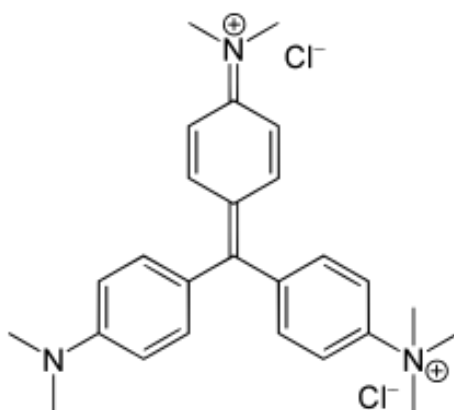


# M is for Methyl Green

**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.

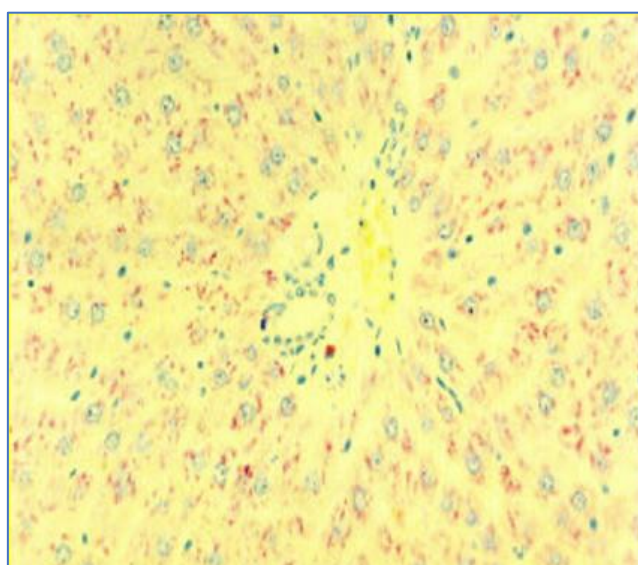


Welcome to the A-Z series of staining where today we will be looking at methyl green, a synthetic, triphenylmethane-type cationic dye (Figure 1). Methyl green is related to ethyl green and is mainly used as a stain for DNA as it binds specifically to the regions rich in adenine-thymine (A-T) nucleotides. The dye is also useful as a nuclear counterstain since the blue-green colour contrasts well with the various coloured products of other staining reactions. Methyl green is also effective as a fluorescent nuclear label for cells and tissues. When used in very low concentrations at physiological pH, the dye has red excitation and emission spectra between 633 and 677 nm and is a convenient alternative to other red-emitting nuclear stains. Commercial methyl green contains methyl (crystal) violet, and this contaminant not only affects the fluorescent properties of the dye, but also makes it particularly difficult to control and consistently reproduce effective tinctorial.

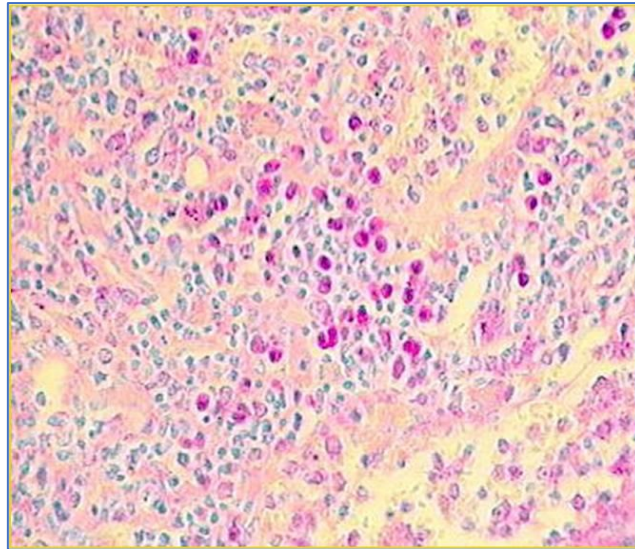


**Figure 1. The structure of methyl green (C<sub>26</sub>H<sub>33</sub>Cl<sub>2</sub>N<sub>3</sub>)**

To obtain pure methyl green, the dye must be washed thoroughly in chloroform to remove the methyl violet impurity. This is usually performed in a separating funnel by shaking a solution of the dye in several changes of chloroform until the solution becomes colourless. Since the 19<sup>th</sup> century, methyl green has been used for staining DNA and this led to the development of the classic Unna-Pappenheim stain. A contrasting dye solution comprising methyl green and pyronin (a red, fluorescent xanthene dye), this method stains DNA green and RNA red (Figure 2). The methyl green-pyronin (MGP) technique was first published by Pappenheim in 1899 and modified a few years later by Unna. Since that time, many more modifications of the procedure have been documented for use in routine staining methods for demonstrating nucleic acids and apoptotic cells. Additionally, the technique has proved to be effective in staining oral exfoliated cells for the early diagnosis of oral cancer.



**Figure 2. MGP stain showing green DNA and red RNA**



**Figure 3. Plasma cells staining red with the MGP method**

The underlying theory of the MGP method suggests that when the dyes are used in combination, the methyl green binds specifically to DNA while the pyronin binds RNA. Although both dyes are positively charged (cationic), the reaction of the methyl green is credited to the position of the amino groups of the dye in relation to the phosphate groups of the DNA. In contrast, pyronin does not show any special affinity and binds to the negatively charged RNA. However, other negatively charged components such as Nissl substance, lipofuscin, acid mucins, cartilage, mast cell granules and plasma cells also stain red (Figure 3). Consequently, pH of the solution, dye concentrations and staining times are critical since an increase in stain time intensifies the pyronin staining while a decrease in stain time intensifies the methyl green staining.

### **Further reading**

1. Ahlqvist J & Andersson L. Methyl green-pyronin staining: Effects of fixation; Use in routine pathology. *Biotech Histochem* 2009;47(1):17-22
2. Godavarthy D, Naik R et al. Tobacco-induced alterations in exfoliated oral epithelial cells: A comparative image analysis study. *J NTR Univ Health Sci* 2018;7:168-173
3. Preto P & Lyon H. Methyl green-pyronin staining of nucleic acids: studies on effects of staining time, dye composition and diffusion rates. *Biotech Histochem* 2003;78:27-33
4. Prieto D, Aparicio G et al. Methyl green fluorescence: an emerging alternative for DNA staining. *Histochem Cell Biol* 2014;142:335–345

5. Raman RK, Kamboj M & Narwal A. The diagnostic role of methyl green-pyronin Y staining in oral leukoplakia and oral squamous cell carcinoma: An exfoliative cytology-based cytomorphometric analysis. *Acta Cytol* 2019;63(5):401-410
6. Simila CSA, Joseph TI et al. Quantitative analysis of apoptotic cells in normal mucosa, oral epithelial dysplasia and oral squamous cell carcinoma using methyl green-pyronin stain. *Int J Health Sci Res.* 2018; 8(9):52-56.

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