

Bacteria that Affect our Koi

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Many bacteria, just like parasites live in our ponds and on our Koi. This is normal, unless there is some damage to the protective barriers of the fish allowing penetration of the infecting organisms and their proliferation. Bacteria certainly can infect Koi, but more commonly, they are *secondary* to another breach of the slime coat. Knowing what type of bacteria is involved may be critical in determining the proper treatment. Bacteria are characterized by two factors: shape and staining properties. In general, most are either round (called cocci) or straight (called rods). When stained, they appear *gram positive* (dark purple) or *gram negative* (pinkish red). The gram staining method was discovered by and named after Danish bacteriologist Hans Christian Joachim Gram who devised it in 1882 and published the technique in 1884. It is one of the most important and widely used staining techniques in all of microbiology. Gram staining reflects the bacterial cell wall's ability to retain the purple stain *crystal violet*. Gram positive bacteria have thicker cell walls than gram negative bacteria. After the purple stain is placed on the bacteria, a decolorizer is used. When applied to gram positive bacteria, the cell wall shrinks, closing its microscopic pores trapping in the dye. In gram negative bacteria, the cell wall contains a high amount of lipid which dissolves with the decolorizer, and much of the dye leaches out. This is stained with fuchsin as a counterstain, giving a pink-red appearance.

The technique is fairly simple and the supplies are inexpensive to purchase (from a science supply store). It is quite messy, however. A smear from the site of infection is taken onto a glass slide. This may be done with a sterile Q-tip. Once dry, it is *fixed* to the slide by passing the slide through a flame a few times. The specimen then has crystal violet added to it, and it is left on for 30 seconds. This is gently rinsed with water, and iodine is added for another 60 seconds. This is then poured off and it is gently rinsed with water. Decolorizer (a mixture of acetone and ethanol) is then dripped on until no more stain appears to drip off. Finally, basic fuchsin (or safranin) is used as the counterstain for about a minute. This is rinsed off with water, allowed to dry, and examined under 400x power, sometimes needing oil.

Under the microscope both purple and pink specks will be seen. This process not only allows us to see what the gram staining is, but we can also discern the shape of the bacteria (cocci or rod).



A drawing (left) and a photo (right) of cocci.



A drawing (left) and a photo (right) of rods.

Although we can categorize bacteria by the above technique, final identification requires culture of the bacteria, which is growing them on a special nutrient rich agar plate. In addition, various antibiotics are used on the bacterial colony, and kill rates can be easily ascertained.

Just as identification of parasites helps guide what the best therapy is to treat them, knowing more information about bacteria (shape and stain characteristics) can be critical in treating bacterial infections.

This is why it is recommended to get a sample of bacteria from any sore on the Koi, and send it to a lab. The sample is collected via a swab kit that contains a long cotton-tipped swab and a fixative. The Koi is placed in a bowl and lifted so the area with the ulcer is out of the water. The ulcer is swabbed and immediately the swab is placed in the fixative. The kit contains a mailing envelope, and the swab with fixative should be mailed as soon as possible. The lab will culture the bacteria, identify it, and then test several antibiotics against it and send you the results in about 3 days. Knowing which antibiotic will give you the best kill rate is critical for the survival of the Koi. When only some of the bacteria are killed by an antibiotic, the remaining bacteria multiply, and those bacteria are now resistant to the antibiotic, and much harder to kill. This is how deadly strains of bacteria are formed, and why it is so important to always complete the entire treatment of antibiotics prescribed.