**SOP Liquid Sparging Protocol**

**Document Number: XX**

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**Purpose**

This SOP describes the procedure used to prepare pre-reduced liquid media or buffer

**Scope:** Pre-reduced liquid BHI media is use to grow anaerobes and create stock of isolates. Sparging makes liquid media or buffer immediately usable to grow anaerobes.

**Regulatory References:** NA

**Responsibility of experimentalist:** understanding and performing this procedure as described; reporting any deviations or problems to area supervisor; adequately documenting the procedures and results.

**Responsibility of area manager or supervisor:** ensuring that the analyst performing this procedure is qualified; ensuring that the procedure is followed, and updating the procedure as necessary.

**Definitions/Abbreviations**

Brain Heart Infusion: BHI

**Related Documents:**

Anaerobic chamber SOP

Laminar Flow hood SOP

**Required Equipment and Materials / Reagents**

* Nitrogen gas (AirGas, NI300)
* Long needles (VWR, 22 gauge 6 inches long, Cat# 89234-304)
* Short syringe needles (BD; Cat# 305196)
* Serum bottles (VWR, Cat# 16171-385)
* 20mm crimp tool (VWR, Cat# 66030-804)
* Ethanol (Sigma-Aldrich, Cat# 24102)
* Rubber septa
* Serological pipettes
* Flame
* Forceps
* Blotting paper

**Precautions**

Personal protection equipment including sterile gloves and lab coat must be worn when executing this procedure.

Because of the use of pressurized gas, inexperienced operators need to observe the procedure executed by an experienced operator

**Procedure**

In laminar flow hood:

1. Aliquot media/buffer into an autoclaved serum bottle

Note 1: minimum volume ~30mLs (otherwise it can be tricky for the needles to reach); maximum volume = 100mLs

1. Flame forceps
2. Place a rubber septa on top of the serum bottle and then press it down into the mouth of the bottle using the forceps
3. Flame forceps
4. Place aluminum crimp on bottle using forceps
5. Use crimping tool to press the crimp onto the bottle
6. Flame forceps
7. Lift tab of crimp to expose top of rubber septa

Note 2: We use tear-away crimps so try to lift the tab until it is standing up right, pulling it further will tear the crimp off the bottle

1. Bring bottle over to anaerobic chamber where Nitrogen gas lines are set up for sparging

By anaerobic chamber:

1. Check to make sure the Nitrogen gas is running to the syringes and not to the small chamber
2. Place the serum bottle into a secondary container lined with blotting paper
3. Twist an autoclaved long needle into one of the syringes a the end of the gas tubing

Note 3: Do not to touch the needle while doing this

1. Wrap a few fingers/palm from your dominant hand around the very top of the long needle and pinch with your other had just below this to help you steady and guide the needle
2. Aim the needle for the very center of the circle on the top of the rubber septa

Note 4: If you are concerned about the sterility of the top of the septa you can rub it with a little 70% EtOH before piercing it with the needle

1. Gently pierce through the septa and allow the untouched parts of the needle to enter the bottle. Use your fingers pinched below your palm to help you judge this depth.
2. Open and insert a short needle into the top of the rubber septa to serve as a pressure release

Note 5: The bottle should look like this:



1. Bit by bit open the Nitrogen gas on using the yellow handle on the tank’s regulator until you see bubbles in the media/buffer.

Note 6: You should only need to open it a little bit

1. Let the media/buffer sparge for 30 minutes
2. Close the Nitrogen gas line
3. Remove the long needle from the serum bottle and then untwist it form the syringe

Note 7: This needle can be brought to the dirty dishes bin and washed.

1. Remove the short needle and place it into one of the unscrewable lidded sharp tubs.

Note 8: These needles can not be placed directly into the normal sharps burn box

1. Dispose of the blotting paper in the burn box

Opening your media/buffer post-sparging in the anaerobic chamber:

1. Forceps can be used to pull the tab of the crimp back which will tear off the entire crimp
2. Forceps can then be used to pull the septa out of the mouth of the bottle
3. When aliquoting media/buffer/buffer out of the serum bottle it is advised that you use the aluminum foil wrapped around the bottle prior to opening or from another autoclaved bottle to keep the bottle covered in between aliquots

Note 9: Only the 5 or 10mL serological pipettes will fit into the mouth of the bottle. Any sized pipette-man tip will fit

**Version History**: NA

**Worksheets: NA**

**Appendix:** NA