About Science Prof Online PowerPoint Resources

• Science Prof Online (SPO) is a free science education website that provides fully-developed Virtual Science Classrooms, science-related PowerPoints, articles and images. The site is designed to be a helpful resource for students, educators, and anyone interested in learning about science.

• The SPO Virtual Classrooms offer many educational resources, including practice test questions, review questions, lecture PowerPoints, video tutorials, sample assignments and course syllabi. New materials are continually being developed, so check back frequently, or follow us on Facebook (Science Prof Online) or Twitter (ScienceProfSPO) for updates.

• Many SPO PowerPoints are available in a variety of formats, such as fully editable PowerPoint files, as well as uneditable versions in smaller file sizes, such as PowerPoint Shows and Portable Document Format (.pdf), for ease of printing.

• Images used on this resource, and on the SPO website are, wherever possible, credited and linked to their source. Any words underlined and appearing in blue are links that can be clicked on for more information. PowerPoints must be viewed in *slide show mode* to use the hyperlinks directly.

• Several helpful links to fun and interactive learning tools are included throughout the PPT and on the Smart Links slide, near the end of each presentation. You must be in *slide show mode* to utilize hyperlinks and animations.

Alicia Cepaitis, MS
Chief Creative Nerd
Science Prof Online
Online Education Resources, LLC
alicia@scienceprofonline.com

Tami Port, MS
Creator of Science Prof Online
Chief Executive Nerd
Science Prof Online
Online Education Resources, LLC
info@scienceprofonline.com

Image: Compound microscope objectives, T. Port

Laboratory Exercise 4

Bacterial Media & Culture

Collecting, Culturing & Interpreting Bacterial Samples

Images: Liquid TSY; Clinical sample being applied to TSY agar; Arm plate; all by T. Port

What am I going to learn from Lab Topic #4?
Bacterial Media & Culture

- You will practice microbial collection techniques.
- Define and use aseptic technique in microbial culture and media preparation.
- Use enriched, complex, selective & differential media to culture microbes.
- Describe colony morphology and its relationship to microbial identification.
- Interpret results of microbial growth on various culture media based on prepared controls.
- Understand the ubiquitous nature of microbes.

