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.

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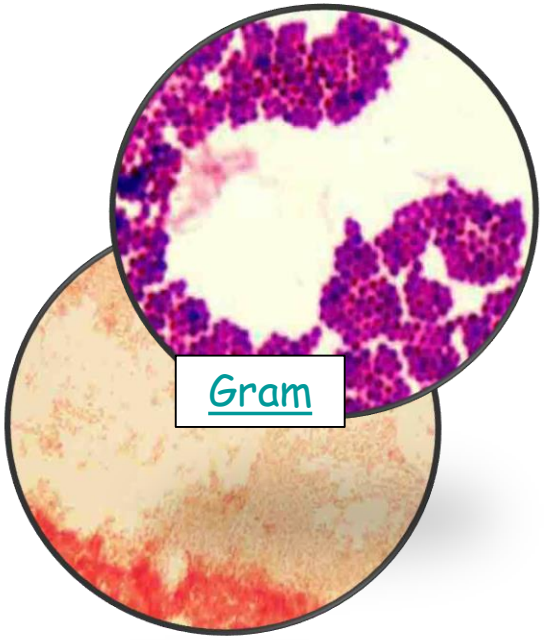
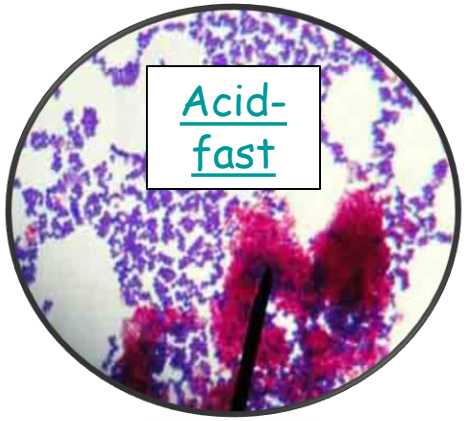
Image: Compound microscope objectives, T. Port

