A Definitive, Rapid Alternative to the Gram Stain Assay

As published in the March/April 2005 issue of *Rapid Microbiology Newsletter,* by Tim J. Farmer, *Director of Business Development, Charles River Laboratories*

The Gram stain test is one of the most widely used microbiological procedures today, yet it has remained virtually unchanged since its invention by Danish physician Hans Christian Gram in 1884. The assay involves a laboratory staining technique that distinguishes groups of bacteria by the identification of differences in the structure of their cell walls.

The Gram stain assay differentiates bacteria into two groups. Gram positive organisms have high levels of peptidoglycan in their cell walls, which retain the primary crystal violet stain and appear purple. The Gram negative cell wall contains small levels of peptidoglycan but also contains an outer membrane composed of lipopolysaccharide. These organisms are counterstained with safranin and appear pink.

While the Gram stain assay has been a very useful tool to differentiate bacteria, it has its shortcomings. The procedure is susceptible to inconclusive or incorrect results due to operator technique, as well as inherent properties of certain organisms. Many companies run additional tests, such as KOH* or aminopeptidase, to confirm the accuracy of the Gram stain results. Since the Gram stain is often the first test for a complete bacterial identification, the cost of erroneous Gram stain results is magnified. Microbiologists also must deal with the issue of stain waste disposal, often requiring treatment as a biohazard.

Charles River Laboratories has addressed the need for a more reliable and less subjective alternative to the Gram stain assay with the introduction of Endosafe®-PTS™ Gram ID, a definitive, stain-free test that decreases the variability and technician time associated with the Gram stain assay. The PTS™ Gram ID is a revolutionary new assay that utilizes a handheld reader and disposable cartridge pre-loaded with all of the materials needed to run a single-step Gram determination.

The PTS™ Gram ID is a fast, simple test that measures the presence of cell walls in a microbial isolate. The measurement is interpreted by the software to indicate whether a sample contains Gram negative bacteria, Gram positive bacteria, or yeast/mold. To perform the test, the user simply suspends a microbial colony from the surface of an agar plate into solution and loads a different sample into each of the four sample reservoirs of the disposable cartridge. The reader draws and mixes each sample and measures the optical density of the reaction. Within about 3 minutes, the PTS™ will indicate if the sample organism is Gram negative or Gram positive. The PTS™ will also identify yeast/mold in about 7 minutes. Documented results, including sample and operator data, can be printed or downloaded to a computer.

The PTS™ Gram ID eliminates technician variability that can occur in a Gram stain determination that requires multiple reagent steps. The PTS™ also reduces the chances of incorrect or Gram-variable results that occur due to physiological properties of the cell wall. The PTS™ makes identifications based on the composition of the cell walls only, not the presence of dyes picked up by the cell walls. Over 70 different organisms have been tested with the PTS™ Gram ID for accuracy and specificity. These organisms have been grown for up to 72 hours and successfully tested, which could mean less sub-culturing of organisms for the microbiologist.

The Gram ID assay is run on the same PTS™ instrument that is currently being used for endotoxin detection. These assays, along with future PTS™ applications that Charles River Laboratories is developing, require only using different reagent cartridges.

For more information please visit our website at www.criver.com or call 1.877.CRIVER.1.

***Editor's Note:** The KOH String Test relies on the differential resistance to 3% potassium hydroxide between Gram positive and negative cells, where a portion of a colony is mixed with a small volume of 3% KOH on a glass slide for no more than 60 seconds. If the cells lyse, the liberated cellular DNA makes the mixture viscous or "stringy." The positive string test indicates a Gram negative organism.*

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