



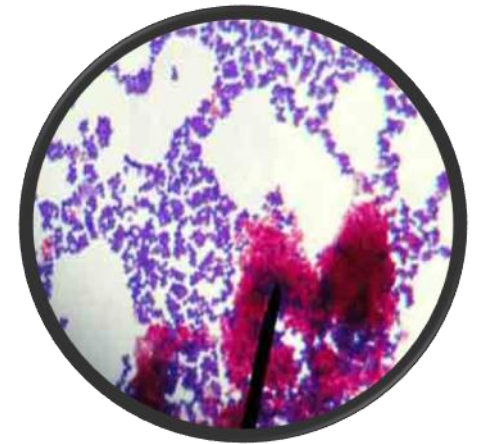
# About Science Prof Online PowerPoint Resources

- 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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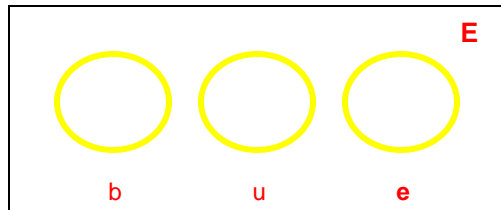
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## Laboratory Exercise 3



# Identification of Unknown Bacteria (Part II):

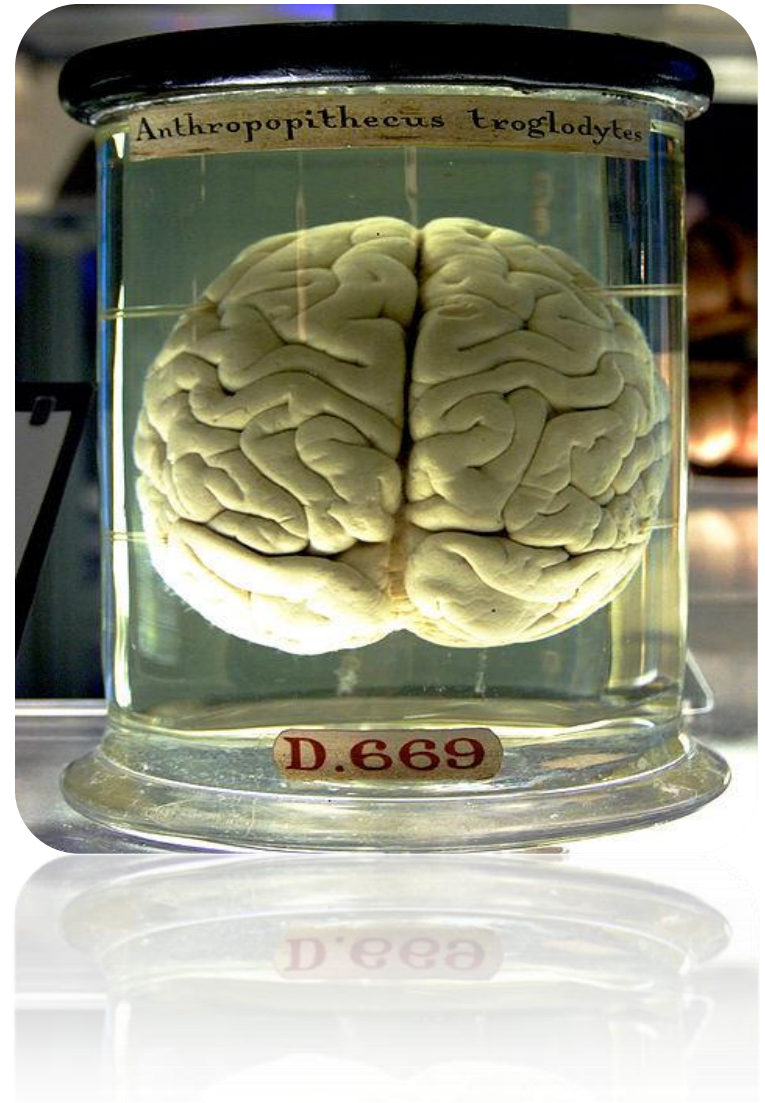
- Differential Staining (Gram, Acid-fast, Endospore)
- Interpret Unknown on Specialized Media
- Identification of Unknown



