

Purple and Red: Gram Stain Test Contributes Important Information to the Sterilization Process

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According to AAMI/ISO 11137, medical device manufacturers need to know the “number and types of microorganisms” found on their devices before sterilization. The bioburden test provides the number of microorganisms, and the Gram stain test can determine the types.

The Gram stain test helps manufacturers differentiate the types of microorganisms on a device before sterilization. Often, medical device manufacturers will know the count because bioburden testing is done, but they don’t always characterize the microorganisms by doing the Gram stain test. The Gram stain test has three components—colony morphology, Gram reaction (purple and red) and microscopic morphology.

Colony morphology is a description of how the microorganism looks when it grows on an agar plate. It includes color, shape and other characteristics of the colony. The membrane and cell wall of a microorganism gives it its shape, which is the

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microscopic morphology. Cells can be coccoid or rod-shaped, which could be compared to the shape of peas and rice, respectively.

A Gram stain test introduces a colored stain (crystal violet) that turns the microorganism's cell wall purple. Iodine is added which binds to the crystal violet to fix the stain. A decolorizer comprised of alcohol and acetone is added, which causes gram negative microorganisms to lose their coloring because the cell wall is thinner which allows the crystal violet-iodine complex to escape. Gram-positive microorganisms retain the purple color because it is harder for the crystal violet-iodine complex to escape the thicker cell wall. A safranin (red) stain is added to counterstain the gram-negative microorganisms so they can be observed. In the end, gram-positive cells are purple and gram-negative cells are red. The differences are due to the composition of the cell walls. This Gram reaction is observed by using a microscope to magnify the cells 1000 times.

The purpose of the Gram stain is to separate the cells into two classifications to see how relevant they are. Gram-negative cells may pose a higher risk because they are more likely to cause a medical issue, disease or negative reaction. Endotoxin is a component of the gram-negative cell wall. Endotoxin can cause problems such as fever whether the bacteria are alive or dead.

Manufacturers need to know Gram stain results before they implement their sterilization process in order to have an idea of how difficult it will be to sterilize the medical device. Additionally, if a test of sterility fails, knowing the type of microorganism present can help to determine if a change is needed to the sterilization method.

Gram stain testing provides a baseline of the types of microorganisms present on the medical device products. If spikes occur in the bioburden, a Gram stain test might be conducted to compare the microorganisms present to the baseline. This allows manufacturers to assess if the bioburden is changing. When changes are made in the manufacturing process, it can be helpful to perform Gram stain testing to characterize any changes to the types of microorganisms. Knowing the types of microorganisms provides manufacturers with valuable information that can help them maintain and improve their sterilization process.

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