The Gram Stain

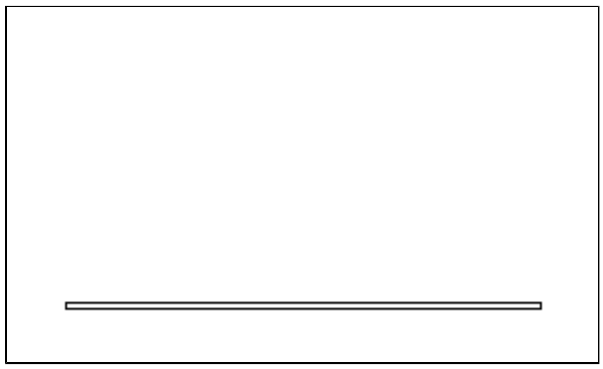
In 1884, Hans Christian Gram, a Danish doctor working in Berlin, accidentally stumbled on a method which still forms the basis for the identification of bacteria. While examining lung tissue from patients who had died of pneumonia, he discovered that certain stains were preferentially taken up and retained by bacterial cells. Over the course of the next few years, Gram developed a staining procedure which divided almost all bacteria into two large groups - the Gram stain.

Individual bacterial cells are hard to see, partly because they are small, but also because they are almost transparent. In addition to magnification under a microscope, optical tricks must also be used to be able to see them:

- Phase contrast microscopy
- Staining

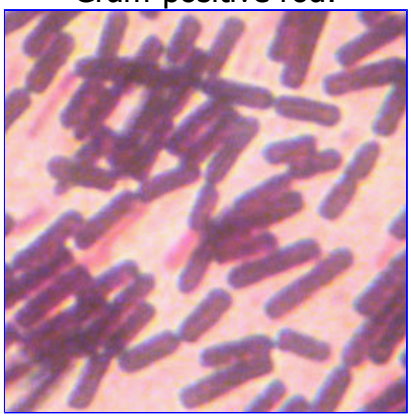
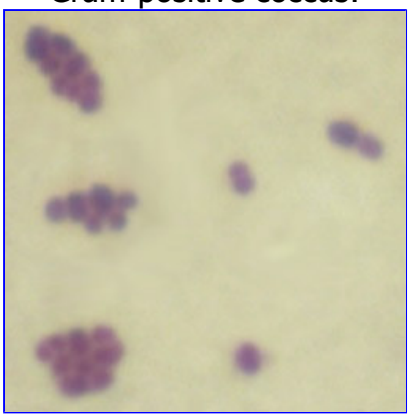
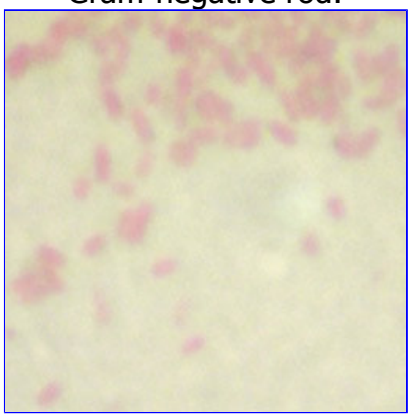
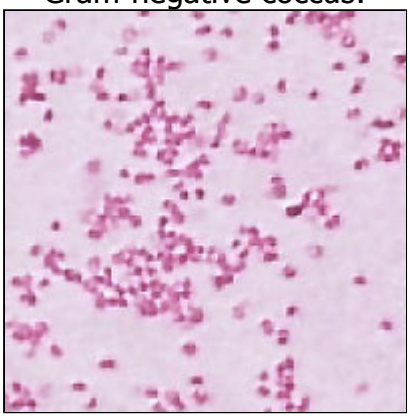
Either of these methods can make bacterial cells visible under the microscope. Other staining methods are described elsewhere in these documents, e.g. the Ziehl-Neelsen acid-fast staining procedure, but the Gram stain procedure is as follows:

1. Place a slide with a bacterial smear on a staining rack.
2. **STAIN** the slide with **crystal violet** for 1-2 min.
3. Pour off the stain.
   Note: fingers stain Gram-positive - use forceps!
4. Flood slide with Gram's iodine for 1-2 min.
5. Pour off the iodine.
6. Decolourize by washing the slide briefly with acetone (2-3 seconds).
7. Wash slide thoroughly with water to remove the acetone - do not delay with this step.
8. Flood slide with **safranin counterstain** for 2 min.
9. Wash with water.
10. Blot excess water and dry in hand over bunsen flame.



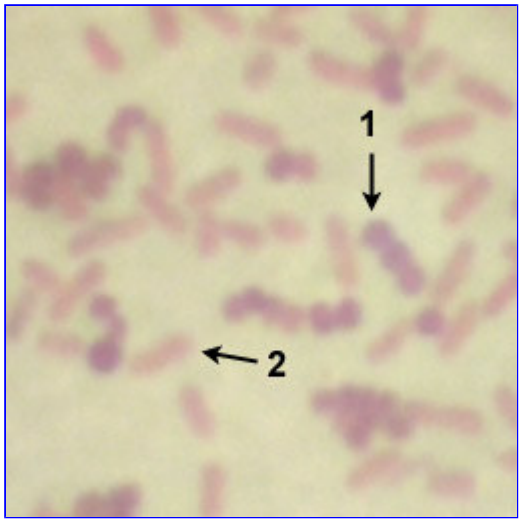
So how does it work? Gram didn't know - he simply worked empirically. We now know that the Gram reaction is based on the structure of the bacterial cell wall.

In Gram-positive bacteria, the **purple crystal violet stain** is trapped by the layer of peptidoglycan which forms the outer layer of the cell.
In Gram-negative bacteria, the outer membrane prevents the stain from reaching the peptidoglycan layer in the periplasm. The outer membrane is then permeabilized by acetone treatment, and the **pink safranin counterstain** is trapped by the peptidoglycan layer.

Gram-positive rod:  e.g. *Bacillus cereus*	Gram-positive coccus:  e.g. *Staphylococcus epidermidis*
Gram-negative rod:  e.g. *Escherichia coli*	Gram-negative coccus:  e.g. *Neisseria*

In the video below you can see two different species of bacteria distinguished both by their morphology and Gram-reaction.

- **Gram-stained bacteria** (0.34Mb QuickTime web quality sample: download FREE QuickTime Player)
- Two species can be distinguished in this mixed culture:
  1. *Micrococcus luteus*: Gram-positive cocci
  2. *Serratia marinorubra*: Gram-negative rods
- You can get a longer, better quality version of this video on the Microbiology Video Library CD.
- Broadcast quality (520 line) digital video: 5



minutes duration, no soundtrack. Supplied on PAL format miniDV tape plus accompanying notes.

- If you are interested in obtaining this video, please contact us.

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