

**STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT**

TITLE: Sterility Testing – Primary Propagation

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A. Scope

This SOP describes the procedure to verify that the Primary Propagator system can be sterilized and held at operating conditions without contamination.

B. Safety and Training Requirements

Refer to UF lab safety policies regarding equipment listed 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:

- Safety Goggles
- Protective Gloves
- Hard Hat

C. Related Documents and SOPs

1. Viable Plate Count SOP- 0507
2. Media Preparation SOP-2155
3. UV Water System Operation SOP-9555
4. Steam Supply SOP-9305
5. Air System Operation SOP-9405
6. Refrigeration System Operation SOP-9210
7. Primary Propagator 2A SOP-3210
8. Primary Propagator 2B SOP-3215
9. Sampling SOP-0511
10. Potable Water SOP-9705
11. Hot Water System SOP-9605

D. Preparation/Materials/Equipment

1. Yeast autolysate (1 lb per tank)
2. Glucose (1 lb per tank)

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3. Bleach solution (0.6 %)
4. Antifoam solution (TBD)

E. Detailed Procedure

1. Ensure the air system is operational according to the Air System Operation SOP-9405.
2. Ensure the potable water is operational according to the Potable Water SOP-9705.
3. Ensure the refrigeration system is operational according to the Refrigeration System Operation SOP-9210.
4. Ensure the hot water system is operational according to the Hot Water System SOP-9605.
5. Ensure the steam is operational according to the Steam Supply SOP-9305.
6. Ensure the UV water system is operational according to the UV Water System Operation SOP-9555.
7. Ensure that the desired propagator is clean and ready for operation according to the respective SOP.
8. Open the propagator vent valves: 3202-V-36 for Propagator 2A or 3210-V-44 for Propagator 2B.
9. Charge the tank with glucose, yeast autolysate, and antifoam by:
 - a. Add 50 gal UV water to the Media Prep tank according to SOP-2155.
 - b. Add 2 mL of antifoam solution.
 - c. Add 1 lb of yeast autolysate to the Media Prep tank according to SOP-2155.
 - d. Add 1 lb of glucose to the Media Prep tank according to SOP-2155.
 - e. Mix for 5 minutes to assure the ingredients are evenly suspended.
 - f. Transfer the mixture to the desired Primary Propagator according to SOP-2155 and to the respective Propagator SOP.
 - g. Fill the Media Prep tank with 20 gallons of UV water.
 - h. Transfer water to the primary propagator as in Step 2.e to rinse the line.
10. For Primary Propagator 2A, sterilize by:
 - a. Set the valves according to the initial valve table in SOP-3210.
 - b. On the HMI, turn on agitation using XS-3202A-05.
 - c. On the HMI, using SIC-3202A-05, set speed to 100% in Manual Mode.
 - d. Assure the Primary Propagator 2A Jacket has been fully drained by visual verification at the line downstream of drain valve 3202-V-18.
 - e. Add Low Pressure Steam to the tank jacket by:
 - i. At the HMI, switch the temperature control TIC-3202A-10B to SIP mode.

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- ii. Close Jacket High Point Vent valve 3202-V-32.
- iii. Verify steam pressure in PI-3202A-15 is below 30 PSI.
 - 1. If not, contact supervisor.
- iv. Slowly open steam supply valve 3202-V-34.
- v. When steam exits drain downstream from drain valve 3202-V-18, open the drain to the steam trap (valve 3202-V-19) and close the drain valve 3202-V-18.
- vi. Verify steam pressure in PI-3202A-15 is at 20 ± 2 PSI. If not, contact supervisor.
- f. When the tank reaches 170 °F, close the Propagator vent valve 3202-V-36.
- g. Continue heating until sterilization temperature of 250 °F is reached.
- h. Record the time and maintain this temperature for 45 minutes.
- i. After sterilization time is completed, cool the tank by:
 - i. Stop steam to the jacket by closing valve 3202-V-34.
 - ii. Relieve pressure in the jacket by slowly opening jacket drain valve 3202-V-18.
 - iii. After steam has stopped exiting drain, open jacket high point vent valve 3202-V-32.
 - iv. After the jacket has been drained, close valves 3202-V-18, V-19, V-32.
 - v. At the HMI, set MODE to NORMAL on TIC-3202A-10B.
 - vi. At the HMI, set Temperature control to AUTO and 98.6 °F (37 °C) in TIC-3202A-10A.
 - vii. Monitor the tank pressure on the HMI at PI-3202A-33.
 - viii. Monitor the tank temperature on the HMI at TIC-3202A-10.
 - ix. When the pressure drops below 10 PSI, open the sterile air valve 3202-V-42 to add air to the tank via the vacuum breaker.
- j. Once the tank temperature reaches 98.6 °F (37 °C):
 - i. Open valve 3202-V-17 and V-33 to the Propagator 2A Jacket pump.
 - ii. At HMI, start Propagator 2A Jacket Pump PT-3212.
 - iii. Place the tank into normal operation according to the tank SOP.

