.

B. Safety and Training Requirements

Refer to UF lab safety policies regarding equipment in section D below before starting any process work.

Review the location of fire extinguishers, fire blankets, safety showers, spill cleanup equipment and protective gear before beginning any process work.

During operations in the plant, the following safety gear will be utilized at all times:

- Autoclave Gloves
- Lab Coat
- Safety Goggles or Face Shield
- Protective Gloves (nitrile, neoprene)

To prevent serious burns and injuries:

Always – read and understand operating instructions before starting.
Always – use caution when opening door and removing contents.
Never – attempt to open door with any pressure in chamber.
Never – attempt to run machine with door open.
Never – attempt service or repairs unless qualified and authorized. Failure to do so may cause damage and void manufacturer's warranty.

C. Related Documents and SOPs

Refer to Small Boiler SOP and manual.
Refer to the UF BioSafety Manual for information on how to prevent and treat burns.

D. Preparation/Materials/Equipment

1. Consolidated Autoclave
2. Autoclave gloves
3. 500 ml glass flask
4. Paper towel
5. Rubber band
6. Autoclave tape

E. Detailed Procedure

Small boiler should be up to 30 psi and the main steam valve OPEN before starting the autoclave.

1. Put autoclave gloves on.
2. Make sure autoclave chamber is closed.
3. Open steam valves in the back of the autoclave.
4. Turn jacket switch on in the front panel and push the jacket reset button.
5. Once the jacket pressure is up to 10-12 psi it will remain there and the autoclave door can be opened and closed.
6. Take a 500 ml glass flask and cover it with a folded paper towel secured with a rubber band.
7. Place a piece autoclave tape on top of the paper towel cover. Black lines on the tape will appear if the autoclave heated up inside the chamber correctly during sterilization.
8. Place the covered 500 ml flask inside the autoclave. Remember to do this only with autoclave gloves as the inside contents of the autoclave may be very hot.
9. Set the sterilization time to 20 minutes and 0 minutes for drying.
10. Set the sterilization mode to "Fast."
11. Begin the sterilization by pressing on the "on" button making sure to have selected the right exhaust procedure. The sterilization time will begin counting down once the chamber is up to temperature.
12. The pressure in the chamber should be ~15 psi during sterilization. **Open the autoclave only when it reads 0 minutes left AND "0 psi."**
13. Open the autoclave slowly and carefully after the sterilization procedure has finished and pressure reads "0."

14. Take the flask out of the autoclave and place on a heat resistant surface to allow it to cool before using for seed propagation.

F. Data Archival and Analysis

Record all autoclave runs in the Autoclave log sheet and keep next to autoclave at all times. The autoclave log sheet should contain items autoclaved, sterilization time, drying time, exhaust mode, and autoclave performance.