U is for Uranyl Acetate

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.

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Welcome to the ABC of Staining series where we continue with the letter 'U' for Uranyl Acetate

Uranyl acetate is a yellow-green acetate salt of uranium oxide and is an electron-dense, heavy metal used to stain biological samples. With the formula $UO_2(CH_3CO_2)_2(H_2O).H_2O$, uranyl acetate is primarily used in transmission electron microscopy (TEM) where it gives excellent contrast to ultrastructure morphology by binding strongly to negatively charged phosphate groups in cellular structures such as nucleic acids and cell membranes. This binding makes cellular components more opaque to the electron beam, which increases the contrast in images produced by the electron microscope. Uranyl acetate can be used both as a positive and negative stain when staining ultrathin tissue sections. When used as a positive stain, the salt is typically followed by lead citrate for a double-

staining technique. As a negative stain, the uranyl acetate solution surrounds the specimen to create a high-contrast negative image, where the specimen appears light against a dark background. This method is often used for visualizing small macromolecules such as protein complexes and viruses. At cryogenic temperatures (−195 °C), uranyl acetate can be induced to fluoresce brightly and this characteristic has been utilized as a dual-purpose contrast agent. By combining conventional TEM and fluorescence with correlative light electron microscopy (CLEM), cellular processes can be further studied by allowing direct correlation between fluorescence and TEM images (1). Although uranyl acetate has been the standard contrasting agent in TEM for many years, it is chemically radioactive and toxic if ingested, inhaled or absorbed through the skin. Due to the safety concerns associated with the strict handling and disposal of uranium salts, the use and purchase of uranyl acetate is subject to very strict regulations. Some years ago, the use of platinum complexes were used as staining agents for electron microscopy. Although platinum blue together with lead citrate post-staining showed similar results compared to uranyl acetate and lead citrate staining, its use was discontinued due to the high toxicity of platinum.

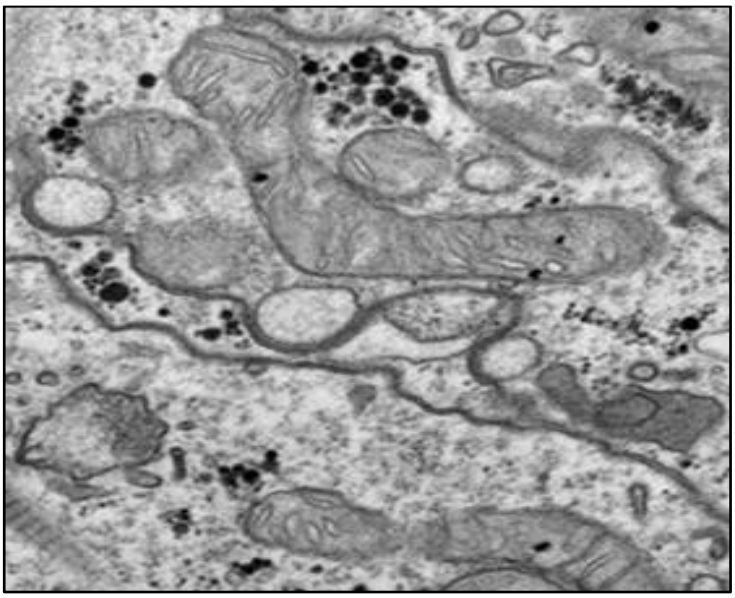

Figure. Transmission electron micrograph showing large, oval mitochondria

Consequently, safer alternative substitutes that are able to achieve similar contrast without the existing hazards are being developed. Uranyl acetate replacement (UAR) stains are non-radioactive, non-toxic substitutes for the traditional stain used in electron microscopy. These substitutes not only avoid the health and safety risks that are associated with uranyl acetate but are formulated (often as

ready to use solutions) to achieve comparable or better contrast than the conventional method. The use of neodymium acetate has been considered as a substitute to using uranyl acetate as it is not toxic, not radioactive and easy to use. Because neodymium (Nd) is directly above uranium (U), it is believed that their chemical properties of both salts would be very similar in binding to tissue in ultrathin sections and lead to a similar levels of contrast (2).

The use of commercial dyes such as haematoxylin followed by lead citrate have shown promise as a replacement to using uranyl acetate. Alternatively, proprietary UAR products such as UAR-EMS, UranyLess and UA-Zero, use lanthanide salts instead of uranium and are designed to be used with minimal or no changes to standard protocols (3). Similarly, Oolong tea extract (OTE) has shown good results as a non-hazardous alternative to uranyl acetate. Containing a high percentage of tannin, the agent can be combined with lead citrate to enhance contrast when double-staining (4).

Further reading

1. Tuijtel MW et al (2017). Inducing fluorescence of uranyl acetate as a dual-purpose contrast agent for correlative light-electron microscopy with nanometre precision. Scientific reports 2017;7:10442. doi: 10.1038/s41598-017-10905-x

2. Kuipers J & Giepmans BNG (2020). Neodymium as an alternative contrast for uranium in electron microscopy. Histochemistry & Cell Biology 2020;153(4):271-277. doi: 10.1007/s00418-020-01846-0

3. Santhana RL et al (2023). En bloc staining with uranyl acetate zero (UA-Zero) for transmission electron microscopy. International Journal of Medical Nano Research 2023;10:040. doi: 10.23937/2378-3664.1410040

4. He X & Liu B (2017). Oolong tea extract as a substitute for uranyl acetate in staining of ultra-thin sections based on examples of animal tissues for transmission electron microscopy. Journal of Microscopy 2017;267(1):27-33. doi: 10.1111/jmi.12544

Dr Phil Bryant

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