Hold the Propagator in normal operation for 24 hours.

11. For Primary Propagator 2B, sterilize by:

- a. Set the valves according to the initial valve table in SOP-3215.
- b. On the HMI, turn on agitation using XS-3202B-05.
- c. On the HMI, using SIC-3202B-05, set speed to 100% in Manual Mode.
- d. Assure the Primary Propagator 2B Jacket has been fully drained by visual verification at the line downstream of drain valve 3210-V-28.
- e. Add Low Pressure Steam to the tank jacket by:
 - i. At the HMI, switch the temperature control TIC-3202B-10B to SIP mode.
 - ii. Close Jacket High Point Vent valve 3210-V-46.
 - iii. Verify steam pressure in PI-3202B-15 is below 30 PSI. If not, contact supervisor.
 - iv. Slowly open steam supply valve 3210-V-40.

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- v. When steam exits drain downstream from drain valve 3210-V-28, open the drain to the steam trap (valve 3210-V-27) and close the drain valve 3210-V-28.
 - vi. Verify steam pressure in PI-3202B-22 is at 20 ± 2 PSI. If not, contact supervisor.
 - f. When the tank reaches 170 °F, close the Propagator vent valve 3210-V-04.
 - g. Continue heating until sterilization temperature of 250 F is reached.
 - h. Record the time
 - i. Maintain this temperature for 45 minutes.
 - j. After sterilization time is completed, cool the tank by:
 - i. Stop steam to the jacket by closing valve 3210-V-40.
 - ii. Relieve pressure in the jacket by slowly opening jacket drain valve 3210-V-28.
 - iii. After steam has stopped exiting drain, open jacket high point vent valve 3210-V-46.
 - iv. After the jacket has been drained, close valves 3210-V-27, V-28, V-46.
 - v. At the HMI, set MODE to NORMAL TIC-3202B-10B.
 - vi. At the HMI, set Temperature control TIC-3202B-10A to 98.6 °F (37 °C) and switch to AUTO.
 - vii. Monitor the tank pressure on the HMI at PI-3202B-33.
 - viii. Monitor the tank temperature on the HMI at TIC-3202B-10.
 - ix. When the pressure drops below 10 PSI, open the sterile air valve 3210-V-07 to add air to the tank via the vacuum breaker.
 - k. Once the tank temperature reaches 98.6 °F (37 °C):
 - i. Open valve 3210-V-26 to the Propagator 2A Jacket pump.
 - ii. At HMI, start Propagator 2A Jacket Pump PT-3212.
 - iii. Begin normal operation according to the tank SOP.
- 12. After 24 hours, take a sterile sample of the Primary Propagator according to Sampling SOP-0511.
- 13. Take the sample to the lab and process immediately for Viable Plate Count according to SOP-0507.
- 14. Shut down and clean the Primary Propagator according to the respective tank SOP.
- 15. The tank must pass the sterility test by having ZERO viable plate count numbers.
- 16. If the tank fails the sterility test, thoroughly clean the vessel by:
 - a. Remove all probes and sensors from the tank.
 - b. Inspect the probes and sensors, fittings, and o-rings for sources of contamination, e.g. dirt, debris, etc.
 - c. Clean the probes and fittings with dilute bleach solution.
 - d. Remove the sampling port and clean with dilute bleach solution.
 - e. Remove all temporary fittings that penetrate the tank in a similar manner.
 - f. Reassemble the tank.
- 17. Repeat the sterility test until successful.