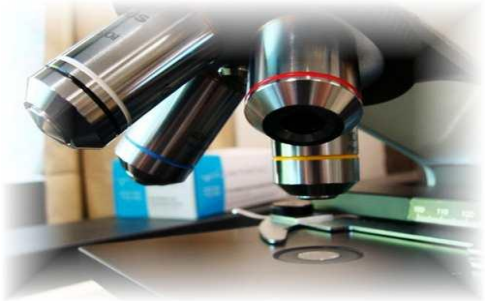

About Science Prof Online PowerPoint Resources

• 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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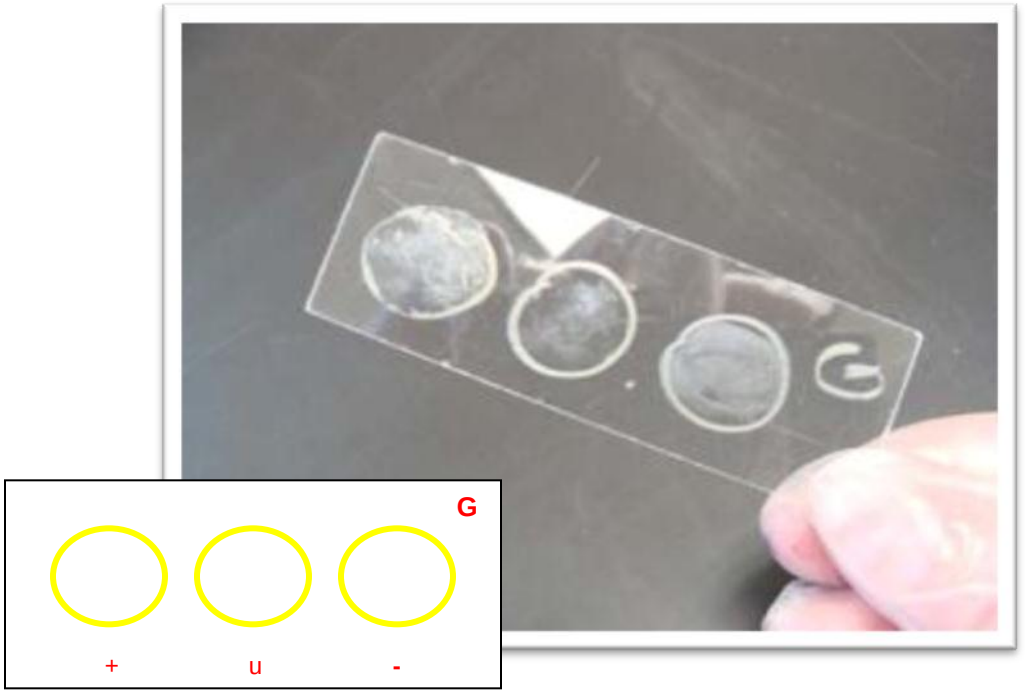
Image: Compound microscope objectives, T. Port

Laboratory Project 2

Differential Staining of Bacterial Cells:

Preparing Bacterial Smears for Differential Staining



Images: Isolation streak plate of *Legionella*, PHIL #7925; Bacterial smear, T. Port

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.

