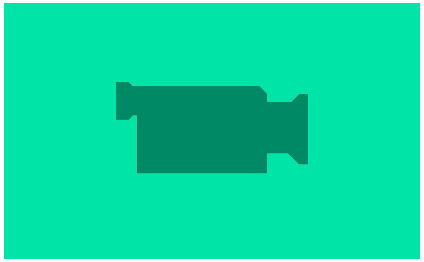
Bacterial Plate Preparation

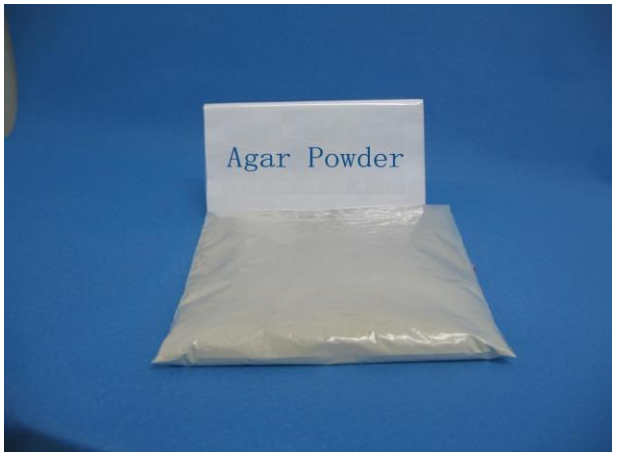
~ Using aseptic techniques ~

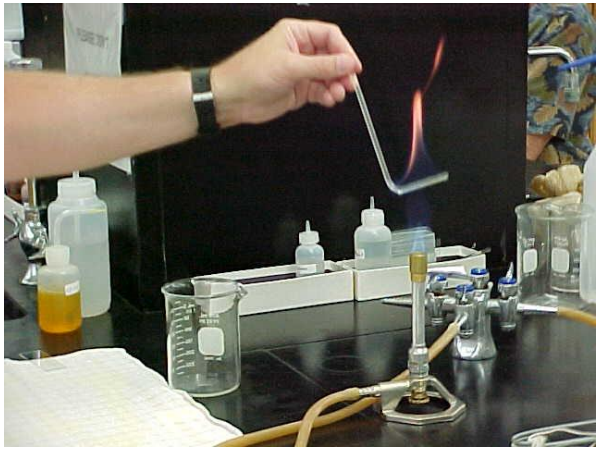
Bacterial Plates

- Laboratory and research scientists have to prepare nutrient media to grow specific strains of bacteria for their research.
- To do this they need Petri dishes, nutrient media, sterilization devices, streaking tools, and incubators.
- Why?



Tools of the trade





Preparation of Bacterial Growth Plate

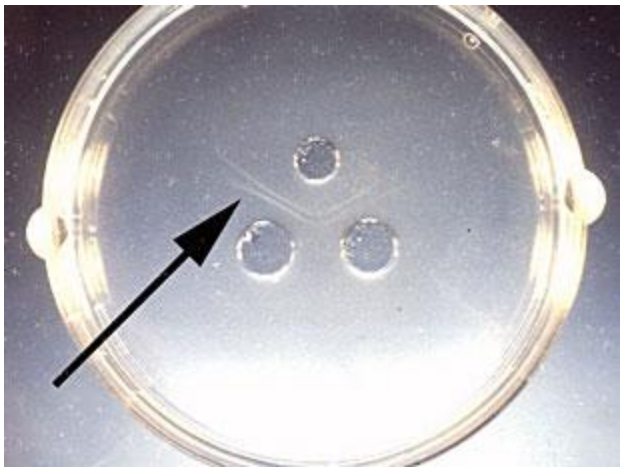
- Depending on the type of bacteria the scientist hopes to grow, they must select a growth media that will provide the correct balance of nutrients to propagate the species.
- 4 Types of Media:
  - Nutrient media
  - Minimal media
  - Selective media
  - Differential media

