

Gram's stain

Gram staining (or Gram's method) is an empirical method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls. The Gram stain is almost always the first step in the identification of a bacterial organism. While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique, thus forming Gram-variable and Gram-indeterminate groups as well.

Materials required:

Microscope slides* / Bunsen burner or hot hair drier / sink / Gram crystal violet* / Gram iodine* / Gram carbol fuchsin or safranin* / immersion oil* / microscope containers*

** Can be obtained from Awanui Veterinary using online ordering.*

How to:

1. Prepare a thin smear, air dry, and heat fix for 3 seconds in a hot flame or 10 seconds under hot hair drier (or dip in methanol for 30secs and let dry prior to staining).
2. Place slides on a rack over a sink, smear side up.
3. Apply Gram crystal violet for 30 secs.
4. Wash with water.
5. Apply Gram iodine for 30 secs.
6. Decolourise with acetone and wash IMMEDIATELY with water. Note: Do not leave acetone on for longer than 3 seconds as decolourisation occurs very quickly.
7. Counter-stain with Gram carbol fuchsin or safranin for 30 secs.
8. Wash with water and drain or blot to dry.
9. Examine microscopically using an oil immersion lens (100x recommended).

Results:

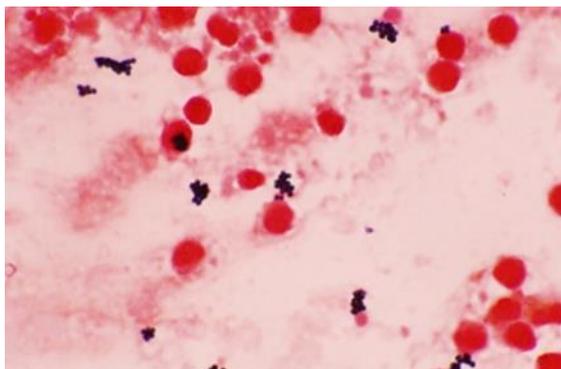
- Gram-positive organisms retain the crystal violet-iodine complex and stain blue/purple.
- Gram-negative organisms are decolourised by the acetone and are stained red by the counter-stain.
- Cells present will stain predominantly red.
- See overleaf for examples of microscopic morphology.



Microscopic morphology

The following information should be used as a guideline only, as bacterial species within the same Gram stain group (e.g. Gram positive bacilli) all have differing microscopic morphology. Please always consult with your local Awanui Veterinary laboratory if you are in doubt.

Please note: These photographs are all taken at varying magnifications and should not be compared with each other for reference.



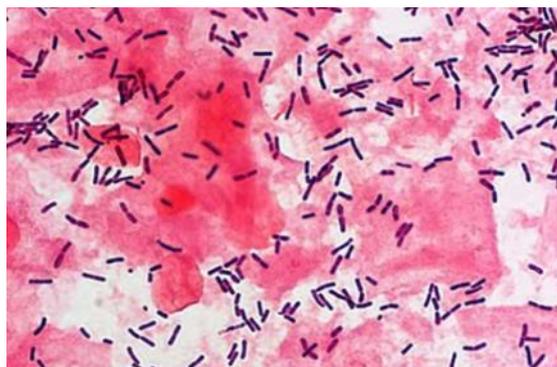
Gram-positive cocci in clusters (Staphylococcus species)



Gram-positive cocci in chains (Streptococcus species)



Gram-negative bacilli (GNB)



Gram-positive bacilli



Malassezia pachydermatis



Budding yeast with pseudo-hyphae plus GNB