

About <u>Science Prof Online</u> 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.

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From the <u>Virtual Microbiology Classroom</u> on <u>ScienceProfOnline.com</u>

Image: Compound microscope objectives, T. Port

Selective & Differential Bacterial Growth Media

and Colony Morphology



From the <u>Virtual Microbiology Classroom</u> on <u>ScienceProfOnline.com</u>

Images: Sterile MAC, BAP and MSA specialized media; Arm plate of normal flora; all by T. Port

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.
- <u>Growth media</u> (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.

(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 guantity 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 _____ (all life forms killed).





Specialized Media:

McConkey's, Mannitol Salt & Blood Agar



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

These specialized <u>selective & differential</u> media provide information about the bacteria that grows.

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?

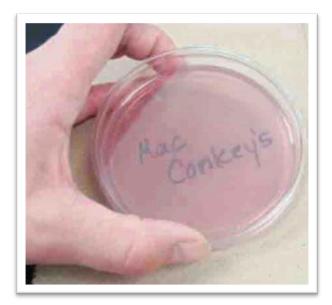
Image: McConkey's media growing *E. coli*, T. Port

MacConkey's (MAC)

<u>MacConkey's</u> media is both <u>selective & differential</u>.

- 1. Selective because it *only grows <u>Gram-negative</u> bacteria*. Inhibits the growth of <u>Gram-positive</u> 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 <u>pH</u> sensitive dye to turn pink.
 - So lactose fermenting bacteria will grow in bright pink colonies while non-lactose fermenters will be colorless and clear.

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





Meet the Microbe: Escherichia coli

GRAM-NEGATIVE

Facultative anaerobe, lactose fermenter (____) bacillus-shaped

Some strains of *E. coli* inhabit gastrointestinal tracts of warm-blooded animals as normal flora and provide a portion of the microbially-derived vitamin K for their host.

While many strains of *E. coli* are harmless commensals, of some are human pathogens.

Common cause of bacterial food poisoning and urinary tract infections.

Bacteria must be able to "stick" to cause infection (otherwise, in case of UTI, bacteria would just get peed out).

Bladder lined with proteins, to prevent this. *E. coli* has <u>fimbriae</u> to help it stick.





Q: Regardless of the color of the plate, what do 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?







Bacterial Genera:

GRAM NEGATIVE Non-lactose fermenters Facultative anaerobes, bacillus-shaped

Food poisoning: Infection in lining of small intestine caused by bacteria (both <u>G+</u> & <u>G-</u>), including <u>Salmonella</u> and <u>Shigella</u>.

Transmission: Ingesting foods and materials that are fecally contaminated.

Symptoms / Course: Diarrhea, fever, and abdominal cramps 12 - 72 hours after infection. Usually lasts 4 to 7 days. Most recover without treatment. Severe infections may last several weeks.

Bacteria shed in feces. Carrier state exists in some people who shed the bacteria for 1 year or more following initial infection.

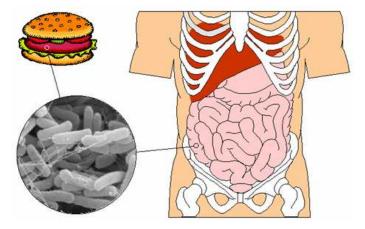
Treatment: Replace fluids. Don't use anti-diarrheals. May prolong illness.

Thorough cooking kills these bacteria. Proper food handling, storage and good hand washing are preventive measures.

From the Virtual Microbiology Classroom on ScienceProfOnline.com

MacConkey's NON-Lactose Fermenter

å



Images: <u>MacConkey's media</u>, one growing Salmonella, the ther *E. coli* (lactose fermenter); <u>Food poisoning diagram</u>, Shirley Owens, Michigan State

Mannitol Salt (MSA)

<u>Mannitol Salt</u> media 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 _____, and grow well on this media.

2. Differential because this medium contains a pHsensitive dye to identify organisms that ferment mannitol. O

Organic acids wastes mannitol fermenters produce change the medium from red to yellow.

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

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





Meet the Microbes: Staphylococcus

GRAM-POSITIVE

Facultative anaerobe, halophile coccus-shaped

PATHOGEN

- Staphylococcus aureus (golden staph), most common cause of staph infections.
- Approximately 20-30% of general population "Staph carriers."
- S. aureus can cause illnesses ranging from minor skin infections to life-threatening diseases, such as meningitis, Toxic shock syndrome (TSS) & septicemia.
- MRSA = Methicillin-resistant *Staphylococcus* aureus
- One of the four most common causes of **nosocomial infections**, often causing postsurgical wound infections.

NORMAL FLORA

- *S. epidermidis* is <u>normal flora</u> which inhabits the skin of healthy humans.

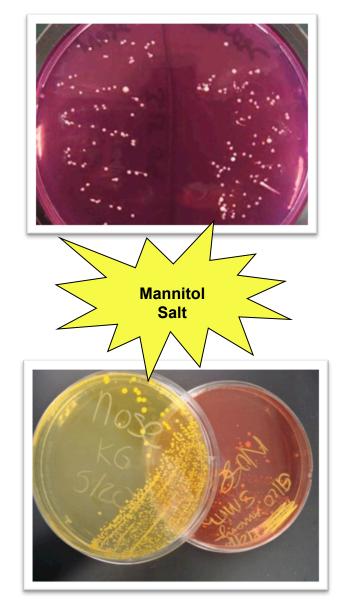


Image: Mannitol salt plates, T. Port; *S. aureus,* Janice Haney Carr , <u>PHIL</u> #10046; <u>Gram stain Staph</u>, T. Port

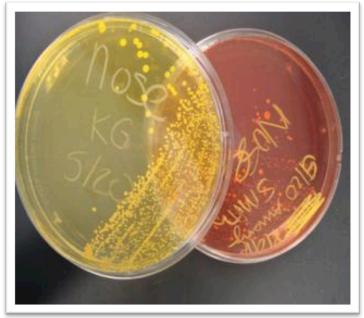
Mannitol Salt (MSA)

Q: Is Mannitol Salt selective? Explain.