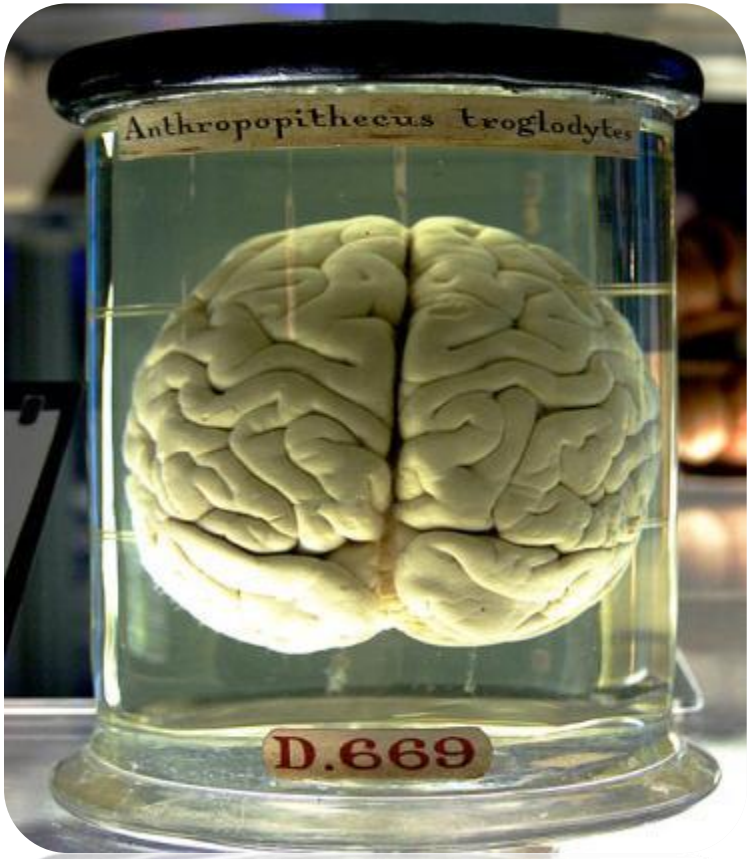


Image: Chimp brain in a jar, Gaetan Lee

Growth Media

- Bacteria and other microbes have particular requirements for growth.
- In order to successfully grow bacteria in lab, we must provide an environment suitable for growth.
- **Growth media** (singular = medium) are used to cultivate microbial growth.
- **Media** = mixtures of **nutrients** that the microbes need to live.
  Also provides a **surface** and the necessary **moisture** and **pH** to support microbial growth.
- Before being used, media must be autoclaved, so that it is sterile (all life forms killed.)
- **Tryptic Soy Agar** (TSY) is the medium that we most often use. Complex nutrient media which supports the growth of a wide variety of microbes.

Image: Streak plate of *E. coli*, T. Port

Specialized Media:

McConkey's, Mannitol Salt & Blood Agar

Look at the plates on your lab bench that are red/pink in color.

McConkey's = lighter, purplish-pink
Mannitol Salt = orangish-pink
Blood Agar = very dark red

Unlike TSY media, these specialized selective & differential media plates are already prepared for you.

Image: McConkey's, Mannitol Salt & Blood Agar specialized media, T. Port

Differential
&
Selective
Specialized Media

Q: What does **selective** mean?

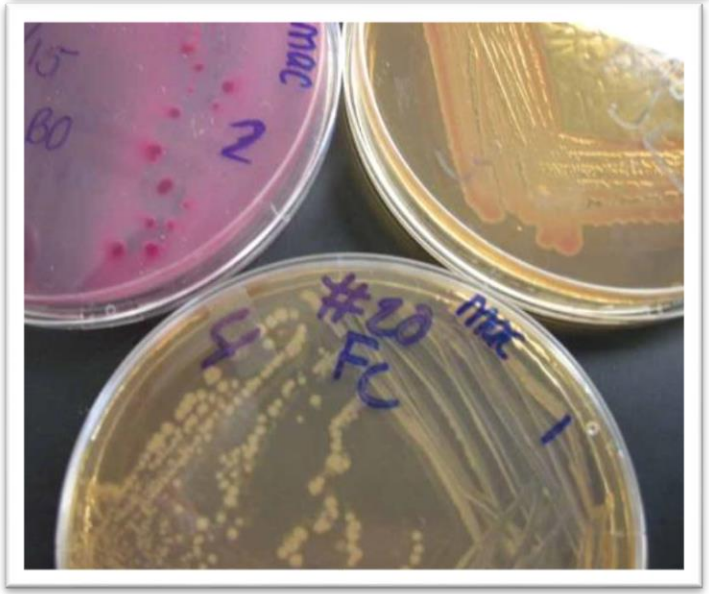
Q: What does **differential** mean?

Image: McConkey's media growing *E. coli*, T. Port

MacConkey's (MAC)

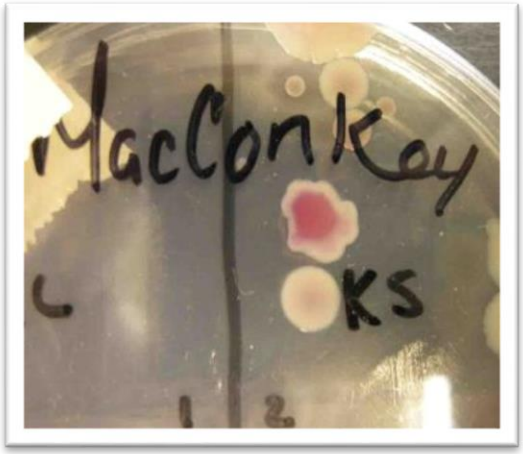
Q: Is MacConkey's selective? Explain.

Q: Is MacConkey's differential? Explain.



