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EDITORIAL

Classic Spotlight: How the Gram Stain Works

George A. O'Toole

Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, New Hampshire, USA

From the teaching laboratory to the clinical microbiology laboratory, one of the best-known tools to study microbes is the Gram stain. This stain, originally developed to help distinguish bacteria from host cells in tissue, has evolved as a key assay to help clinicians decide which antibiotics should be used to treat infections, given that Gram-positive and Gram-negative organisms display differential responses to some classes of antimicrobial agents. While not providing any phylogenetic information, the purple versus pink staining mirrors the different biologies of these two groups of organisms and reflects relationships between many clinically relevant microbes as defined by 16S rRNA gene-based analyses.

Since its development in the late 1800s, evidence has accumulated that the Gram stain differentiates between microbes based on their ultrastructure; typically the thick cell wall of Gram-positive organisms is credited with helping to retain the purple crystal violet-iodine complex upon treatment with ethanol. In a 1983 publication in the *Journal of Bacteriology*, Beveridge and Davies (1) used a variation of Gram stain with an electron-dense substitute of iodine to directly visualize the crystal violet precipitate via electron microscopy. This work confirmed the differential retention of the crystal violet precipitate and thus helped establish the mechanism by which this ubiquitous assay functions. Furthermore, the authors' diagrams that model how *Bacillus subtilis* versus *Escherichia coli* responds to the ethanol-destaining step are a must-see for any student of microbiology.

REFERENCE

1. **Beveridge TJ, Davies JA.** 1983. Cellular responses of *Bacillus subtilis* and *Escherichia coli* to the Gram stain. J Bacteriol **156:**846–858.

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Address correspondence to georgeo@dartmouth.edu.

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