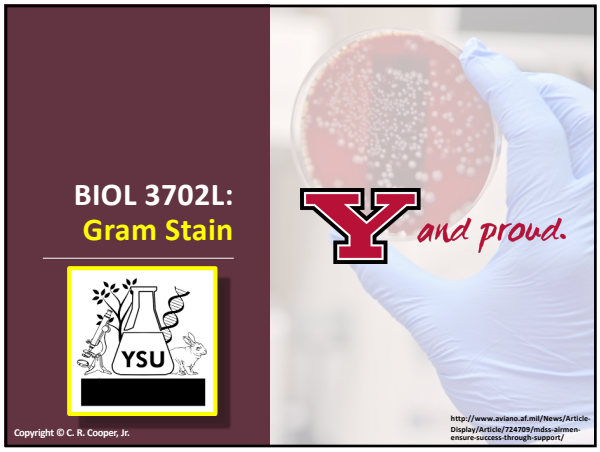


1

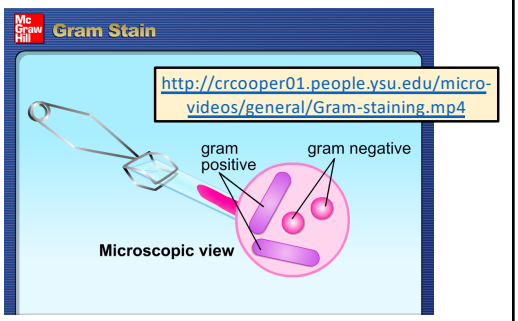
Today's Technique

- The purpose of today's laboratory exercise is correctly learn to perform the Gram stain.
- The Gram stain will differentiate between two general cell types:
  - Gram negative (**pink/red** after staining)
  - Gram positive (**purple/blue** after staining)
- The staining results are dependent upon the bacterial cell-wall structure.
- The most critical step in the Gram stain procedure - ***decolorization***

https://www.thoughtco.com/crystal-violet-for-the-gram-stain-608139

2

Overview of the Gram Stain Procedure

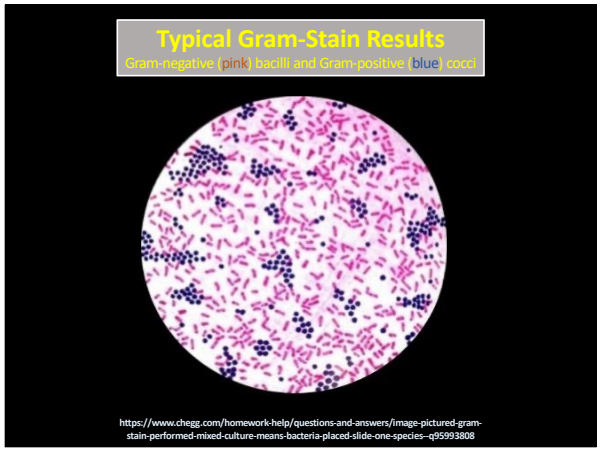


3

Gram Stain Procedure

- Today's procedure uses broth cultures grown for 18-24 hours:
  - *Staphylococcus aureus* (Gram positive);
  - *Escherichia coli* (Gram negative); and
  - A mixed broth culture of both bacteria.
- Note: A proper Gram stain is always performed with 18-24 hour-old cultures. Older cultures may not stain correctly.

4



5

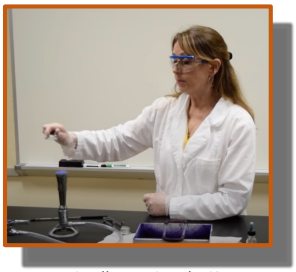
Gram Stain Procedure (cont.)

- Prepare a three-smear slide as follows:
  - On one side of the slide, prepare a smear of *S. aureus*.
  - One the other (far) side, prepare a smear of *E. coli*.
  - In the middle of the slide, prepare a smear of the mixed culture.
- **DO NOT ALLOW SMEARS TO MIX TOGETHER.**

6

Gram Stain Procedure (cont.)

- Heat fix the prepared slide.
- Place the slide on the stain rack over the bench-top sink.



https://www.youtube.com/watch?app=desktop&v=JWDFR8Zjd1A

7

Gram Stain Procedure (cont.)

Use the Hardy Diagnostics Gram Stain Kit

8

Gram Stain Procedure (cont.)

- Flood the slide with **crystal violet** and allow it to sit undisturbed for 30 seconds.
- Tip the slide to drain off the dye and rinse the slide for 5 seconds using the water bottle on the bench top.
- Place the slide on the stain rack again, then flood the slide with **Gram's Iodine**. Let the slide sit undisturbed for 60 seconds.

9

Gram Stain Procedure (cont.)

- Tip the slide to drain off the dye and rinse the slide for 5 seconds using the water bottle on the bench top.
- With the slide now held at an angle, **decolorize** the slide by adding acetone/95% ethanol (1:1 v/v) drop-by-drop and allow the solvent to drain off until no additional crystal violet washes off.

NOTE: DO NOT DECOLORIZE TOO LONG!

10

Gram Stain Procedure (cont.)

- Tip the slide and rinse the slide for 5 seconds using the water bottle on the bench top.
- Place the slide on the stain rack again, then flood the slide with **Safranin**. Let the slide sit undisturbed for 60-80 seconds.
- Tip the slide to drain off the dye and rinse the slide for 5 seconds using the water bottle on the bench top.

11

Gram Stain Procedure (cont.)

- Drain the water from the slide and carefully blot the slide dry using **PAPER TOWEL**. (Bibulous paper is not available.)

Note: Although the typical procedure calls for the use of bibulous paper, used properly, paper towel works just as well.

12

Gram Stain Procedure (cont.)

- Observe the stained slide using the oil-immersion objective. If the *S. aureus* smear have stained **purple/blue** and the *E. coli* cells have stained **red/pink**, then you may assume that you have correctly performed the Gram stain procedure. If these controls did not stain correctly, then you need to repeat the procedure with a new, heat-fixed slide with all three types of smears.

13

Special Notes

- Initially, you may wish to make more than one heat-fixed slide with multiple smears in case your first staining attempt is not successful.
- Slides of stained or unstained, heat-fixed smears can be stored in a slide storage box for later processing.

14

Special Notes (cont.)

- Do not "stop and start and stop again". Once you start the staining process, do not pause to see how much time a step consumes. **Know the procedure!!!**

www.countryside-maryville.org/the-importance-of-timing/

15

Lab Report Expectations

- Complete the drawings and the discussion questions on the laboratory report sheets. YOUR ANSWERS MUST BE CLEAR, CONCISE, AND GRAMMATICALLY SOUND SENTENCES!
- Retain this lab report. Your laboratory instructor may or may not call for it in the near future for submission via Blackboard.

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