1. Procedure summary

This procedure describes how to mix and then filter sterilize media for carboys at the LCTS.

1.1. Related Procedures

Media Preparation	LC-01-003-014
Carboy Assembly	LC-01-001-007
Autoclave Usage	LC-01-001-019
Flask to Carboy Transfer	LC-01-001-002
Carboy to Carboy Transfer	LC-01-001-008

1.2. Procedure impacts and concerns

Safety	Proper PPE for this procedure: safety glasses, safety toe shoes and gloves. Also consider all PPE needed to remove items from autoclave and while handling items under hood. 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

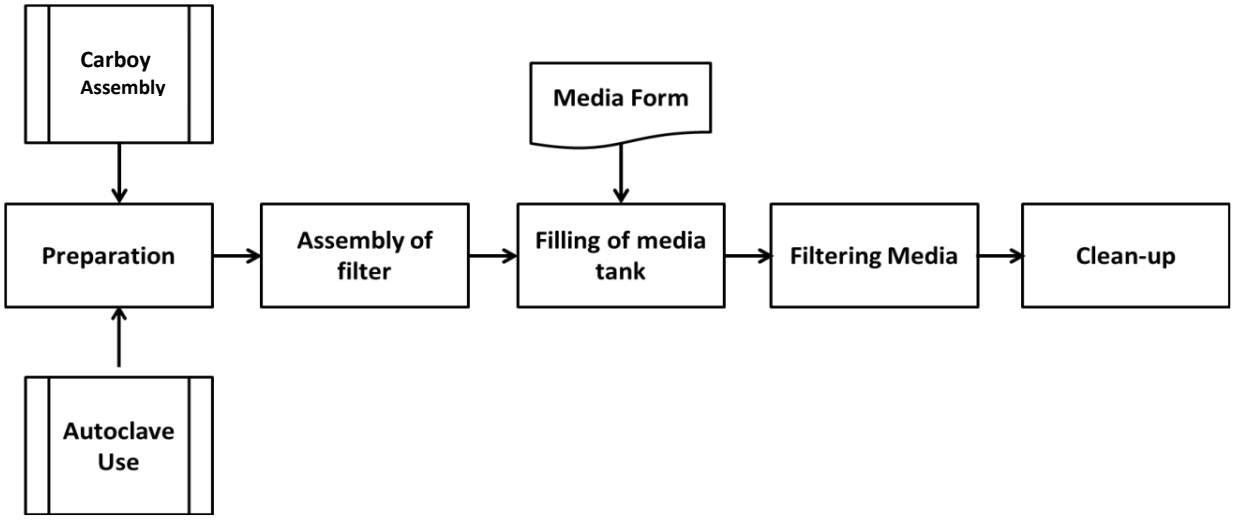
Document Owner	Manage content and distribution	Thomas Holguin
Process Owner	Responsible for content and process validation	Abe Anderson
Site Manager	Responsible for implementation and conformance	Becky Ryan

2. Process

2.1. Process description

Everything in Appendix 1 needs to be gathered to start the process of mixing and ultimately filtering media for carboys. The water and media components will first be mixed in the media tank to a uniform solution. The filter will then be used to sterilize the media as carboys are filled.

2.2. Process diagram: Work Instruction



2.3. Process steps

2.3.1. Preparation

2.3.1.1. Gather all supplies, and equipment. Please see 6.1-Appendix 1 for list.

2.3.2. Filling media tank

2.3.2.1. Refer to Media Preparation SOP to develop appropriate media sheets needed. LC-01-003-014

2.3.2.1. Fill 227 Liter/60 Gallon tank with appropriate amount of water needed. See **Figure 1**.

2.3.2.2. The mixing tank has wheels. After filling up tank, transport to the filtering area.

2.3.2.3. Plug the chord attached to the mixing motor in, and flip the red tab up to access the power switch to the motor. Turn on the motor so the mixer can start mixing the water before you add any components. See **Figure 2.**

2.3.2.4. Add appropriate media components to make the desired media.

Note: *The 60 gallon tank is used for large amounts of media (multiple carboys). For smaller volume, there is a 7 gallon tank and/or other containers in the Media prep room and Culture room.*

Figure 1. Mixing tank and 1” valve on bottom of mixing tank.

Figure 2. Power switch to the motor for the mixer.