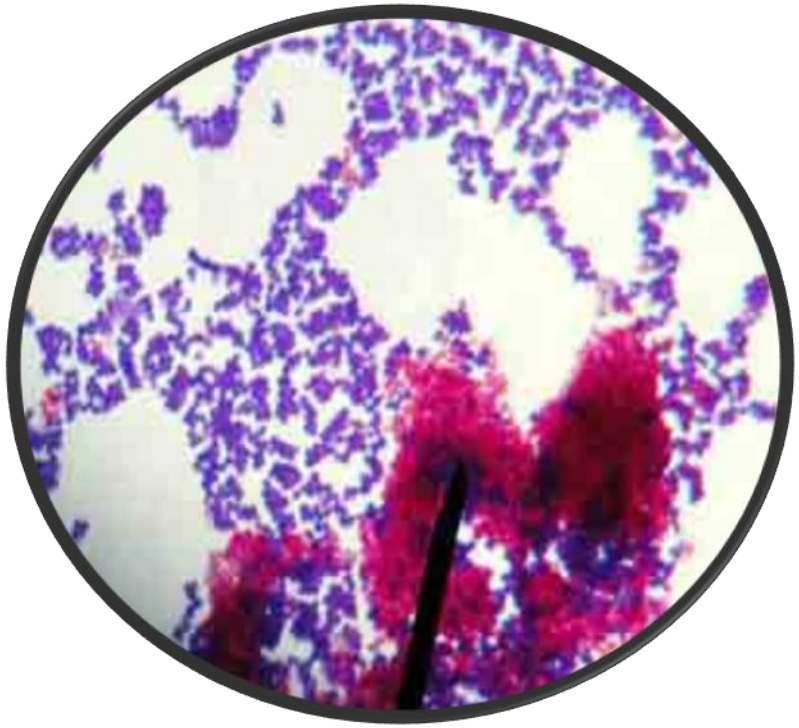
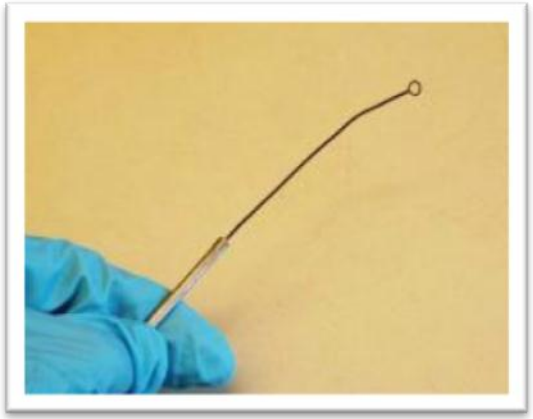
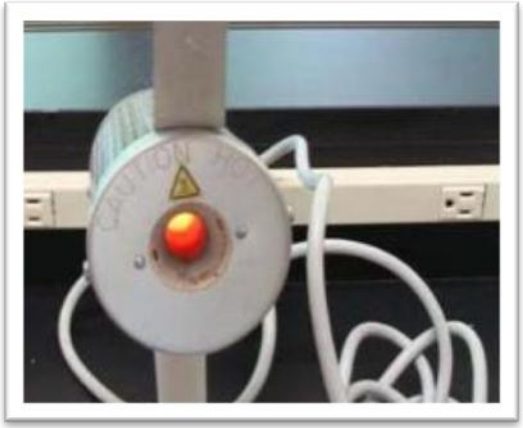


Image: Acid fast stain, T. Port

Inoculation Loop & Aseptic Technique



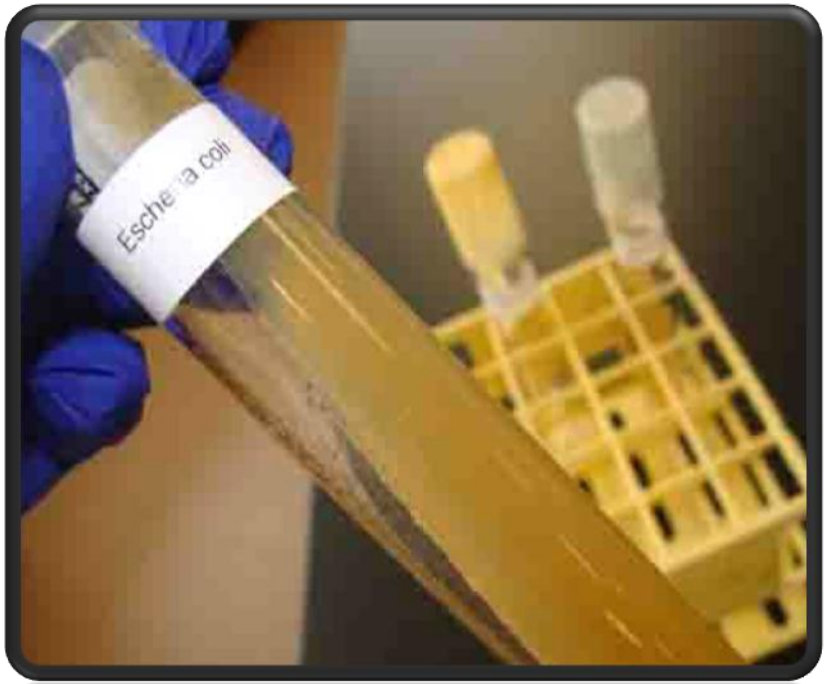
- You will be using an **unknown bacteria** that you will be identifying in a future lab.
- To transfer the bacteria to your slide and make bacterial the smears, you will use an **inoculation loop** (aka smear loop, inoculation wand or microstreaker).
- Simple tool used to retrieve an inoculum from a culture of microorganisms.
- Always sterilize in microincinerator until loop becomes red hot ***before*** and ***after*** each use.
- By doing this, the same tool can be reused in different experiments without fear of cross-contamination.
- Be sure that your inoculation loop has **cooled** before using it to retrieve inoculum or streak a plate!
- If you hear medium *sizzle* when you touch it with loop, the loop is too hot!



