Azur-methylene blue staining of resins sections

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Paraffin embeddeding methods are the most used procedures in histology, histopathology and immunohistology. An alternative to the classical paraffin method is embedding of tissues in one of the modern resins (Epon, methacrylates etc.). Even if such preparations are specifically made for scientific purpose rather than for routine diagnostics, they offer several advantages inasmuch as cellular details are preserved to be studied at both light and electron microscopic levels. Furthermore, very thin sections can be cut from resin embedded tissues, the so-called semithin sections in the order of 0.5 µm thickness. Semithin sections enable classical histological stainings and also immunostainings. In the case of immunostainings, a general histological staining is required to obtain informations on the morphology under study and for the selection of appropriate tissue blocks.

Histological staining of semithin sections is readily achieved with Azure B and methylene stains. This procedure was originally introduced by KC RICHARDON et al. (1960) and gives an excellent information of the tissue morphology. Azure B is formed by the oxidation of methylene blue and used in Azure-eosin stains of blood smears. Methylene blue is a commonly used dye in histology. Following 'ripening' which is a process of atmospheric or chemical oxidation, the three metachromatic dyes Azure A, B and C are formed (polychrome methylene blue).*

Azure-methylene blue (semithin resin sections)

Chemicals	**Chemical solution**
Azure B (C.I. 52010) Methylene blue (C.I. 52015) Periodic acid Sodium tetraborate Ethanol Distilled water	• Periodic acid solution: 1.0 g periodic acid dissolved in 100.0 mL distilled water • Sodium tetraborate solution: 1.0 g sodium tetraborate dissolved in 100.0 mL distilled water • Azure B stock solution: 1.0 g Azure B dissolved in 100.0 mL distilled water • Methylene blue stock solution: 1.0 g Methylene blue dissolved in 100.0 ml sodium tetraborate soltion • Azur B-Methylene blue dye solution: 1.0 mL Azure B stock *plus* 1.0 mL Methylene blue stock

* Dyes and other chemicals in histological staining can be toxic. They must be handled with care

Staining procedure

Semithin sections from resin (e.g. Epon) embedded tissue are cut and deposited with a drop of distilled water on acetone cleaned glass slides. Sections are dried for 30 min at 90ºC, then glass slides are brought to the cold (room temperature). Staining procedure:

–	Periodic acid solution (when tissue is fixed/postfixed with OsO_4)	5 min
–	distilled water	several rinses
–	transfer slides to 90ºC for drying	
–	Azure B-Methylene blue dye solution	3-4 min at 90ºC
–	transfer slides to room temperature	
–	distilled water	several rinses
–	differentiate in 70% ethanol	10 sec under microscopic control
–	absolute ethanol	2 x 2min

Slides are cleared in xylene or xylene substitute and mounted in resinous medium under coverglass

References for further readings

Richardson KC *et al*. (1960)

Full citation of publication is given in chapter *References*
link: https://www.kuhlmann-biomed.de/wp-content/uploads/2020/12/References.pdf

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