

# I is for Iodine

**A-Z of Staining** - a series of articles where we share a little extra information about stains, staining techniques and some of the interesting chemicals associated.



**Welcome to our continuing series on the A to Z of staining where today we will be looking at the chemical iodine and its applications in the clinical and laboratory settings.**

Iodine is a lustrous, purple-black, crystalline solid that sublimates to form a purple vapour when heated. With the chemical symbol I and atomic number 53, iodine is one of the halogens, chemical elements that form salts upon reaction with metals. Although iodine can be used topically to treat and prevent infections in mild cuts and scrapes, the first commercial use of iodine was in photography. Today, iodine is used in medicines, disinfectants, printing inks, animal feeds and in the manufacture of LCD displays. In the human body, iodine is an essential component for the production of thyroid hormone which helps to regulate both growth and

body temperature. Deficiencies of iodine causes the thyroid gland to swell (goitre), but since it is not produced within the body, iodine becomes an essential dietary requirement. Both fish and dairy produce contain large amounts of iodine; so too does laverbread, a nutritious Welsh delicacy made from seaweed, another rich source.

As a biological stain, iodine is useful for studying plant cells because it stains starch blue-black and in animal tissue it can help in the demonstration of nuclei and cell membranes. However, the most common application of iodine is in the preparation of Lugol's iodine which comprises iodine, potassium iodide and water. In the clinical setting, Lugol's iodine is used to demonstrate glycogen by the vital staining of oesophageal mucosa in patients being prepared for endoscopy. When the dye reacts with glycogen in normal squamous epithelium, it appears blue-black. This helps to emphasize abnormal mucosa since high cellular proliferation results in the degradation of glycogen. Consequent failure of staining by the iodine thereby improves the sensitivity of endoscopy. By taking biopsies from the non-stained areas, it allows the detection of early malignancies. This is also true in studies of other mucosa such as that of the cervix where Lugol's (or Schiller's) iodine can be applied to the cervical epithelium prior to examination (Schiller test). In the histology laboratory, Lugol's iodine is used as a rapid, non-specific dye for demonstrating helminths and protozoa in wet mount preparations of faecal material. In addition, Lugol's iodine is useful for the gross staining of amyloid which appears as a waxy, yellow deposit. However, the most popular application of Lugol's iodine is in the staining of bacteria by the method of Gram. This procedure is a powerful diagnostic tool and is usually the first step in the preliminary identification of a bacterial organism. Although this method has been described previously under the heading 'G is for Gram,' this additional information will hopefully justify its inclusion in this section.

In the pathology laboratory, it is common knowledge that the Gram stain is used to distinguish and classify bacteria as Gram-positive (purple) and Gram-negative (red) following the critical decolourizing step (Figure 1). The historic mechanism of the Gram stain associates itself with the properties of bacterial cell walls where it was advocated that the crystal violet stain readily traversed the peptidoglycan layer and cytoplasmic membrane before equilibrating within the cytosol (Figure 2).

