1. Procedure summary

This procedure details the proper uses and methods of using the autoclaves to sterilize lab ware, media, lab waste, or other solids/liquids that need autoclaving

1.1. Related Procedures

Making Media/Solutions from Recipe Sheets	LC-06-003-005
Carboy assembly	LC-01-001-007

1.2. Procedure impacts and concerns

Safety	The autoclave uses high heat and high pressure to sterilize. The user must be trained by a person authorized to conduct such training and must be sure to follow the correct procedures to ensure safe use. PPE: Safety glasses, Orange Full-forearm Oven Gloves Compliance with OSHA's Hazardous Waste Operations and Response, and Hazardous Communication Standard in addition to the Sapphire Energy, Inc. Chemical Hygiene Plan is require (see 29 CFR 1910.120 and 1200).
Quality	It is the user's responsibility to ensure the autoclave runs the full desired cycle. Incomplete cycles can result in non-sterilized articles.
Delivery	N/A
Environmental	N/A
Cost	N/A
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 require (see 29 CFR 1910.120 and 1200).

1.3. Responsibilities and owners

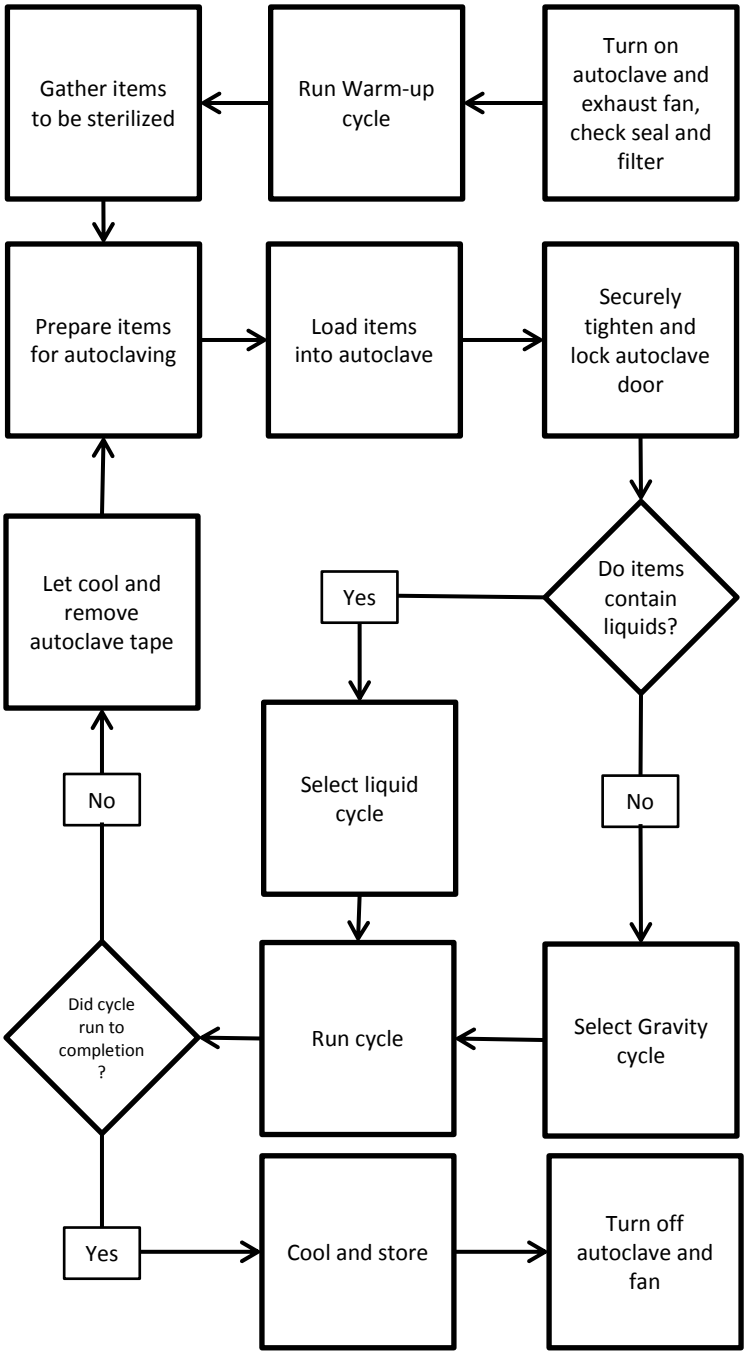
Document Owner	Manage Content and Distribution	Phil DeBroux
Process Owner	Responsible for content and process validation	Phil Lee
Site Manager	Responsible for implementation and conformance	Becky Ryan