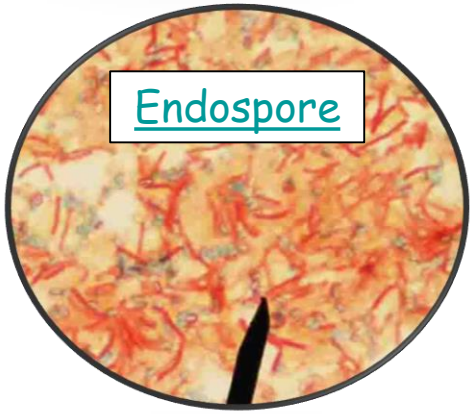
Laboratory Exercise 3

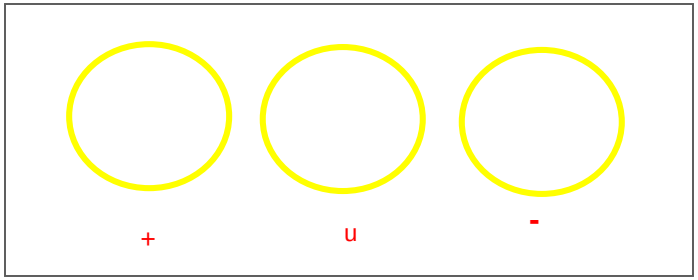
Differential Staining

- Gram Stain
- Acid-fast Stain
- Endospore Stain





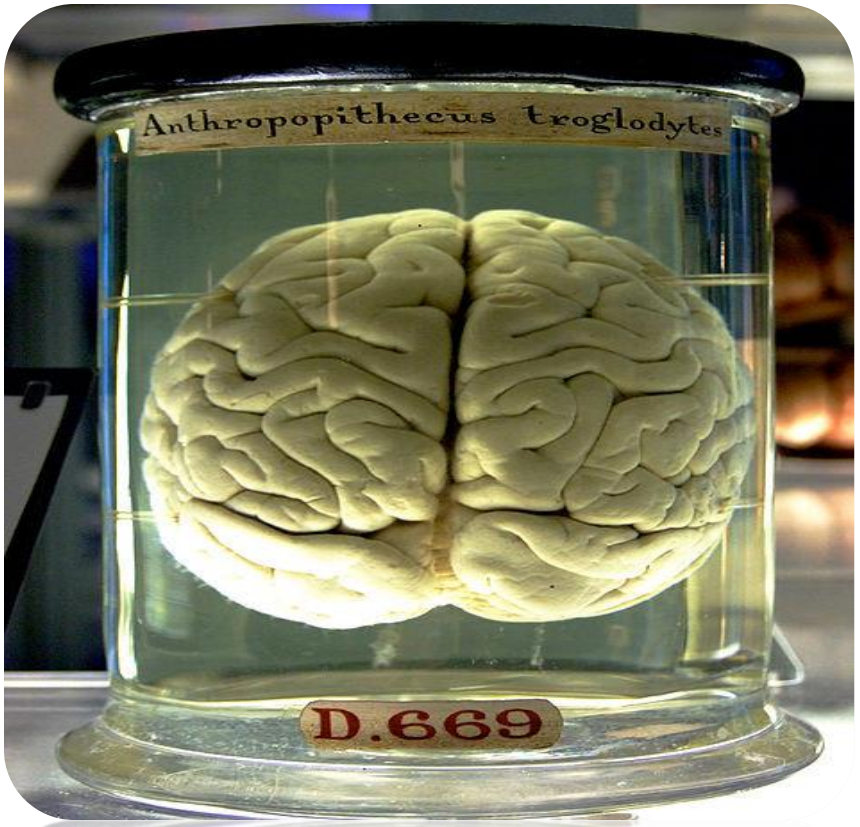




Images: Acid fast stain & mordant step of Gram stain, both by T. Port

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

- Perform Gram, Acid fast and Endospore stains.
- Compare and contrast differential staining procedures and the clinical information obtained from performing them.
- Practice viewing bacteria under oil immersion and taking photo micrographs of bacterial samples.



Please plug in your microincinerators.

Image: Chimp brain in a jar, Gaetan Lee

Differential Stains

- Most stains used in microbiology are differential.
- Differential stains involve use of more than one dye, so that certain differences between cell type or structures can be distinguished.

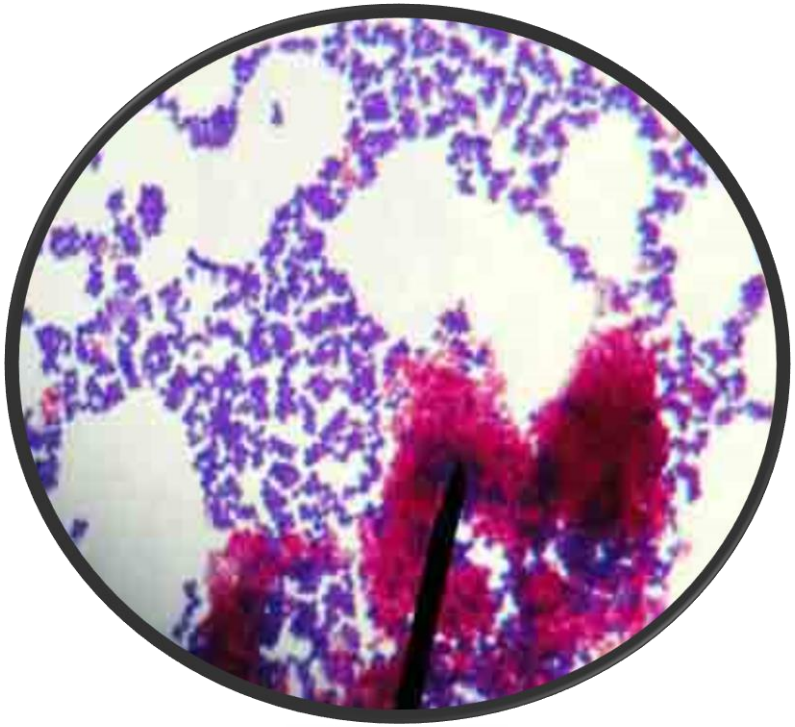


Image: Acid fast stain, T. Port

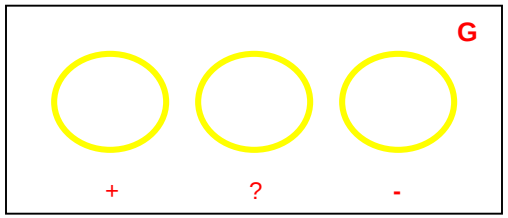
Gram Stain

- Distinguishes between two large groups of microorganisms:
  - purple staining, Gram-positive cells
  - pink staining, Gram-negative cells