Images:; Isolation streak plate of *Legionella*, PHIL #7925
Inoculation loop, Jeffrey M. Vinocur; Microincinerator, T. Port

From the Virtual Microbiology Classroom on ScienceProfOnline.com

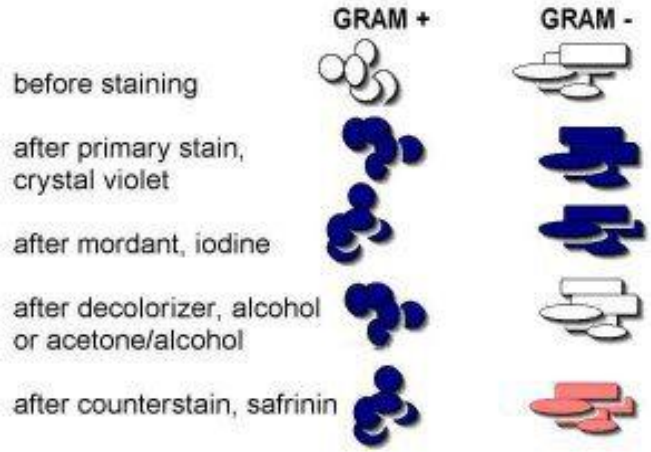
When obtaining a bacterial sample from a tube or plate of media do so **gently**! The bacteria is growing as a thin film on top of the media! Don't scrape so hard that you have pieces of agar in your sample!



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.

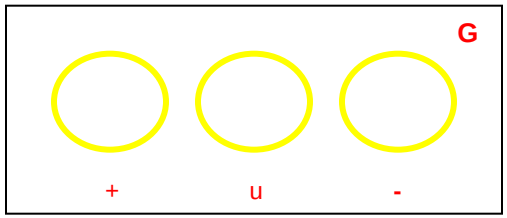
Image: E. coli growing on TSY agar in slant tube and in Petri dish, T. Port

Gram Stain



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

- What is the difference in cell structure of Gram+ vs Gram- baacteria?



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

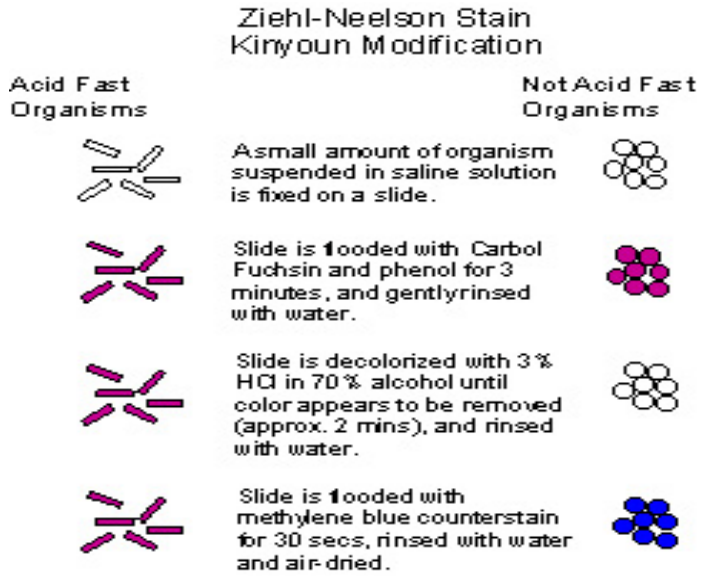
To prepare Gram bacterial smear for staining:

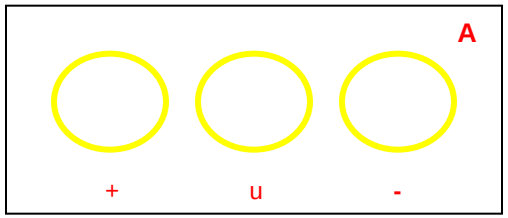
- Draw three circles on slide using wax pen.
- Also include a "G" to identify that slide will be Gram stained.
- Flip slide over.
- Use DI water dropper to place very small drop of water inside each circle.
- Using a sterilized inoculation loop, take a small sample of your unknown. *Be gentle!* The bacteria 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.

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)

- What is the difference in cell structure between acid-fast and nonacid-fast bacterial cells?