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

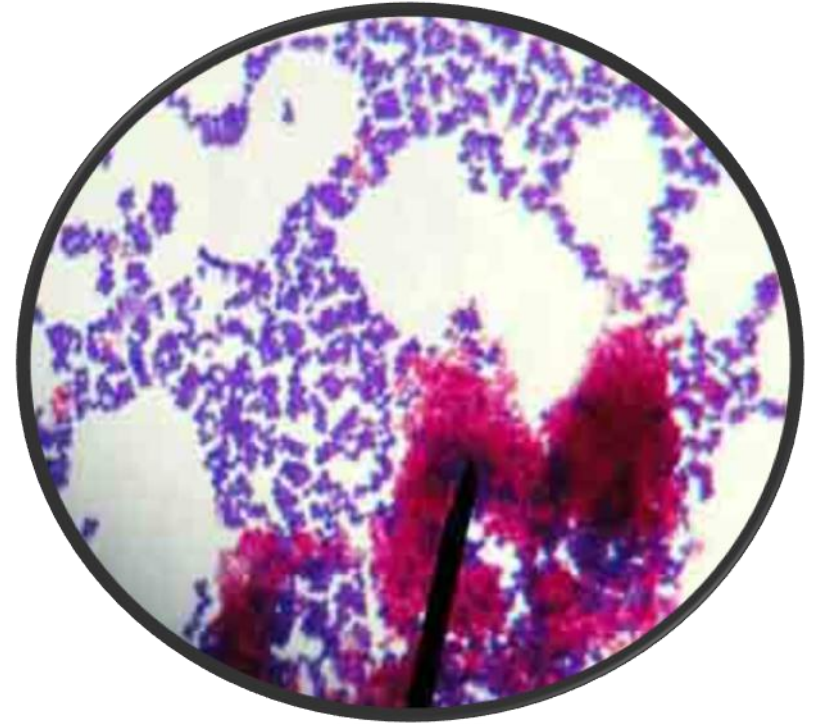
## Identification of Unknown Bacteria

- Perform [Gram](#), [Acid fast](#) and [Endospore](#) stains.
- Compare and contrast [differential stains](#) procedures and the clinical information obtained from performing them.
- Use a dichotomous flow chart for identification of an unknown bacterium.
- Practice viewing bacteria under [oil immersion](#) and taking photo micrographs of bacterial samples.
- Read and interpret growth on [selective and differential media](#).
- View and understand demonstration of API 20 E bacterial test.



# 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.



# 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](#)?
- The structure of the thinner cell walls of Gram negative bacteria cannot hold primary stain, once the decolorizer is applied.

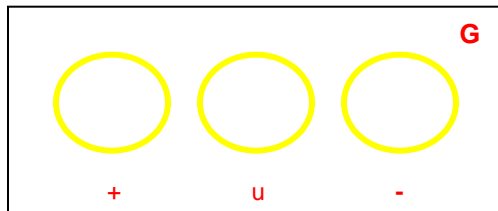
## GRAM STAINING PROCEDURE

Crystal violet (1 min) > *rinse*

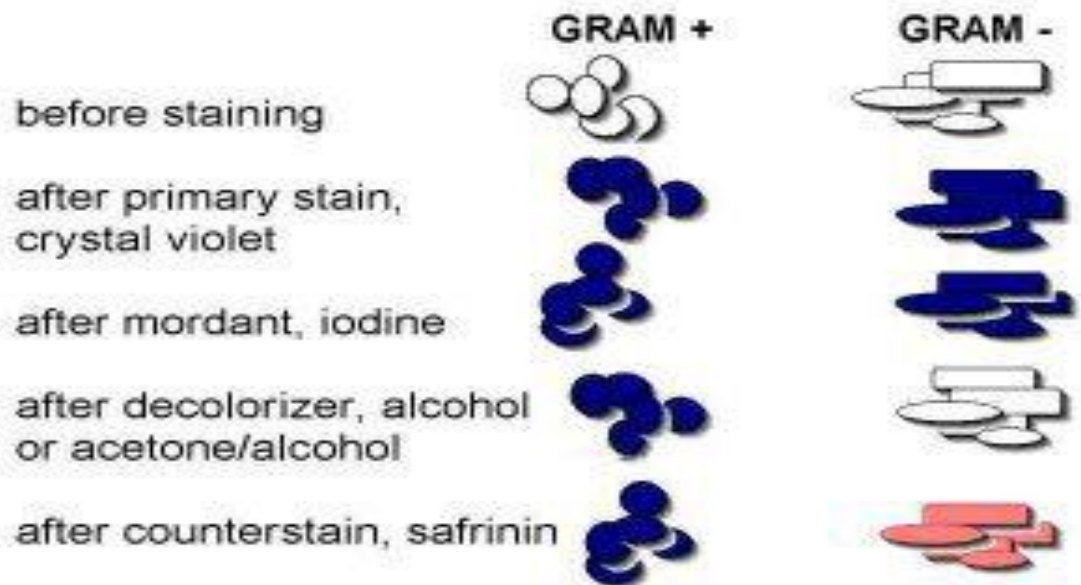
Iodine (1 min) > *rinse*

Acetone Alcohol (10-15 sec) > *rinse*

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

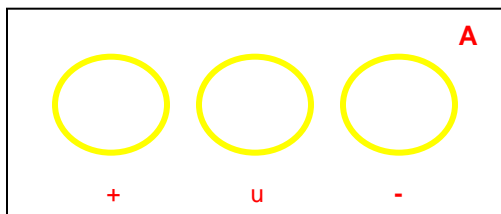


Watch **video** of  
[How to Do a Gram Stain](#)



