HISTOPATOLOGIA

Contrast in transmission electron microscopy: emerging alternatives to uranyl acetate - Systematic Review

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Transmission electron microscopy (TEM) is a key technique for nanometric ultrastructural analysis in biomedical research. Uranyl acetate (UA) has long been the contrast agent of choice due to its high electron density and affinity for cellular components. However, UA is radioactive, nephrotoxic, potentially carcinogenic, and subject to strict regulation by the European Chemicals Agency, prompting the need for safer alternatives.

This systematic review aimed to evaluate whether non-radioactive substitutes for UA preserve ultrastructural image quality while improving laboratory safety.

A review was conducted following PRISMA 2020 guidelines. Searches were performed in October 2025 in PubMed, Scopus and ScienceDirect. Original studies published from 2000 onwards assessing UA alternatives for TEM contrast in biological samples were included. Methodological quality was assessed using Joanna Briggs Institute critical appraisal tools.

From 1,355 records identified, 22 studies met the inclusion criteria. Alternatives were classified into four groups: (1) commercial substitutes, which showed contrast comparable to UA but often required double staining; (2) lanthanide-based agents, which consistently achieved similar ultrastructural contrast without radioactive risk under strict pH control; (3) natural extracts, which were effective and low-cost when combined with lead citrate; and (4) heavy metals, which demonstrated superior staining of specific targets

such as polysaccharides and glycogen. Most studies relied on qualitative visual assessment of representative micrographs, and only nine included objective quantitative measures, such as contrast density or signal-to-noise ratio. Moreover, substantial methodological heterogeneity across studies, including sample type, resin, staining protocols and concentrations, limited direct comparability and robust statistical inference.

Despite the limitations, replacing UA in TEM protocols is technically feasible. Lanthanides emerge as the most versatile substitutes for routine applications, while natural extracts represent viable low-cost alternatives. For specific targets, including glycogen, hafnium- and platinum-based stains outperform UA, provided that staining conditions are carefully optimised.

Keywords: Transmission Electron Microscopy, Uranium Compounds, Contrast Media