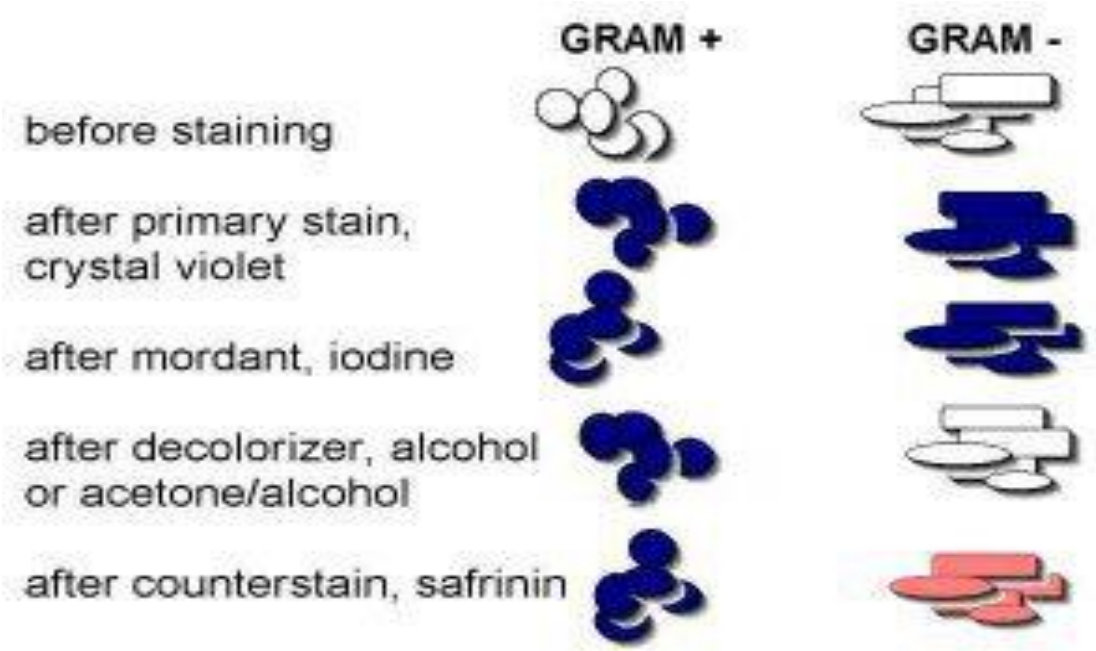
GRAM STAINING PROCEDURE

Crystal violet (1 min) > *rinse*
Iodine (1 min) > *rinse*
Acetone Alcohol (10–15 sec) > *rinse*
Safrinin (1 min) > *rinse & blot dry*

- **Q: What is the difference between Gram+ and Gram- cell wall structure?**



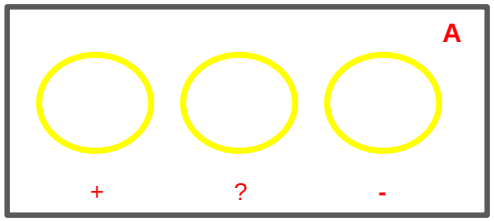
Watch **video** of
How to Do a
Gram Stain



Acid-fast Stain

Q: What does an acid-fast cell have that a non acid fast cell does not?