# 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?*



Watch **video** of  
[How to Do an  
Acid Fast Stain](#)

## ACID-FAST STAINING PROCEDURE

### Blotting paper

Ziehl's carbol fuchsin (3 – 5 min heat) > *rinse*

Acid Alcohol (10 – 15 sec) > *rinse*

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

	Acid Fast Organisms	Not Acid Fast Organisms
Create a smear of organism you are testing. Cover smear with a blotting paper.		
Saturate paper with Ziehl's carbol fuchsin (say fyook-sin). Heat 3 – 5 minutes. Remove blotting paper.		
Rinse slide with tap water, then decolorize the smear for 10 - 15 seconds with acid alcohol. Rinse.		
Apply crystal violet for 1 minute, wash, blot dry.		

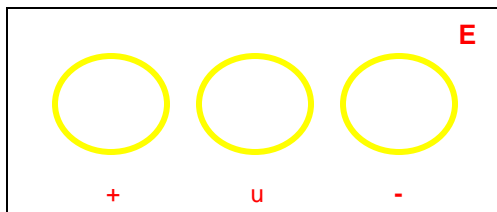
# Endospore Stain

- 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.
- After cooling, the sample is decolorized with water and counter stained with safranin.
- Results in green stained endospores and red-colored vegetative cells.

