

L is for Luxol Fast Blue

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 A-Z series of staining where we will be looking at the letter L for Luxol Fast Blue (LFB). This dye is an alcohol soluble, copper phthalocyanine derivative which is attracted to the lipoproteins found in myelin. In the central nervous system (CNS), myelin is rich in lipid and appears white (the white matter of the brain). This region consists primarily of nerve fibres surrounded by a myelin sheath which not only protects them from injury but also enhances the speed and transmission of electrical impulses along them. In contrast, the grey matter has a copious supply of nerve cell bodies but only small numbers of myelinated nerve fibres. When myelin becomes damaged, the nerve impulses slow down or stop altogether and this can cause symptoms of diseases such as multiple sclerosis (MS), the most common demyelinating disease of the CNS. Other diseases such as myelitis, Guillain-Barre syndrome and Parkinson's disease are also characterized by abnormal changes in the white matter. Although the brain

has a natural ability to regenerate myelin through special cells called oligodendrocytes, regeneration becomes less frequent as the brain ages.

In the histology laboratory, there are various methods available for staining both normal and regenerative myelin. In the haematoxylin and eosin (H&E) method for example, myelin is eosinophilic while the nerve cell nuclei and Nissl substance (the discrete granular structures in the cytoplasm) stain blue with the haematoxylin. However, the gold standard for staining myelin is with LFB which takes advantage of a simple acid-base reaction. Tissue sections are treated in a dye solution at a temperature of around 60°C for an extended period (usually overnight). During this time, the base of the sulphonated copper phthalocyanine dye exchanges with the base of the phospholipid present in the myelin, forming a dark blue precipitate. Excess stain is removed with 95% alcohol and the sections differentiated with lithium carbonate and 70% alcohol until the grey matter becomes colourless. With this technique, myelin stains blue and the neurones (nerve cells) appear purple. The LFB method is often counterstained with cresyl fast (or cresyl echt) violet to highlight important structural features of neurones (the Kluver-Barrera method). Cresyl violet is a basic aniline dye that selectively stains ribonucleic acid (RNA) blue. Since Nissl substance contains a high content of ribosomal RNA, the staining of it gives the cytoplasm a dark blue mottled appearance (see Figure). Additionally, a modified Kluver method applies an eosin step between the LFB and cresyl violet stages and this stains myelinated fibres blue, neuropil (areas composed of mostly unmyelinated nerve fibres) pink and neurones purple.

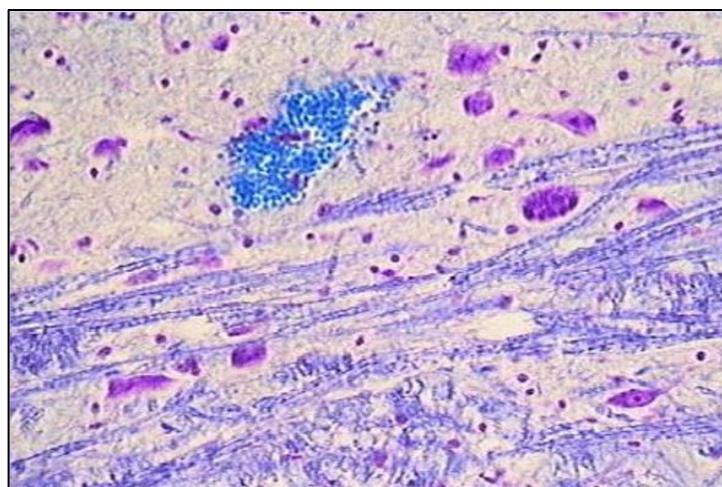


Figure. Section of brain showing blue staining of myelin in longitudinal and cross sections of myelinated nerve fibres. The granular Nissl substance stains dark blue to purple in the cytoplasm of the nerve cells (Luxol Fast Blue / Cresyl Fast Violet)

The traditional LFB has often been combined with a variety of other common staining methods and these have proved reliable in the diagnosis of certain pathological processes of the CNS. These have included the H&E, periodic acid Schiff, oil red O and silver impregnation such as the Bodian and Bielshowsky methods. Another stain combination uses LFB with the dye picosirius red (the MCOLL method) and this simultaneously stains the myelin sheath and tissue collagen fibres. This offers an integrated overview of the histology and myelin content of nervous tissue before and during the regeneration process.

Further reading

1. A method for the combined staining of cells and fibers in the nervous system (Kluver & Barrera). *J. Neuropath Exp Neurol* 1953;12:400-403
2. Evaluation of myelin sheath and collagen reorganization pattern in a model of peripheral nerve regeneration using an integrated histochemical approach (Carriel et al). *Histochem Cell Biol* 2011;136:709-717
3. The modified method of Luxol Fast Blue for paraffin-embedded myelin sheath staining (Sajadi et al). *Int J Morphol* 2020;38:1197-1200

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