Q: Is Mannitol Salt differential? Explain.







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

Blood agar (BAP)

Most specimens received in a clinical microbiology lab are plated onto <u>Blood Agar</u>. 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 <u>enzymes</u> called ______that act on red cells to produce either:
 - * _____ hemolysis: Enzymes lyse the blood cells completely, producing a clear area around the colony.
 - * **hemolysis**: Incomplete hemolysis produces a greenish discoloration around the colony.
 - _____ 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.

From the <u>Virtual Microbiology Classroom</u> on <u>ScienceProfOnline.com</u>







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

Bacterial Genus:

GRAM-POSITIVE, Facultative anaerobe, coccus-shaped

Diverse genus, some normal flora, some pathogens that produce **toxins**.

Pairs or chains of cocci.

Classified by **hemolysis pattern** on <u>blood agar</u>; alpha, beta and gamma hemolysis.

Beta-hemolytic Strep fall into two groups:

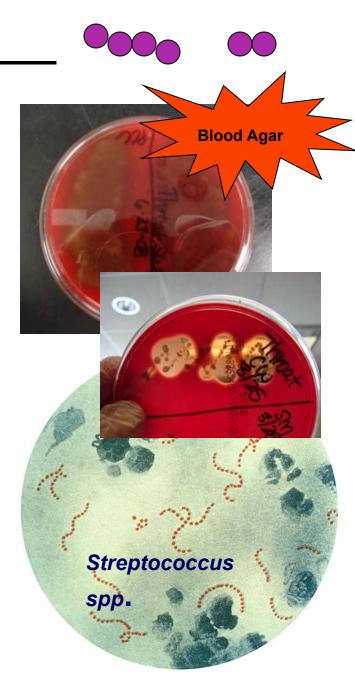
- Group A streptococci (5. pyogenes) cause diseases including strep throat, necrotizing fasciitis (flesh-eating disease), scarlet fever, postpartum fever, and streptococcal toxic shock syndrome.
- Group B streptococci (*S. agalacitiae;* say a-ga-LAC-tea-ae) can cause life-threatening pneumonia and meningitis in newborns the elderly and adults with compromised immune systems.

Group B strep infections are different from other strep infections. Individual can be colonized by the bacteria before any symptoms are obvious.

Women screened for GBS during pregnancy. Approx 10-30 percent carry GBS in vagina or surrounding area. Usually harmless in healthy adults, but may cause stillbirth and serious infections in babies.

Group A and B distinguished based on **antigens** (specific chemicals that our immune system reacts to) in their cell walls.

Images: Hemolysis patterns on Blood Agar, T. Port; *Streptococcus* bacteria <u>Public Health Image Library</u> 900x,, #2110.





Q: Is Blood agar selective?

Q: Is Blood Agar differential? Explain.





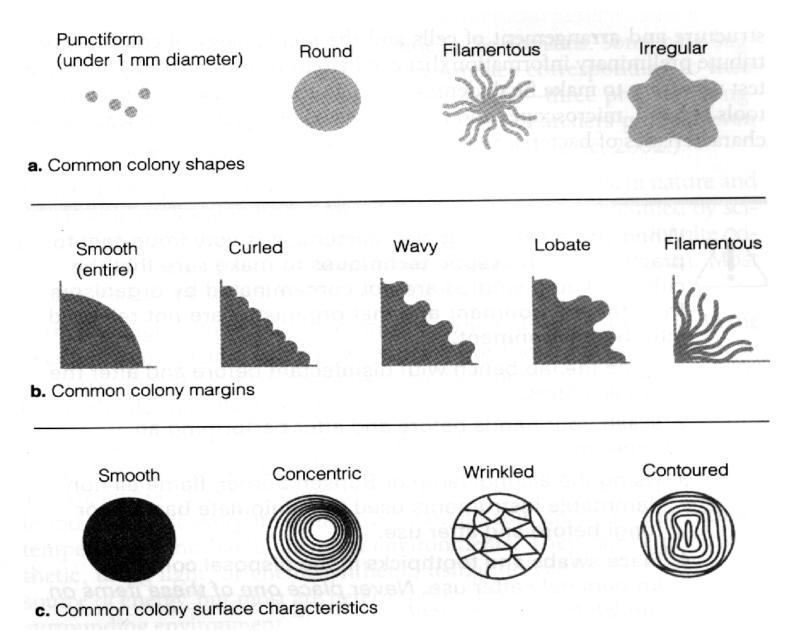
Images: Beta-hemolysis, Alpha-hemolysis and a sterile plate of <u>Blood Agar</u>, T. Port

Microbial Colony Morphology



Q: What is the difference between colony morphology and cell morphology?

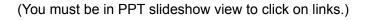
Microbial Colony Morphology

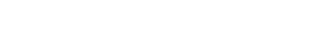


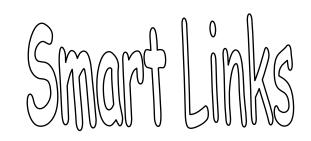
Confused?

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

- Media & Culture Laboratory Main Page on the Virtual Microbiology Classroom of <u>Science Prof Online</u>.
- "<u>Germs</u>", 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 population growth</u> 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 <u>www.ScienceProfOnline.com</u>