

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

**TITLE: Sterility Testing – Secondary Propagation**

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**APPROVALS: Process Change Committee**

**DATE:**

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**A. Scope**

This SOP describes the procedure to verify that the Secondary 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 Glasses
- Protective Gloves
- Hard Hat

**C. Related Documents and SOPs**

1. Viable Plate Count SOP- 0507
2. Secondary Propagator 3A SOP-3220
3. Secondary Propagator 3B SOP-3225
4. Sampling SOP-0511
5. Media Preparation SOP-2155
6. Phosphoric Acid System Operation SOP-8110
7. Base B System Operation SOP-8565
8. Refrigeration System Operation SOP-9210
9. Steam Supply SOP-9305
10. Air System Operation SOP-9405
11. Wastewater System SOP-9530
12. Potable Water SOP-9705

**D. Preparation/Materials/Equipment**

1. Yeast autolysate (10 lb per tank)
2. Glucose (220 lb per tank)
3. Antifoam solution (20 mL)

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4. Bleach solution (0.6 %)

**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: 3203-V-52 and V-56 for Propagator 3A or 3204-V-56 and V-60 for Propagator 3B.
9. Charge the tank with yeast autolysate and glucose by:
  - a. Add 622.5 lbs UV water to the Media Prep Tank according to SOP-2155.
  - b. Add 10 lb of yeast autolysate to the Media Prep Tank according to SOP-2155.
  - c. Add 220 lb of glucose to the Media Prep Tank according to SOP-2155.
  - d. Add 20 mL of antifoam to the Media Prep Tank according to SOP-2155.
  - e. Turn on the prep tank agitator and mix for 5 minutes to ensure the ingredients are evenly dissolved.
  - f. Transfer the solution to the desired propagator according to SOP-2155 and its respective Secondary Propagator SOP.
  - g. Fill the Media Prep Tank with 622.5 lbs of UV water.
  - h. Transfer the water to the propagator as in Step 9.f to rinse the line.
10. Add UV water to the propagator according to the respective SOP in order to fill the tank up to 50%.
11. For Primary Propagator 3A, sterilize by:
  - a. At the HMI, switch the temperature control TIC-3203A-10B to SIP mode
  - b. Set the valves according to the initial valve table in SOP-3220.
  - c. On the HMI, turn on agitation using XS-3203A-05.
  - d. On the HMI, using SIC-3203A-05, set speed to 100% in Manual Mode.
  - e. Ensure the Primary Propagator 3A jacket has been fully drained by visual verification at the line downstream of drain valve 3203-V-27.
  - f. Add low pressure steam to the tank jacket by:
    - i. Close the jacket high point vent valve 3203-V-49.

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- ii. Verify steam pressure in PI-3203A-15 is below 30 PSI. If not, contact supervisor.
    - iii. Close the drain valve 3203-V-27.
    - iv. Slowly open steam supply valve 3203-V-48.
    - v. Make sure the steam trap is open (T1-3203-03).
    - vi. Verify steam pressure in PI-3203A-15 is at  $15 \pm 2$  PSI. If not, contact supervisor.
  - g. At the HMI, set the temperature control TIC-3203A-10B to 250 °F and switch to AUTO.
  - h. Open valve 3203-V-35 to allow steam into the tank.
  - i. When the tank reaches 180 °F, close the propagator vent valve 3203-V-56.
  - j. Once the temperature of 250 °F is reached, record the time and maintain this temperature for 60 minutes.
  - k. After sterilization time is completed, cool the tank by:
    - i. Stop steam to the tank by closing valve 3203-V-35.
    - ii. At the HMI, switch the temperature control TIC-3203A-10B to MANUAL and set the OUTPUT to -5.
    - iii. Stop steam to the jacket by closing valve 3203-V-48.
    - iv. Relieve pressure in the jacket by slowly opening jacket drain valve 3203-V-27.
    - v. After steam has stopped exiting drain, open jacket high point vent valve 3203-V-49.
    - vi. After the jacket has been drained, close valves 3203-V-27 and V-49.
    - vii. At the HMI, set MODE to NORMAL on TIC-3203A-10B.
    - viii. At the HMI, set Temperature control to AUTO and 98.6 °F (37 °C) in TIC-3203A-10A.
    - ix. Monitor the tank pressure on the HMI at PI-3203A-33.
    - x. Monitor the tank temperature on the HMI at TIC-3203A-10.
    - xi. When the pressure drops below 10 PSI, open the sterile air valve 3203-V-15 to add air to the tank via the vacuum breaker.
  - l. Once the tank temperature reaches 98.6 °F (37 °C):
    - i. Open valve 3203-V-25 and V-50 to the Propagator 3A Jacket pump.
    - ii. At HMI, start Propagator 3A Jacket Pump PT-3213.
    - iii. Place the tank into normal operation according to the tank SOP-3220.
12. For Primary Propagator 3B, sterilize by:
- a. Set the valves according to the initial valve table in SOP-3225.
  - b. On the HMI, turn on agitation using XS-3203B-05.
  - c. On the HMI, using SIC-3203B-05, set speed to 100% in Manual Mode.
  - d. Ensure the Primary Propagator 3B jacket has been fully drained by visual verification at the line downstream of drain valve 3204-V-29.
  - e. Add low pressure steam to the tank jacket by:
    - i. At the HMI, switch the temperature control TIC-3203B-10B to SIP mode.
    - ii. Close jacket high point vent valve 3204-V-54.
    - iii. Verify steam pressure in PI-3203B-15 is below 30 PSI. If not, contact supervisor.
    - iv. Close the drain valve 3203-29.