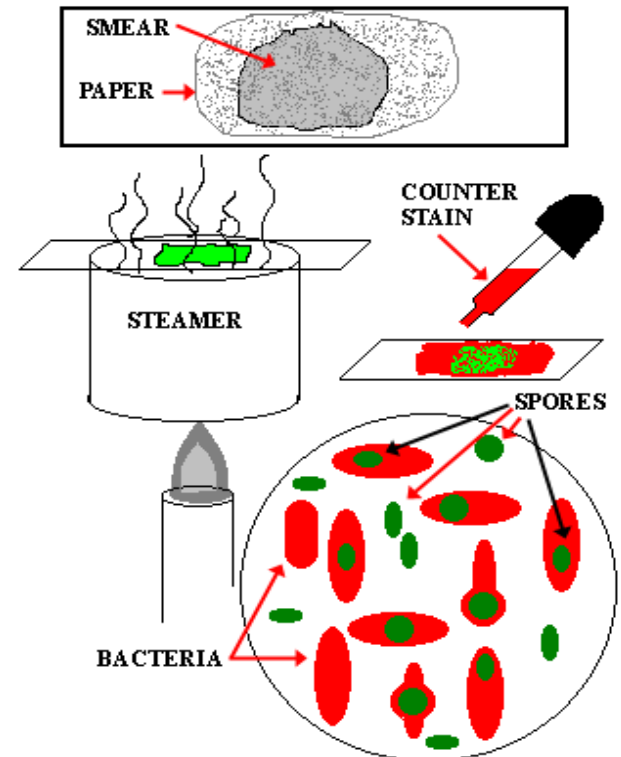
## ENDOSPORE STAINING PROCEDURE

Malachite Green (5 min heat) > *rinse*

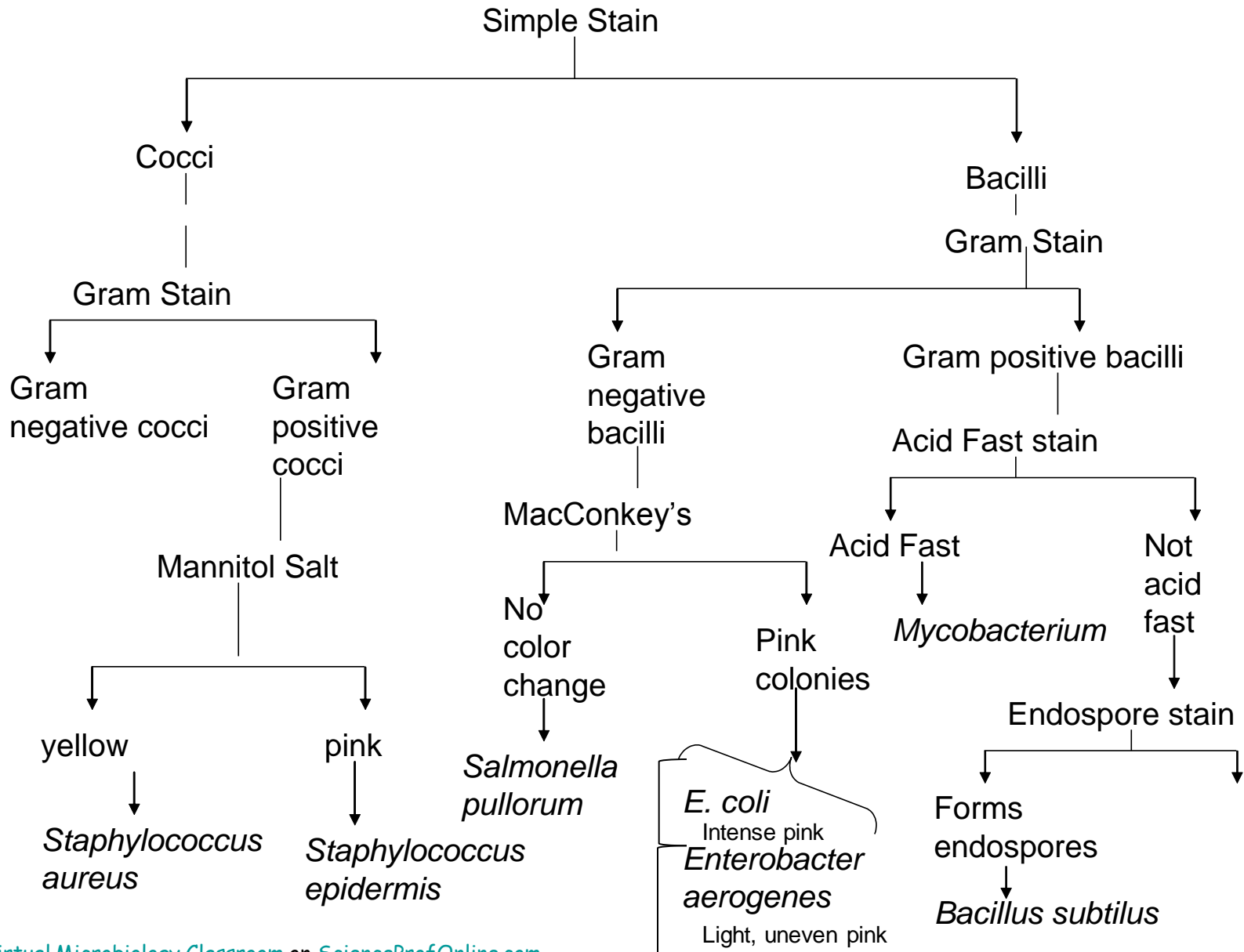
Safrinin (1 min) > *rinse & blot dry*



Watch **video** of  
[How to Do an  
Endospore Stain](#)



