A. Media and Contamination

Microbiologists grow organisms on media (singular: medium). Media contain nutrients, salts, and other factors to support the growth of bacteria and other microbes. There are many types of media available for the culture of different microbes. For example, tryptic soy medium is a mixture of partially digested soybean protein and mineral salts that supports the growth of many bacterial species.

Media must be sterilized before use. Sterilization is the removal of all life from a substance or an object. Most media are sterilized by autoclaving, a process in which it is heated at 120°C for about 30 min.

Broth medium, such as tryptic soy broth, consists of nutrients dissolved in water and sterilized. Microbiologists keep broth cultures in glass culture tubes covered with plastic caps. The caps fit loosely to allow free exchange of air while preventing contaminants from entering the tube.

Semisolid medium, such as tryptic soy agar, is formed by adding a gelling agent to a broth medium. When the medium is boiled or autoclaved, the gelling agent dissolves in the broth. As the medium cools, the agent forms a firm gel that molds into the shape of its container. The most commonly used gelling agent is agar, a polysaccharide from seaweed. Very few bacteria can digest agar, so an agar gel will remain stable even in the presence of bacterial growth. In addition, agar gel is stable at a wide range of temperatures. Molten agar remains a liquid until it is cooled to about 50°C. Once it has gelled, it will not melt again unless it is heated to 100°C.

Once it is dissolved and sterilized, semisolid medium is poured into appropriate containers and allowed to gel. Microbiologists often use petri dishes, or plates, to hold semisolid media. Petri dishes are flat, round dishes that are covered with loosely fitting lids. In a petri dish, semisolid medium forms a flat surface that can be used to isolate individual bacterial species from a mixture of organisms.

Microbes are everywhere in nature: in soil and water, on dust particles floating in the air, on the surfaces of laboratory bench tops, and even on the hands of a microbiologist! Therefore, one must handle sterile media properly to prevent contamination by unwanted microbes. Aseptic technique is a way to manipulate cultures to minimize the chance of contamination.

Petri dishes:

When inoculating a petri dish, do not lay the lid on the lab bench or touch the inside of the lid or the dish. You should open the dish just enough to perform the inoculation. When observing colonies on a petri dish, avoid opening the dish. Observe the dish by holding it up to the light, and looking through the transparent plastic.
**Labels:**

Petri dishes are labeled on the bottom, near the edge of the plate. You can use labeling tape, or you can write directly on the plate with a permanent fine-tipped marker. The label must include the name of the sample (for example, “Bench top contaminants”), the initials of the person who inoculated the plate, the group number (each group of four persons will be assigned a number by the instructor), and the date. Always label a culture before you inoculate it.

**Cotton-tipped swab:**

These are useful for sampling microbes on surfaces such as bench tops. The swab can be moistened with sterile water to help remove microbes from the surface.

**Confluent inoculation:**

In this method, the entire surface of agar in a petri dish is swabbed with a cotton-tipped swab. Hold the swab at a small angle (as shown in the picture above) to avoid tearing or digging into the agar.

**Incubation:**

Petri dishes are incubated in an inverted, or “upside-down,” position. In this way, condensation will remain in the lid of the plate, and will not drop onto the surface of the agar and ruin the colonies. Each lab section will be assigned a different shelf in the incubator. Be sure to put your plates on the correct shelf.

---

**B. Broth Cultures**

Broth cultures are a convenient way to grow small quantities of bacteria. In addition, the characteristics of growth in broth culture may be a clue to the identity of the organism. These characteristics include turbidity, sediment, and surface growth.
**Turbidity:**

This is visible growth within a broth culture, often having a cloudy appearance. Turbidity may be spread throughout the tube, or it may be localized in one region of the tube (top, middle, or bottom). It may be uniform, or it may be flocculent, appearing as small “chunks.”

![Turbidity images]

**Sediment:**

Sediment is growth that has settled to the bottom of the tube.

**Surface growth:**

This is growth at the surface of the broth culture. Flocculent surface growth appears as small pieces or chunks of bacterial growth at the surface. A pellicle is a continuous, membrane-like growth that completely covers the surface of the broth. A ring of growth is growth all around the edge of the surface where it touches the inside of the tube.

![Surface growth images]

**Inoculating Loop:**

The loop is sterilized by heating it in a Bunsen burner flame until it becomes red-hot, burning off any cells present. Hold the loop with the thumb and two fingers. Flame the wire completely along its length.
**Culture Tubes:**

When you remove the cap from a culture tube, do not put it down. Instead, hold the cap with the same hand that holds the loop, as shown. This will prevent the cap from being contaminated.

When the cap is removed, briefly warm the lip of the tube in the burner flame. The lip is not heated red-hot, but is warmed only slightly to prevent cold air from being drawn into the tube by convection currents.

