

## 1. Procedure summary

This procedure describes how to filter sterilize media.

### 1.1. Related Procedures

Laminar Flow Hood Use

LC-01-001-018

### 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	Using proper sterile technique will eliminate contamination.
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's and label information will be available for easy reference in a binder in the administration building.
Anomaly Reporting	NA

### 1.3. Responsibilities and owners

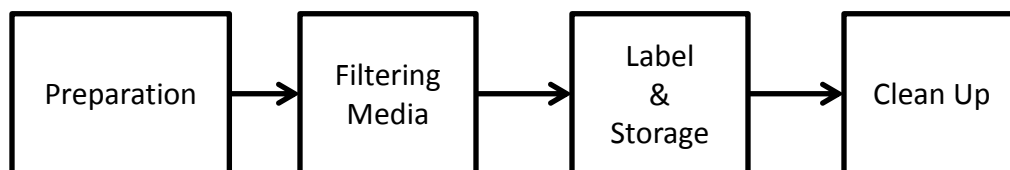
Document Owner	Manage content and distribution	Thomas A. Holguin
Process Owner	Responsible for content and process validation	Abraham Anderson
Site Manager	Responsible for implementation and conformance	Becky Ryan

## 2. Process

### 2.1. Process description

All of the supplies needed should be gathered and sprayed generously and wiped with ethanol. Media is added to the top cup of the bottle top vacuum sterile filter and then a vacuum attached to the neck. Sterilized media will be collected in the lower bottle and is ready to be capped labeled and stored.

## 2.2. Process diagram: Work Instruction



## 2.3. Process steps

### 2.3.1. Preparation

**2.3.1.1.** Gather all supplies and equipment, (prepared media, air pump, bottle top vacuum sterile filter, desired media containers, two spray bottles of 70% Ethanol and label maker) making sure everything is staged near the Laminar Flow Hood. See **Figures 1 and 2.**



**Figure 1.** 70% Ethanol spray bottle, bottle top vacuum sterile filter and label maker.



**Figure 2.** Air pump; electrical outlet.

**2.3.2. Sterilize Filtering media for flasks**

**2.3.2.1.** See Laminar Flow Hood Use SOP for Proper Hood Use.

01-001-018

**2.3.2.2.** Turn on electric switch for hood allowing lights and vent to turn on. If hood has a UV light, it should be turned off at this point.

**2.3.2.3.** Spray all supplies with 70% ethanol then immediately place into hood.

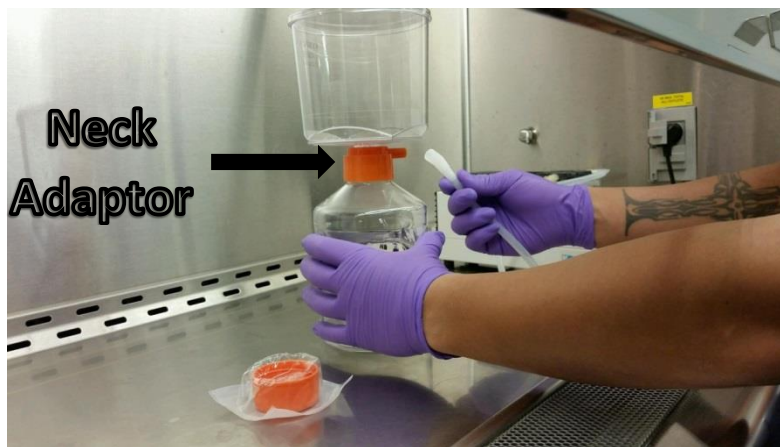
**2.3.2.4.** Spray hands (with nitrile gloves on) generously with 70% ethanol, then immediately place in hood.

**2.3.2.5.** Plug air pump into electrical outlet inside hood.

**2.3.2.6.** Remove bottle top vacuum sterile filter from bag.

**2.3.2.7.** Make sure the neck adaptor in between top filter and desired bottle on bottom is screwed on tight. **Figure 3.**