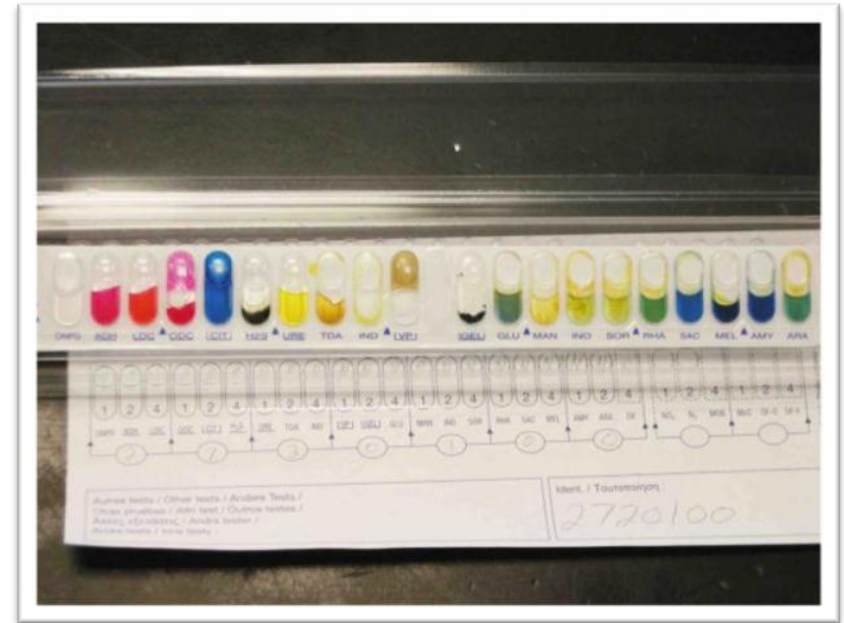
# Dichotomous Key

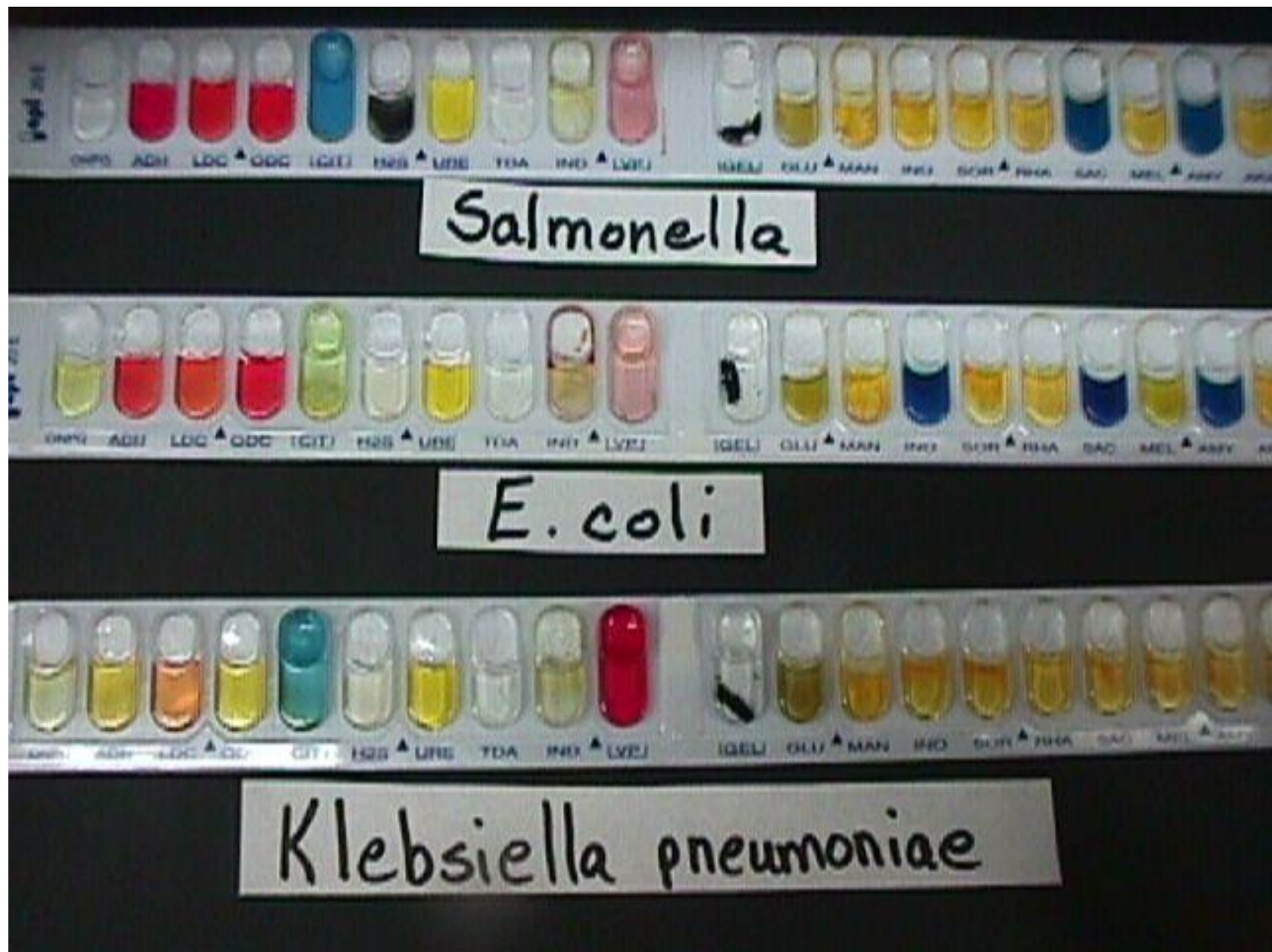




# API - 20E

- The stains and media that we use to help ID bacteria are mainly useful for preliminary ID, not usually to the species level.
- Once we have some idea as to the identity of a bacteria, we can then perform more sensitive tests for more specific identification.
- The **API-20E** test is used to ID Gram-negative bacilli from the family Enterobacteriaceae.
- *Q: So what test would someone need to do on a bacterial sample before utilizing the API-20E?*
- The API-20E is a system of 20 individual, miniaturized tests used to determine the metabolic capabilities of the organism.
- From identification of metabolic capabilities, we can zero in on the identification of the genus and species.
- The tests allow us to come up with a numerical 7-digit profile, based on which tests are positive and which are negative. You then look up that magic number, to find the species identification of your sample.





