Module: **The Gram Stain**

Procedure - click "start lab"

From a liquid culture, take a loopful of bacteria emulsify it in a small drop of water or saline on the slide. This should be a thin, not milky, suspension or it will not stain properly. Air dry the slide. *This is done automatically in the virtual module.*

To begin:
1. Heat fix the slide: click on the Bunsen burner, pass the slide gently two or three times (1-2 seconds) through the flame. Do not overheat - this will cause distortion of the cells.
2. Flood the slide with crystal violet for 1 minute
3. Rinse with H_20
4. Flood the slide with iodine for 1 minute
5. Rinse with H_20
6. Decolorize with alcohol for 5-10 seconds
7. Rinse with H_20
8. Flood the slide with safranin for 1 minute
9. Rinse with H_20
10. View slide under the microscope

The "slide" contains *E. coli* and *Staph. aureus* – is that what you see?
If not, think about what you might have done incorrectly
Then, repeat the exercise.

When you are finished with the exercise, click on "Examine Examples" to see actual micrographs of several bacteria that have been gram stained. You will recognize the names of many of the bacteria from lecture.

Some Pitfalls:

1. Slide not heat-fixed: smear will wash off → what would you expect to see?
2. Slide over heat-fixed: cellular morphology may be distorted
3. Slide over-decolorized: gram-positive bacteria will appear gram-negative
4. Slide under-decolorized: gram-negative bacteria will appear gram-positive
5. Smear too thick: cells in very thick areas will not decolorize properly and gram negative bacteria will appear gram-positive
6. Insufficient time for safranin counterstain: gram-negative bacteria may be very faint and difficult to see