



# 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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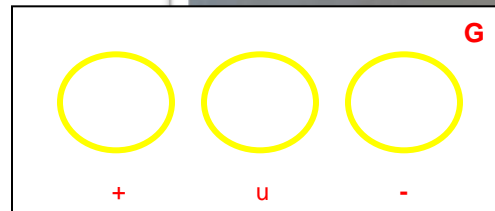
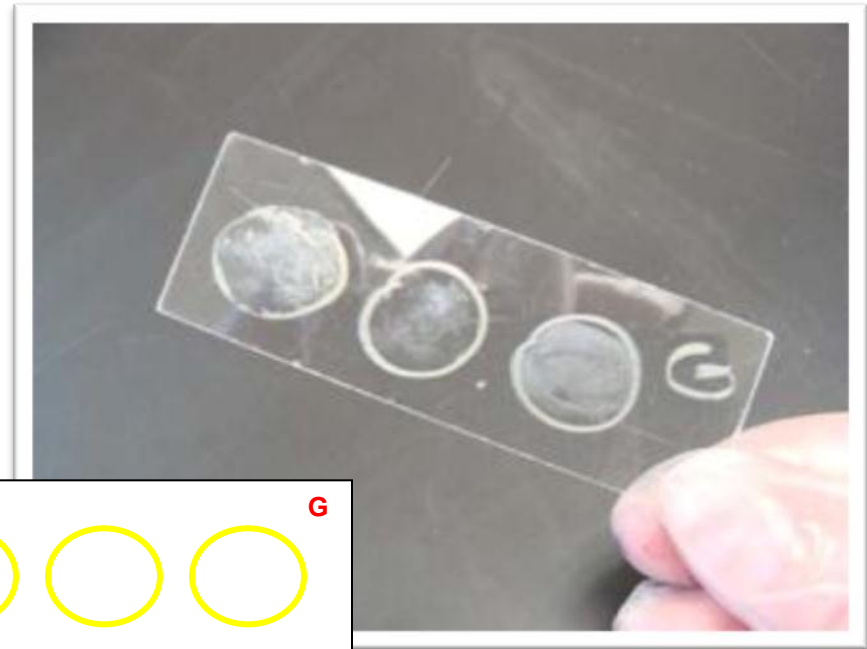
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## Laboratory Exercise 3a

# Identification of Unknown Bacteria (Part I):

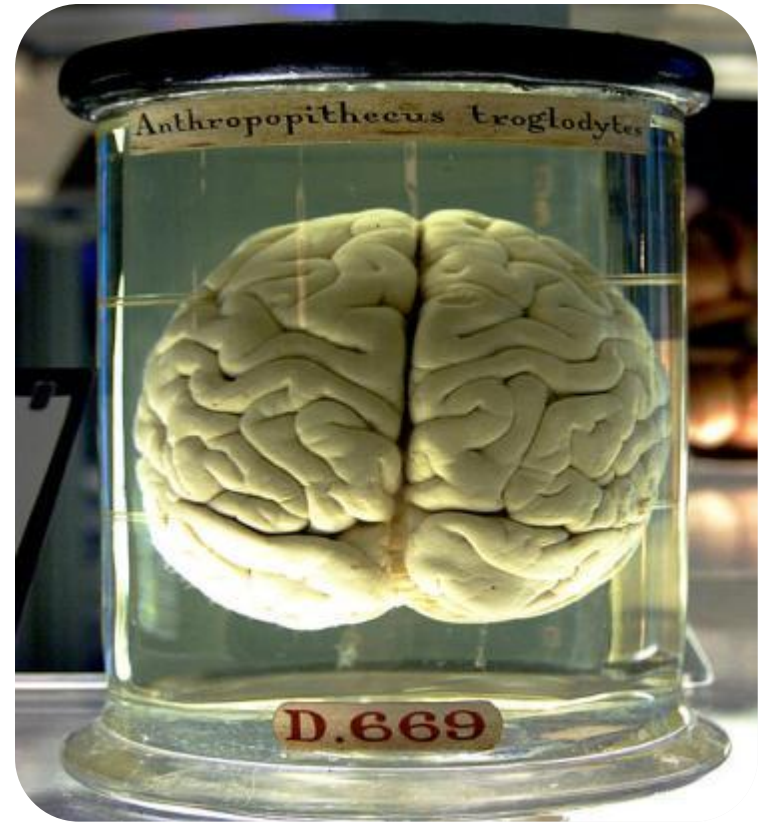
## Preparing Bacterial Smears for Differential Staining



