

1. Procedure summary

This process describes how to assemble carboys for use in culture room at the LCTS.

1.1. Related Procedures

Autoclave Usage

LC-01001-019

1.2. Procedure impacts and concerns

Safety	Proper PPE for this procedure: safety glasses, safety toe shoes and gloves. Nitrile gloves should be worn when handling pond samples. The MSDS/SDS for chemicals used in this SOP should be reviewed.
Quality	Sterile carboys are vital in order to prevent contamination so that the integrity of our cultures can be maintained.
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 users list, MSDSs and label information will be available for easy reference in a binder in the administration building.

1.3. Responsibilities and owners

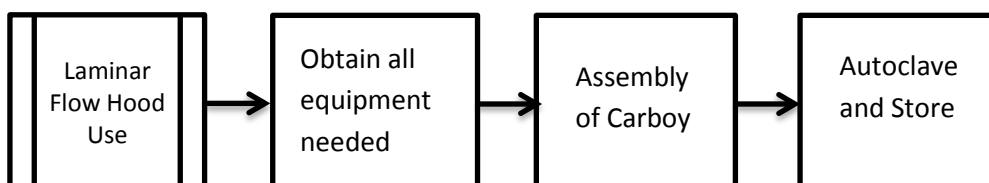
Document Owner	Manage contentment and distribution	Thomas A. Holguin
Process Owner	Responsible for content and process validation	Abraham Anderson
Plant Manager	Responsible for implementation and conformance	Becky Ryan

2. Process

2.1. Process description

The first step in the process is to obtain all the necessary cleaned equipment listed in Appendix 1. The top side of the carboy lid is then assembled and prepped for the autoclave. Then the underside of the carboy lid is assembled and screwed lightly on to the carboy. Lastly the carboys are autoclaved, Autoclave Usage SOP should be referenced, and are stored ready to be used in the LCTS culture room.

2.2. Process diagram: Work Instruction



2.3. Process steps

2.3.1. Obtaining all equipment needed.

2.3.1.1. See Appendix 1 for list of equipment needed and photos. Obtain clean equipment off of the carboy rack in the media prep room. A carboy is cleaned with Liquinox Soap and water and thoroughly rinsed three times. Clean equipment consists of equipment that has been cleaned with diluted bleach and has been rinsed several times to ensure there is no residual bleach.

**Do not use bleach when cleaning carboy.*

2.3.2. Assembly of Carboy Lid.

2.3.2.1. Obtain two, 5" segment of tubing and a purple double check valve. On the first 5" segment of tubing, wrap one end of the tube with aluminum foil and use autoclave tape to affix the aluminum foil to the tube. On the second 5" segment of tubing, place an AcroVent air filter on one end. Then rap both ends of the double check valve with aluminum foil and use autoclave tape to affix both ends of the double check valve.

2.3.2.2. On the top of the Carboy lid is the word "NALGENE". Place the tube with the AcroVent air filter on the carboy lid on the nipple end near the N on the carboy lid. Place the tube wrapped in aluminum foil on the lids nipple on the other end near the E. Place the double check valve in the nipple between the tubes, make sure that the narrow end of the check valve is on the inside on the carboy lid. See **Figure 1**.



Figure 1. Assembly of Carboy Lid with arrows pointing to where tubes should be placed, where the double check valve is inserted, and how the foil and tape are applied.

2.3.2.3 On the underside of the carboy lid, place the sample tube on the nipple corresponding with the double check valve. Then place the aeration glass tube on the nipple corresponding with the air filter tube. See **Figure 2**.



Figure 2. Assembly of sample tube underside lid.

2.3.2.4. See **Figure 3** for complete carboy lid assembly.

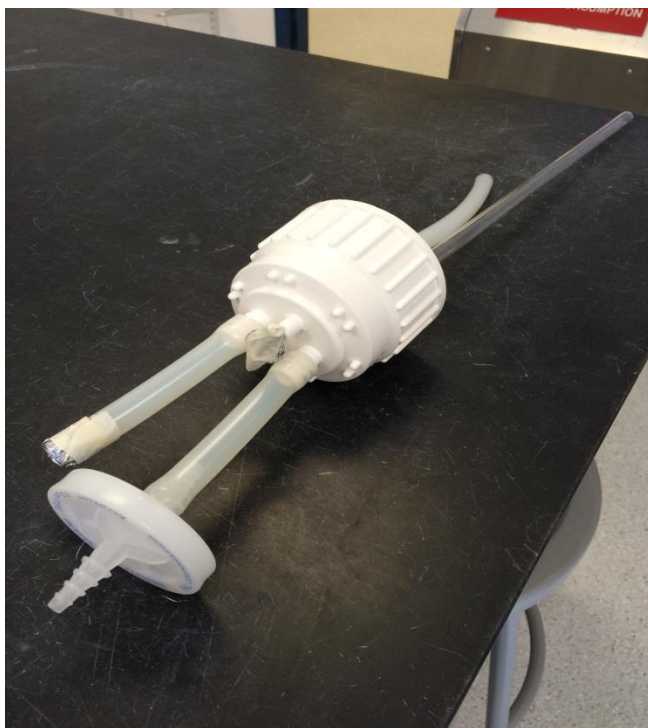


Figure 3. Carboy Lid

2.3.2. Carboy assembly

2.3.2.1. Take the completed assembled carboy lid and place it on to a clean carboy with sample and aeration tube inside carboy. Then place the lid on the

carboy and turn 3 to 4 times. Do not tighten lid all the way. The pressure and heat from the autoclave can damage the carboy. See **Figure 4**.



Figure 4. Completed Carboy

2.3.3. Autoclave and Storage

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2.3.3.1. In order to operate autoclave you **MUST** be trained by facilities. If you are not trained **STOP** and ask for assistance from someone who is. If you are trained, proceed to **2.3.3.2**.

2.3.3.2. Place up to five carboys into autoclave. Ensure they are not contacting each other nor the autoclave. See **Figure 5**.



Figure 5. Carboys placed into Autoclave.

2.3.3.3. Run the autoclave on gravity 1 setting.

2.3.3.4. Remove the carboy from autoclave after cycle is complete. Allow carboy

to cool to room temperature, then tighten down lid to just hand tight.

2.3.3.5. Place the finished carboy on the racks in media prep room until needed in the culture room.



Figure 6. Carboys on Rack

3. Required documents

3.1. Input documents

NA

3.2. Output documents

NA

4. Document control

4.1. Revision history

R0 – Thomas A. Holguin

11/20/2015

4.2. Document approval

Becky Ryan

1/29/2016

4.3. Document reviewers

Abe Anderson

01/28/2016

5. Risk analysis

NA

6. Appendix**6.1.** List of equipment required:

1. Carboy (A)
2. Carboy Lid (B)
3. Sample tube (C)
4. Aeration tube (D)
5. Two tubeflex tubing cut around 5" (E)
6. Purple double check valve (F)
7. AcroVent air filter (G)

