

1. Procedure summary

This procedure describes how to pour media into a petri dish.

1.1. Related Procedures

Autoclave usage

LC-01-001-019

Using the Laminar Flow Hood

LC-01-001-018

1.2. Procedure impacts and concerns

Safety	Proper training for Autoclave and Hood use needs to take place prior to pouring media.
Quality	Using proper sterile technique will eliminate contamination on plates.
Delivery	NA
Environmental	NA
Cost	NA
Compliance	All PPE needed to remove items from autoclave and while handling items under hood. 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.

1.3. Responsibilities and owners

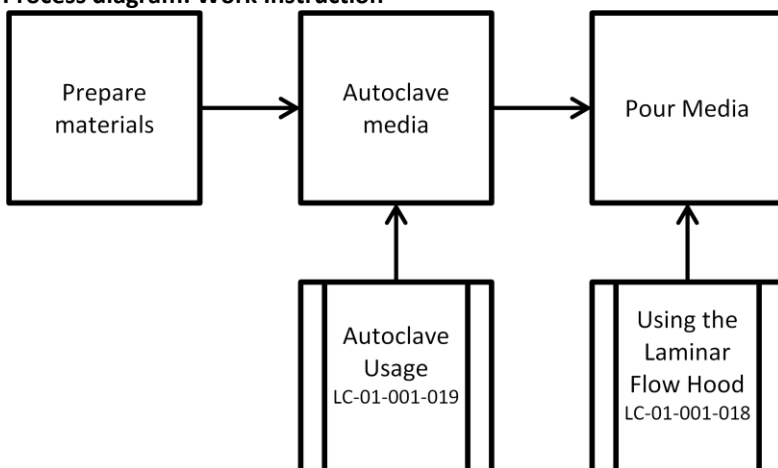
Document Owner	Manage content and distribution	Irene Calderon
Process Owner	Responsible for content and process validation	Alex Diffley
Site Manager	Responsible for implementation and conformance	Bryn Davis

2. Process

2.1. Process description

This process describes the method used to pour media into petri plates, using a sterile method. Sterile method is used to keep contaminants from growing on the agar plates.

2.2. Process diagram: Work Instruction



2.3. Process steps

2.3.1. Preparation and Transport.

2.3.1.1. Gather all supplies, equipment, and supplies.

2.3.1.1.1. Appropriate media bottles, sleeves of round petri plates, tape, 2 spray bottles of 70% ethanol.

2.3.1.2. If materials are in other areas on site make sure to transport in autoclave bag to culture room.

Transporting of any supplies, equipment, or media from other buildings needs to be placed in autoclave bag. This helps to reduce contaminants introduced to culture room.

2.3.2. Autoclaving Media.

2.3.2.1. Fill glass media bottle with desired media. Make sure media bottle cap is loosened before placing into autoclave.

2.3.2.2. Place in autoclave and turn on using the liquid setting. Refer to Autoclave Usage SOP.

2.3.2.3. Use gloves to remove media bottle from autoclave. Bottle will be very hot, use caution when handling.

2.3.2.4. Tighten lid on media bottle.

2.3.2.5. Allow bottle to cool at room temperature until media bottle is warm to touch. Monitor bottle temperature closely, if media is cooled to a low temperature it will solidify.

2.3.2.6. Once media is warm to touch, spray media bottle with ethanol, and place into hood.

Media bottle should be warm to the touch before pouring. If media is too hot when poured it will cause large amounts of condensation.

2.3.3. Pouring of Media into Petri Plates.

2.3.3.1. All media pouring into plates must be done in a sterile flow hood. See Using Laminar Flow Hood SOP. Gloves are required from this point on in the process.

2.3.3.2. Spray sleeves of petri plates and spray bottle. After spraying immediately place item into hood.

2.3.3.3. Spray arms and hands with ethanol. After spraying immediately place into hood.

2.3.3.4. Tear open top of sleeve and gently remove petri plates. Stack plates to one side.

2.3.3.5. Loosen lid on media bottle.

2.3.3.6. Place the petri plates into 3 stacks.

2.3.3.7. Use right hand to hold media bottle.

2.3.3.8. Use left hand to remove lid of plate.

2.3.3.9. Gently pour media into plate and fill to line on the inside of the plate. After pouring a few plates gently swirl media bottle to keep media mixed thoroughly. Repeat until all media is poured into plates.

2.3.3.10. Plates should remain in hood for at least 2 hours, to allow media to solidify.

2.3.3.11. Remove media bottles and trash in hood. Keep plastic bag from sleeves of petri plates in hood, bags will be used for storage.

2.3.3.12. After 2 hours, place plastic sleeve over plates. Seal opening of bag with tape.

2.3.3.13. Label sleeve with media type, date, and initials.

2.3.3.14. Place into autoclave bag and transport to storage in deli fridge located in lab.

When pouring you observe bubbles in media, lightly spray ethanol in the air above the plate. This should remove all bubbles from media before placing lid on plate.

3. Required documents**3.1. Input documents**

NA

3.2. Output documents

NA

4. Document control**4.1. Revision history**

R0 – Irene Calderon

04-27-2012

R1 – Robert McBride

04-30-2012

R2 – Irene Calderon

05-07-2012

4.2. Document approval

Robert McBride

05-07-2012

4.3. Document reviewers

Megan Allen, Irene Calderon, Alex Diffley, Robert McBride

04-30-2012

5. Risk analysis