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

## Identification of Unknown Bacteria

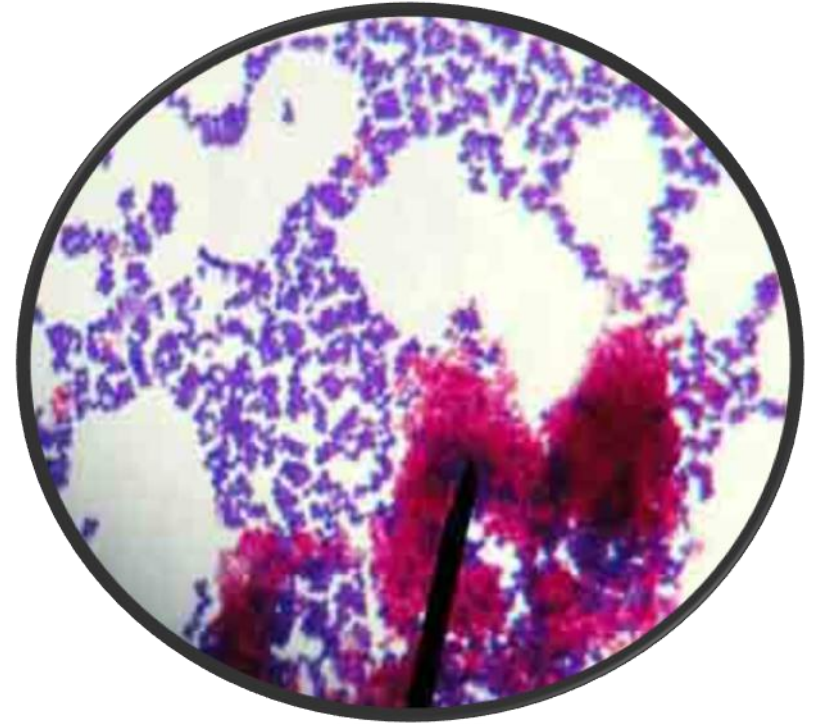
- You will practice performing isolation streak plates using aseptic technique.
- How to prepare bacterial smears which include controls and an unknown.
- You will be introduced to three differential staining techniques: Gram stain, Acid-fast stain & Endospore stain.



**Please plug in your  
microincinerators.**

# 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.



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!

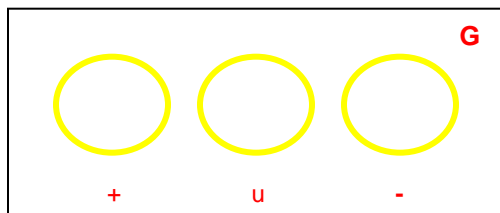
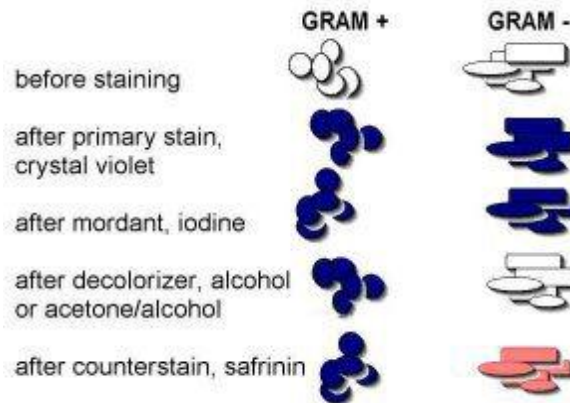


If obtaining bacterial sample from slant tubes:

- 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.

# Gram Stain

- Distinguishes between two large groups of microorganisms:
  - purple staining, Gram-positive cells
  - pink staining, Gram-negative cells
- These two types of cells differ significantly in the chemical and physical structure of their cell wall.
- The structure of the thinner cell walls of Gram negative bacteria cannot hold the dyes previously used, once the decolorizer is applied.



Watch **video** of  
[How to Prepare a Bacterial Smear for Gram Staining](#)

To prepare Gram bacterial smear for staining next week:

- 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.

# Acid-fast Stain

- Distinguishes cells that have mycolic acid in cell wall, from those that do not:
  - purple staining, Nonacid-fast cells (NAF)
  - bright pink staining, Acid-fast cells (AF)
- Cell are considered to be Acid-fast if they have mycolic acid in their cell wall.
- *Q: What is mycolic acid, and which types of bacteria have it?*

## Ziehl-Neelson Stain Kinyoun Modification

### Acid Fast Organisms



### Not Acid Fast Organisms



A small amount of organism suspended in saline solution is fixed on a slide.