Watch
VIDEO:

How to Interpret MacConkey's Agar (MAC)



Images clockwise from top: McConkey's agar sterile; growing *E. coli, Enterobacter* & *Salmonella* (plates clockwise from top left); Lactose fermenting and non-lactose fermenting Gram – colonies on MAC. All by T. Port

Mannitol Salt (MSA)

Q: Is Mannitol Salt selective? Explain.

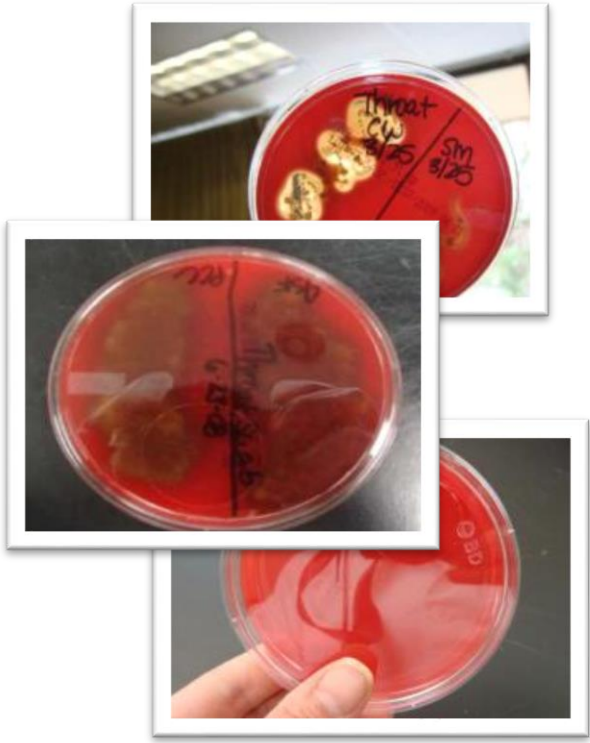
Q: Is Mannitol Salt differential? Explain.

Watch
VIDEO:

How to Interpret
Mannitol Salt Agar
(MSA)

Images: Sterile Mannitol Salt Agar, Positive & negative differential reaction on Mannitol Salt Agar, T. Port

Blood agar (BAP)



Most specimens received in a clinical microbiology lab are plated onto **Blood Agar**. It is an enriched medium that will grow even fastidious bacteria.

Also contains 5% sheep blood.

This media is *not selective*. It is enriched and **differential**:

Certain bacteria produce enzymes (hemolysins…say *hemo-lice-ins*) that act on red cells to produce either:

* **Beta hemolysis**: Enzymes lyse the blood cells completely, producing a clear area around the colony.
* **Alpha hemolysis**: Incomplete hemolysis produces a greenish discoloration around the colony.
* **Gamma hemolysis**: No effect on the red cells.

Blood agar is usually inoculated from a patient's throat swab.

Microbiologist are trying to detect Group A **beta** hemolytic *Streptococcus pyogenes* (a Gram-positive cocci-shaped bacteria that causes Beta hemolysis on blood agar.)

Normal flora of the throat will exhibit alpha or gamma hemolysis.

Watch **VIDEO**:

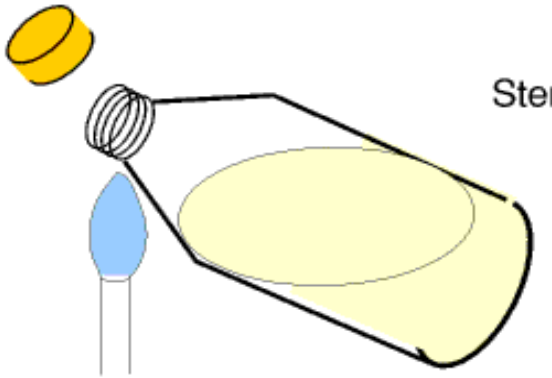
How to Interpret Blood Agar (BAP)

Images: Beta-hemolysis, Alpha-hemolysis and a sterile plate of Blood Agar, T. Port

"Pouring a Plate"

