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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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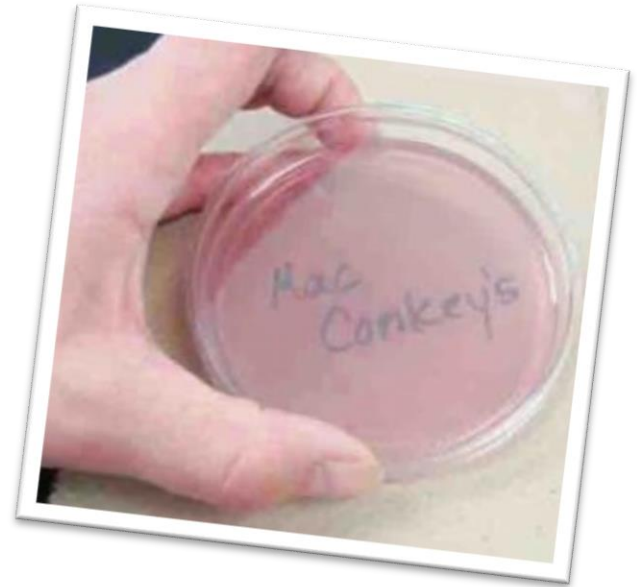
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IDENTIFICATION OF UNKNOWN BACTERIA

Laboratory Exercise 3

Specialized Bacterial Growth Media

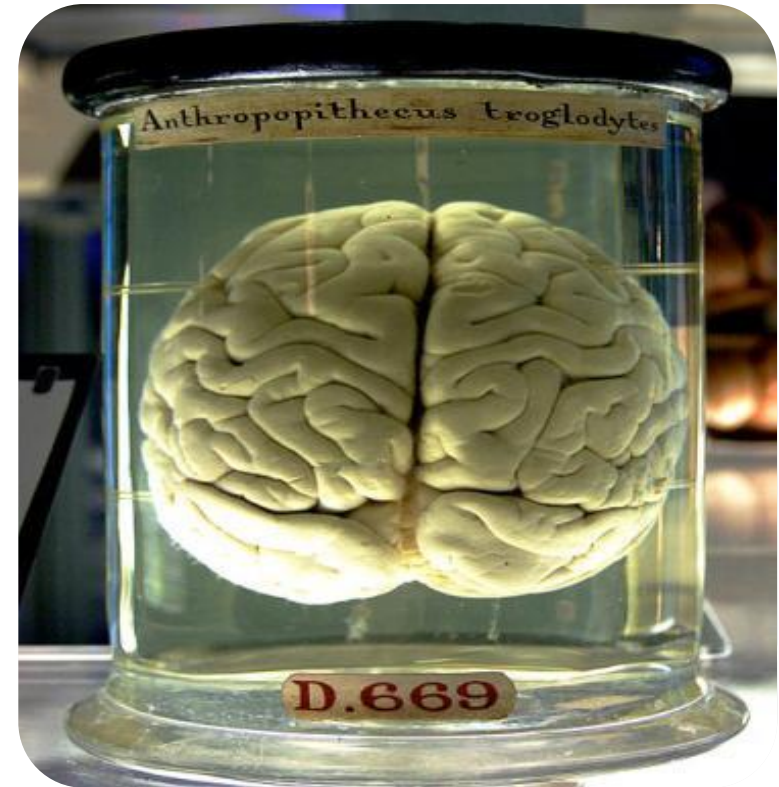
- MacConkey's Agar
- Mannitol Salt Agar



What am I going to learn from Lab Topic #3?

MacConkeys & Mannitol Salt Agars

- You will practice microbial collection techniques.
- Define and use aseptic technique in microbial culture and media preparation.
- Use selective & differential media to culture and identify microbes.
- Describe colony morphology and its relationship to microbial identification.
- Interpret results of microbial growth on various culture media.



Please plug in your microincinerators.

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.



How is media made?

- When lab personnel make media they measure out a quantity of **dry powdered nutrient media**, add **water** and **check the pH**.
- They pour the media into bottles, cap it and **autoclave**.
- This is a process similar to home canning techniques in food preservation.
- The autoclave exposes the media to high temperature (121°C) and pressure (15 psi) for 20 minutes.
- Once the media is autoclaved it is considered **sterile** (all life forms killed).



