# 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.



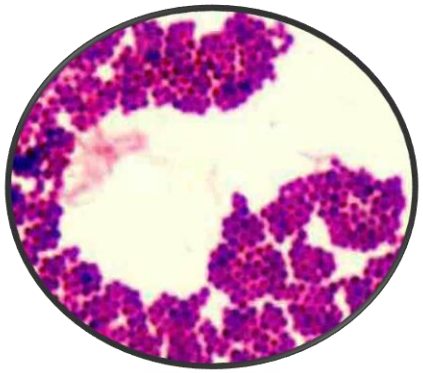
Alicia Cepaitis, MS
Chief Creative Nerd
Science Prof Online
Online Education Resources, LLC
alicia@scienceprofonline.com

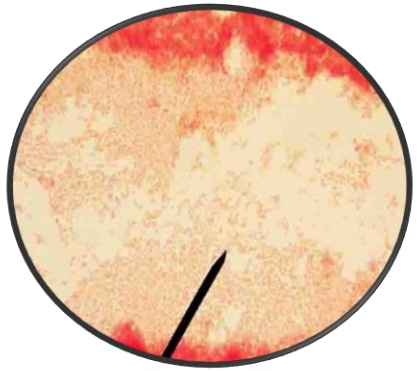
Tami Port, MS
Creator of Science Prof Online
Chief Executive Nerd
Science Prof Online
Online Education Resources, LLC
info@scienceprofonline.com



Image: Compound microscope objectives, T. Port

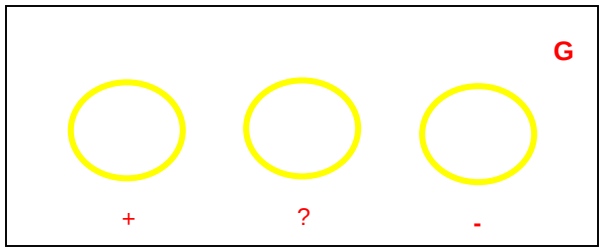
Laboratory Exercise 2





# Gram Stain

- ✓ Prepare Bacterial Smear for Gram Staining
- ✓ Gram Stain Procedure
- ✓ Identification of Unknown Bacteria







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

# What am I going to learn from Lab Topic #2?
# Gram Staining

- Provide student with opportunity to perform a Gram stain with controls and an unknown.
- Practice evaluating Gram stain results.
- Practice viewing bacteria under oil immersion and taking photo micrographs of bacterial samples.