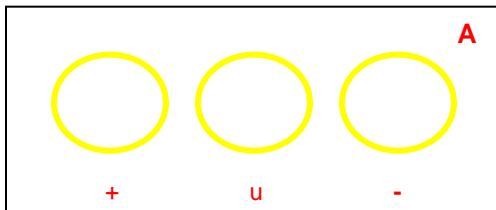
Slide is flooded with Carbol Fuchsin and phenol for 3 minutes, and gently rinsed with water.

Slide is decolorized with 3% HCl in 70% alcohol until color appears to be removed (approx. 2 mins), and rinsed with water.

Slide is flooded with methylene blue counterstain for 30 secs, rinsed with water and air-dried.

## To prepare Acid-fast bacterial smear for staining next week:

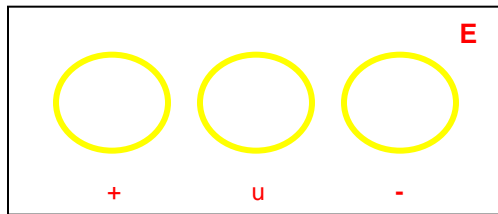
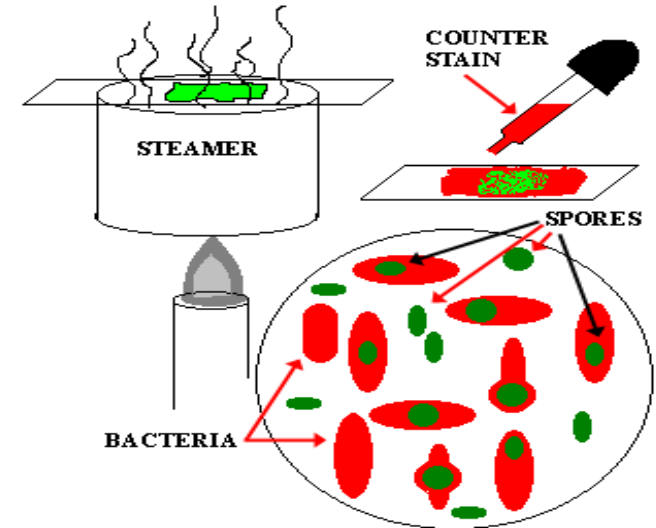
- Draw three circles on slide using wax pen.
- Also include an "A" to identify that slide will be Acid-fast stained.
- Flip slide over.
- Use DI water dropper to place very small drop of water inside each circle.
- Using a sterilized [inoculation loop](#), take a small sample of your unknown. **Be gentle!** The bacteria is on the surface of the medium.
- Swirl into the water in the center circle of your slide.
- *Q: What (+) and (-) control can we use for this stain?*
- 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.



Watch **video** of  
[How to Prepare a Bacterial Smear for Acid Fast Staining](#)

# Endospore Stain

- Distinguishes between two things:
  - endospores, which stain green
  - vegetative cells, which stain pink
- *Q: What is an endospore?*
- *Q: What two genera of endospore-producing bacteria have we studied in class?*



Watch **video** of  
[How to Prepare a Bacterial Smear for Endospore Staining](#)

**To prepare Endospore bacterial smear for staining next week:**

- Draw three circles on slide using wax pen.
- Also include an "E" to identify that slide will be Endospore 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: What (+) and (-) control can we use for this stain?*
- 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.



# Confused?



Here are links to fun resources that further explain streak plate technique and differential staining:

- [How to Prepare a Bacterial Smear for Gram Staining](#), video from 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.
- [How to Prepare a Bacterial Smear for Acid Fast Staining](#), video from Science Prof Online.
- [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.
- [How to Prepare a Bacterial Smear for Endospore Staining](#), video from Science Prof Online.
- [Endospore Stain](#) PowerPoint. Although this is just a PPT, it does have useful information and images for students learning about the endospore stain.

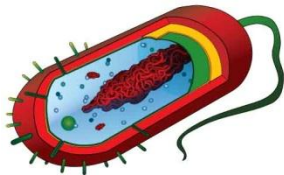


# Are microbes intimidating you?

*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](http://www.ScienceProfOnline.com)