**2.3.2.8.** Connect hose from air pump to hose connector on filter. **Figure 3 and 4.**



**Figure 3.** Neck adaptor and air hose connection.



**Figure 4.** Connection of air pump hose to filter.

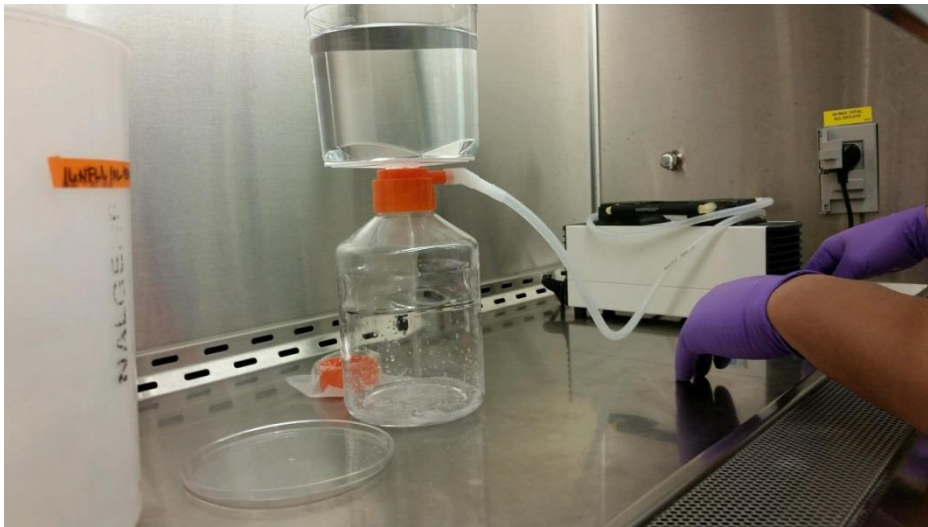
**2.3.2.9.** Remove thin plastic lid from top of filter.

**2.3.2.10.** Carefully pour media to fill line on filter cup. **Figure 5**



**Figure 5.** Media being poured into filter cup.

**2.3.2.11.** Turn on pump, monitoring media as it filters. If media is pulled through filter too fast, the filter may tear or rip. **Figure 6**



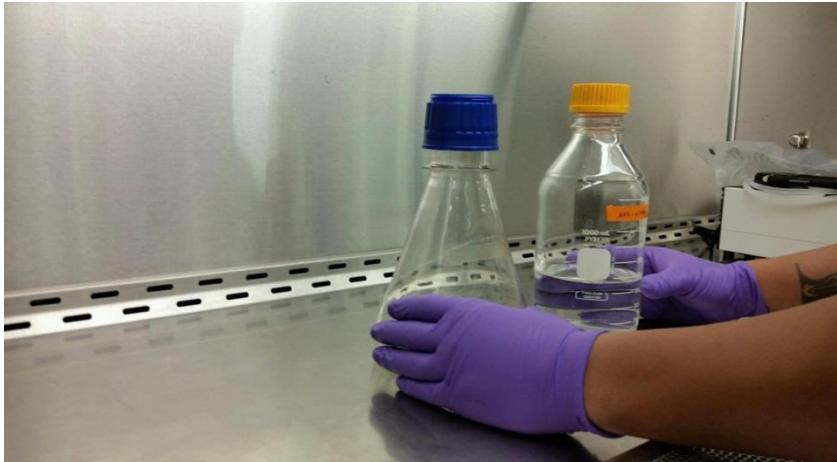
**Figure 6.** Turn pump on and observe media being filtered into container.

**2.3.2.12.** Once media is completely filtered, turn off pump and inspect filter membrane for rips or tears. If rips or tears are observed, re-filter media with a new filter.

**2.3.2.13.** Remove hose from hose connector on filter.

**2.3.2.14.** Unscrew top filter cup and replace it with a sterile screw top lid.

**2.3.2.15.** If storing media skip to 2.3.3. If you wish to pour media into sterile flasks for inoculation, pour filtered media into desired sterile container(s). See **Figure 7 and 8.**



**Figure 7.** Sterile Flask on left; filtered media on right.



**Figure 8.** Filtered media being-poured into sterile flask.

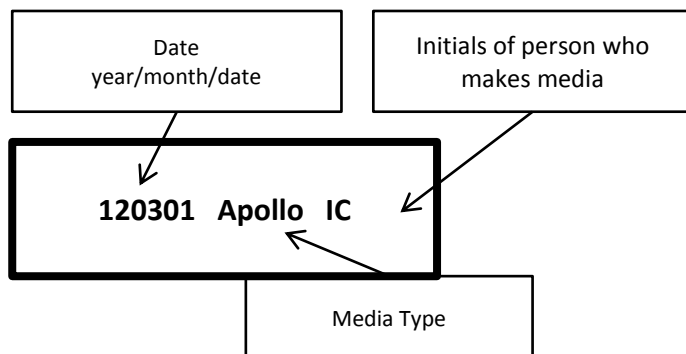
### **2.3.3. Labeling and Storage**

**2.3.3.1.** Ensure tight seal on all media bottles and remove from hood.

**2.3.3.2.** Label with date, media type, and your initials. See **figure 9** for example.



**Figure 9.** Example of label



**2.3.3.3.** Store media in deli fridge or media room located in lab building 200.

**2.3.4. Clean Up**

**2.3.4.1.** Remove all waste from hood and dispose.

**2.3.4.2.** Spray hood with 70% ethanol and wipe down.

**2.3.4.3.** Close hood and turn off electric switch. If hood has a UV light it should be turned on at this point.

**UV light helps prevent contamination inside hood.**

**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