

# Standard Operating Procedure for Performing a Sterility Test on Lysogeny Broth

SOP#: PPP-01

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## 1. Purpose

This procedure is designed to test the sterility of lysogeny broth (LB). If the broth will be used to grow a specific strain (type) of bacteria, the broth must be sterile. Otherwise, the resulting growth cannot be guaranteed to be the desired bacteria. Bacteria is everywhere, and LB is a media designed to support the growth of bacteria. The results of the sterility test will be recorded on the 'LB Sterility Results Report' document. A failed test would require the technician to trace all experiments that may have used that particular batch of LB. The LB was made previously, transferred into suitable flasks and bottles which are appropriately labeled, and autoclaved as a batch.

Each team member will perform this test using a sterile aliquot of LB that they have been provided. The test will involve a transfer of some of the LB liquid to an LB media plate followed by spreading of the liquid LB on the plate. The spread plate method is a technique to plate a liquid sample onto solid media in order to determine if any bacteria are growing in the liquid media. Bacterial growth on solid media in a plate is easy to see. If the liquid LB media is sterile, there will be no bacterial growth on the LB agar plate. The plates will be incubated for 24 hours at 37°C in an incubator.

## 2. Scope

This Standard Operating Procedure is intended for students using the Shoreline Biotechnology Experience Biomanufacturing Kit.

## 3. Definitions

**Media:** A solution (liquid, gel, or some other type) used to grow bacteria.

**Lysogeny broth (LB):** A liquid growth medium, also called Luria broth, Lennox Broth, or Luria-Bertani medium. The standard recipe contains distilled water plus tryptone, yeast extract, and sodium chloride.

**Batch record:** Any reagent produced in a lab is identified by a batch record so it is traceable in the event of possible contamination or failed experiments. A batch record contains the recipe used to make the reagent, the concentrations of all the ingredients, the source of all the ingredients, the storage conditions and expected shelf life, who made it and when, and information relating to equipment or possible deviations and how they were corrected. This record is kept on file for future reference. The ID

of the batch record is also put on all containers of the reagent produced in that batch. Outside of your lab, you might see it as a lot number.

**Sterility:** The absence of bacteria or other living contaminants.

**Bacteria:** Small single-celled organisms found almost everywhere on earth.

**Bacterial Strain:** A genetic variant or subtype of a microorganism. The example in lab today is the DH5 $\alpha$  strain of *E. coli*, which is one that is commonly seen in biotech labs because it has been bred to have specific mutations useful for inserting plasmids, otherwise known as subcloning or cloning. The DH5 $\alpha$  strain is thus known as a good cloning vector. The DH5 $\alpha$  strain is also non-pathogenic, meaning it will not make humans sick.

**LB Agar Plate:** LB with agar added. The agar allows the media to solidify in the plate. The result is a plate with a layer of a firm gel-like substance called LB agar. The LB agar plate is commonly used in biotech, but particularly for sterility tests.

#### 4. References

Microbiology—004—Spread Plate Method. (n.d.). Microbiology Undergraduate Program. Retrieved April 11, 2022, from <https://www.micro.iastate.edu/video/microbiology-004-spread-plate-method>

Sezonov, G., Joseleau-Petit, D., & D'Ari, R. (2007). *Escherichia coli* Physiology in Luria-Bertani Broth. *Journal of Bacteriology*, 189(23), 8746–8749. <https://doi.org/10.1128/JB.01368-07>

Sterility Test. (n.d.). International Society of BioProcess Technology. Retrieved April 11, 2022 from <https://www.isbiotech.org/about.html>

*SODIUM HYPOCHLORITE* | *CAMEO Chemicals* | NOAA. (n.d.). Retrieved February 14, 2022, from <https://cameochemicals.noaa.gov/chemical/4503>

#### 5. Materials, Reagents, and Equipment

##### 5.1. Materials

- 5.1.1. A plastic or glass container around 500mL in volume for holding the 10% bleach solution.
- 5.1.2. Paper towels
- 5.1.3. Sharpie marker for labelling

##### 5.2. Reagents

- 5.2.1. Lysogeny broth (LB) to be tested
- 5.2.2. LB Agar Plate
- 5.2.3. Bleach
- 5.2.4. 70% Ethanol spray (70% EtOH)
- 5.2.5. Dish soap spray (a spray bottle filled with a little dish soap added to water)

##### 5.3. Equipment

- 5.3.1. Sterile Bacterial spreader
- 5.3.2. Micropipettor and tips
- 5.3.3. Shaker Incubator

#### 6. Responsibilities of all team members

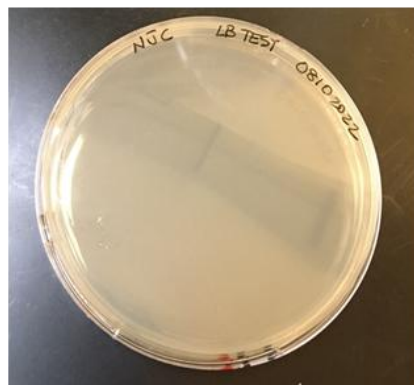
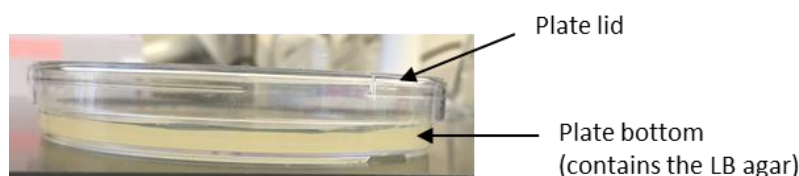
Lab personnel overseeing and performing the procedure are responsible for adhering to current lab safety procedures. Lab technicians must be trained to operate equipment and follow precautions listed below for handling potentially hazardous materials. Aseptic technique should be used. Bacterial waste should be disposed of in the proper manner.