- purple staining, Non acid-fast cells (NAF)
- bright pink staining, Acid-fast cells (AF)



Watch **video** of
How to Do an
Acid Fast Stain

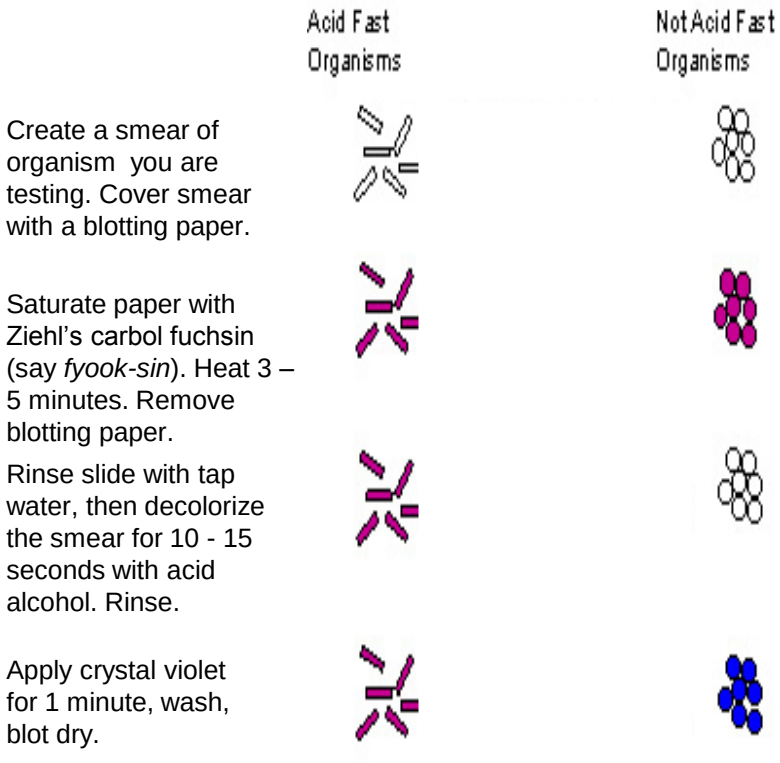
ACID-FAST STAINING PROCEDURE

Blotting paper

Ziehls carbol fuchsin (3 – 5 min heat) > *rinse*

Acid Alcohol (10 – 15 sec) > *rinse*

Crystal violet (1 min) > *rinse & blot dry*



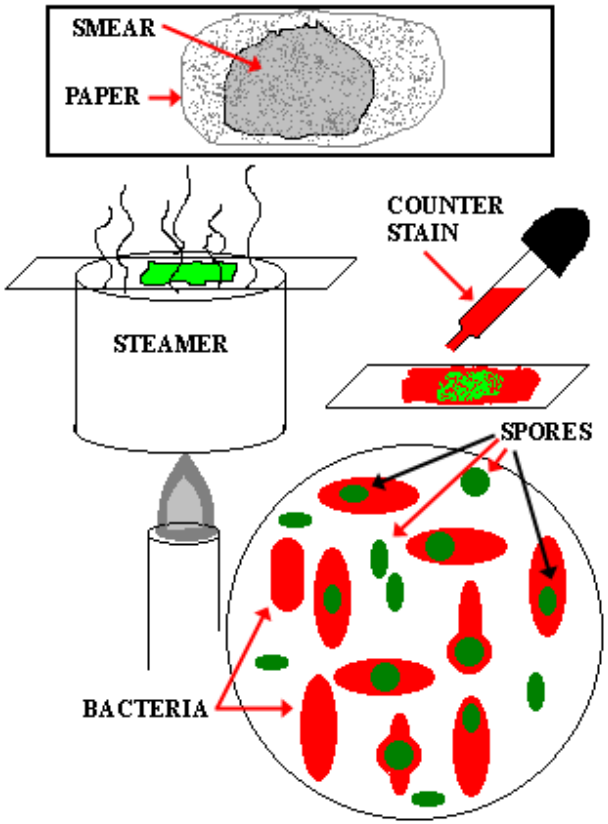
Endospore Stain

ENDOSPORE STAINING PROCEDURE

Malachite Green (5 min heat) > *rinse*

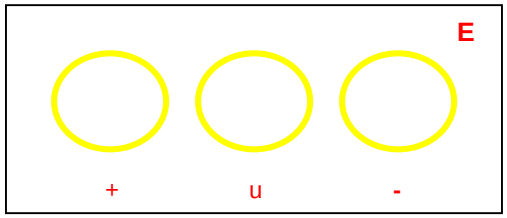
Safrinin (1 min) > *rinse & blot dry*

- Distinguishes between two things:
  - endospores, which stain green
  - vegetative cells, which stain pink
- Some bacteria produce endospores, dormant, highly-resistant structures that can survive environmental extremes (desiccation, heat, harmful chemicals).
- Most notable genera: *Bacillus* and *Clostridium*
- Endospores cannot be stained by normal staining procedures because their walls are practically impermeable.
- Endospore stain uses heat to drive the primary stain, (malachite green) into the endospore.



