Same-Day EM-Diagnosis of Tissues and Potential Bioterrorism Samples by the Use of Microwave-Assisted Processing

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The purpose of this study was to compare the turnaround time as well as the section and image quality of specimens processed for electron microscopic diagnostic examination by microwave technology [1] versus conventionally processed samples.
In the collaborative study the following specimens addressing critical infectious or potential bioterrorism/emerging agents [2] and tissues with crucial pathologic diagnosis were supplied: RKI/Berlin: cell cultures infected with SARS, West-Nil-Virus, Elephant-Poxvirus, and Herpesvirus; AF/Koblenz: suspensions of E. coli and Acanthamoeba causing diarrhoea, BNI/Hamburg: suspensions containing malaria, leishmania, and microsporidia pathogens as well as C. elegans; Patho./Regensburg: agar culture with B. anthracis, Drosophila-flies infected with microsporidia, experimental 3D-tumor spheroids with three different cocultured cell lines, biopsy material of human fresh adrenal and previously formalin fixed neuro-secretory tumors, a bronchial biopsy of a patient with suspected ciliar dyskinesia disease, and liver samples of a laboratory mouse.
All samples were routinely fixed in 2% buffered GA or Karnovsky-fixative overnight or longer, fresh samples of bronchial cilia, liver and the flies were fixed in the Karnovsky-fixative by microwaving (20 min.). Subsequently, one half of each sample was OsO_4-postfixed, ethanol-dehydrated, and Epon-infiltrated (overnight) in an automated tissue processor (LYNX, Leica/Germany), polymerization was carried out at 60°C for 48h; the other half was parallel processed including the resin block polymerization in the REM (*Rapid Electron Microscopy*) Microwave Processor (Milestone/Italy) [2].
This device applies the same sample baskets as the LYNX for all processing steps and special vials with a magnetic stirrer for each of the solutions. The REM processor automatically regulates microwave power with the temperature of the reagent vial precisely monitored via an infrared non-contact temperature sensor. The preset programs in the software can be easily customized and the microwaving conditions of each step (temperature, time, stirring) modified according to the needs of the sample. The total time of the microwave-assisted run (fixation, washings, postfixation, dehydration, resin polymerization) was 4h 25 min.
The microwave processed resin blocks were uniformly polymerized and had good sectioning properties (diamond knife) for preparing semi- and ultrathin sections. After routine uranyl and lead staining, sections examined in a LEO912AB electron microscope (operated at 80 kV in zero-loss mode) were stable in the electron beam. Preservation of the ultrastructure was excellent and the crisp images (independent of darkroom or digital processing) displayed well recognizable details of the examined cells and pathogens to render a diagnosis. The recognition of pathogens, for example SARS virions is demonstrated in Fig. 1, the superb ultrastructure of the liver cell components after microwave fixation is shown in Fig. 2.