Examples of Growth Media

- eosin-methylen blue agar (EMB) that contains methylene blue – toxic to Gram-positive bacteria, allowing only the growth of Gram negative bacteria
- YM (yeast and mold) which has a low pH, deterring bacterial growth
- blood agar (used in strep tests), which contains beef heart blood that becomes transparent in the presence of hemolytic *Streptococcus*
- MacConkey agar for Gram-negative bacteria
- Hektoen Enteric (HE) which is selective for Gram-negative bacteria
- Mannitol Salt Agar (MSA) which is selective for Gram-positive bacteria and differential for mannitol
- xylose lysine desoxyscholate (XLD), which is selective for Gram-negative bacteria
- Mannitol Salt Agar (MSA), which is differential for mannitol fermentation
- X-gal plates, which are differential for lac operon mutants

Media Forms

- Media can come in 2 forms:
  - Gel media
  - Liquid media (broth)

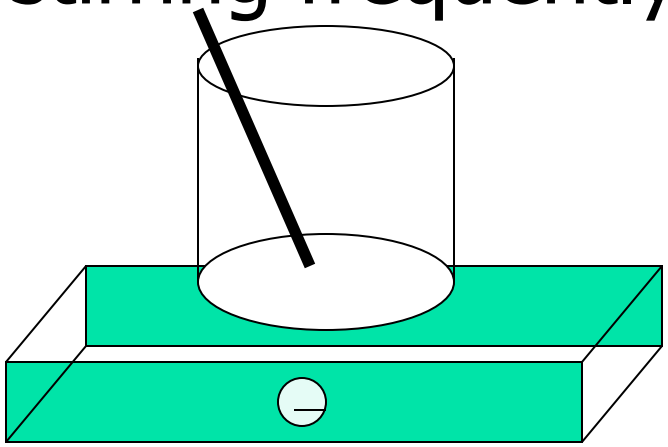


Preparing a Gel Plate

- First, wear gloves or wash hands using aseptic techniques discussed in last class (to prevent cross contamination).
- Acquire Petri dishes, agar gel, beaker, water, autoclave (sterilizer), bacterial sample, hot plate, and glass stirring rod.

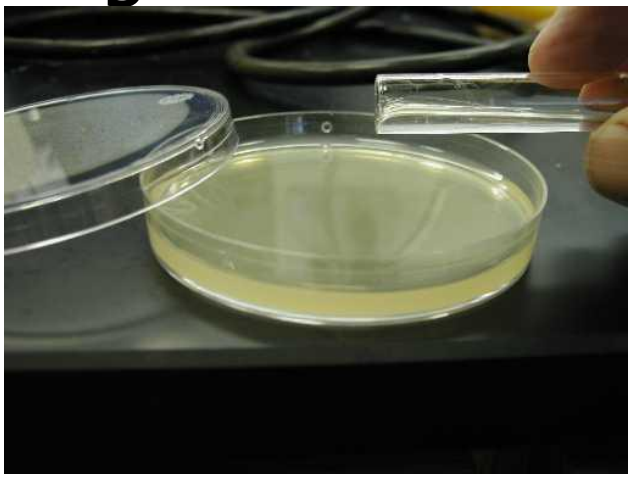
Step 1 – Making the Agar

- Add 1 liter of distilled H_20 into a beaker, and then add the powered agar gel. Heat over a hot plate until just boiling, stirring frequently.



Step 2 – Pouring the Gel

- Once the agar gel solution has been appropriately heated, carefully pour the solution into new clean Petri dishes, to depth of about ¼ inch – making sure to avoid making bubbles.



Step 3 -Autoclaving

- This step is necessary for some agar gels in order to prevent contamination growth on the media before intended inoculation.
- Steams all contents of container to high temp and pressure.

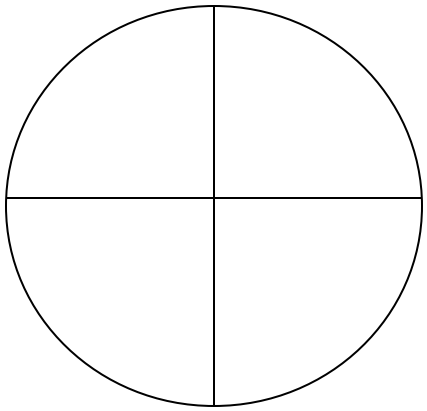
Step 4 – Cooling and inverting the plates

- Plates should be allowed to cool before removing them from the hot autoclave.
- Once they have cooled and the gel is no longer supple or pliable, seal the edges of the container with tape, and invert them to prevent bacterial growth due to condensation within the dish.