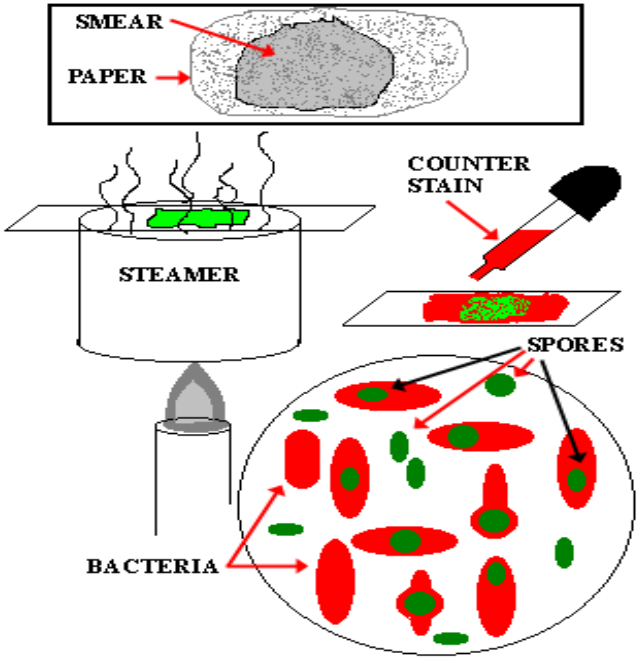
Watch **video** of [How to Prepare a Bacterial Smear for Acid Fast Staining]

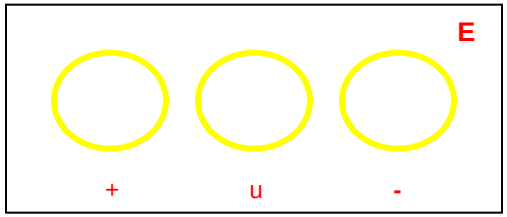
To prepare Acid-fast bacterial smear for staining:

- 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.

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]

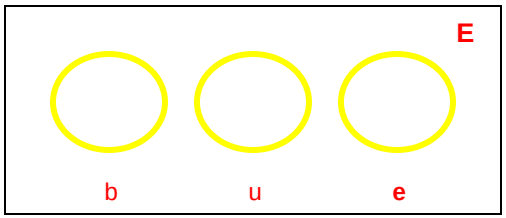
To prepare Endospore bacterial smear for staining:

- 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.

Laboratory Project 2

Differential Staining of Bacterial Cells

Preforming the Gram, Acid-fast & Endospore stains



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

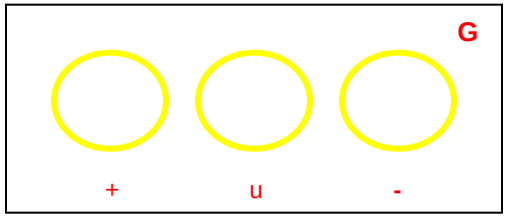
Gram Stain

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

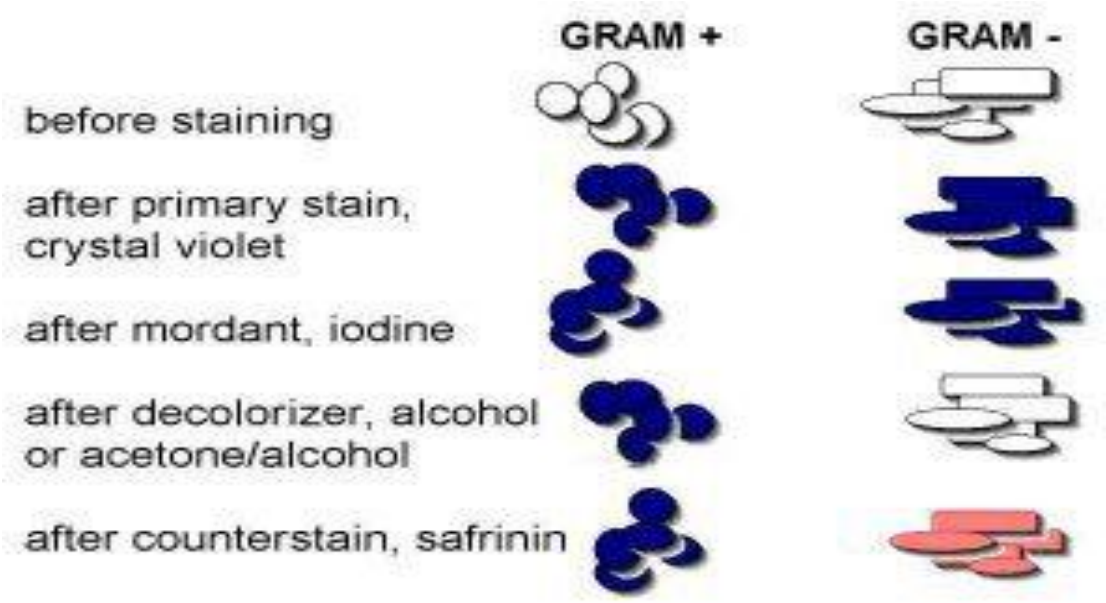
GRAM STAINING PROCEDURE

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- bacterial cell wall structure?