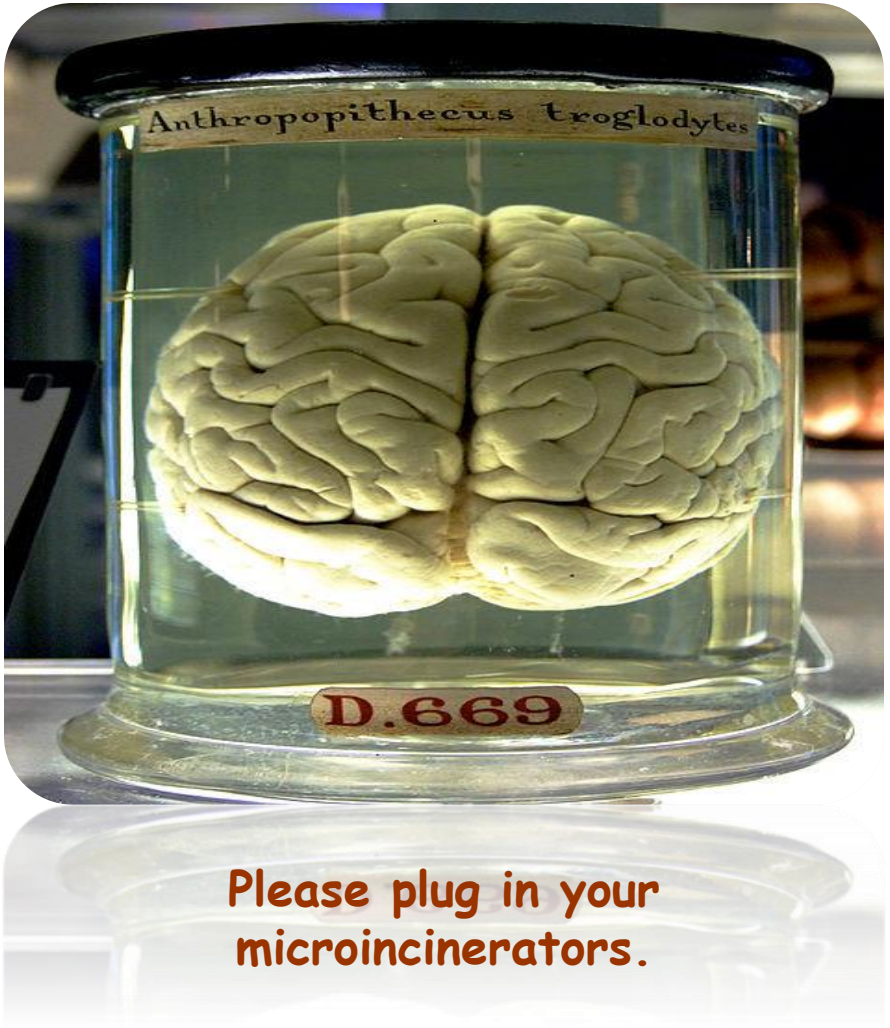





Image: Chimp brain in a jar, Gaetan Lee

# 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.

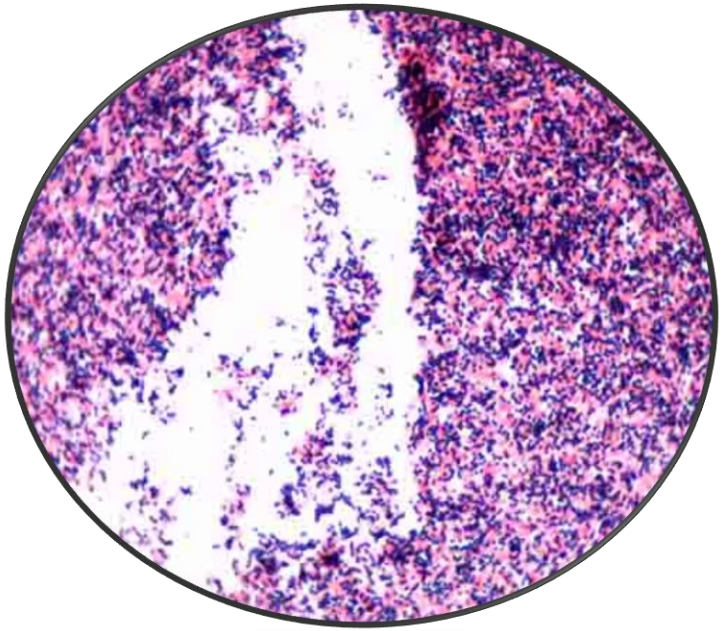
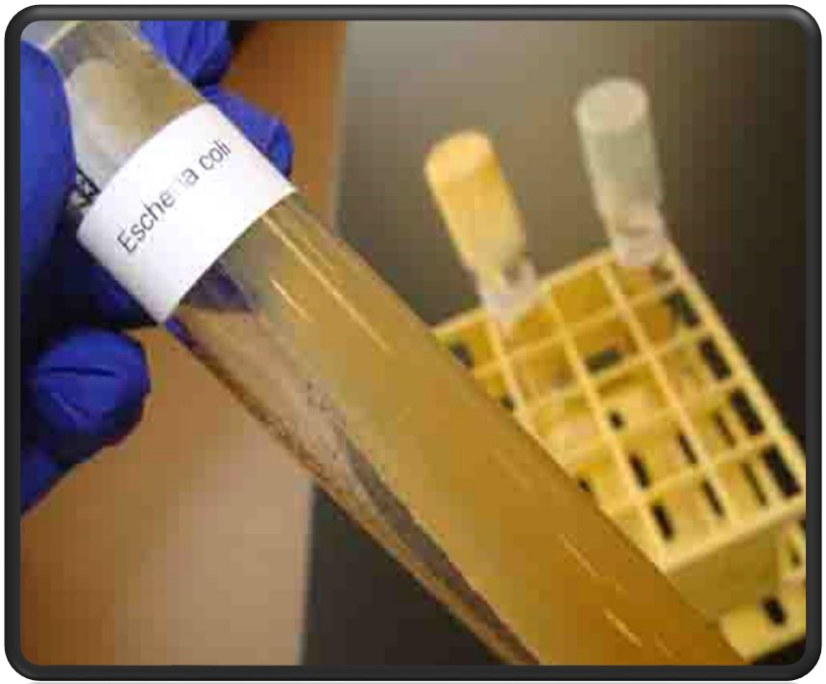


Image: Acid fast stain, T. Port

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.

