Low-Voltage Electron Microscopy for Viral Diagnostics

Low voltage electron microscopy (LVEM) is a more accessible option for routine viral diagnostic use, successful in visualizing large and small viruses, with the added benefits of lower costs, compact size, simplified operation, and speed.

Transmission Electron Microscopes (TEM) have been the benchmark for diagnostic electron microscopy due to their high resolution for imaging of viral structures. However, the significant cost, complex infrastructure, and high maintenance expenses associated with TEM limit its accessibility for many laboratories. A study by Robert Koch Institute evaluated whether LVEM can offer similar diagnostic capabilities to high-voltage TEMs in the identification and analysis of viruses, while presenting a more accessible option for diagnostic laboratories due to its affordability, speed, ease of use, and compact size. Möller, Holland, & Laue (2020) compared a high-voltage TEM to low-voltage TEM for this purpose.

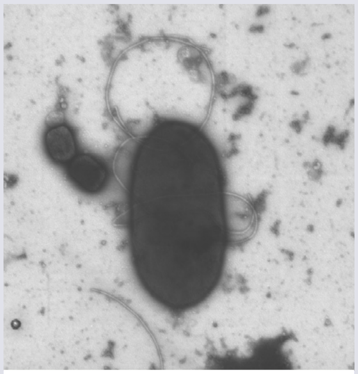

Fig A. *Human rotavirus and Vaccinia virus, negatively stained (0.5% UA) and deposited on carbon film (LVEM 25, TEM)*

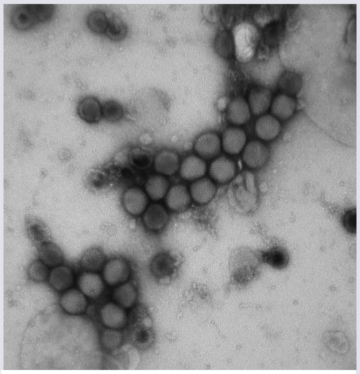

Fig B. *Adenovirus and Human rotavirus, negatively stained (0.5% UA) and deposited on carbon film (LVEM 25, TEM)*

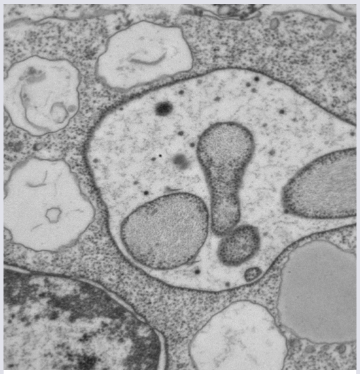

Fig C. *Insect cells infected with Culex theileri flavivirus, 70 nm thin section poststained with 2% UA and 1% lead citrate (LVEM 25, TEM)*

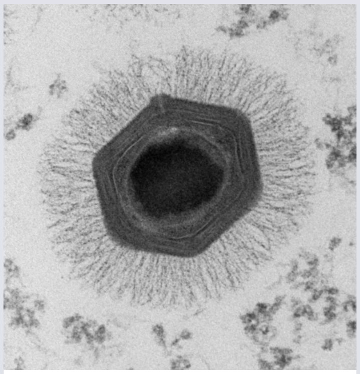

Fig D. *Acanthamoeba polyphaga mimivirus, 70 nm thin section post-stained with 2% UA/1% lead citrate, coated with carbon (LVEM 25, TEM)*

A JEOL JEM 2100 operated at 200 kV in TEM mode was compared to the LVEM25 compact transmission electron microscope, operated at 25 kV in TEM mode, and 15 kV in STEM mode. Samples included negatively stained viruses such as Vaccinia virus (Fig A), Human rotavirus (Fig B), Yellow fever virus, Tobacco mosaic virus (TMV), as well as ultrathin sections of Culex theileri flavivirus-infected cell cultures (Fig C) and Acanthamoeba polyphaga mimivirus (Fig D).

LVEM was able to successfully visualize both small and larger viruses. The LVEM 25 provided sufficient resolution for virus detection in the TEM, with STEM mode providing additional benefits for thicker or denser samples.

LVEM performed comparably to high-voltage TEM in terms of screening speed. Sample exchanges on the LVEM 25 were generally swift, with an average time of three minutes (improved to few seconds on new model LVEM 25E). The LVEM technique provides for easier operations due to simplified and intuitive, computer-based controls. Minimal manual adjustments were needed, facilitating faster training and use.

The study also tested a scanning electron microscope with a transmission detector (STEM in SEM). While the image data was acceptable for these purposes, STEM in SEM had appearances of scan artifacts, such as darkened imaging areas or lines which significantly prolonged sample inspection process. Additionally, STEM in SEM required more time to acquire image data due to complex software and many adjustments. Pump down times due to the large samples chamber reduced sample throughput.

Conclusion

The study concludes that LVEM offers a viable alternative to high-voltage TEM for viral diagnostics. The LVEM 25 provides sufficient resolution and image quality for routine diagnostic use, with the added benefits of lower costs, compact size, simplified operation, and speed. Their findings show that LVEM can enable more laboratories to conduct routine viral diagnostics while maintaining necessary diagnostic standards.

References

Möller, L., Holland, G., & Laue, M. (2020). Diagnostic electron microscopy of viruses with low-voltage electron microscopes. Advanced Light and Electron Microscopy, Centre for Biological Threats and Special Pathogens, Robert Koch Institute, Berlin, Germany.