Lab 2 – Aseptic Technique and Streak Plates

The 5 I’s of Culturing Microbes

- Inoculation
- Incubation
- Isolation
- Inspection
- Identification
Inoculation

Introduction of a small sample *inoculum* into a container of media to produce a *culture* of observable growth
Culturing Microorganisms

• Obtaining Pure Cultures
  – Cultures composed of cells arising from a single progenitor
    • Progenitor is termed a CFU
  – Aseptic technique prevents contamination of sterile substances or objects
  – Two common isolation techniques
    • Streak plates
    • Pour plates
Figure 6.9  Streak plate method of isolation-overview
Incubation

• Temperature-controlled chamber
• Microbe multiplies and produces macroscopically observable growth
Isolation

• If an individual bacterial cell is separated from other cells and has space on a nutrient surface, it will grow into a mound of cells—a colony.

• A colony consists of one species.
**Figure 6.8** Characteristics of bacterial colonies - overview

<table>
<thead>
<tr>
<th>Shape</th>
<th>Circular</th>
<th>Rhizoid</th>
<th>Irregular</th>
<th>Filamentous</th>
<th>Spindle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Margin</td>
<td>Entire</td>
<td>Undulate</td>
<td>Lobate</td>
<td>Curled</td>
<td>Filiform</td>
</tr>
<tr>
<td>Elevation</td>
<td>Flat</td>
<td>Raised</td>
<td>Convex</td>
<td>Pulvinate</td>
<td>Umbonate</td>
</tr>
<tr>
<td>Size</td>
<td>Punctiform</td>
<td>Small</td>
<td>Moderate</td>
<td>Large</td>
<td></td>
</tr>
<tr>
<td>Texture</td>
<td>Smooth or rough</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appearance</td>
<td>Glistening (shiny) or dull</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigmentation</td>
<td>Nonpigmented (e.g., cream, tan, white)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pigmented (e.g., purple, red, yellow)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optical property</td>
<td>Opaque, translucent, transparent</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

(a) [Image of bacterial colonies]

(b) [Image with a scale of 20 mm]
Inspection

Inspection – observation; macroscopic and microscopic

- **Pure culture** – grows only single known species of microorganisms

- **Mixed cultures** – hold two or more identified species or microorganisms

- **Contaminated culture** – once pure or mixed culture that has unwanted microbes growing
Various conditions of cultures
Identification

Identification – macroscopic and microscopic appearance, biochemical tests, genetic characteristics, immunological testing
Ex. 1.4 Aseptic Transfers

• Aseptic – What does it mean?
• Inoculation Methods
• Inoculation Tools
Sterilizing Instruments

Bunsen Burner Flame

Flaming Loop – angle is wrong – should be inverted

1/10/2018

MDufilho
Mixing Broth Cultures

Vortex Mixer

Hand Mixing
Aseptic Transfer

Video

Removing Cap

Flaming Tube

Holding Tube
Broth to Broth Transfer

Move the tube/not loop

Film on loop
Plate inoculation Ex. 1.5

**Video**

Invert Plate

Streaking Plate
Streaking Plate