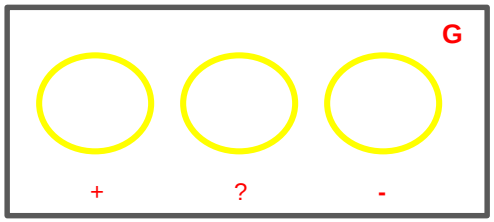


Image: E. coli growing on TSY agar in slant tube and in Petri dish, T. Port

# Gram Stain

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

- **Q**: What is the difference between Gram+ and Gram- cell wall structure?
- **Q**: Why do we draw three circles on the slide?

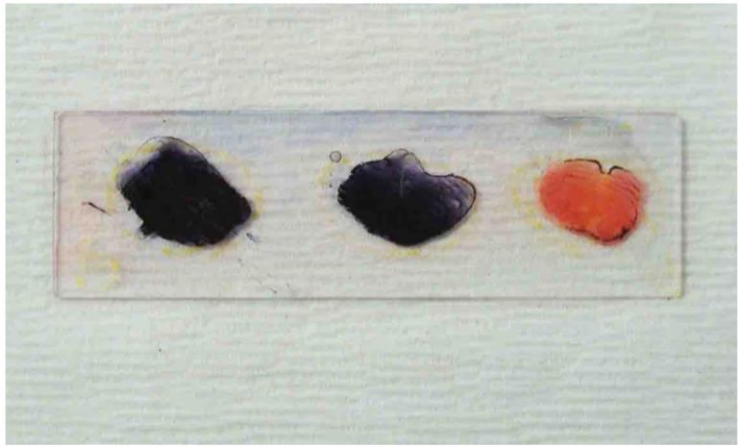



Watch **video** on
How to Prepare a Bacterial Smear for Gram Staining

## How to prepare bacterial smear for Gram staining:

- Draw three circles on slide using wax pen
- Also include a "G" to identify that slide will be Gram stained.
- Flip slide over.
- Use DI water dropper to place very small drop of water inside each circle.
- Using a sterilized inoculation loop, take a small sample of your unknown. *Be gentle!* The bacteria is on the surface of the medium.
- Swirl into the water in the center circle of your slide.
- ***Q**: Why are there two additional circles on our slide?*
- Use same method to add controls to circle on left and right.
- Heat fix the slide on top of your microincinerator. Allow it to stay in the platform for 5 minutes after water has completely evaporated.