2. Process

2.1. Process description

Using an autoclave is a technique to properly sterilize lab items that may be contaminated by biological matter. Items to be sterilized can include lab glassware, media that will be used to grow pure biological samples, and biological waste resulting from experiments. The autoclave works by pressurizing its chamber to 30 psi and heating water into steam. This combination will completely incapacitate any living organism inside, resulting in a sterile container, media, or waste. However, care must be taken to ensure this pressure and steam cannot escape, which would be very unsafe.

2.2. Process diagram: Work Instruction



2.3. Process steps

1. Check the door seal and remove any build up that could prevent a good seal with the door gasket.
2. Check the filter inside the autoclave and remove any debris present by unscrewing the filter and placing debris in appropriate waste container.
3. If autoclave has already been run today and is warmed up, skip to step 6. Otherwise, continue to step 4 for set up.
4. Turn on the autoclave jacket by pressing the button which says “Jacket is off”. This should turn on the jacket and the generator as well. The jacket button should now saw “Jacket is on” and to the right of it, the generator button should say “Generator is on”. If it does not, press the generator button too. Turn the ceiling hood fan on to high with the knob on the wall to the right of the autoclave.
5. Fully close the door by sliding the lever that will close the locking mechanism. Then twist the 3 handles of the securing mechanism to hold the door in place. Tighten this handle to ensure a tight seal.
6. Run the warm up cycle. To find the cycle, press ‘Select Cycle’, then enter your user passcode, select ‘Gravity Cycles’, select ‘More Gravity Cycles’ and select ‘Warm Up cycle’. Confirm the door is closed and run the cycle.
7. Gather together all of the items you wish to autoclave. These will need to be separated on the basis of whether or not they contain liquids. There are special cycles for items that contain liquids. Dry items can be run on these liquid cycles too, but items with liquids cannot be run on Gravity cycles.
8. Prepare items for autoclaving. Specific items have individual requirements but some steps apply to everything that goes in. These can be found in the appendix along with cycle run times.
    a. Tape a small (one to two inch long) piece of autoclave tape to each individual item. Turn one end of the tape up and stick it over on itself to create a non-sticky tag end that can be easily removed.
    b. Loosen any lids so that air can escape, but not so much that they can come off. Tighten the lid fully and then turn back a half to a full turn to accomplish this.
    c. Place loose items in plastic trays but ensure they are not touching each other. Items with sides touching can superheat and melt. If a liquid cycle is being run, put 1-2 inches of water in the bottom of the tray to distribute heat.
9. Load everything into autoclave.
10. Close the door as previously described in step 5.
11. After the door is locked, input the selected user password and proceed to selecting the desired cycle. If any of the objects inside contain liquids (such as media to be sterilized, or carboys of water) a liquids cycle must be chosen. Vessels empty of liquids can also be run in the liquids cycle with these objects. If the individual volumes of liquid are a liter or less, Liquids Cycle 1 is the appropriate cycle to select. For larger volumes, refer to the autoclave manual (pp. 36). If none of the objects contain liquids, select the Gravity Cycle 1. The autoclave screen will ask for confirmation that the door is closed. If it is locked and secured tightly, press yes.
12. The autoclave will run. If there is a problem during the operation, such as a loss of pressure or it is unable to maintain the required temperature in the interior, it will abort the cycle. If this happens, wait until it cools down. Then open it, remove the items and re-tape them (normally the tape will change

Note: Before you can get a user passcode and be authorized for autoclave use, you must undergo training.

color even in an aborted cycle). Replace the items and go back to step 9.