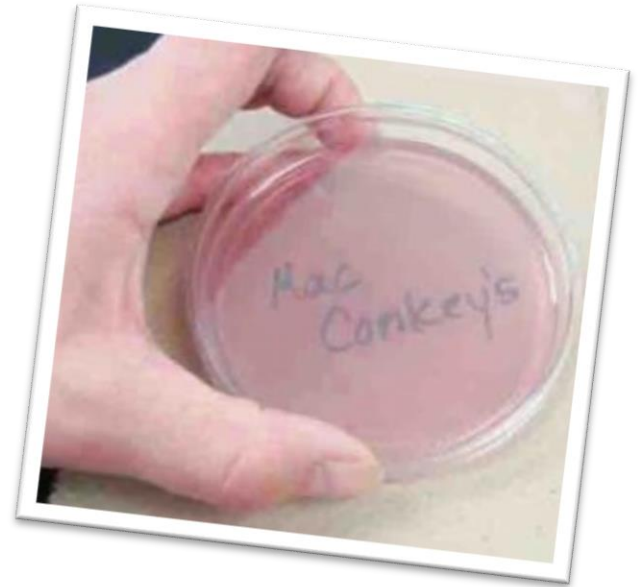
Specialized Media:

- McConkey's Agar
- Mannitol Salt Agar

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

McConkey's = lighter, purplish-pink
Mannitol Salt = orangish-pink

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



Differential & Selective Media



Q: What does **selective** mean?

Q: What does **differential** mean?

MacConkey's (MAC)

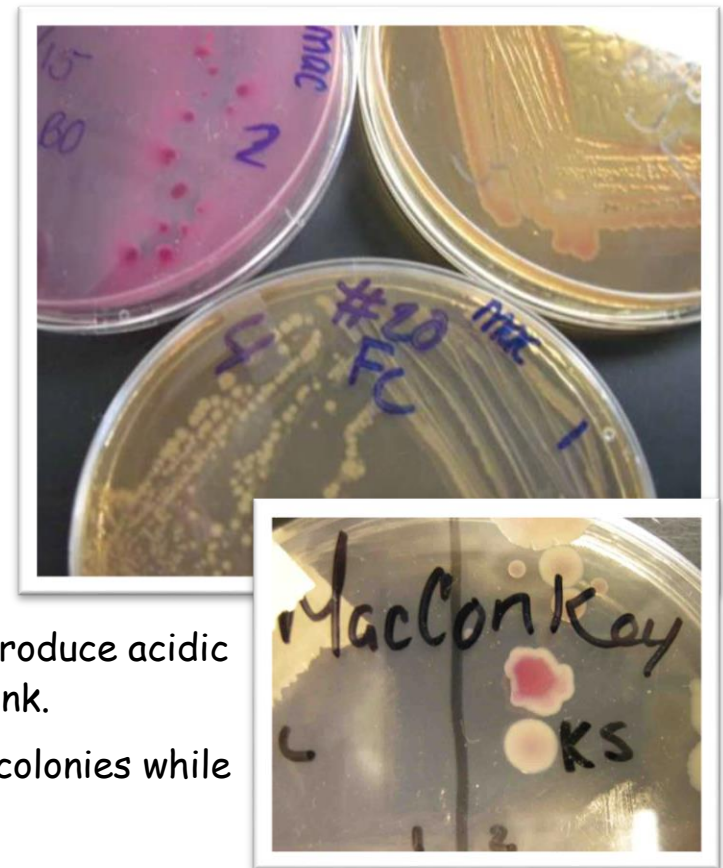
MacConkey's agar is both selective & differential.

1. **Selective** because it *only grows* Gram-negative bacteria. Inhibits the growth of Gram-positive bacteria.
2. **Differential** because neutral red (pH-sensitive dye) and lactose (type of sugar) have been added to media.
 - Bacteria that use lactose for food (lactose fermenters), produce acidic metabolites that trigger the pH sensitive dye to turn pink.
 - So lactose fermenting bacteria will grow in bright pink colonies while non-lactose fermenters will be colorless and clear.

Q: Regardless of the color of the plate, what do you know about bacteria found growing on MacConkey's?

Q: If there is growth, what additional information is provided when the color of the bacteria is examined?

Enteric bacteria are the most frequently encountered bacteria isolated from many types of clinical specimens. They are most commonly lactose fermenters.