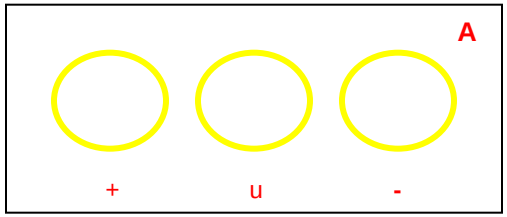
Watch **video** of How to Do a Gram Stain



Acid-fast Stain

- Distinguishes between two groups of microorganisms:
  - purple staining, Nonacid-fast cells (NAF)
  - bright pink staining, Acid-fast cells (AF)

- ***Q:*** *What is the difference between AF and NAF bacterial cell structure?*



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

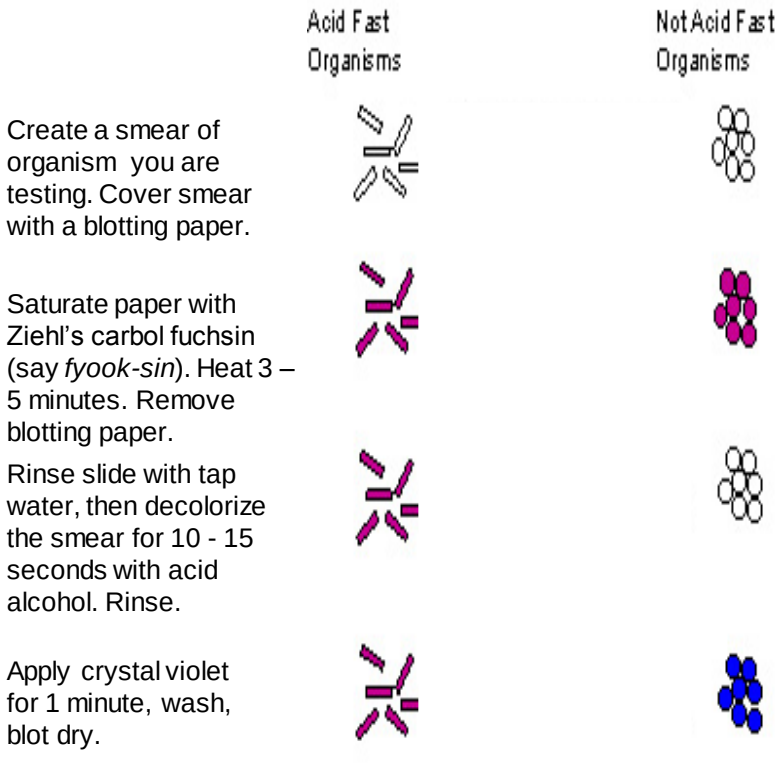
ACID-FAST STAINING PROCEDURE

Blotting paper

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

Acid Alcohol (10 – 15 sec) > *rinse*

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



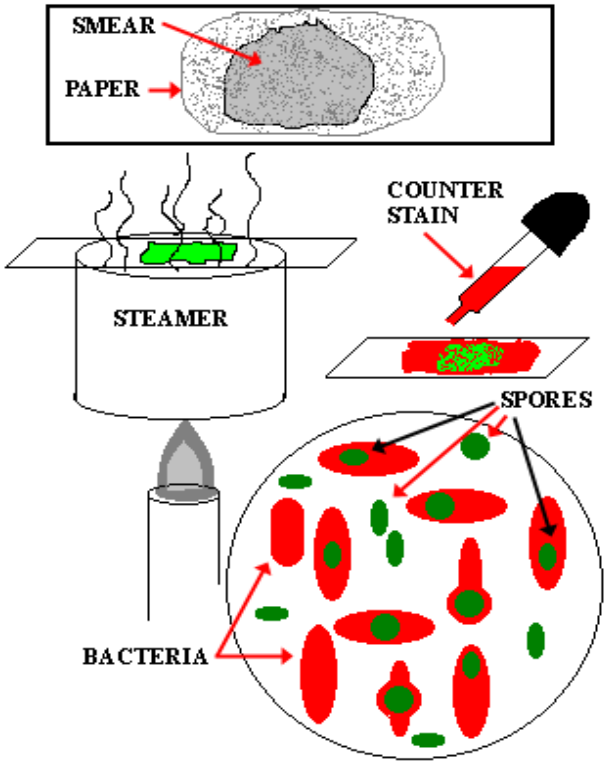
Endospore Stain

