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... Gram's stain is a differential and selective stain that is used to identify bacteria. It is a simple, rapid, and reliable method. The most useful is the Gram stain, which separates organisms into 2 groups: gram-positive bugs and gram-negative bugs. This stain allows the clinician to determine whether the organism is round or rod-shaped.

For any stain you must first ensure the microscope is clean. The most useful is the Gram stain, which separates organisms into 2 groups: gram-positive bugs and gram-negative bugs. This stain allows the clinician to determine whether the organism is round or rod-shaped.

- 1. There are 4 steps to the Gram stain.
- 2. Pour on crystal violet stain (is blue dye) and wait 60 seconds.
- 3. Wash off with water and flood with iodine wash time. Wait 60 seconds.
- 4. Wash off with water and then "decolorize" with 95% alcohol.
- 5. Finally, counter-stain with safranin (is red dye). Wait 30 seconds and wash off with water.

When the slide is studied microscopically, cells that absorb the crystal violet and hold onto it will appear blue. These are called gram-positive organisms. However, if the crystal violet is washed off by the alcohol, these cells will absorb the safranin and appear red. These are called gram-negative organisms.

Gram-positive = BLUE
I'm positively BLUE over you!!

Gram-negative = RED
No inorganic RED conduct!!

The different stains are the result of differences in the cell walls of gram-positive and gram-negative bacteria.

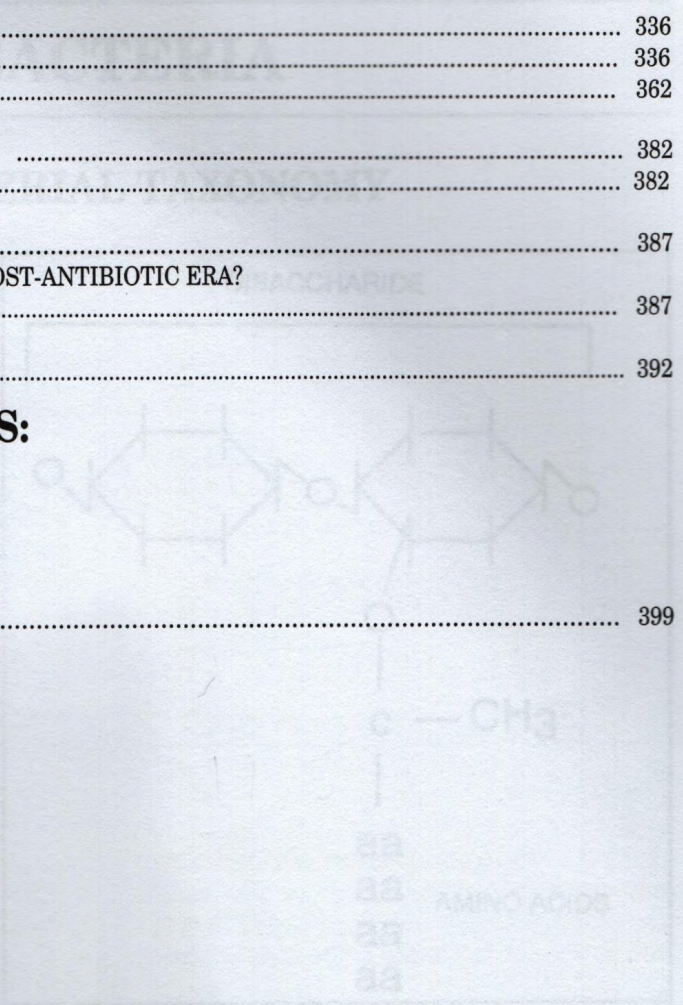


Figure 1-1

Both gram-positive and gram-negative organisms have more than 1 layer protecting their cytoplasm and keeping them from the extracellular environment, unlike animal cells, which have only a single cytoplasmic membrane composed of a phospholipid bilayer. The layer outside the bacterial cytoplasmic membrane is the peptidoglycan layer or cell wall. It is present in both gram-positive and gram-negative organisms.

The NAM units of the peptidoglycan layer or cell wall are composed of repeating disaccharides with 4 amino acids in a side chain extending from each NAM unit.

The amino acid chains of the peptidoglycan cross-link to other amino acid chains from neighboring chains. This results in a stable cross-linked structure. The enzyme that catalyzes the formation of this linkage is called transpeptidase and is located in the inner cytoplasmic membrane. The antibiotic penicillin binds to and inhibits this enzyme. For this reason, the process is also called penicillin-binding protein (see page 100).