Watch
VIDEO:
[How to Interpret
MacConkey's Agar
\(MAC\)](#)

Images: [MacConkey's](#) growing *E. coli*, *Enterobacter* & *Salmonella* on plates clockwise from top left; MAC with lactose fermenting colony above a non-lactose fermenting colony. All photos by T. Port

Mannitol Salt (MSA)

Mannitol Salt agar is both **selective** & **differential**.

1. **Selective** because it has a high NaCl (7.5%) concentration, and few types of bacteria can grow on this hypertonic medium.

Members of genus *Staphylococcus* are halophilic, and grow well on this media.

2. **Differential** because this medium contains a **pH**-sensitive dye to identify organisms that ferment mannitol. Organic acids wastes mannitol fermenters produce change the medium from red to yellow.

MSA works well for identifying **pathogenic staphylococci**, such as *Staphylococcus aureus*, which will ferment mannitol.

Most non-pathogenic staphylococci (*Staphylococcus epidermidis*) will not ferment mannitol.

Q: Regardless of the color of the plate, what do know about bacteria found growing on Mannitol Salt?

Q: If there is growth, additional information can then be obtained about bacteria growing based on color of the medium.



Watch
VIDEO:

[How to Interpret
Mannitol Salt
Agar \(MSA\)](#)

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 $\frac{1}{2}$ of the Petri plate with media faces up. The empty $\frac{1}{2}$ of the Petri plate is down.

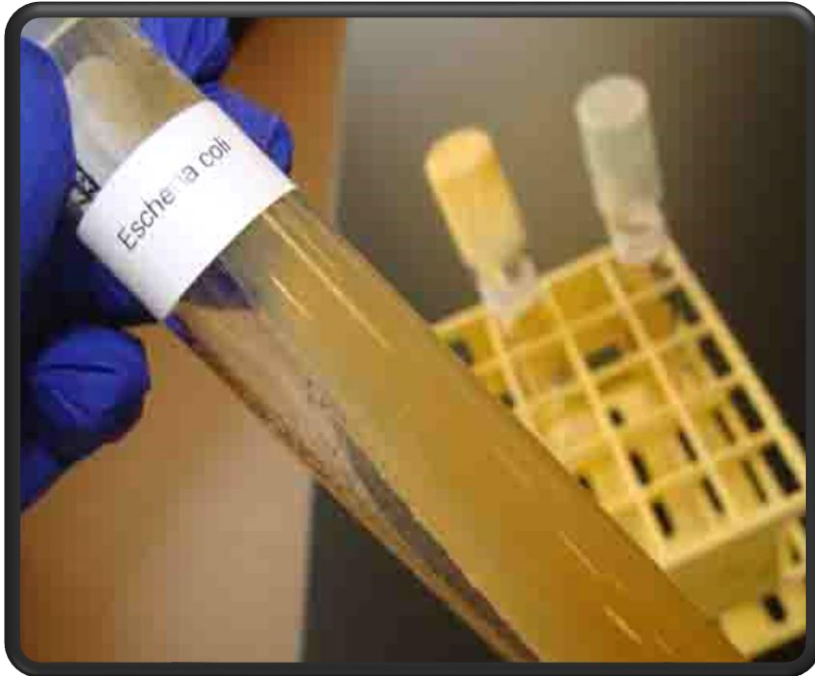
We do **not** use rubber bands to hold lids in place.
(Except for the plates that you may transport home)

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



Images: Positive & negative differential reaction on [Mannitol Salt Agar](#), T. Port

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!

