

GRAM STAIN

This is a simple test used to check for the presence and quantities of various types of bacteria and yeast from the cloaca (feces), oral cavity, or some other site.

To perform this test, a cotton-tipped applicator stick is used to swab the collection site. The sample is applied to a microscope slide and then “heat-fixed” by gently moving a flame back and forth underneath the slide. The slide is transported back to the laboratory where it is then stained with several dyes in order to “color” the bacteria and yeast for identification.

There are 4 steps to the staining procedure:

1. The microscope slide is flooded with Crystal Violet which stains everything purple. The bacteria take up the stain through tiny holes in their wall known as pores. Certain types of bacteria have large pores, others have only small pores.
2. The excess Crystal Violet is rinsed off of the slide and then iodine is added. This has an astringent or “closing” action on the pores. The smaller pores are closed completely, the larger pores only partially.
3. The slide is decolorized with a chemical that removes all the crystal violet from the bacteria whose pores are only partially closed. However the crystal violet is trapped in the bacteria whose pores are totally closed.
4. A pink dye called Safranin is added to the slide for 30 seconds and then rinsed off. It stains the inside of those bacteria whose pores are only partially closed but not those whose pores are completely closed (these bacteria remain purple).

When the process is complete, those bacteria with large pores stain pink and are called Gram negative bacteria while those bacteria with small pores stain purple, and are called Gram positive bacteria.

Bacteria occur in several shapes. The two primary shapes are cocci (round or oval) and bacilli (rod-shaped). Other less common shapes are coccobacillic (rounded, slightly rod-shaped), and spirochetes (curved spirals).

All cocci are Gram positive bacteria. These include Streptococcus and Staphylococcus spp., most of which are part of the normal bacterial flora seen in healthy psittacine birds.

Bacilli can be either Gram positive or Gram negative. Gram positive rods are known as Bacillus spp. These are generally considered part of the normal flora. Lactobacillus is an example of this type of bacteria which is found in commercial products known as Probiotics which are used to repopulate a bird's GI tract with "healthy" bacteria.

Gram negative rods consist of most of the bacteria considered "potentially pathogenic" or disease producing in birds. Examples include E. coli, Pseudomonas, Proteus, Citrobacter, and Enterobacter spp.

Coccobacillic shaped bacteria are Gram negative and the most common example that is disease producing is Klebsiella spp. Spirochetes are also Gram negative. These are rare but they have been associated with disease in small hook bills, especially cockatiels.

It is useful to estimate the number of bacteria and general proportions of Gram positive to Gram negative bacteria present on each slide. Estimates are classified as follows:

rare	only a few bacteria present
+1	occasional bacteria present
+2	5-15 bacteria per oil immersion field
+3	15-75 bacteria per oil immersion field
+4	more than 75 bacteria per oil immersion field

By determining an estimate of the number and types of bacteria present, the veterinarian can better determine if specific cultures or other tests, treatments, or management changes should be carried out

In general, most healthy psittacines have primarily Gram positive bacteria in their GI tract. 90% or more is considered normal. Gram negative bacteria may be found but these are usually in much smaller numbers than Gram positive bacteria. 10% or less is considered normal. Thus in a potentially sick bird, we are looking for increased numbers and proportions of Gram negative bacteria compared to Gram positive bacteria.

Yeast are unicellular fungi. The most common example in pet birds is *Candida albicans*. Spores of this fungus are ubiquitous; however they may become pathogenic in debilitated or immune-suppressed individuals. Their presence and in what numbers and form help determine if treatment is necessary.

***Candida* spp. are oval in shape and can appear in three forms; single, budding, or chains (pseudohyphae). These all stain Gram positive and are up to four times larger than bacteria.**

Small numbers of single yeast cells are common in normal healthy psittacines. Large numbers of budding yeast or the presence of pseudohyphae is abnormal and indicates the yeast is multiplying in the GI tract and that a disease state may exist. Examples include young birds with sour crop and/or infection in the mouth, and birds on long term antibiotics which kill normal bacteria causing a yeast overgrowth.

Megabacteria are another type of yeast occasionally seen on a fecal gram stain. These are large, Gram-positive rod-like structures that are 10x larger than normal rod-shaped bacteria. They are most significant in budgerigars and other small hook bills in which they can cause gastrointestinal disease.