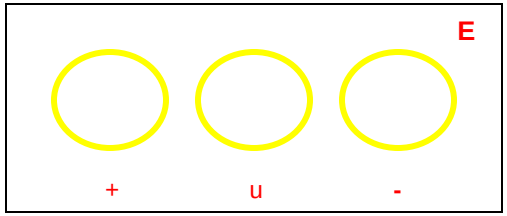
ENDOSPORE STAINING PROCEDURE

Malachite Green (5 min heat) > *rinse*

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

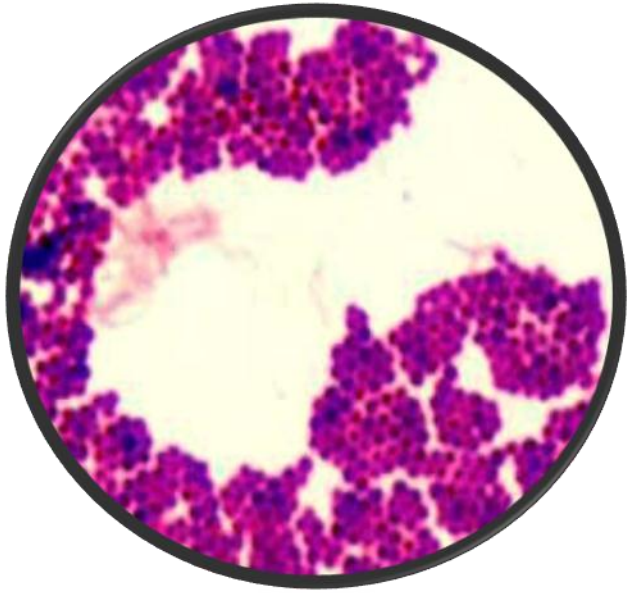
- Distinguishes between two things:
  - endospores, which stain green
  - vegetative cells, which stain pink
- Some bacteria produce endospores; dormant, highly-resistant structures that can survive environmental extremes (desiccation, heat, harmful chemicals).
- Most notable genera: *Bacillus* and *Clostridium*
- 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 or colors will I see in my endospore + control?*
  What color or colors will I see in my endospore – control?



