

# Gram Staining Tips

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## 1. Slide preparation

Purulent material should be selected whenever possible.

Apply the sample evenly and thinly to the slide. Smears that are too thick will be difficult to decolorize and impossible to read.

Do not cover the entire slide with the sample. This will make handling difficult and areas may be missed during decolorization. An area the size of a nickel usually is adequate.

## 2. Slide fixation

The material must be fixed to the slide to prevent it from washing off during staining. This can be done by quickly passing the slide over a gentle flame (the slide should not become too hot to touch) or on a slide warmer. Overheating may alter cell morphology or cause organisms to decolorize more quickly.

An alternative and superior method of fixation is to flood the slide with methanol for 1 minute. Methanol fixation prevents liquid specimens from washing off the slide better than heat fixing, preserves blood cell morphology and results in a clearer background.

## 3. Staining

Flood the entire slide when crystal violet, iodine and safranin are applied. This will ensure that all areas are stained evenly.

## 4. Decolorization

The critical step of the Gram staining procedure is the decolorization step. Hold the slide in a tilted downward position and allow the decolorizer to flow over the smear. Be careful not to miss any portion of the smear. Usually a few seconds will suffice.

95% ethanol will decolorize slower than acetone/alcohol, than does acetone.

## 5. Reading the Gram stain

Begin reviewing the slide using the 10x objective. This will allow you to focus quickly and to look for areas of purulence. The 100x oil immersion lens is essential for viewing individual bacteria.

When reviewing a Gram stain, look at many microscopic fields and in different areas of the slide.

The presence of many squamous epithelial cells in the smear usually indicates a poorly collected sample that will contain normal flora, whereas, the presence of polymorphonuclear leukocytes and no squamous epithelial cells indicates a good sample and an inflammatory process.

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Some specimens on preparation will have areas on the slide that are thicker than others. This can result in areas that are under decolorized, thus organisms that are truly Gram-negative will appear Gram-positive. Microscopic fields that contain crystal violet precipitant, or PMNs, macrophages or epithelial cells that are staining purple are areas that are under decolorized.

Gram positive bacteria will usually stand out easily against the pinkish background. Gram negative organisms because of their lower contrast can be missed, particularly in thicker smears. *Haemophilus*, *Bacteroides* and *Fusobacterium* are Gram-negative organisms that are often overlooked due to their size, pleomorphic morphology or faintly staining qualities.