Method of preparation of Gram stain slide

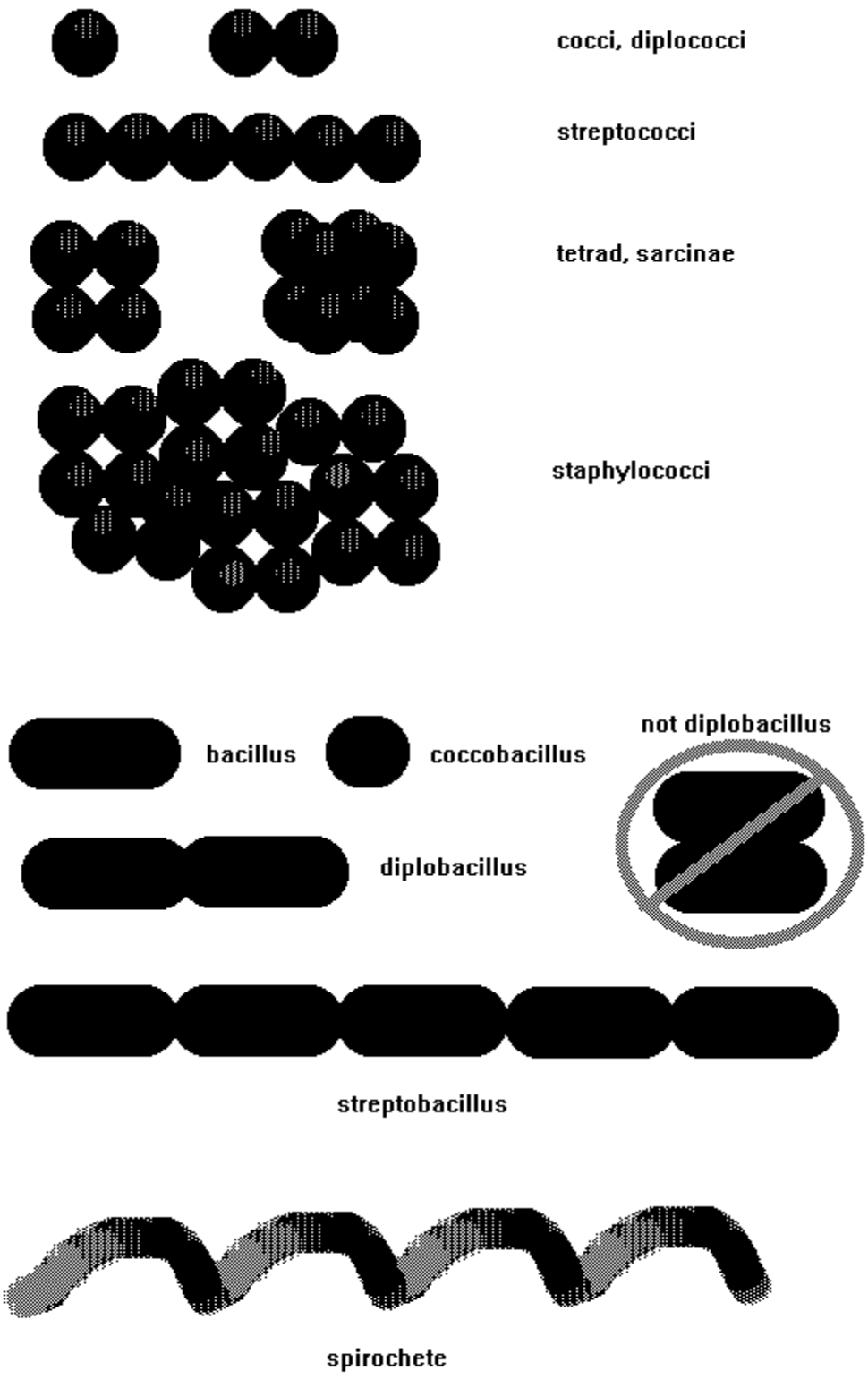
1. Centrifuge 15mL of sample in a 15mL centrifuge tube at 1000rpm for 5 min.
2. Pour off 10mL of supernatant and re-suspend pellet in remaining 5mL.
3. Place a drop of suspension on a microscope slide using a disposable inoculation loop and smear to a thin layer.
4. Allow slide to dry completely.
5. When dry, pass slide over Bunsen burner 3 times to fix cells to slide.
6. Flood slide with crystal violet, let stand for 1 min.
7. Pour off crystal violet (Blue). Flood slide with iodine, let stand 1 min.
8. Pour off iodine. Flush slide briefly with decolorizer, then immediately flush briefly with water.
9. Flood slide with safranin (Red), let stand for 1 min.
10. Briefly flush slide with water then let slide dry.
11. Place a small drop of immersion oil on the slide in an area that is visibly stained.
12. Place oil immersion slide on the microscope, adjust the mechanical stage so that the oil drop is directly below objective lens (Ensure that the 100X objective lens is in the vertical position). Turn the microscope light on. Adjust lamp brightness to high.
13. Using the coarse adjustment knob, rotate clockwise until objective lens touches the oil drop.
14. Look into the eye-piece and start to turn the fine adjustment knob clockwise slowly until you see pink or purple objects.
15. A microbiological contamination should be clearly visible using this method.

If a contamination exists, bacteria will fall into two main categories:

1. Gram positive. (Purple to dark blue objects)
2. Gram negative. (Pink to red objects)

Bacteria will then fall into 3 sub categories:

1. Cocci. (perfectly spherical small objects that clump (look like grapes) or are isolated from each other).
2. Rod. (cigar shaped. Often form chains or could be separated)
3. Spirochete. (Very rarely seen, looks like a spring.)


cocci, diplococci
streptococci
tetrad, sarcinae
staphylococci
bacillus
coccobacillus
not diplobacillus
diplobacillus
streptobacillus
spirochete