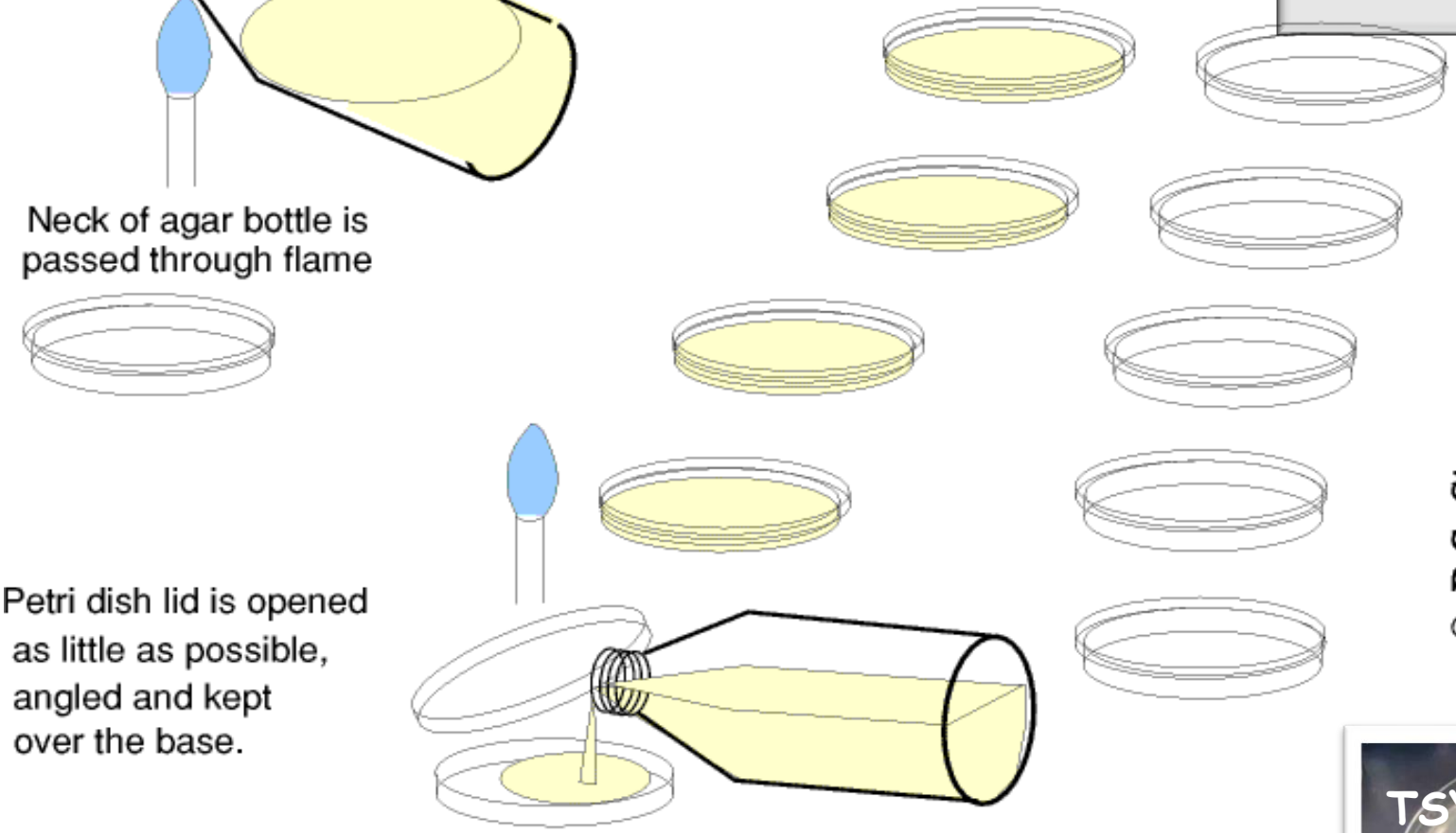
Watch
VIDEO:

How to Aseptically Pour Bacterial Growth Media



Neck of agar bottle is passed through flame

Sterilised molten agar is poured in and left to set.



Petri dish lid is opened as little as possible, angled and kept over the base.

Each Petri dish hold about 20 ml, so 200ml will do for 10.