Salmonella

E. coli

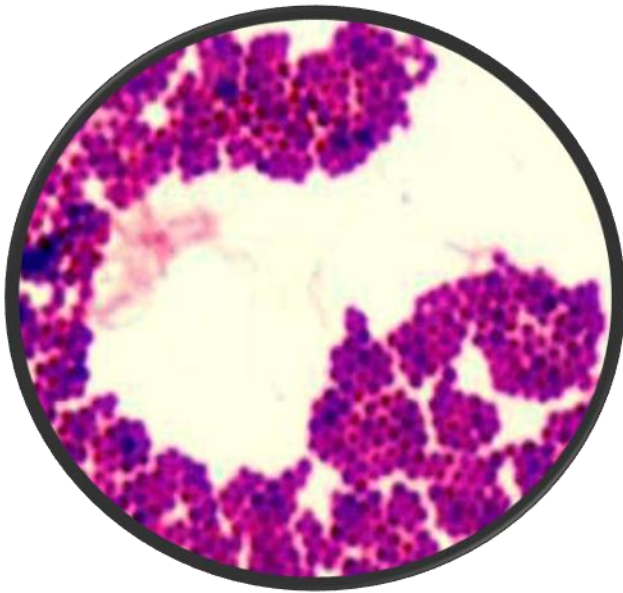
Klebsiella pneumoniae

# Now go be a bacteria detective!

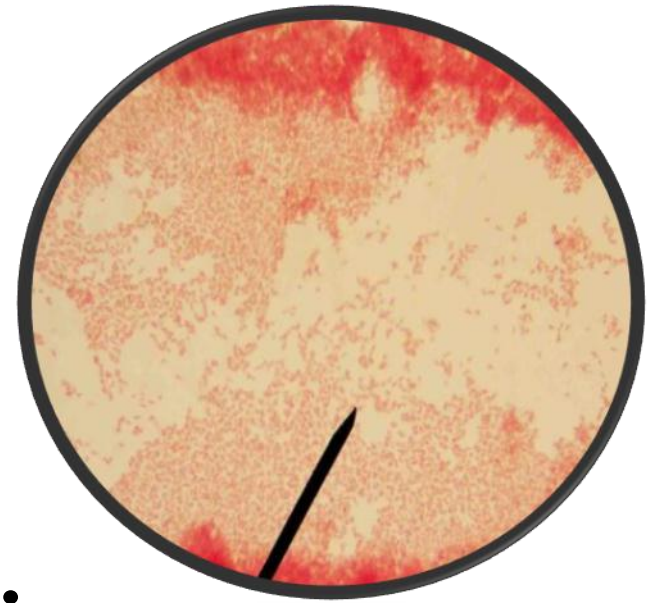
In order to figure out what organism your unknown is, you will need to...

1. Examine your [Gram](#), [Acid fast](#) and [Endospore](#) stain results.
2. Examine the [MacConkey's](#) and [Mannitol Salt](#) plates that you inoculated last week.
3. Use the dichotomous key I have placed on your bench to figure out what your unknown is.
4. Once you have identified your unknown, write the unknown number and its scientific name on your Lab Report 3b.