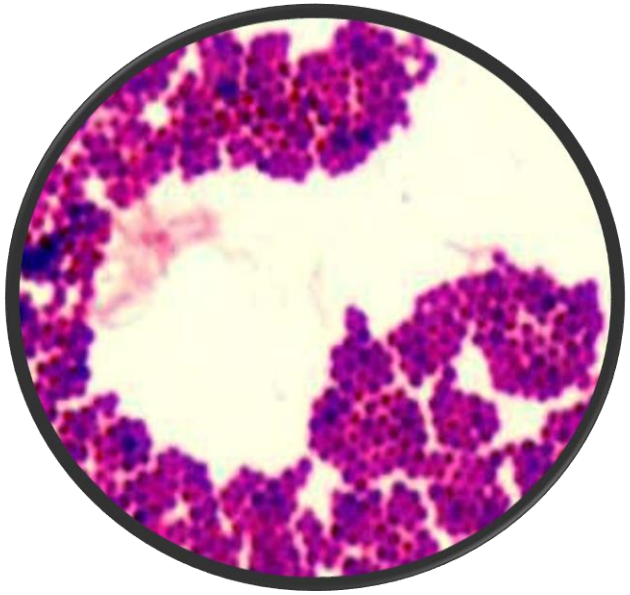
Q: What color(s) will I see if sample is negative?

Q: What color(s) will you see if sample is positive?