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- v. Slowly open steam supply valve 3204-V-52.
  - vi. Open the drain to the steam trap (valve 3204-V-28).
  - vii. Verify steam pressure in PI-3203B-15 is at  $15 \pm 2$  PSI. If not, contact supervisor.
- f. At the HMI, set the temperature control TIC-3203B-10B to 250 °F and switch to AUTO.
- g. Open valve 3204-V-37 to allow steam into the tank.
- h. When the tank reaches 180 °F, close the Propagator vent valve 3204-V-54.
- i. Once the temperature of 250 °F is reached, record the time and maintain this temperature for 60 minutes.
- j. After sterilization time is completed, cool the tank by:
  - i. Stop steam to the tank by closing valve 3204-V-37.
  - ii. At the HMI, switch the temperature control TIC-3203B-10B to MANUAL and set the OUTPUT to -5.
  - iii. Stop steam to the jacket by closing valve 3204-V-52.
  - iv. Relieve pressure in the jacket by slowly opening jacket drain valve 3204-V-29.
  - v. After steam has stopped exiting drain, open jacket high point vent valve 3204-V-54.
  - vi. After the jacket has been drained, close valves 3204-V-28, V-29, V-54.
  - vii. At the HMI, set MODE to NORMAL on TIC-3203B-10B.
  - viii. At the HMI, set Temperature control on TIC-3203B-10A to 98.6 °F (37 °C) and switch to AUTO.
  - ix. Monitor the tank pressure on the HMI at PI-3203B-33.
  - x. Monitor the tank temperature on the HMI at TIC-3203B-10.
  - xi. When the pressure drops below 10 PSI, open the sterile air valve 3204-V-64 to add air to the tank via the vacuum breaker.
- k. Once the tank temperature reaches 98.6 °F (37 °C):
  - i. Open valve 3204-V-26 and V-55 to the Propagator 3B Jacket pump.
  - ii. At HMI, start Propagator 3B Jacket Pump PT-3213.
  - iii. Place the tank into normal operation according to the tank SOP-3225.
- 13. Hold the propagator in normal operation for 24 hours according to their respective SOP.
- 14. After 24 hours, take a sterile sample of the secondary propagator according to Sampling SOP-0511.
- 15. Take the sample to the lab and process immediately for Viable Plate Count according to SOP-0507.
- 16. The tank must pass the sterility test by having ZERO viable plate count numbers
- 17. If the viable plate count is not ZERO (there are several bacterial or fungal colonies on the plates);
  - a. Raise the pH of the corresponding secondary propagator to 9.0 using ammonium hydroxide (19 % w/w) through the Base B system according to the Base B System SOP-8565 and the respective Secondary Propagator SOP.
  - b. At the HMI, change the set point of the controller TIC-3203A-10A to 140 °F.

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- c. Once the temperature and pH have been reached, maintain these conditions for at least 3 h.
  - d. Make sure the wastewater tank is ready to receive the broth according to the Wastewater System SOP-9530.
  - e. Drain the respective Secondary Propagator into the sump by:
    - i. For Secondary Propagator 3A, open the valves 3203-V-34, V-30 and V-29.
    - ii. For Secondary Propagator 3B, open the valves 3204-V-30, V-33 and V-34.
  18. Shut down the respective Secondary Propagator and run CIP (clean in place) for Secondary Propagator 3A and 3B according to the Secondary Propagator 3A SOP-3220 and Secondary Propagator 3B SOP-3225 respectively.
  19. 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 sensors, fittings and o-rings 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.
  20. Repeat the sterility test until successful.

**F. Data Archival and Analysis**

Date of sterility test:

Vessel: Date	Time of Day	Time Elapsed	Temp (°F)	pH	Viable Counts	Comments

Take notes of all calculations and measurements. Store the data obtained in the appropriate Log Book.