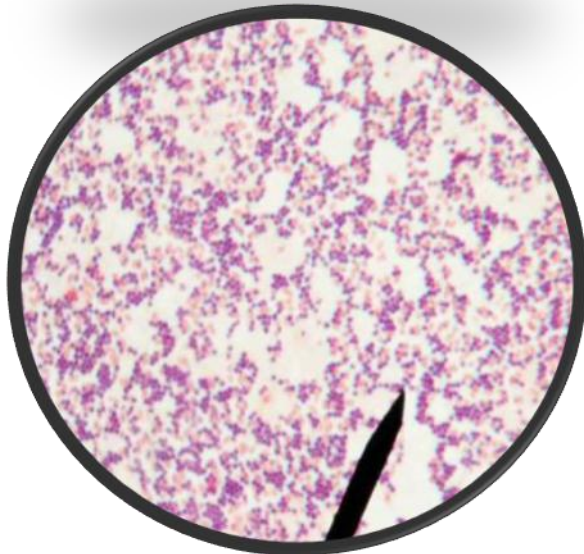


*Staphylococcus epidermidis*

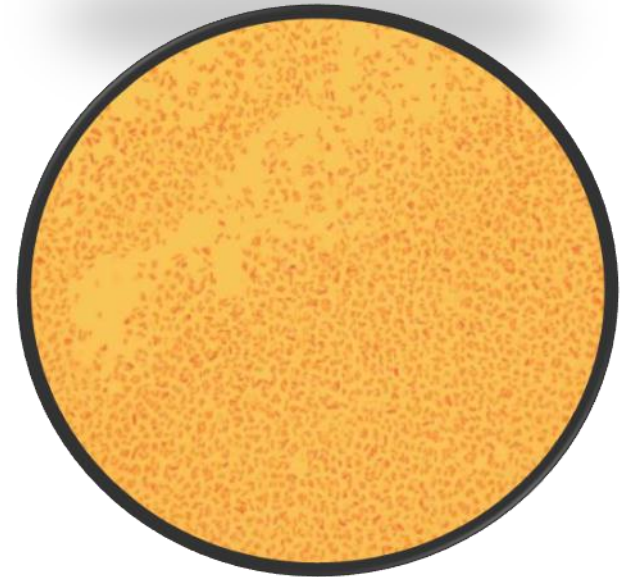


*Escherichia coli*

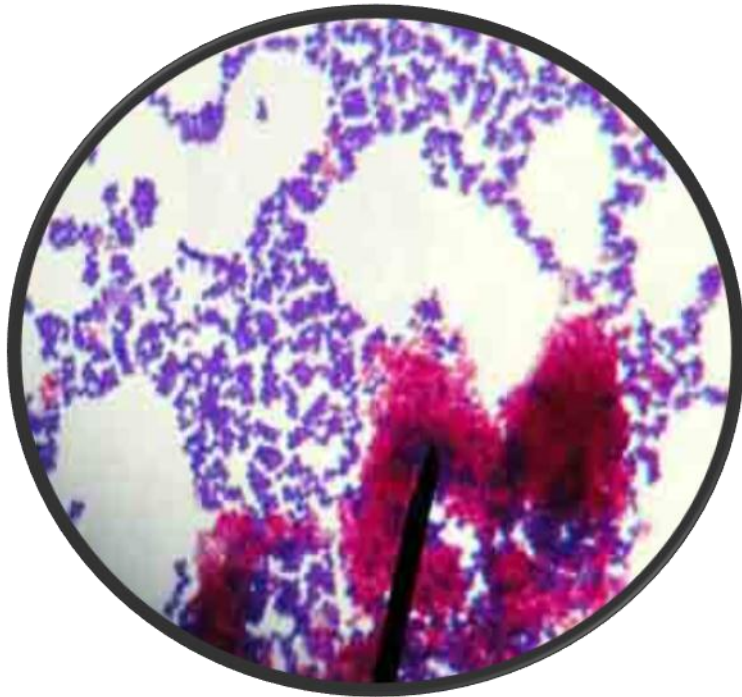
# Gram Stain Examples



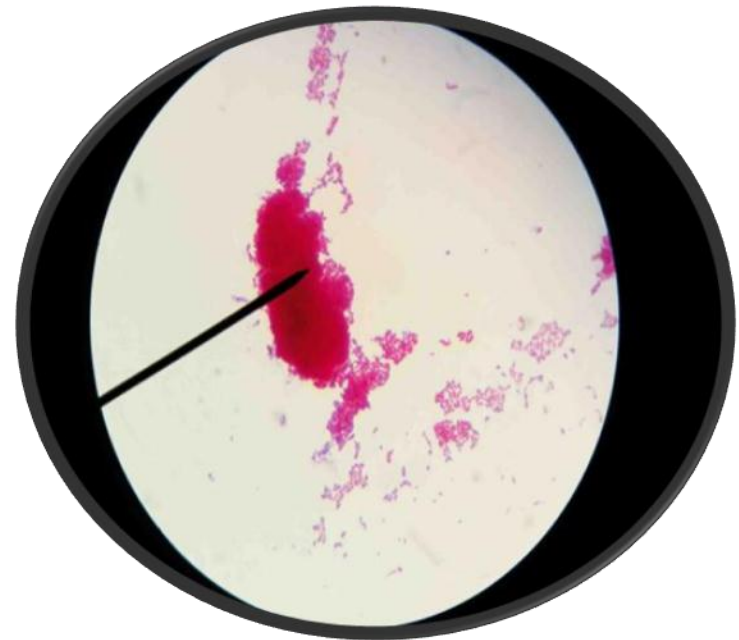
Mixed Sample of *S. epidermidis* & *E. coli*