13. After a successful run and the pressure inside has dropped to .1 psi, open the autoclave door and let the steam escape. Allow to door to sit semi-opened for 10-15 minutes, or as long as it takes for the items to cool down. Sometimes the pressure will drop negative and in this case, the door will not open immediately. Allow the chamber pressure to match atmospheric pressure (0 psi) and then open the door. The items inside will be very hot, so do not attempt to remove them immediately. Glass bottles containing liquids may still be very hot at this point and will need extra time to cool down.
14. Items can now be removed and placed on the counter to finish cooling down. Take the orange forearm heat gloves from the rack next to the autoclave and put them on before attempting to remove any items.
15. Once cooled down to the point where they can be comfortable touched with bare hands, tighten lids finger tight.
16. At the end of the day, turn off the autoclave by pressing the “jacket off” button and turn off the exhaust fan.
17. Other parts to note on the autoclave
    a. Removable rack – There is a removable rack to divide the space in half for shorter items, like trays of flasks, to allow more to fit.
    b. Print feed – The autoclave keeps a record every minute it is in use of the chamber temperature and pressure, along with what cycles are running.
    c. Fan – The fan above the autoclave should always be on while the autoclave is running to help dissipate steam and heat.

3. Required documents

3.1. Input documents

N/A

3.2. Output documents

1. Autoclave print roll will automatically record autoclave activity for later documentation. It records users selecting cycles, temperature and pressure during operation of any cycle, and opening and closing of doors.

4. Document control

4.1. Revision history

R0 – Initial release – Phillip DeBroux	10/16/2013

4.2. Document approval

Becky Ryan	11/8/2013
Eric Ruvalcaba.	11/8/2013

4.3. Document reviewers

John Fissel	10/28/2013
Phil Lee	10/31/2013
Jeff Huges	11/8/2013

5. Risk analysis

6. Appendix

Individualized preparation of different autoclavable items

a. **Empty Flasks and Glass bottles** – Attach a piece of autoclave tape on each lid. Ensure the lid is loose. Load into plastic tray so sides of flasks and bottles are not touching other flasks or bottles. Run on Gravity 1.
b. **Glass bottles containing media to be sterilized** – Attach a piece of autoclave tape on each lid. Ensure the lid is loose. Load into plastic tray so sides of bottles are not touching other bottles. Fill bottom of tray with 1-2 inches of tap water. This will allow the glass to heat more evenly and reduce cracking. Run on Liquids 1.
c. **Carboys of Deionized water** – Attach a piece of autoclave tape on the lid. Ensure the lid is loose. Place directly onto the floor rack of the autoclave. Run on Liquids 3.
d. **Empty Assembled Carboys** – Make sure the filter apparatus on top of the carboy is assembled and all hoses, valves and places where are could escape are taped closed. Ensure lid is loose. Load up to 6 carboys directly onto the floor rack of the autoclave. Run on Gravity 1.
e. **Bags of biological waste from lab** – Twist the top of the bag shut and tape closed. Fold over and tape again with autoclave tape. Place bag in plastic autoclave tray. Run on Gravity 1.

Gravity Cycles			
Name	**Temperature**	**Time**	**Dry Time**
Gravity 1	250.0° F	30 minutes	5 minutes
Gravity 2	250.0° F	45 minutes	20 minutes
Gravity 3	250.0° F	60 minutes	30 minutes
Warm Up	250.0° F	5 minutes	5 minutes
Liquid Cycles			
Name	**Temperature**	**Time**	**Exhaust Cycle**
Liquids 1	250.0° F	30 minutes	Variable time
Liquids 2	250.0° F	45 minutes	Variable time
Liquids 3	250.0° F	60 minutes	Variable time