Cell Wall Staining Procedure

1. Make smear, air dry, then heat fix slides.
2. Add 3 drops of cetylpyridinium chloride (on desk top) solution to each smear.
3. Add 1 drop of congo red (share drawer) to each smear. Mix the drops gently using the transfer loop. Do not scratch the smears. A gentle rotating and tilting motion of the slide will also help with this process. This should take approximately one minute.
4. Rinse with water.
5. Counterstain with methylene blue stain for 20 seconds.
6. Rinse, blot dry and observe under low, high dry and then oil immersion lens.

Note:

This procedure is used to highlight the cell wall and therefore provide an improved look at cell morphology. Although there is swelling of the cells, this enlargement occurs proportionally so that the shape of the cell is maintained. This technique is useful in determining morphology of smaller cocci and coccobacilllary forms.

Most bacterial cell walls have a net anionic (negative charge). Cetylpyridinium chloride is a cationic solution that places a positive charge on the cell wall exterior by sticking to the negative charge on the cell wall. The Congo Red is negatively charged and when introduced can now stick to the positive charge of the added cetylpyridinium chloride.