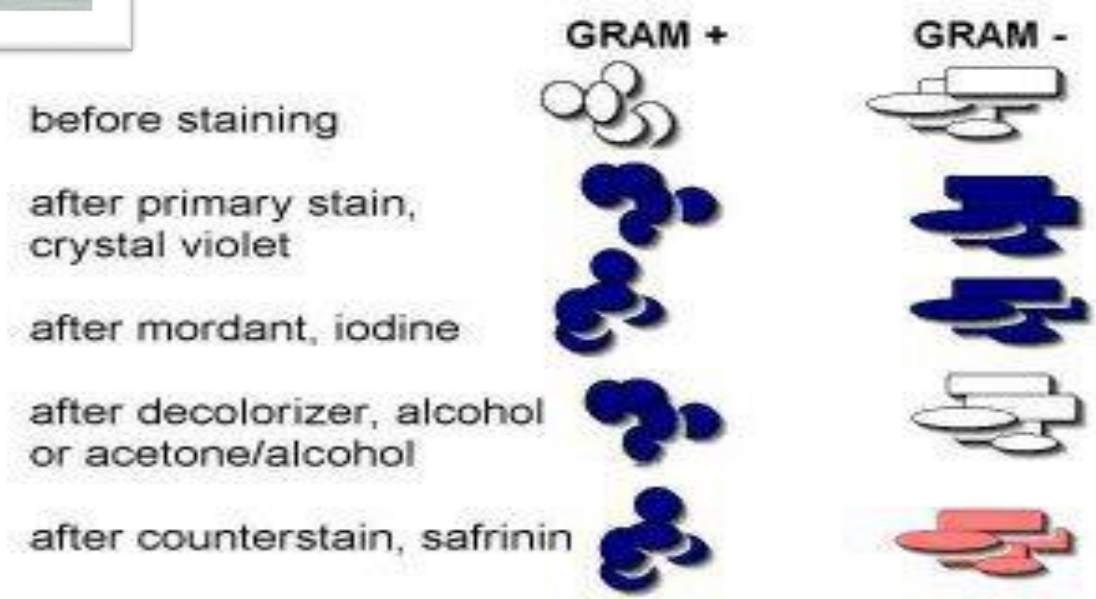
# Gram Stain



## GRAM STAINING PROCEDURE

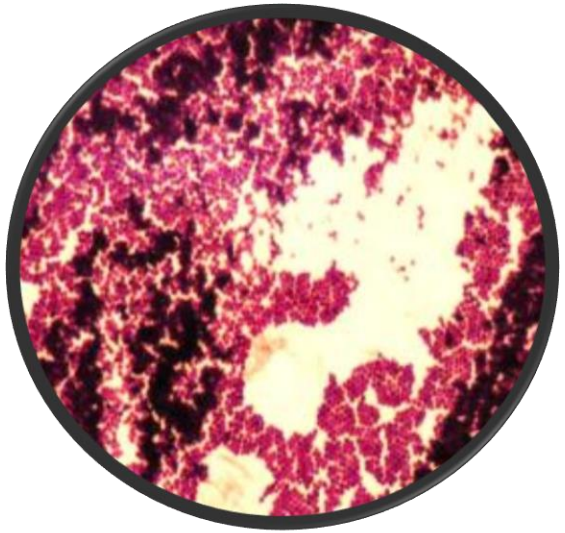
**Crystal violet** (1 min) > *rinse*
**Iodine** (1 min) > *rinse*
**Acetone Alcohol** (10–15 sec) > *rinse*
**Safrinin** (1 min) > *rinse & blot dry*

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



Although you will be using 100xTM to get your specimen in focus, you will not be able to see individual bacteria until you use the 1000xTM oil immersion lens.

**Bacterial smear at 100xTM**



**Bacterial smear at 1000xTM**

**(oil immersion)**

FYI: The example above is *Staph*.

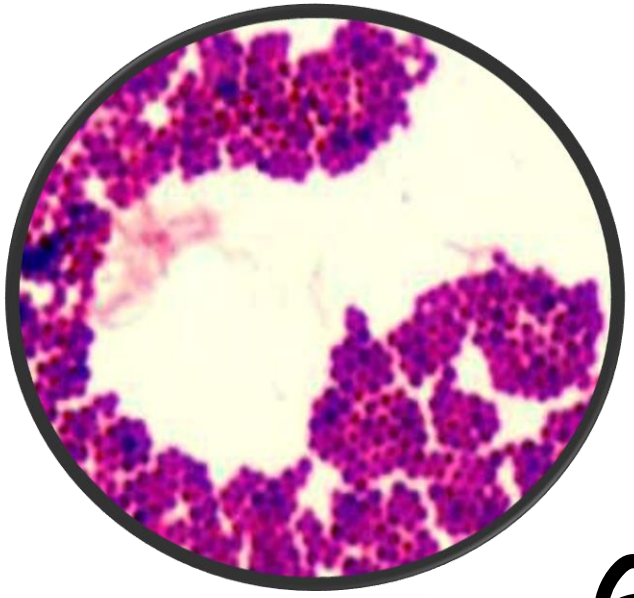
**Remember, you want to use the microscope with a built in camera so that you can take pictures of the bacteria you see at 1000xTM.**



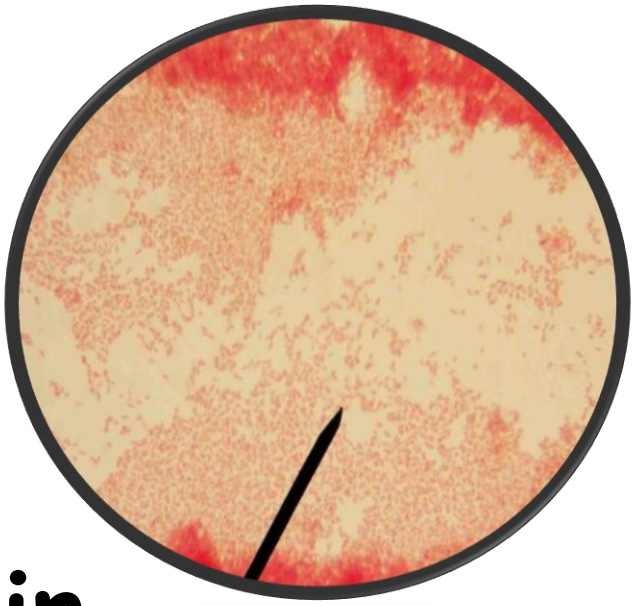
Images: Both images *Staphylococcus*, by T. Port