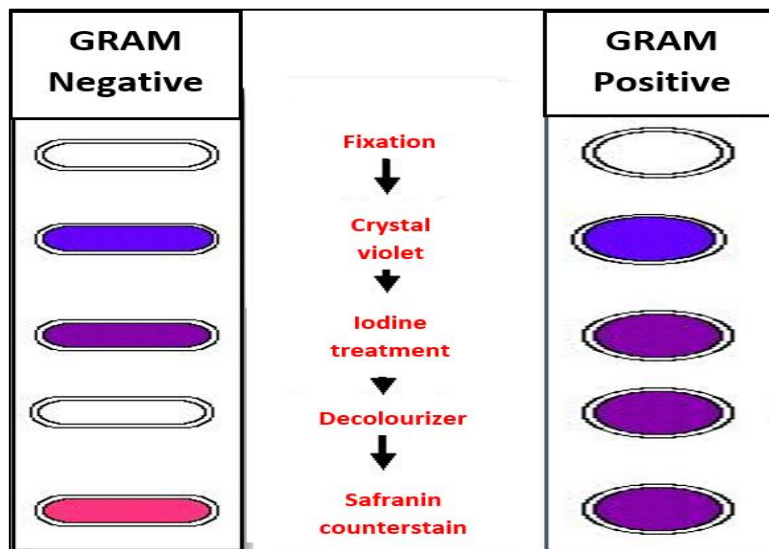


Figure 1 - The application stages and results of Gram staining

Bacteria that have a single inner cytoplasmic membrane coated in a thick peptidoglycan layer trap the dye-iodine complex inside (Gram-positive). Bacteria with an outer and inner cytoplasmic membrane separated by a thin peptidoglycan layer reject the dye (Gram-negative). However, a new mechanism recently proposed contradicts the established theory of how the Gram stain works without affecting result interpretation. The hypothesis suggests that following the timescale of the procedure, the crystal violet is unable to traverse the cytoplasmic membrane as originally thought but is kinetically trapped within the peptidoglycan matrix.

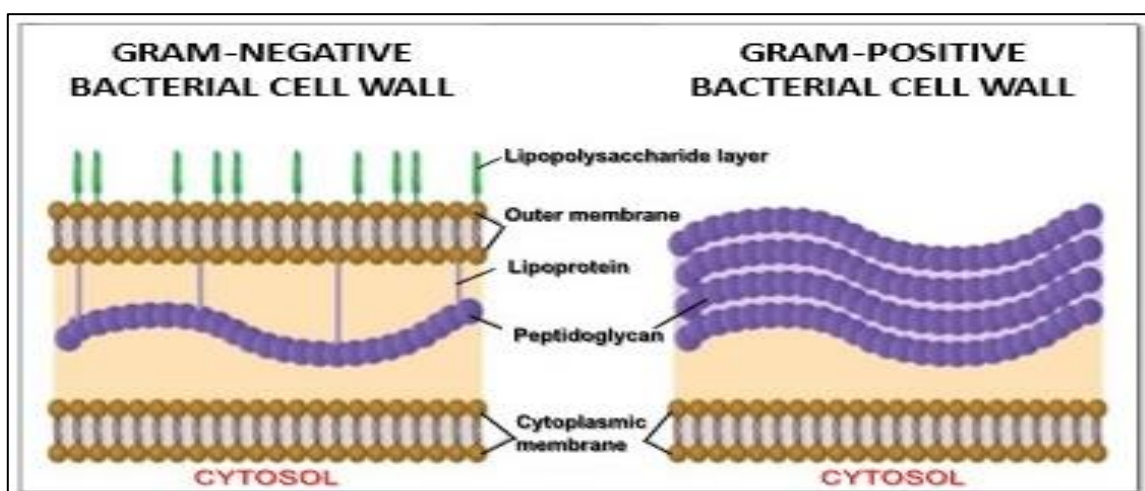


Figure 2 - Diagram showing the composition of cell walls in Gram-negative and Gram-positive bacteria

And finally, one last note to remember about iodine if your tissues have been fixed in a mercuric chloride-containing fixative! Lugol's (or alcoholic) iodine when combined with sodium thiosulphate (hypo) aids removal of the mercury pigment that occurs in tissue sections fixed in agents such as B-5 and Zenker's fixative.

### **Further reading**

Gram's stain does not cross the bacterial cytoplasmic membrane. Wilhelm MJ; Sheffield JB et al. ACS Chem Biol. 2015;10(7):1711-1717

Absence of iodine staining associates with progression of esophageal lesions in a prospective endoscopic surveillance study in China. Liu M; Liu Z et al. Clin Gastroenterol Hepatol. 2020;18(7):1626-1635

100 years of iodine testing of the cervix: A critical review and implications for the future. Reich O & Pickel H. Eur J Obstet Gynecol Reprod Biol. 2021;261:34-40

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