Labeling Plates

All Petri plates for this and future lab exercises should be labeled and stored in the following manner:

1. Make certain that all plates are labeled on the **bottom half** (i.e. the portion of the Petri plate that contains the media).
2. You can label **plastic** with a **sharpie**; **glass** with a **wax pencil**.
3. Include the following:
   a. Your initials or identifying mark
   b. Date
   c. Type of specimen
4. All plates are incubated in the green storage bin (which is identified as "SAVE") in the **"upside down" position**.

"Upside down" means that the ½ of the Petri plate with media faces up. The empty ½ of the Petri plate is down.

We **do not** use rubber bands to hold lids in place.
(Except for the plates that you may transport home)

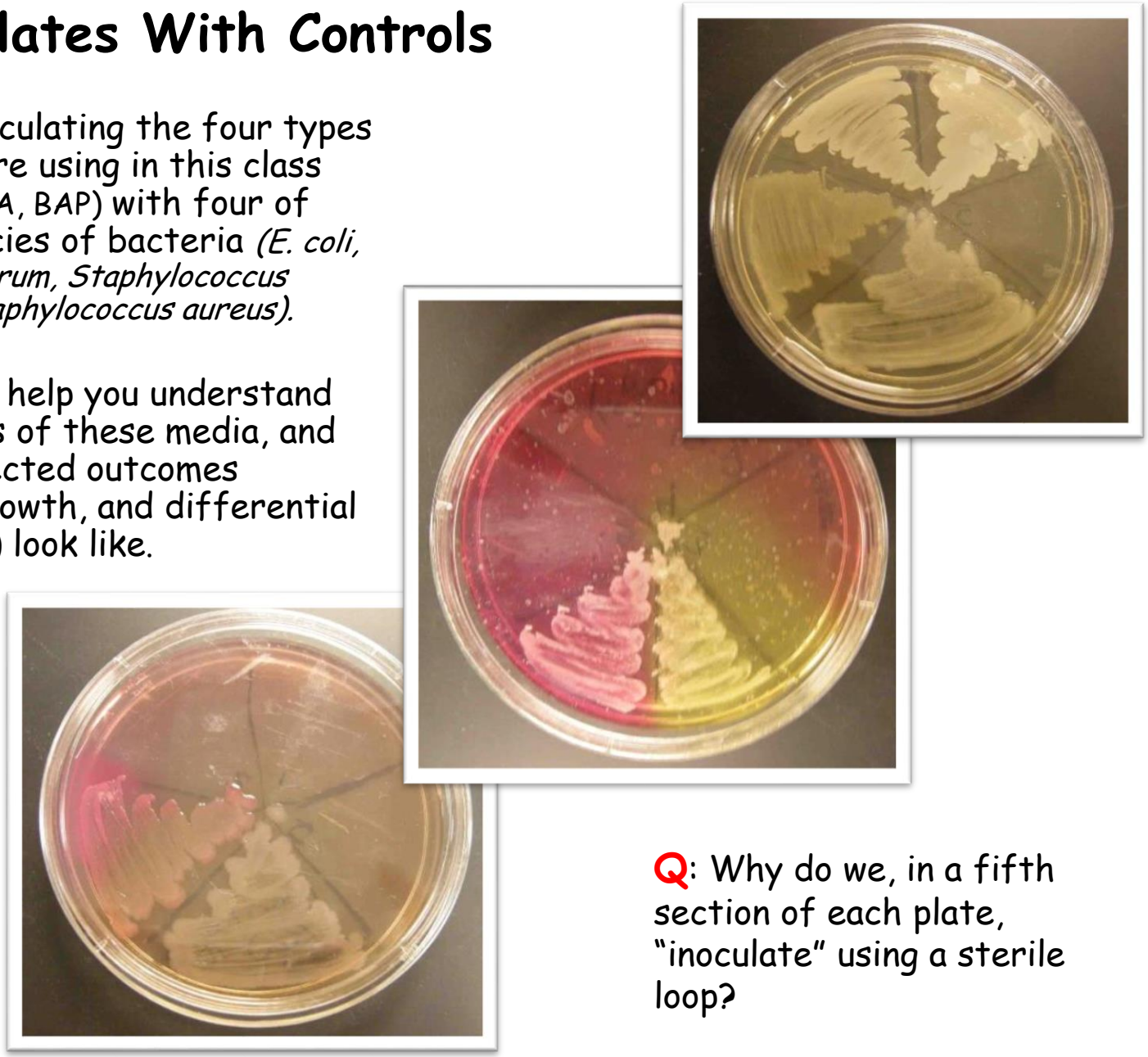
Plates will be incubated at 37° C for 24 hrs, then stored at room temperature until next week, when you will observe for results.