# Acid Fast Stain Examples

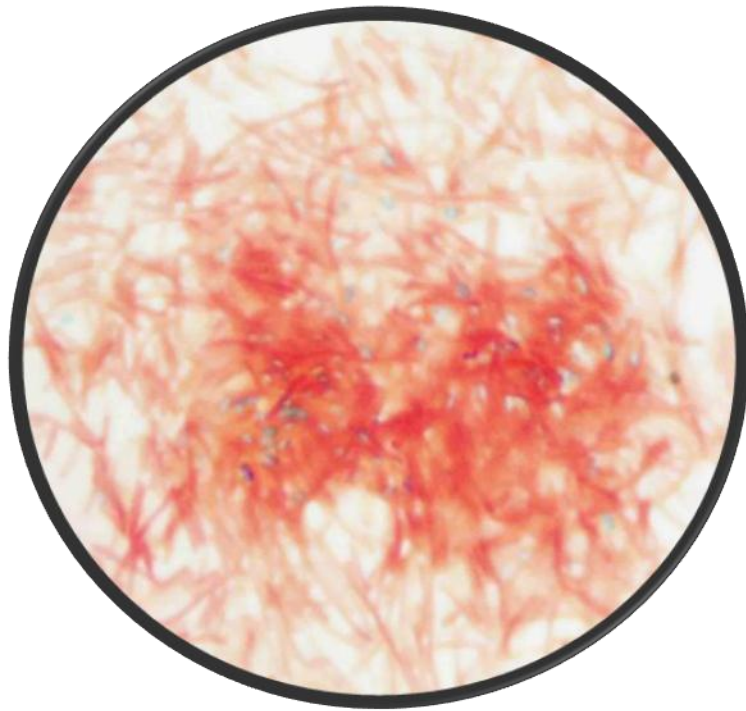


Mixed sample of *Mycobacterium smegmatis* & *Micrococcus luteus*

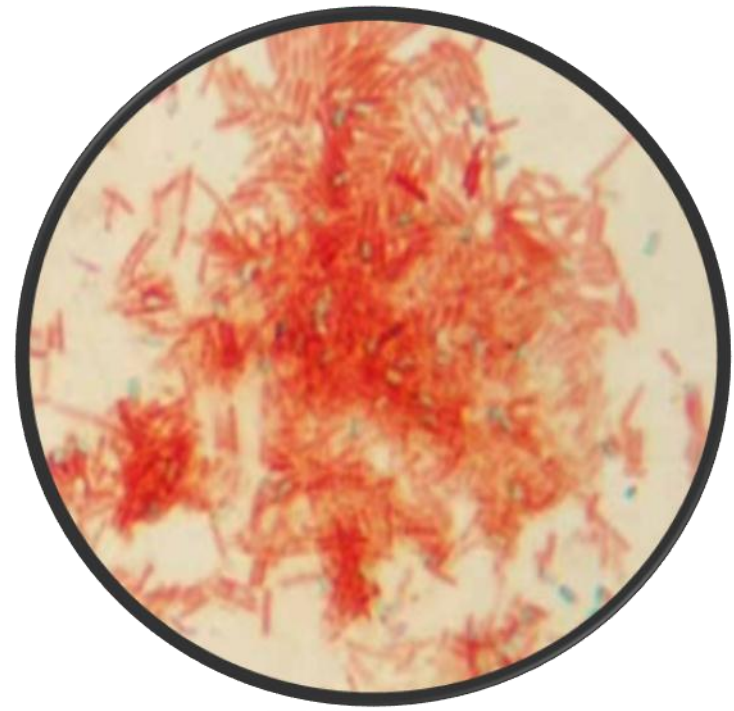


*Mycobacterium smegmatis*

# Endospore Stain Examples



*Bacillus  
cereus*



When you're done with your MAC & MSA plates, put them in the discard bin at back of lab.



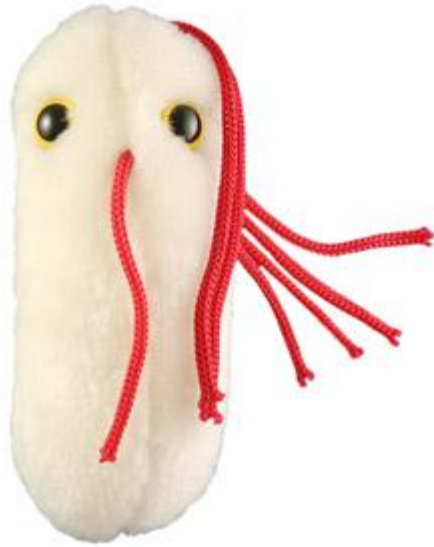
# Confused?



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

- **Bacterial Identification Laboratory** 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.
- [API-20E Animation](#) from Microbe Library.
- [Reading the API-20E](#), a YouTube video from Dr. Kimmitt.
- Videos of differential staining procedures: [Gram](#), [Acid-fast](#), [Endospore](#)



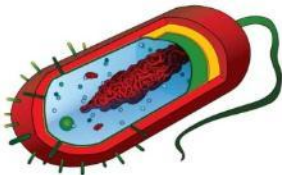


# Are microbes intimidating you?

*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)