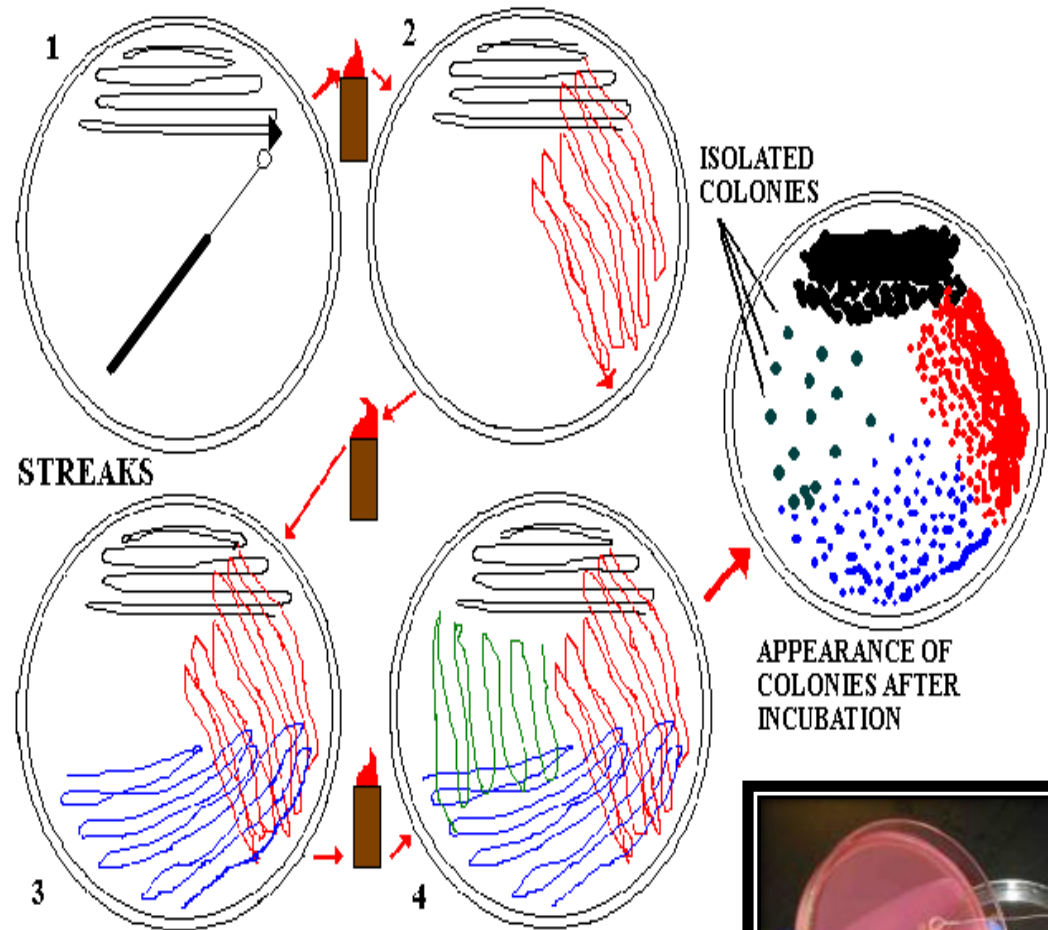


If obtaining bacterial sample from slant tubes:

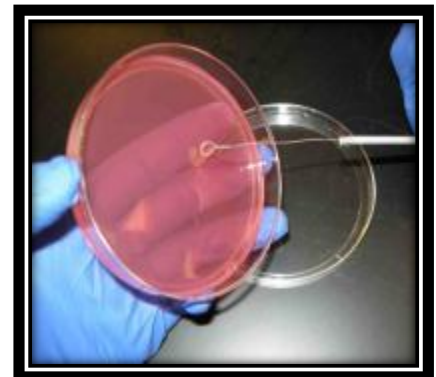
- never pick up test tube by the cap.
- do NOT set cap down on lab bench
- flame neck of the test tube before & after obtaining sample.

Isolation Streak Plates & Aseptic Technique

- Streak plating 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.