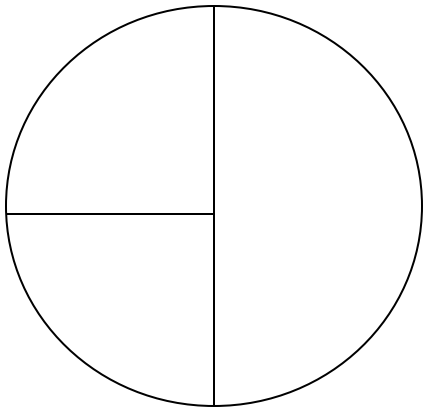
Sectioning the Plates

- Before inoculating the growth plates, scientists usually divide the plate into quadrants (or portions).
- There are 2 main ways of doing this.

4 Quadrants



3 Zones

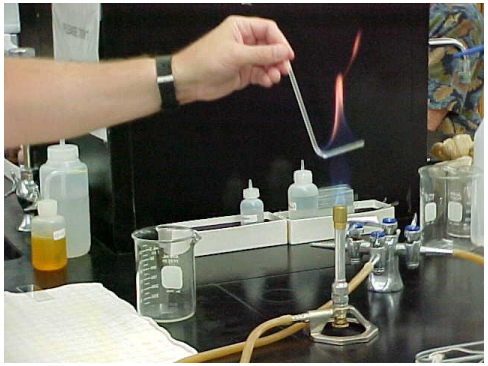


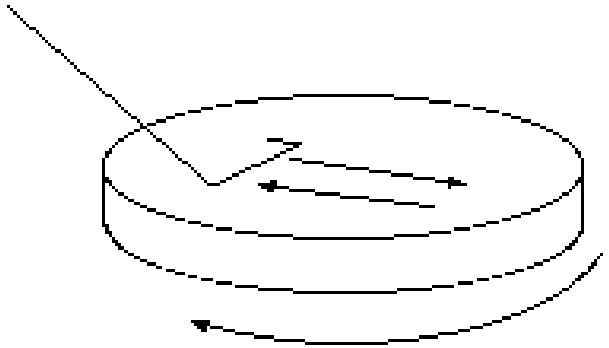
Why divisions?

- Depending on the purpose of the growth media plate, scientists will choose one of the 2 techniques to either make confluent mass growth (Confluency streaking) or attempt to draw the growth into individual colonies (Isolation streaking).

Confluency Streaking

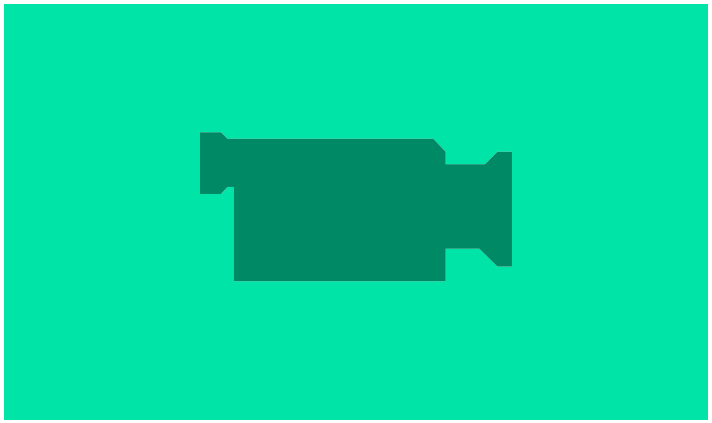
- Used by scientists to grow massive amount of bacteria on the plate.
- The tool used to streak (spread) the bacteria onto the plate is known as a "hockey stick" – a solid bent glass tube.