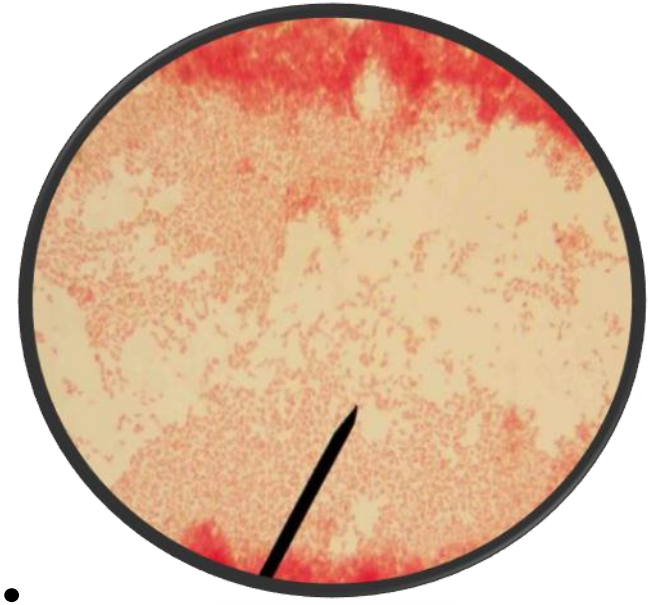


Watch **video** of
How to Do an
Endospore Stain

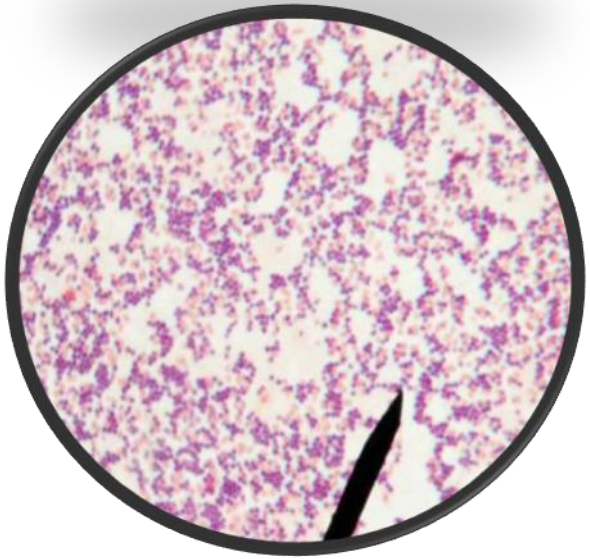
Gram Stain Examples



Staphylococcus epidermidis

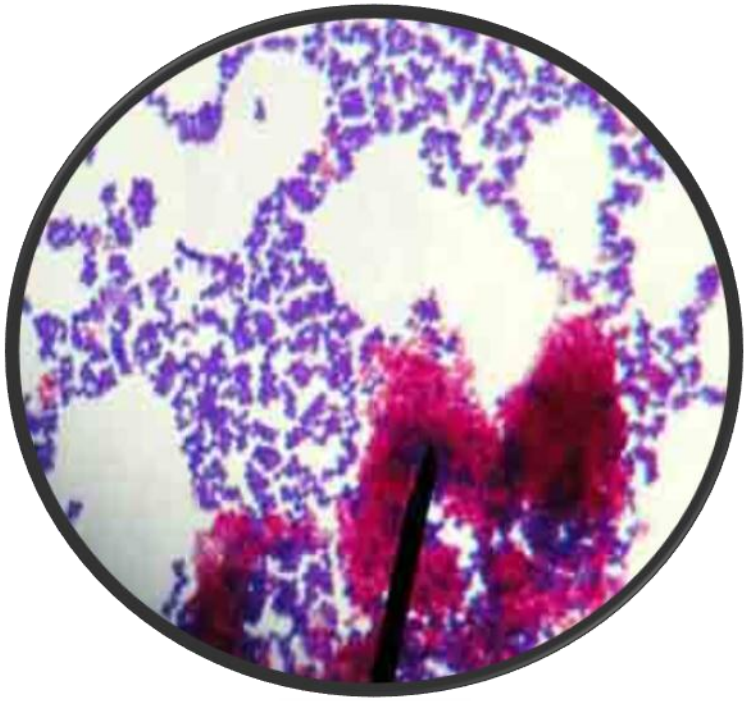


Escherichia coli

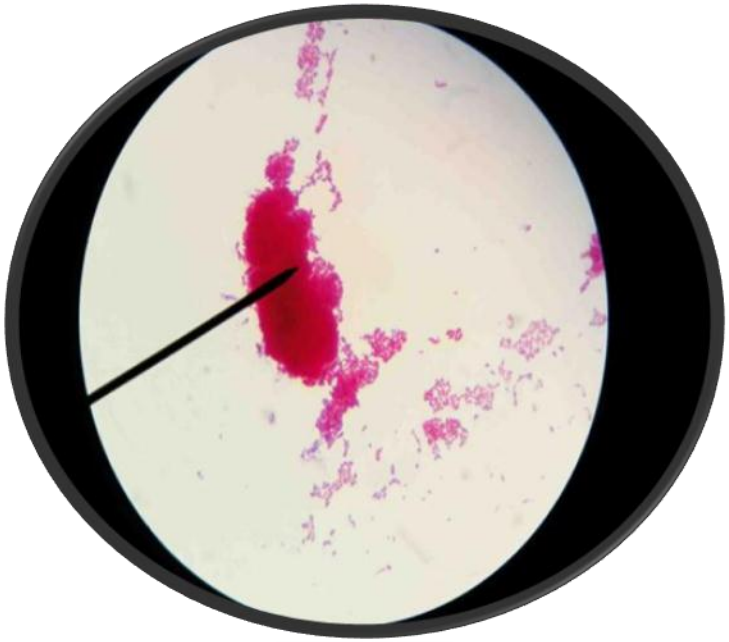


Mixed Sample of *S. epidermidis* & *E. coli*

Acid Fast Stain Examples

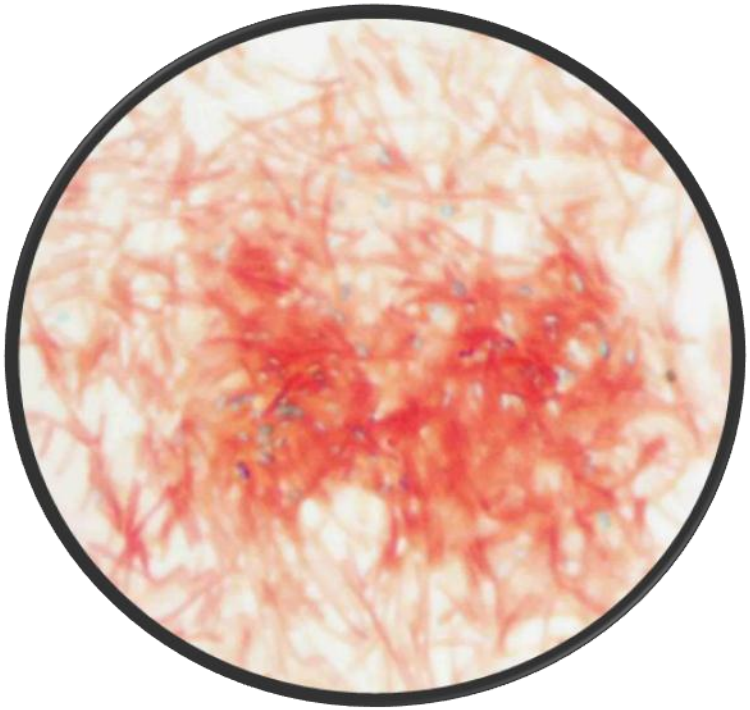


Mixed sample of *Mycobacterium smegmatis* & *Micrococcus luteus*

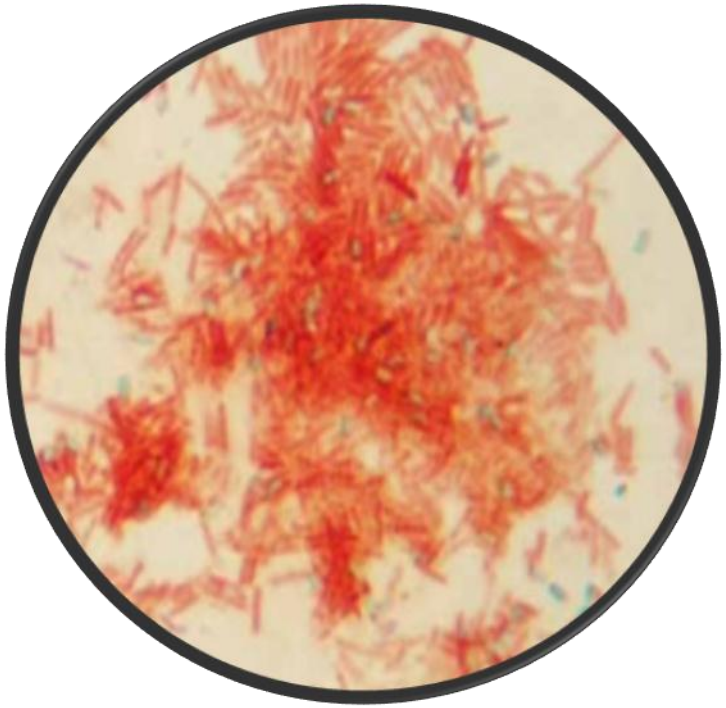


Mycobacterium smegmatis

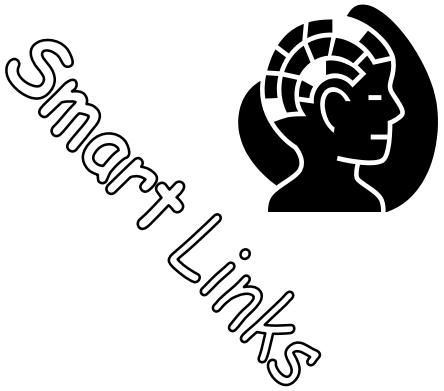
Endospore Stain Examples



Bacillus cereus



Confused?



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

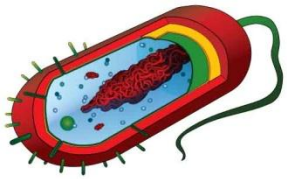
- **Bacterial Identification Laboratory** Main Page on the Virtual Microbiology Classroom of Science Prof Online.
- How to Prepare a Bacterial Smear for Gram Staining, video from Science Prof Online (SPO).
- Gram Stain Interactive Tutorial. This is an extremely useful tutorial that shows, step-by-step, what happens in Gram-positive and Gram-negative cells during Gram staining.
- How to Prepare a Bacterial Smear for Acid Fast Staining, video from SPO.
- 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 SPO.
- 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

Images: C. diff., Giant Microbes; Prokaryotic cell, Mariana Ruiz