Impact of Microwave-Assisted Sample Preparation in Diagnostic Electron Microscopy Today.

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The primary basis for pathologic tissue diagnosis is the morphological analysis: a pathologist assesses by light microscopy cell and matrix appearances including architecture on an H&E-stained section of tissue embedded in paraffin wax, in context of gross findings and clinical data, to render a diagnosis. Additional stains and techniques, like immunohistochemistry (IHC), flow cytometry, cytogenetics and "molecular" techniques (gene rearrangement analysis, fluorescence in situ hybridization and polymerase chain reaction (PCR) analysis) provide additional information to refine the understanding of disease and diagnosis. Electron microscopic (EM) examination is a method of extending morphologic analysis to the ultrastructural level providing information not discernible by the other methods, e.g. on the basis of the antigens expressed by a neoplasm [1]. The advent and continual development and automation of the ancillary techniques, especially in IHC and now in molecular biology, resulted in reduced importance of the EM in pathology since the early 1980s. Also a number of limitations of the EM (e.g. sample error, need for adequate fixation, expensive and sophisticated instrumentation, long turnaround time, high level of staff skills and interpretation expertise) comes along. Today, EM-diagnostic expertise is generally available only in larger laboratories or centres with specific interest in EM [2, 3].

We report the results of routine use of microwave-assisted (MW) rapid tissue sample processing collected with a semi-automatic (Milestone/Sorisole, Italy, fig. 1) and a latest developed full-automated (Leica/Vienna, Austria, fig. 2) MW-tissue processor. This technology cuts the usual 3-5 days turnaround time down to approx. 3–6 hours, enabling a "same-day" EM-diagnosis in urgent clinical settings or potential bioterrorist scenario and/or emerging infectious agents (e.g. SARS) [4]. Examples of MW-assisted processed tissues of excellent quality preserved ultrastructure will be presented (fig. 3).

In the Milestone Rapid Electron Microscope microwave device (REM) the vial, containing baskets with the samples immersed in the process solution, is placed in a special designed carrier which locates the vial in a defined position in the microwave cavity. A non-contact infrared temperature sensor measures the current solutions temperature in the vial, which is the critical parameter to monitor the magnetron wattage power output (max. 700W) controlled via a feedback loop during the continuous microwave irradiation of the sample [5]. The slope of the temperature rise/stabilization and the time for each processing step could be defined on a dedicated touch screen monitor, each solution change to the next process step must be done manually by the user.

This change is carried out fully automated by a robotic reagent system in the Leica automatic microwave tissue processor (AMW) which is a great benefit for saving laboratory manpower. The implemented mono-mode microwave chamber provides for homogeneous microwave distribution at the sample location without hot and cold spots. Thus water loads are not needed and virtually 100% of the MW-radiation energy (restricted to 30W) is absorbed by the processing fluids and the specimens. Additionally a dedicated pulse mode is available to maximize the benefits of the microwave -assisted processing.

In both devices the whole MW-assisted processing is controlled by a microprocessor and dedicated software, both are running with the same sample baskets that are applied in the EM tissue processors (e.g. LYNX) for routine conventional tissue embedding. We observed, that this shared equipment is a benefit for the EM-lab workflow because one can combine MW-assisted steps (fixation 20 minutes, polymerization 80 minutes) with conventional overnight processing generating more flexibility in handling clinical urgent samples.

References

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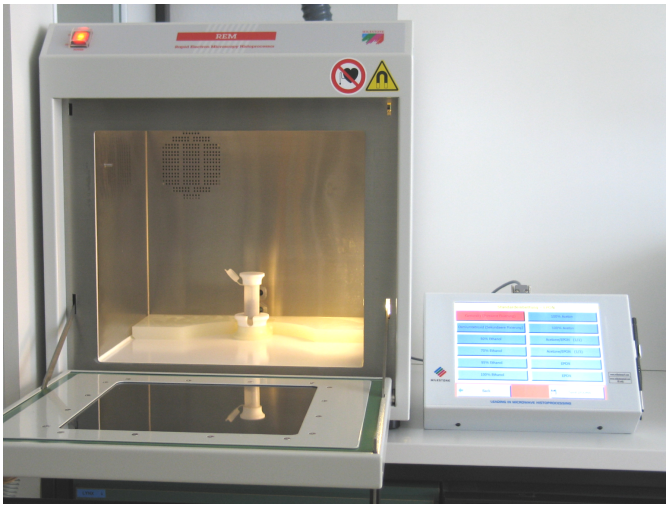

Figure 1: Rapid Electron Microscope Microwave Device (Milestone REM)

Figure 2: Automatic Microwave Tissue Processor (Leica EM AMW)

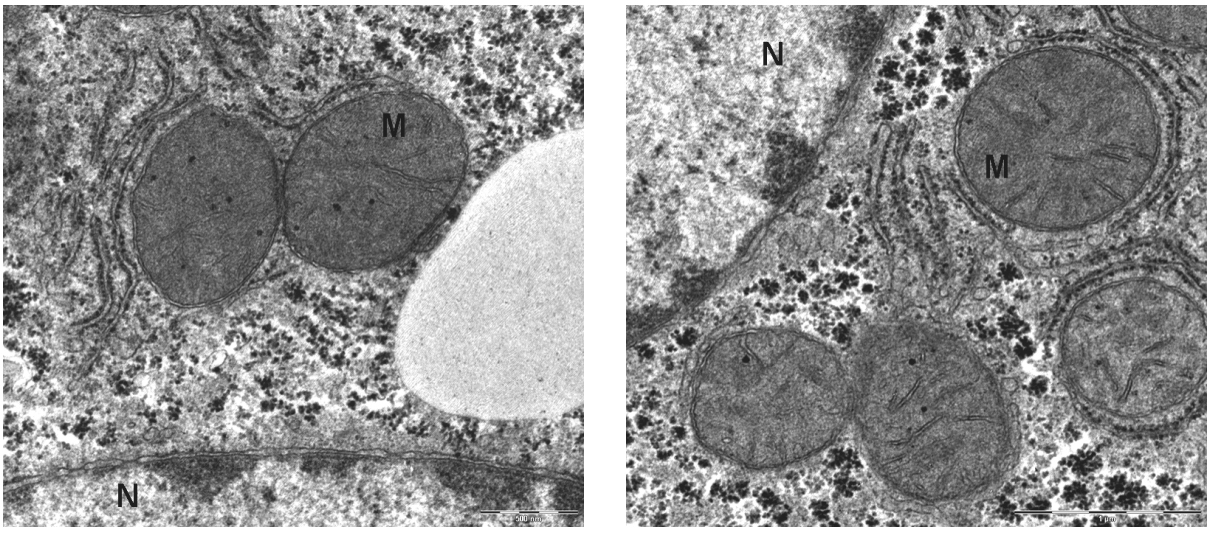

Figure 3: Liver cell detail. Left: microwave embedding. Right: conventional embedding. Total MW-assisted processing time: 5 hours. Note the excellently displayed membranes of the mitochondria (M) and nucleus (N) in both images. Original magnification: 5,000x.