Images: Positive & negative differential reaction on Mannitol Salt Agar, T. Port

Inoculate Plates With Controls

- You will be inoculating the four types of media we are using in this class (TSY, MAC, MSA, BAP) with four of our stock species of bacteria *(E. coli, Salmonella pullorum, Staphylococcus epidermidis, Staphylococcus aureus).*

- Doing this will help you understand the properties of these media, and what the expected outcomes (growth, no growth, and differential color changes) look like.



Q: Why do we, in a fifth section of each plate, "inoculate" using a sterile loop?

Image: Arm plate, Source unknown

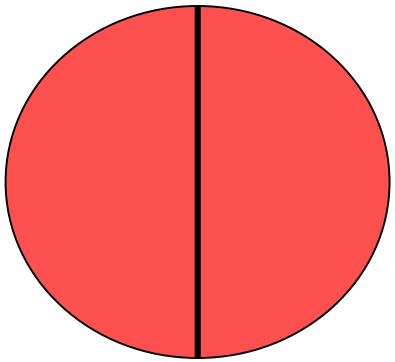
Normal Flora Samples

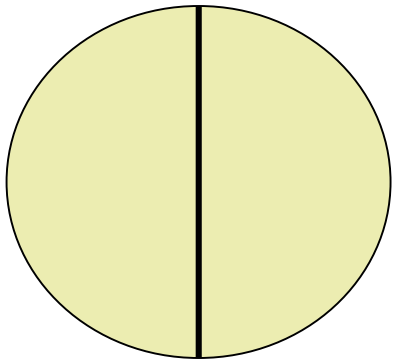
1. **Arm Plate** will demonstrate the microorganism inhabiting the surface of the skin.

2. **Touch Plates** will demonstrate the bacteria in the epidermis of our hands.

3. **Throat Swab** on Blood agar.

4. **Nasal Swabs** on TSY & MSA.





REMEMBER…

- When placing one sample on two plates, inoculate the *non-selective* medium **first**!
- Very gently transfer your sample to the plate. You want to avoid gouging the surface of the media.