**Steps in a Broth Inoculation:**

- Using label tape, label a tube of sterile broth medium with the name of the sample, your initials, your group number, and the date.
- Sterilize an inoculating loop.
- Remove the cap from a turbid stock culture of bacteria.
- Briefly warm the lip of the tube in the burner flame.
- Dip the sterile loop into the broth culture, and remove it.
- Briefly warm the lip of the stock tube, and replace the cap.
- Remove the cap from a tube of sterile broth medium, and briefly warm the lip of the tube.
- Dip the loop, containing the inoculum, into the sterile broth.
- Briefly warm the lip of the tube, and replace the cap.
- **Important:** Always sterilize the loop by flaming before you put it down.
C. Colony Isolation: Streak Plate Method

In order to identify and study a particular bacterial species, microbiologists must first isolate the organism into pure culture. A pure culture is a culture that contains only one microbial species. The most common technique to isolate a pure culture is the streak plate method. In this method, an inoculum is streaked onto the surface of an agar medium in a petri dish. If the streak is successful, individual bacterial cells will adhere to isolated sections of the plate and form bacterial colonies.

A colony is a small area of growth that ideally represents the progeny of a single bacterial cell. Because it came from a single parental cell, a colony should contain only one species of bacteria. A single colony can be transferred to a fresh plate of sterile medium to produce a pure culture.

Colony morphology refers to the appearance of a bacterial colony. Colonies vary with respect to their size, shape, color, margins, elevation, and texture. Most species have a characteristic colony morphology that can be an important clue to the identity of the species. Microbiologists examine the following characteristics to describe the morphology of bacterial colonies. **Remember always to keep the plate covered with the lid as you look at it. Lift the lid only slightly to observe elevation and texture.**

**Colony Size:**

The average size, in millimeters, is measured with a small metric ruler. Colonies that are less than about 0.5 mm are described as pinpoint. Notice that colony size may vary on different areas of a plate. In particular, closely spaced or crowded colonies are much smaller than well-isolated colonies.
Colony Shape:

Round colonies are circular in shape. Some bacteria develop oval colonies. Others grow in irregularly shaped colonies. Filamentous colonies are fibrous or cotton-like in appearance.

Round Colonies  Oval (Oblong) Colonies  Irregular Colonies  Filamentous Colonies

Margin:

The margin is the edge of a bacterial colony. An entire margin, or smooth margin, is one that is completely smooth with no irregularities. An undulate margin is a slightly wavy edge. A lobate margin has large projections or lobes growing from the edge of the colony. An erose margin has jagged, pointed projections on the edge.

Entire Margin  Undulate Margin  Lobate Margin  Erose Margin

Elevation:

Elevation refers to how the colony rises away from the surface of the medium. To observe elevation, look at the edge of the plate, at about eye level, and lift the lid slightly. Tilt the plate until you can see the elevation of the colonies. A flat colony lies completely flat along the surface of the medium. A raised colony has its top above the medium but still parallel with the surface. A convex colony curves across the top, much like a drop of water. An umbonate colony has a higher center than the edges. A crateriform colony, by contrast, has a depression in the center of the colony. A hilly colony has an irregular surface with many elevations and depressions.
Color:
Bacteria vary greatly with respect to colony color. Many bacteria have colonies that are light tan or cream-colored. Some species produce pigments that make the colonies white, red, yellow, or other colors. In addition, bacteria may secrete pigments or other substances that change the color of the medium surrounding the colonies.

Opaqueness:
Colonies differ in the way that they transmit light. Transparent colonies are almost completely clear. Translucent colonies are not completely clear, but permit some scattered light to get through. Opaque colonies completely block light transmission, and are often heavily pigmented. Hold the petri dish up to the light to observe this property.

Texture:
Texture refers to the smoothness or roughness of a colony. As you look at the edge of a plate and lift the lid slightly, tilt the plate so that the light reflects from the colonies. Do not touch the colonies. Smooth colonies have a shiny surface. Rough colonies have a dull, rough appearance. Filamentous colonies appear to be a mass of small threads or fibers.

Variation in colony morphology:
All bacteria will exhibit at least some degree of variation in their colony morphology.
Streak plate method:

- Flame the loop and take a small amount of bacteria. A tiny bit of culture on the end of the wire usually is ideal. Streak the inoculum across about one-fourth of the plate, as shown.

- Flame the loop again and allow it to cool for a few seconds. Using the sterile loop, streak into the first area several times, then continue streaking without going into the first area. You should make a single zig-zag, using the pattern as shown.

- Flame the loop again, allow it to cool, and streak a third area. Streak into the second area several times, and then continue streaking without going into the first or second areas.

- Incubate the plate in an inverted (upside-down) position.

How much inoculum?

Using the right amount of inoculum is critical in getting good colony isolation on a streak plate. Most of the time, beginners get too much bacterial inoculum on their loop. Here are some general guidelines. You may have experiment some to get the right amount in a particular application.

<table>
<thead>
<tr>
<th>Starting Material</th>
<th>Amount of inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A turbid broth culture</td>
<td>Dip the sterilized loop in the broth culture. Before you take the loop out of the tube, tap the loop on the inside wall of the test tube to break the drop out of the loop. Use only the amount of liquid that sticks to the wire to inoculate the streak plate.</td>
</tr>
<tr>
<td>Growth from a petri dish culture</td>
<td>Get a tiny amount of the growth on the edge of the loop.</td>
</tr>
<tr>
<td>Sources with scanty bacterial amounts (e.g., bacteria that have been resuspended from freeze-dried stocks)</td>
<td>You may need to use several loopfuls in the first quadrant, streaked one on top of another. (Note: Students will rarely, if ever, have to do this.)</td>
</tr>
</tbody>
</table>