GRAM STAINING OF BACTERIA

List of Reagents and Instruments

- **Equipment:**

❖ Bunsen burner

❖ alcohol-cleaned microscope slide

❖ water

- **Reagents:**

❖ Crystal violet,

❖ Gram's iodine solution,

❖ acetone/ethanol (50:50 v:v),

❖ 0.1% basic fuchsin solution

Procedures

1. Prepare a Slide Smear:

A. Transfer a drop of the suspended culture to be examined on a slide with an inoculation loop.

If the culture is to be taken from a Petri dish or a slant culture tube, first add a drop or a few loopful of water on the slide and aseptically transfer a minute amount of a colony from the Petri dish.

Note that only a very small amount of culture is needed; a visual detection of the culture on an inoculation loop already indicates that too much is taken.

If staining a clinical specimen, smear a very thin layer onto the slide, using a wooden stick. Do not use a cotton swab, if at all possible, as the cotton fibers may appear as artefacts. The smear should be thin enough to dry completely within a few seconds. Stain does not penetrate thickly applied specimens, making interpretation very difficult

Cont..

B. Spread the culture with an inoculation loop to an even thin film over a circle of 1.5 cm in diameter, approximately the size of a dime. Thus, a typical slide can simultaneously accommodate 3 to 4 small smears if more than one culture is to be examined.

C. Air-dry the culture and fix it or over a gentle flame, while moving the slide in a circular fashion to avoid localized overheating. The applied heat helps the cell adhesion on the glass slide to make possible the subsequent rinsing of the smear with water without a significant loss of the culture. Heat can also be applied to facilitate drying the the smear. However, ring patterns can form if heating is not uniform, e.g. taking the slide in and out of the flame.

2. Gram Staining:

- A. Add crystal violet stain over the fixed culture. Let stand for 10 to 60 seconds; for thinly prepared slides, it is usually acceptable to pour the stain on and off immediately. Pour off the stain and gently rinse the excess stain with a stream of water from a faucet or a plastic water bottle. Note that the objective of this step is to wash off the stain, not the fixed culture.

- B. Add the iodine solution on the smear, enough to cover the fixed culture. Let stand for 10 to 60 seconds. Pour off the iodine solution and rinse the slide with running water. Shake off the excess water from the surface

Cont..

C. Add a few drops of decolorizer so the solution trickles down the slide. Rinse it off with water after 5 seconds. The exact time to stop is when the solvent is no longer colored as it flows over the slide. Further delay will cause excess decolorization in the gram-positive cells, and the purpose of staining will be defeated.

D. Counterstain with basic fuchsin solution for 40 to 60 seconds. Wash off the solution with water. Blot with bibulous paper to remove the excess water. Alternatively, the slide may shaken to remove most of the water and air-dried.

3. Quality control:

- It is a simple matter to prepare a control slide by breadking a clean wooden applicator stick and picking a small amount of material from the interproximal space of one's teeth.
- This should be smeared into a drop of clean tap water on a clean glass slide. The slide may be stained as above.
- This material will consistently display a few neutrophils and a mixture of Gram (+) and (-) organisms. Neutrophil nuclei should be pink.

3. Examine the finished slide under a microscope.

- A caveat in the examination of the Gram smears is the distortion in morphology that can be caused by antimicrobial therapy.
- This is especially likely to occur in urine speciments. Filamentous and pleomorphic forms may be observed among the Gram (-) rod species.
- Gram reaction of the organism may also change after antimicrobial therapy, Gram (+) bacterial may become gram variable.
- Look at areas that are one cell thick only; observation of thick areas will give variable and often incorrect results.
- White blood cells and macrophages should stain Gram-negative, whereas sqamous epithelial cells are Gram-positive.