2.3.3. Assembly of filter (refer to figure 1.)

2.3.3.1. Remove top of filtering canister.
2.3.3.2. Push filter into round opening.
2.3.3.3. Connect filter canister.
2.3.3.4. Connect hoses to both elbow openings.
2.3.3.5. Place foil over all openings and use autoclave tape for reinforcement.
2.3.3.6. Place filter canister into autoclave tray.
2.3.3.7. Autoclave filter: Refer to autoclave usage SOP LC-01-001-019

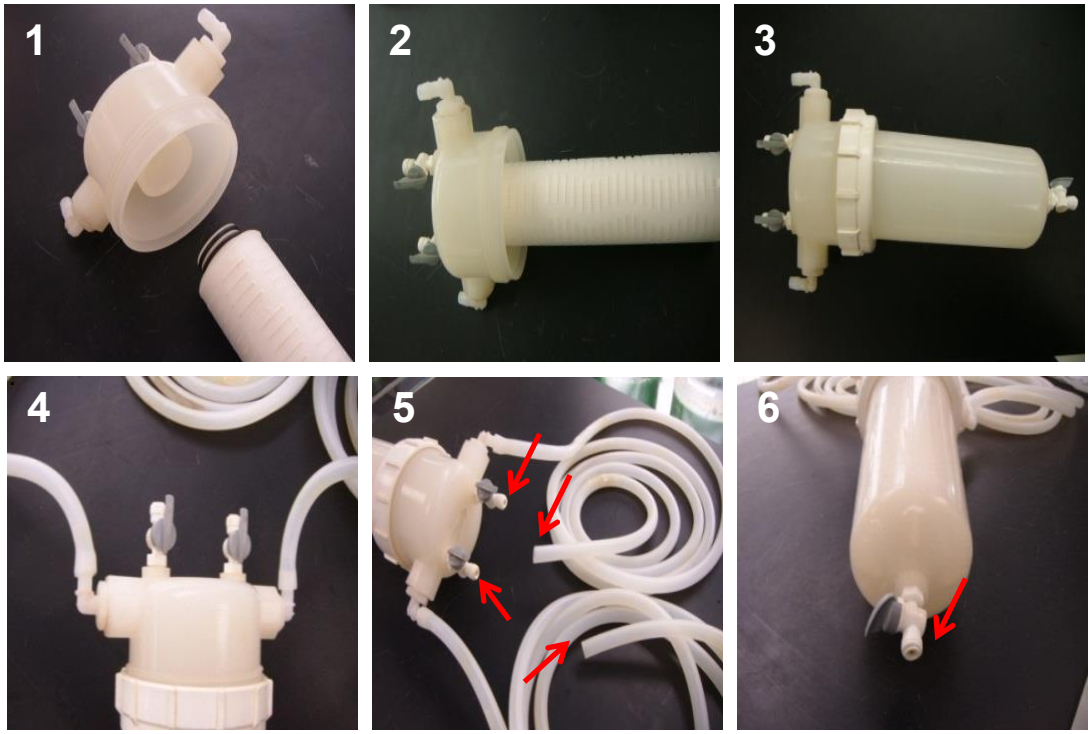


Figure 3. Assembly of media filter.

2.3.3.8. After filter canister has been autoclaved, tighen connection between lid and canister. Complete this before transporting to culture room to eliminate contamination during transport.
2.3.3.9. Transport to culture room and remove filter from autoclave tray; place on rack in culture room dish room.
2.3.3.10. Connect filter to cart. See **Figure 4**.
2.3.3.11. Place filter canister in between your knees for support and snap in brackets as seen in image 1 **of Figure 4**.
2.3.3.12. Tighten screws to connect brackets.
2.3.3.13. Repeat **2.3.2.11-2.3.2.12** on opposite side of filter canister.

Figure 4. Attaching the filter case.

2.3.3.14. Using the arrow on Top of filter canister, connect hose located on the back of arrow into P-pump and close. Connect end of hose on to 1" valve on the bottom of the media mixing tank. Refer to **Figure 1.** to locate valve. If you are not using the large mixing tank, and are using a smaller tank, place end of hose into container and clamp hose on to container. Refer to **Figure 5.** images 2 and 5.

2.3.3.15. From this point in the process, gloves should be worn for sterility and safety. Connect hose located at the point of the arrow to the carboy. Spray ethanol on hose as soon as autoclave tape is off and as you are attaching hose to carboy. Refer to **Figure 5** image 3 & 4.