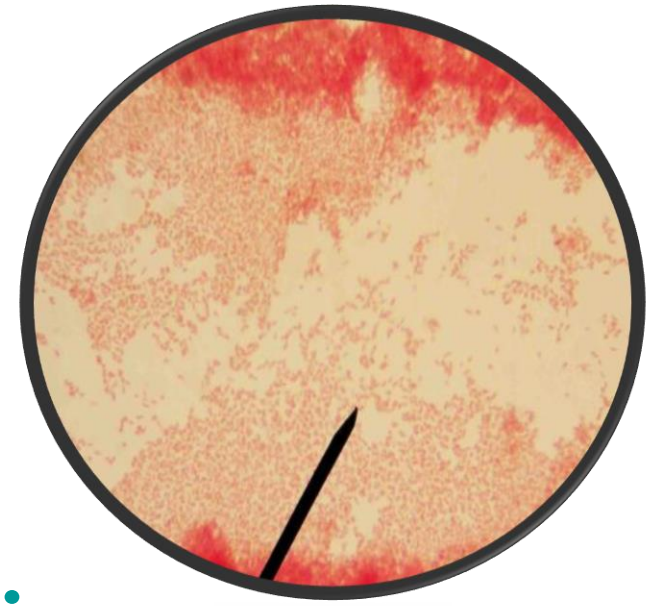


Watch **video** of How to Do an Endospore Stain

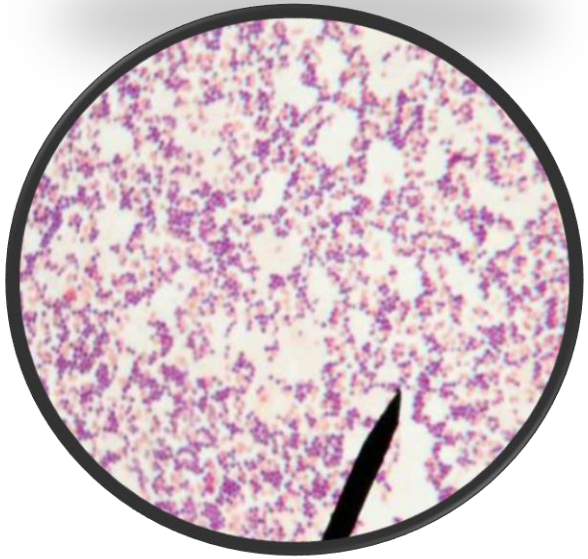
Gram Stain Examples



Staphylococcus epidermidis



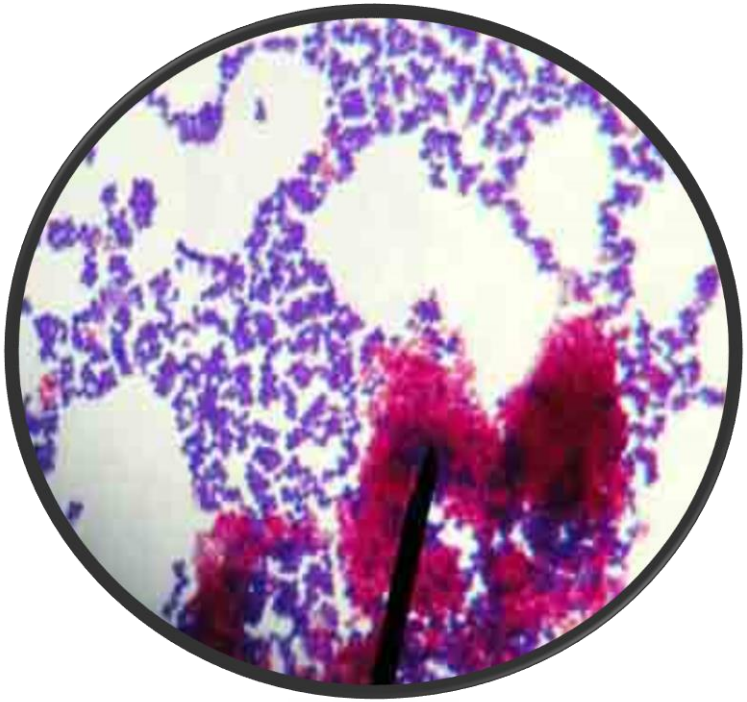
Escherichia coli



Mixed Sample of *S. epidermidis* & *E. coli*

Acid Fast Stain

Examples

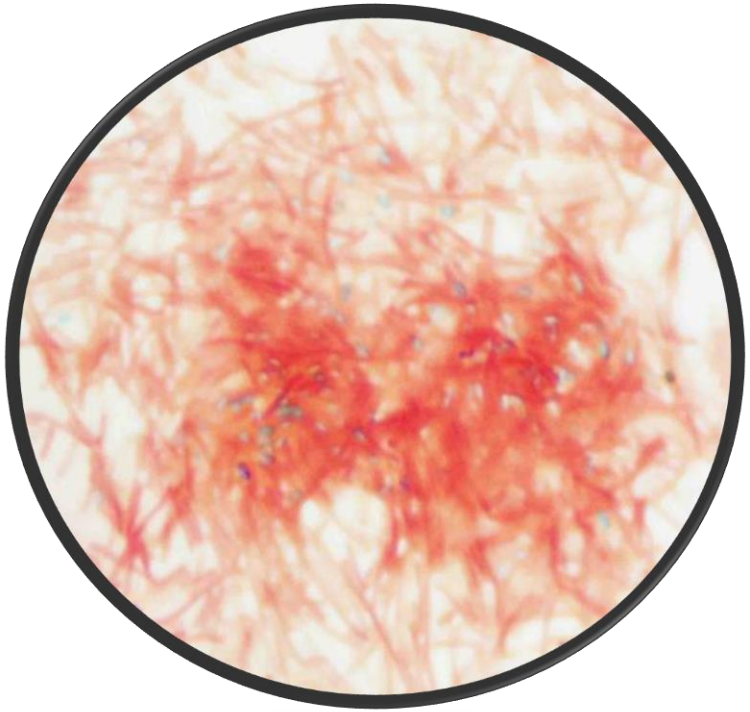


Mixed sample of *Mycobacterium smegmatis* & *Micrococcus luteus*

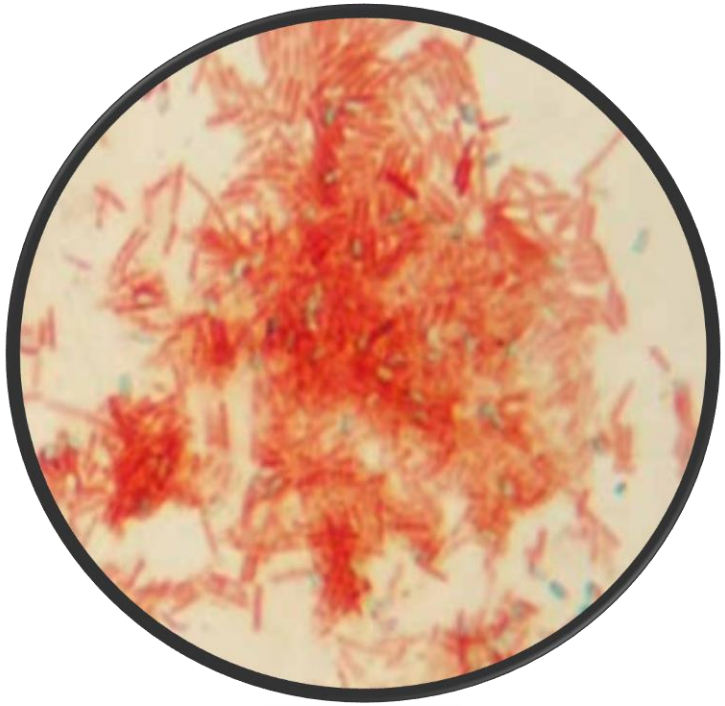
Mycobacterium smegmatis

Endospore Stain

Examples

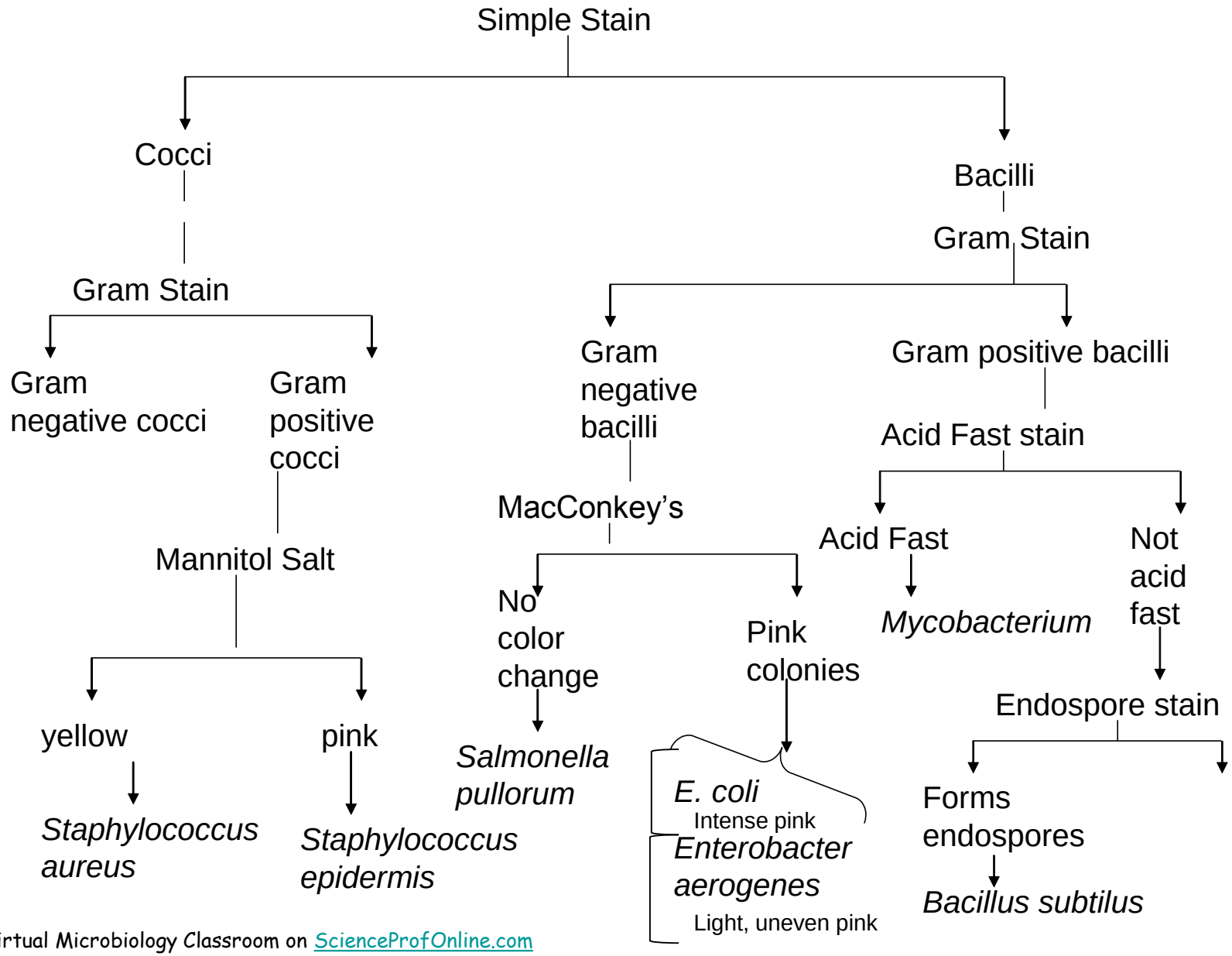


Bacillus cereus

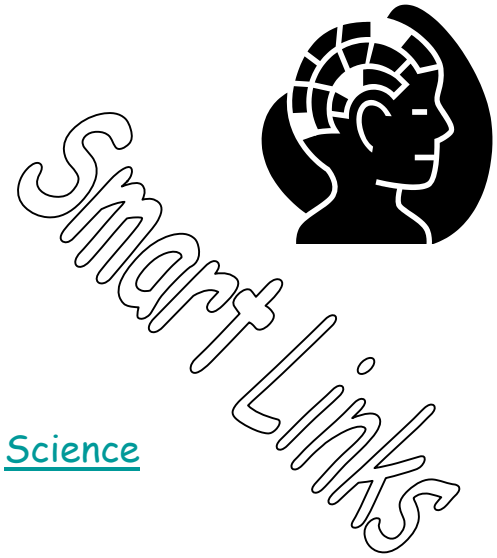