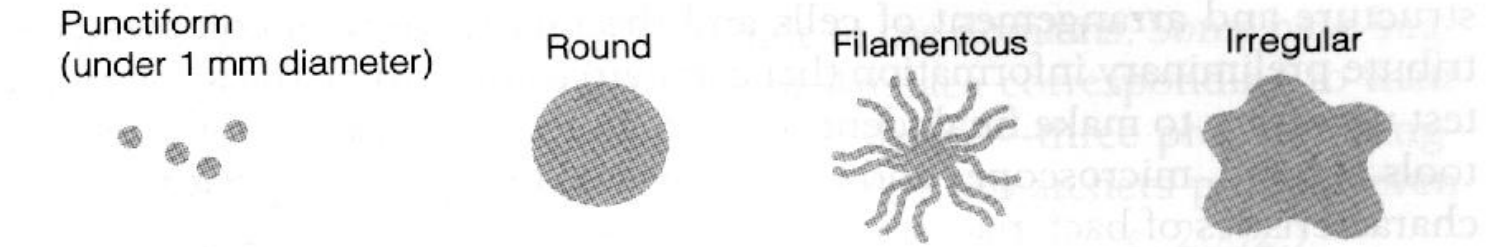
Image: Arm plate, Source unknown

Microbial Colony Morphology

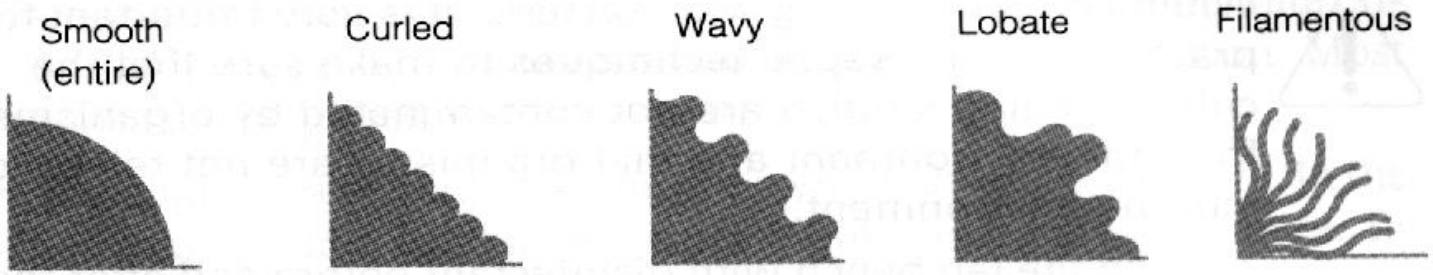
From the Virtual Microbiology Classroom on ScienceProfOnline.com

Images: Arm plate, TSY with sample from dish washer, MacConkey's with variety of colonies, all by T. Port

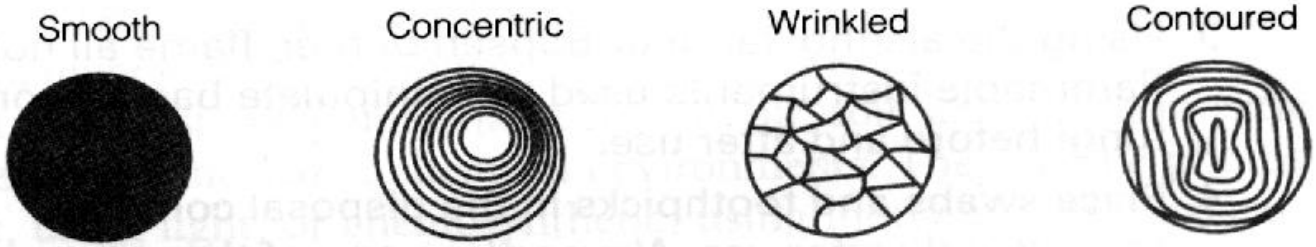
Microbial Colony Morphology



a. Common colony shapes



b. Common colony margins



c. Common colony surface characteristics

Discard Bin at Back of Lab

Confused?

Here are links to fun resources that further explain microbiology media & culture:

Smart Links

- **Media & Culture Laboratory** Main Page on the Virtual Microbiology Classroom of Science Prof Online.
- "Germs", music by Weird Al Yankovic. Video by RevLucio.
- Normal Flora webpage, by Douglas F. Fix. Interactive page where you can select an area of the body and learn which normal flora typically colonize that location.
- How to Interpret: MacConkey's (MAC), Mannitol Salt (MSA) and Blood Agar (BAP) videos from Science Prof Online.
- How to Pour Bacterial Growth Media into Petri Dishes, video from Science Prof Online.
- Bacterial growth video and narration, YouTube, Dizzo95..
- **Microbial Growth & Metabolism** Main Page on the Virtual Microbiology Classroom of Science Prof Online.
- *E. coli* population growth time lapse video.

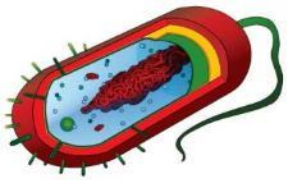
(You must be in PPT slideshow view to click on links.)

Are microbes intimidating you?

Do yourself a favor. Use the…

Virtual Microbiology Classroom *(VMC)* !

The VMC is full of resources to help you succeed, including:



- practice test questions
- review questions
- study guides and learning objectives

You can access the VMC by going to the Science Prof Online website
www.ScienceProfOnline.com

Images: Staph, Giant Microbes; Prokaryotic cell, Mariana Ruiz