# Gram Stain Examples

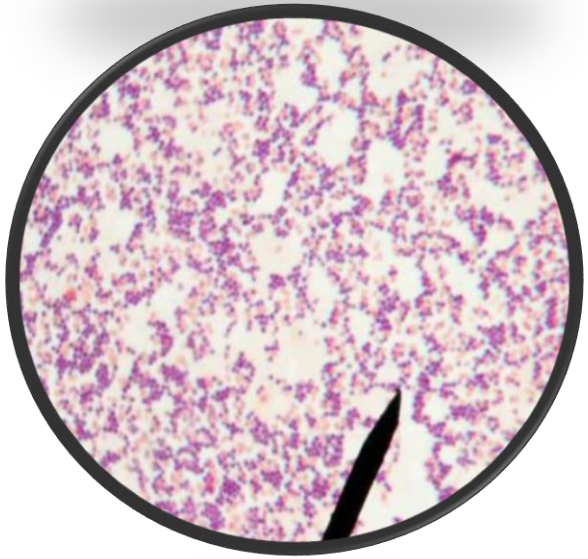
@ 1000xTM



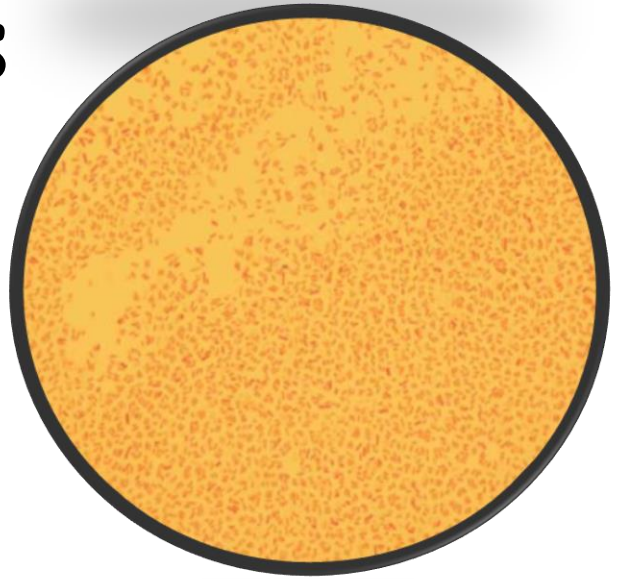
*Staphylococcus epidermidis*



*Escherichia coli*



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



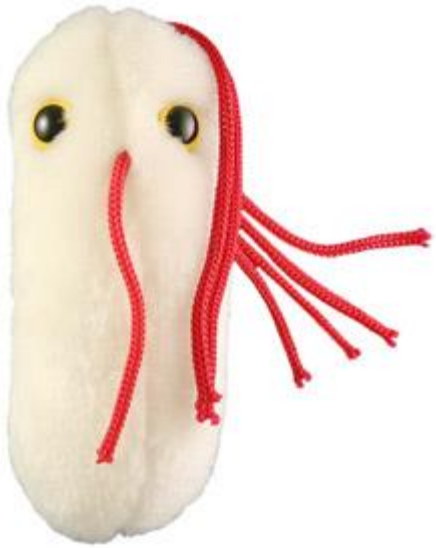
# Confused?

Here are links to fun resources that further explain Gram Staining:

- **Gram Stain** Lab 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.
- Video on How to Prepare a Bacterial Smear for Gram Staining
- Video of Gram Stain Procedure

Smart Links

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

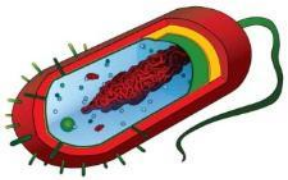


# Are microbes intimidating you?

*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