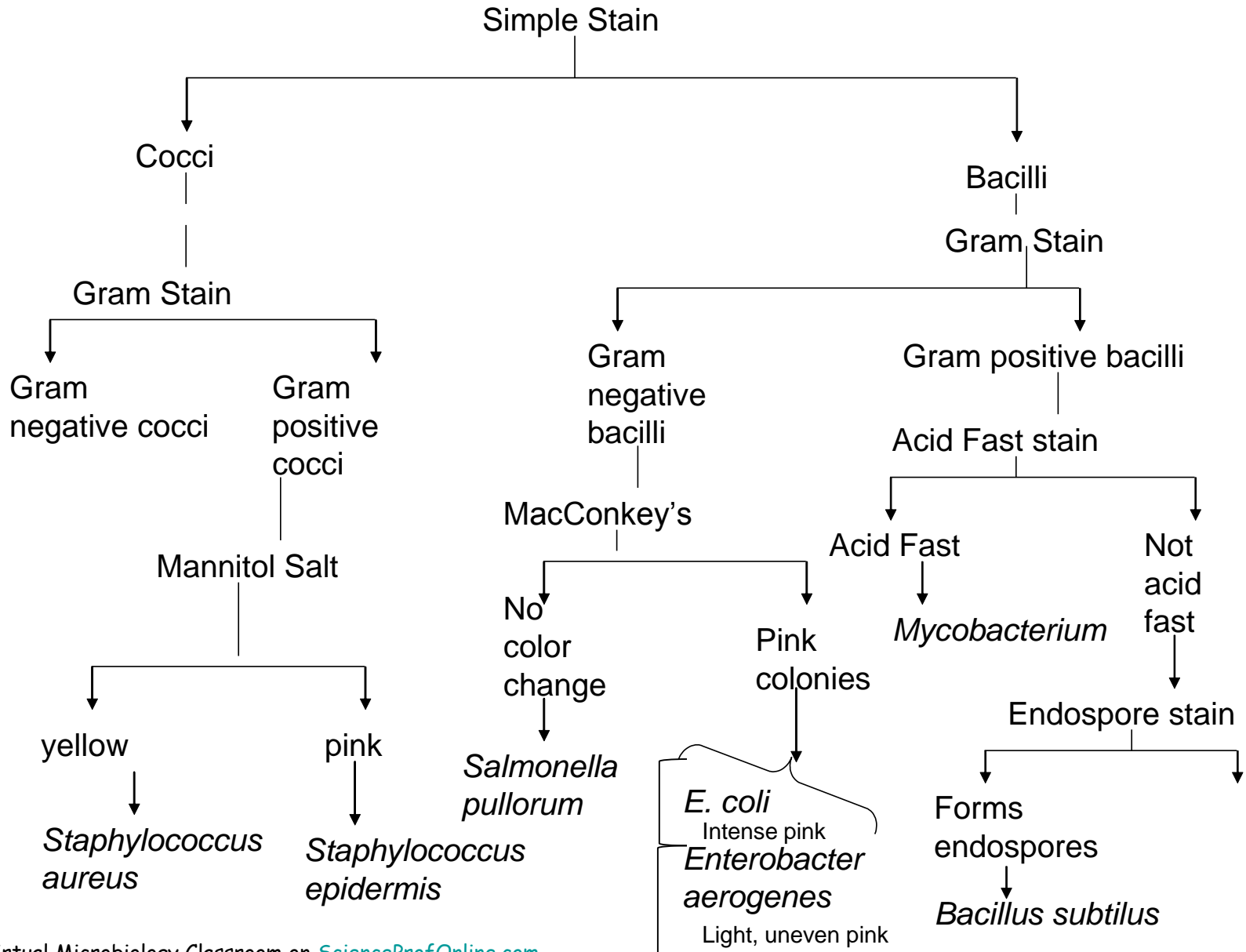


* **IMPORTANT!!!**: Be very gentle when streaking the sample onto the plate. Try not to gouge the surface of the medium with your inoculation loop.

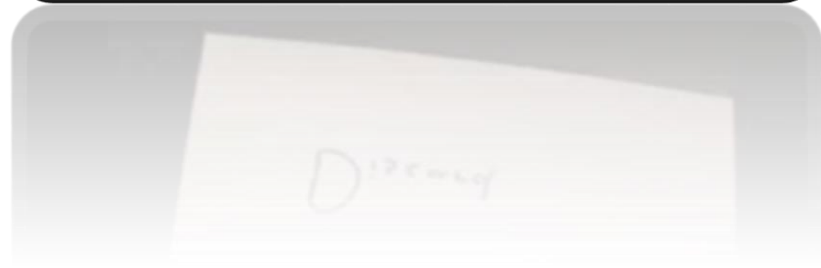


Q: Why are we plating our bacterial unknown onto MacConkey's & Mannitol Salt Agar?

Dichotomous Key



Discard Bin at Back of Lab



Confused?

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

- **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 \(MSA\)](#) videos 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.

Smart Links



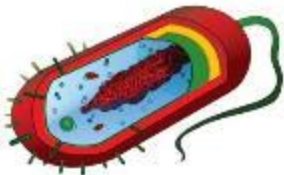
Are microbes intimidating you?



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