STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PLANT

TITLE: Culture Sampling Operating Procedure

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APPROVALS: **Process Change Committee** **DATE:**
UF EH&S **DATE:**

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A. Scope

This procedure describes how to aseptically sample cultures for analysis by HPLC, GC, Spectrophometer, etc.

B. Safety and Training Requirements

Refer to UF lab safety policies and review the Material Safety Data Sheets (MSDS) for each material 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:

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

Avoid inhalation of vapors and wear nitrile or neoprene rubber gloves. Contain spills by using spill kits next to fermentors.

C. Related Documents and SOPs

1. UF Biosafety manual

D. Preparation/Materials/Equipment

The chemicals/materials used in this SOP are listed below:

1. 3 ml BD syringe (Luer-Lok tip, REF 309657)
2. 10 ml pipet (Falcon individually wrapped 10 ml serological pipet, 50 per bag, 200 per case, REF 357504)
3. Drummond portable pipet-aid
4. 1.5 ml Microcentrifuge tubes (Fisherbrand Premium, 05-408-129, 500 pk)
5. 2 ml self-standing tubes with caps (USA Scientific, 1420-9700, 500/unit)
6. Eppendorf centrifuge 5418 (18 tube capacity)
7. Plastic tube rack

E. Detailed Procedure

1. Connect a 3 ml BD Syringe to the female luer-lok on fleakers or Pom tea vessels. If sampling from 10 L fermentor, use a 10 ml pipet attached to a portable pipet-aid. To take a sample from a 100 L fermentor, open the bottom valve by turning anti-clockwise, then open the ball valve to the left of the bottom valve slowly until culture begins to flow out. Collect 100 L culture sample in a 50 ml centrifuge tube and transfer 1.5 ml of it to an appropriately labeled microcentrifuge tube.
2. Aspirate out (fleakers and Pom tea vessels) or decant out (100 L fermentor) 1.5 ml of culture and transfer to an appropriately labeled microcentrifuge tube on a rack.
3. Quickly cover the sample port with either a lug (10 L vessels), a syringe (fleakers and Pom tea vessels), or by closing opened valves.

4. Sterilize the sampling port for the 100 L fermentor by first putting autoclave gloves on and making sure valves at the bottom of the fermentor are closed.
5. Slowly open the black steam valve to the right of the bottom valve BV-1201.
6. Slowly open the yellow ball valve leading out the right drain and let steam flush through for 1 minute.
7. Close the ball valve.
8. Open the yellow ball valve (located furthest to the left) and let steam flush through for 1 minute.
9. Centrifuge the microcentrifuge tubes for 2 minutes at 14,000 rpm.
10. Decant contents into 2 ml self-standing tubes and cap.
11. Store samples at -20°C until further analysis.

F. Data Archival and Analysis

Store samples at -20°C until further analysis.