2.3.3.16. Remove tape from valves on the top of fitler canister and turn the valve to the open position. Refer to **Figure 5**. image 5.

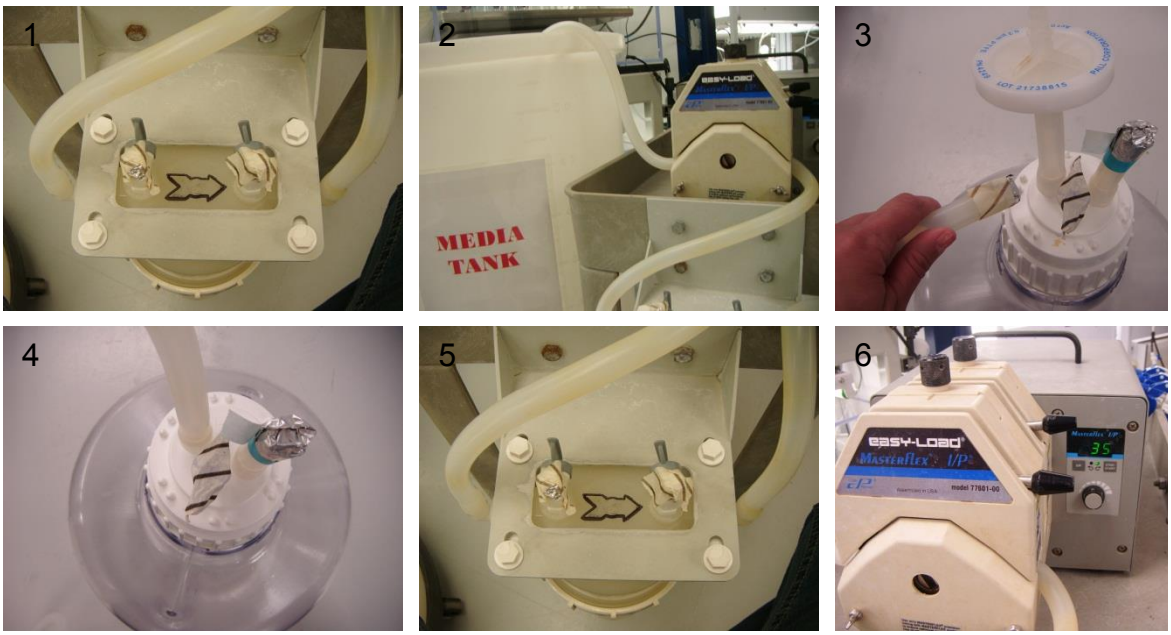


Figure 5. Pump set up.

2.3.4. Filtering Media

2.3.4.1. Turn on pump to speed 35. Refer to **Figure 5**. image 6.

2.3.4.2. Filter will fill up first and water will come out of the top valves. As soon as water

starts to come out turn valve to the close position.

2.3.4.3. When carboy is filled to desired volume press the off botton on the p-pump.

2.3.4.4. Bring a empty carboy and place next to full carboy.

2.3.4.5. In one hand, hold the filter for the carboy and remove the hose, while using open hand to spray 70% Ethanol as hose is being removed and carboy filter is being replaced.

2.3.4.6. Immediately move hose to empty carboy and attach.

2.3.4.7. Refer to SOP for Carboy to Carboy transfer or Flasks to Carboy transfer for any desired inoculation. LC-01-001-002 LC-01-001-008

2.3.4.8. Repeat until all media is filtered.

2.3.5. Clean Up

2.3.5.1. Unplug p-pump.

2.3.5.2. Remove hosing from filter canister and drain out any excess media into media tank.

2.3.5.3. Unscew filter canister and open over sink to drain excess media.

2.3.5.4. Filter canister and hosing should be taken to dish room for cleaning.

2.3.5.5. Media tank needs to be triple rinse and left to dry.

3. Required documents

3.1. Input documents

NA

3.2. Output documents

NA

4. Document control

4.1. Revision history

R0 – Initial release - Thomas A. Holguin 1/25/2016

4.2. Document approval

Becky Ryan 2/1/2016

4.3. Document reviewers

Abe Anderson 1/29/2016

5. Risk analysis

NA

6. Appendix 1

6.1. List of Supplies and Equipment:

1. Filter canister and filter cartridge
2. Hoses for filter canister
3. 2 spray bottles of ethanol
4. P-pump
5. Cart with filter canister connectors
6. Media components