## 7. Hazards and Safety Precautions

- 7.1. *Warning: 10% bleach causes serious eye irritation, potential skin irritant.*
- 7.2. Prevention: Wear Personal Protective Equipment (PPE), avoid contact, ingestion.
- 7.3. Response: Flush eyes with water for up to 30 minutes after exposure, wash hands after exposure.

## 8. Procedure

- 8.1. Pre-procedure preparation
  - 8.1.1. Put on personal protective equipment
  - 8.1.2. Make sure long hair is tied back.
  - 8.1.3. Clear clutter off the lab bench.
  - 8.1.4. Organize your reagents, materials and equipment so they are close at hand.
  - 8.1.5. Spray work surfaces with 70% ethanol (EtOH). Spread the EtOH evenly with a paper towel. Let surfaces dry.
  - 8.1.6. Make sure the windows and doors of the room are closed.
- 8.2. LB Plating
  - 8.2.1. Label a pre-warmed LB plate. The label should include the team member's initials, the date, and the purpose (LB test). Be sure to label the half of the plate containing the LB agar. Write the label on the edge of the plate (not across the middle). See **Figure 1** for an explanation of how to label your plate.



Turn the plate upsidedown.  
Write the label on the bottom of the plate.  
Write the label around the edge of the plate.

Figure 1: How to properly label a bacterial plate.

- 8.2.2. Using a p200 micropipette, remove 50uL of the LB from the container.
- 8.2.3. Carefully open your plate using the clam shell technique with the opening facing away from you. You may want a co-worker to do this for you.
- 8.2.4. Add the LB media to the surface of the LB Agar drop-wise.
- 8.2.5. Remove a sterile spreader from the package.
- 8.2.6. Carefully spread the LB over the surface of the plate.

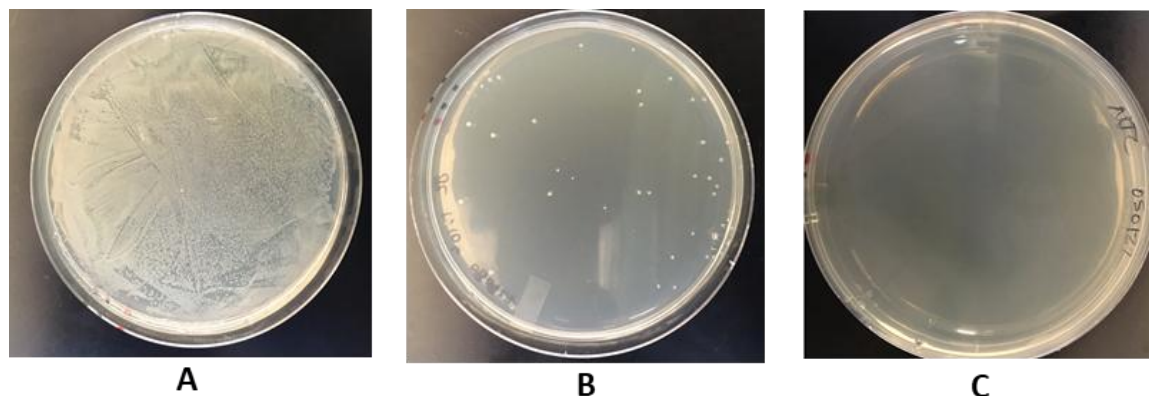


Figure 2: (A) Plate showing a lawn of bacteria. (B) Plate with individual bacterial colonies. (C) Plate with no bacterial growth.

### 8.3. Incubation

- 8.3.1. Incubate the plates in a 37 degree °C incubator for 24 hours. Make sure your plate is upside down in the incubator.
- 8.3.2. After the 24 hour incubation examine your plate and record your results on the LB Sterility Test Results Report. **Figure 2** shows examples of what plates might look like. If you have bacterial contamination, your plate may look like example A or B. If you have no contamination, your plate will look like example C.
- 8.3.3. Each team member should sign and date the Aseptic Technique Check Report
- 8.3.4. Give the Report to the QA Technician to review, sign, and date
- 8.3.5. The QA Technician files the LB Sterility Test Report in the team file.

### 8.4. Waste Disposal

- 8.4.1. Used bacterial spreaders should be disposed of in biohazard trash.
- 8.4.2. Remaining LB should be poured into a beaker containing 10% bleach. This beaker should be kept in the sink to minimize the risk of spills. This mixture should incubate at room temperature for 10 minutes and can then be poured down the sink.
- 8.4.3. After plates have been examined for colonies they should be disposed of in the biohazard trash.
- 8.4.4. All used micropipette tips should be disposed of in the biohazard waste.