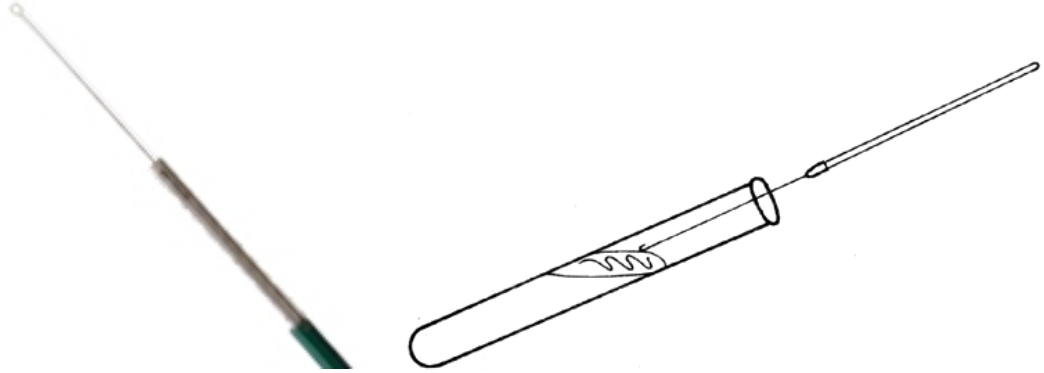
Steps to Confluency Streaking

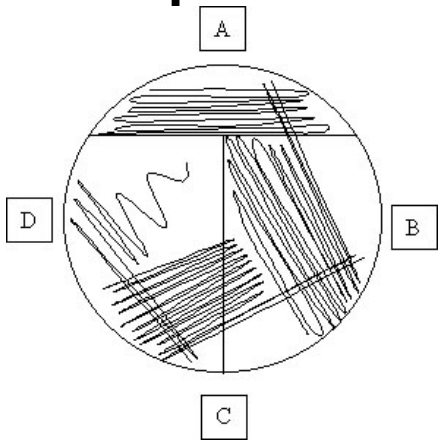
- 1st – Flame heat the hockey stick – allow to cool briefly
- 2nd – Lift the Petri dish in one hand and quickly place a couple drops of growth from a liquid media (broth) onto the center of the growth plate.
- 3rd – With lid in one hand, use the cooled bacterial hockey stick gently swiping it from top to bottom of the gel, turning it about 25 degrees, and streaking it again, like painting.
- 4th – Replace lid of Petri dish, and flame heat the hockey stick to kill any bacteria.
- 5th – Seal the edges of the plate with tape, and invert it.
- 6th – Place into an incubator at desired temperature to encourage maximum bacterial growth.



Isolation Streaking

- Used by scientists to grow only a few colonies, allows scientists to do more selective tests (ex – genetics).
- Tool usually used to produce isolation streaking is called an inoculation loop.





Steps to Isolation Streaking

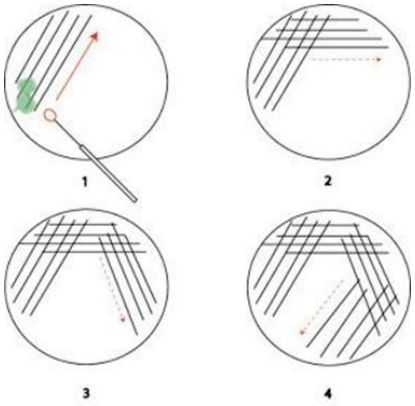
- 1st – Flame heat the inoculation hoop (until red) – allow to cool briefly
- 2nd – Take source container and flame heat the rim of the vial to avoid airborne contamination.
- 3rd – Dip cooled loop into broth or bacterial colony, carefully remove from the source container.
- 4th – Remove lid of Petri dish, carefully swipe the surface of quadrant #1 in close, thick pattern.
- 5th – Remove the loop and heat flame it (until red) to kill any bacteria – let cool.

