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## Gram's Stain Procedure

The technique for preparing a Gram-stained smear is as follows.

1. Prepare a thin smear from tissue exudates or bacterial suspension on a clean slide and allow smear to air dry.
2. Fix material to the slide so that it does not wash off during the staining procedure by passing the slide, right side up, through a flame three or four times.
3. Flood smear with crystal violet solution and let stand for 1 minute.
4. Wash smear briefly with tap water.
5. Flood smear with Gram's iodine solution, and let stand for 1 minute.
6. Wash with tap water and decolorize until solvent flows colorlessly from the slide. This usually requires 5 to 10 seconds.
7. Wash briefly with tap water and flood the slide with safranin counterstain for 30 to 60 seconds.

Wash briefly with tap water, blot dry and examine the stained smear under the 100 X (oil immersion) objective of the microscope. *Gram-positive bacteria* stain dark blue to purple. *Gram-negative bacteria* appear pink to red.

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