Q: Why are we doing **Differential Stains** of our bacterial unknown?

Dichotomous Key



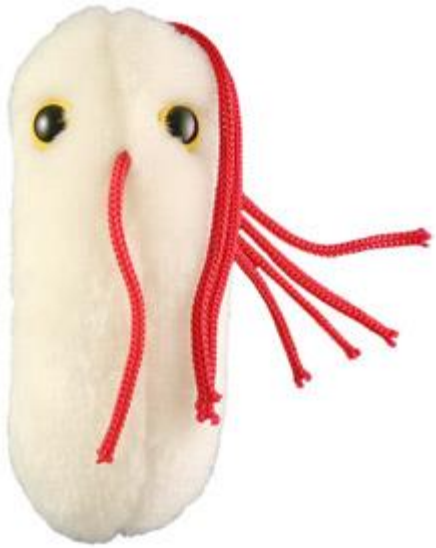
Confused?



Here are links to fun resources that further explain bacterial identification:

- **Differential Stain** Main Page on the Virtual Microbiology Classroom of Science Prof Online.
- Gram Stain Interactive Tutorial. This is an extremely useful tutorial that shows, step-by-step, what happens in Gram-positive and Gram-negative cells during Gram staining.
- Acid-fast Stain Animated Tutorial. The staining procedure depicted in this tutorial differs a bit from how we do it in lab, but this tutorial is still very useful. Shows the steps of the staining procedure and the resulting color of Acid-fast and Nonacid-fast cells.
- Endospore Stain PowerPoint. Although this is just a PPT, it does have useful information and images for students learning about the endospore stain.
- Videos of differential staining procedures: Gram, Acid-fast, Endospore

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

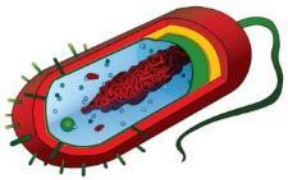


Are microbes intimidating you?

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: Salmonella, Giant Microbes; Prokaryotic cell, Mariana Ruiz