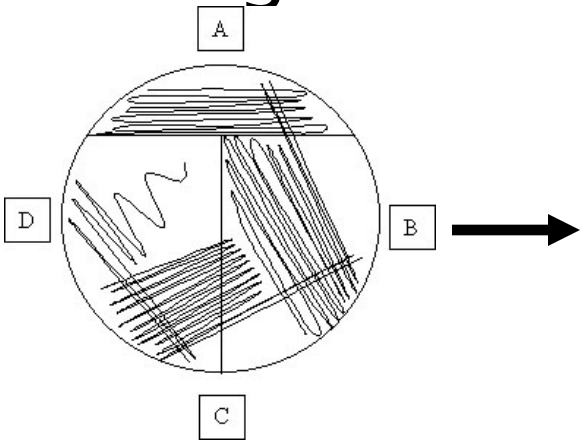
Steps to Isolation Streaking

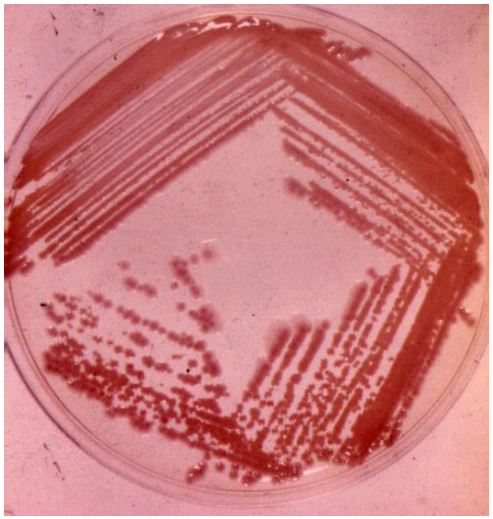
- 6th – Drag a small portion of bacteria from quadrant #1 into quadrant #2 and make a smaller, more spread out pattern.
- 7th – Heat flame loop – let cool
- 8th - Drag a small portion of bacteria from quadrant #2 into quadrant #3 and make an even smaller, more spread out pattern.
- 9th – Repeat process into Quad. 4 if needed.

Steps to Isolation Streaking

- 10th – Replace lid of Petri dish. Flame heat loop.
- 11th – Seal edges of Petri dish, invert and incubate at desired temperature to encourage maximum growth.

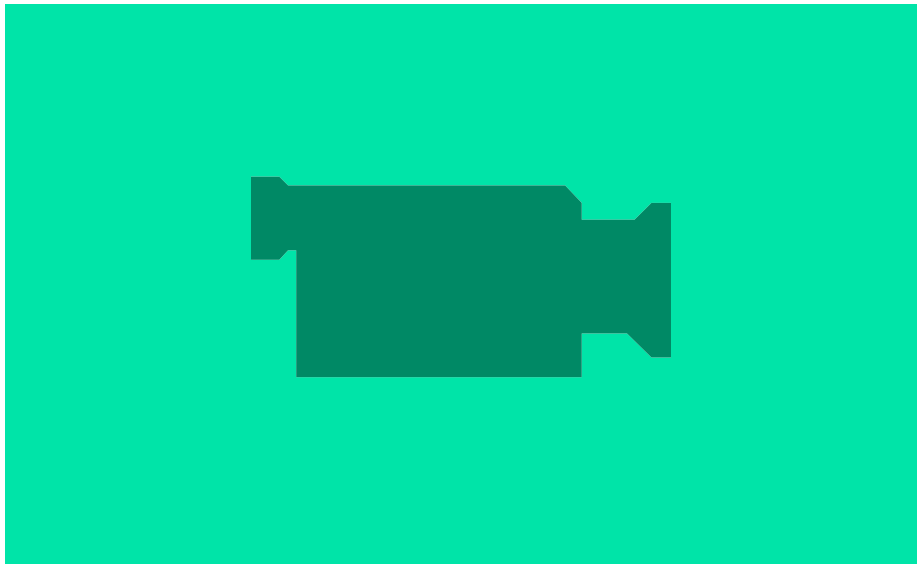






Reflection on Plate Preparation

- Following these plating procedures will help scientists to growth the maximum amount of the desired bacteria without contamination from airborne bacteria, or from you.
- This ensures the safety of lab scientists and students that work with bacteria, so that no harmful, non desired, bacteria will propagate in the plates.



Our Lab (Tomorrow)

- Your table will be responsible for preparing a bacterial plate or 1 of 2 possible bacterial species.
- You will not be following the exact processes detailed above, but will use a sterile cotton swab and follow the basic streaking techniques of Isolation streaking and Confluency streaking.
- At the end of the week we will study our bacterial growth and prepare slides to view them under the microscope.

Resources

- http://en.wikipedia.org/wiki/Growth_medium
- www.google.com/images/