1. Procedure summary

This procedure describes how to check the health of a culture based on PAMs, settling, and microscope observations at the LCTS.

1.1. Related Procedures

Pond Sampling and Data Collection	LC-01-003-001
Microscopy	LC-06-001-003
Preview Tube Assays	LC-06-001-005
After Action Review	LC-06-001-010

1.2. Procedure impacts and concerns

Safety	Proper PPE for this procedure: safety glasses, safety toe shoes and nitrile gloves. The MSDS/SDS for chemicals used in this SOP should be reviewed.
Quality	NA
Delivery	NA
Environmental	NA
Cost	NA
Compliance	Compliance with OSHA's Hazardous Waste Operations and Response, and Hazardous Communication Standard in addition to the Sapphire Energy, Inc. Chemical Hygiene Plan is required. See 29 CFR 1910.120 and 1200. An authorized user list, MSDS/SDS's and label information will be available for easy reference in a binder in the administration building.

1.3. Responsibilities and owners

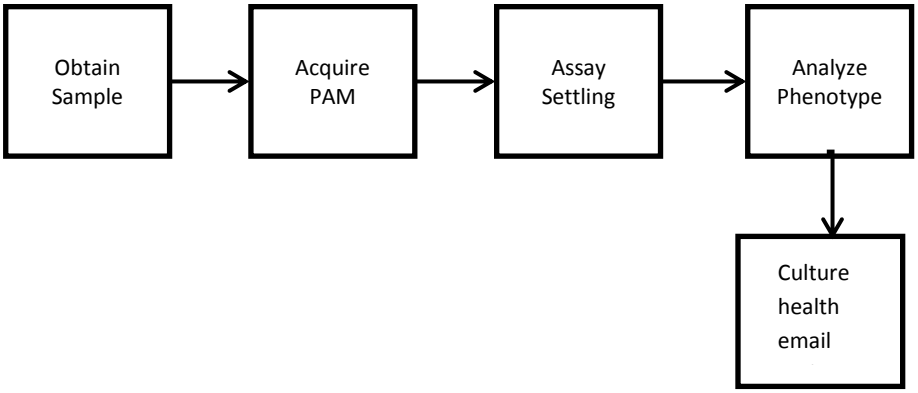
Document Owner	Manage content and distribution	Alex Diffley
Process Owner	Responsible for content and process validation	Alex Diffley
Site Manager	Responsible for implementation and conformance	Becky Ryan

2. Process

2.1. Process description

This process explains the steps that need to be followed when determining the health of an algal culture. The health of a culture can be negatively affected by either unfavorable environmental conditions or by biological contaminations. It is important for the operator of this SOP to distinguish between two measures that are objective (PAM and fluorescence) and two measures that are subjective (microscope observations and settling). The subjective measures will require a degree of training to ensure the user is familiar with criteria mentioned in this SOP.

2.2. Process diagram: Work Instruction



PAM and fluorescence data is collected daily by QAQC personnel.

2.3. Process steps

2.3.1. Receive Sample for Health Check

Obtain a minimum 500 mL sample from the appropriate raceway, minipond and/or PBR.

2.3.2. Acquire PAM on Culture

Submit this sample to QAQC, see **Pond Sampling and Data Collection** SOP.

2.3.2.1. A healthy green algae culture has a PAM yield between 0.6 and 0.7. PAM yields below these levels may suggest culture stress.

2.3.2.2. It is also important to note individual Fo and Fm values. Low Fo and Fm values can sometimes result in artificially high yield.

2.3.2.3. Look at recent data that has been collected on the culture and assess the trend in PAM yield. A healthy culture has a stable or increasing PAM Yield trend.

2.3.3. Perform a Settling Assay on Culture

2.3.3.1. Place 50 mL of culture in a 100 mL glass test tube and let the sample sit for 60 minutes.

2.3.3.2. Take a picture of the sample every 15 minutes.

2.3.3.3. Measure the amount of sediment that has settled to the bottom of the test tube.

2.3.3.4. Since this assay is largely qualitative, report observations to the raceway manager and allow them to interpret the settling rate of the culture.

2.3.4. Analyze the Phenotype of the Cells

2.3.4.1. Reference the **Microscopy** SOP to set up the microscope and load a 20 uL sample on a slide.

2.3.4.2. Begin by viewing the sample using the 10X objective. This objective should be used to search for larger eukaryotic organisms such as rotifers and grazing ciliates. This objective can also be used to document any flocculation that is observed. Record all observations in a scope observation sheet log located here: N:\Production\Scope Observations. Log sheet information will additionally be added to daily data forms for each production group.

2.3.4.3. Switch the objective to 40X. This objective should be used to observe algae characteristics such as pigmentation and morphology. This objective is also useful to search for smaller pests that tend to attach to the cell wall of algae

(e.g. chytrids) or pests that consume smaller algae (e.g. amoeba). Record all observations.

2.3.4.4. After completing microscope observations, consult with the raceway manager and a member of the crop protection team. Final discretion on actions to take based on qualitative observations is up to the crop protection manager and the raceway manager.

2.3.5. Write a Brief Summary on Culture Health

2.3.5.1. After consulting with the appropriate managers, a brief summary of the observations that were made and any actions that were taken should be communicated to the site manager, all production managers, and the crop protection manager through email.

2.3.6. Process Summary

2.3.6.1. The purview of this SOP is to designate a culture as healthy or not. What to do if a culture is unhealthy is in the purview of the Crop protection manager and pond manager's discretion. A number of courses can be initiated at the discretion of the crop protection and pond manager which range from crop protection experiments such as preview tubes, see Preview Tube SOP. Depending on the severity of health, a process interruption may be declared in which an after action review will be initiated, see After Action Review SOP.

3. Required documents

3.1. Input documents

LC-01-003-001.R3 - Pond Sampling and Data Collection - Sample Submission Form	(N:\Cultivation Group\SOP's\Active Documents)
LC-01-003-001.R3 - Pond Sampling and Outdoor Data Collection – Data Collection Form	

3.2. Output documents

n/a

4. Document control

4.1. Revision history

R0 – Initial Release – Salvador Lopez	02-04-2014

4.2. Document approval

Becky Ryan	02-21-2014
Eric Ruvalcaba	02-21-2014

4.3. Document reviewers

Heather Fancher	2-4-14

5. Risk analysis

NA