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- 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.
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Laboratory Project 3

Differential & Selective Bacterial Growth Media

- Streak Plate Technique
- Collecting and Culturing Bacterial Samples
- Positive & Negative Experimental Controls



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.
- 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.

Specialized Media:

McConkey's, Mannitol Salt & Blood Agar



McConkey's = lighter, purplish-pink

Mannitol Salt = orangish-pink

Blood Agar = very dark red

These media will be used to introduce you to the <u>selective & differential</u> properties of specialized agars.

MacConkey's (MAC)

Q: Is MacConkey's selective? Explain.

Q: Is MacConkey's differential? Explain.

Watch **VIDEO**:

How to Interpret

MacConkey's Agar

(MAC)





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 <u>Mannitol Salt Agar</u>, Positive & negative differential reaction on Mannitol Salt Agar, T. Port

Blood agar (BAP)

Q: Is Blood agar selective? Explain.

Q: Is Blood Agar differential? Explain.

Watch **VIDEO**:

How to Interpret
Blood Agar (BAP)



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

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 plates with either a sharpie or 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 $\frac{1}{2}$ of the Petri plate with media faces up. The empty $\frac{1}{2}$ 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)

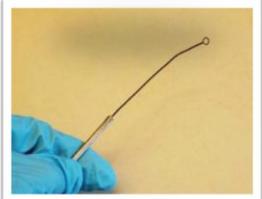
Plates will be incubated at 37° C for 24 hrs, then stored at room temperature until next week, when you will observe for results.



Isolation Streak Plates & Aseptic Technique

- To do a <u>streak plate technique</u>, we will use an <u>inoculation</u> <u>loop</u> (aka smear loop, inoculation wand or microstreaker).
- Simple tool used to retrieve an inoculum from a culture of microorganisms.
- Always sterilize in <u>microincinerator</u> until loop becomes red hot *before* and *after* each use.
- By doing this, the same tool can be reused in different experiments without fear of cross-contamination.
- Be sure that your inoculation loop has cooled before using it to retrieve inoculum or to streak a plate!
- If you hear medium sizzle when you touch it with loop, the loop is too hot!







When obtaining a bacterial sample from a tube or plate of media do so **gently**! The bacteria is growing as a thin film on top of the media! Don't scrape so hard that you have pieces of agar in your sample!



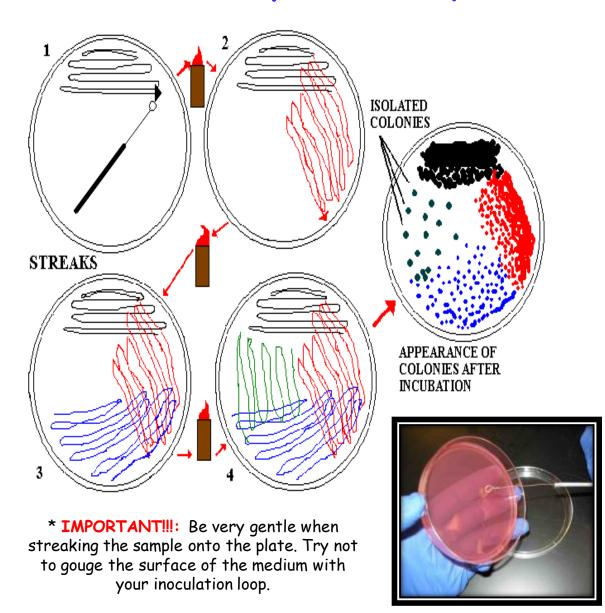
If obtaining bacterial sample from slant tubes:

- never pick up test tube by the cap.
- do NOT set cap down on lab bench
- flame neck of the test tube before & after obtaining sample.



Isolation Streak Plates & Aseptic Technique

- <u>Streak plating</u> is used to isolate a single type of bacteria
- This technique spreads out original "parent bacteria" in a sparse pattern that ,after growth, results in individual colonies.
- After incubation, the 4th quadrant of your plate should have dots.
- These small "dots" are individual colonies, and represent millions of bacteria of the same type.

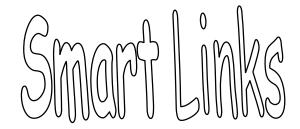




Confused?

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

- Differential & Selective Bacterial Growth Media
 <u>Lecture</u> & Laboratory Main Pages 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: <u>MacConkey's</u> (MAC), <u>Mannitol Salt</u>
 (MSA) and <u>Blood Agar</u> (BAP) videos from Science Prof Online.
- How to Pour Bacterial Growth Media into Petri Dishes, video from Science Prof Online.
- <u>Bacterial growth</u> video and narration, YouTube, Dizzo95...
- Microbial Growth & Metabolism Main Page on the Virtual Microbiology Classroom of <u>Science Prof Online</u>.
- <u>E. coli</u> population growth time lapse video.







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