Thin section electron microscopy – fast embedding methods versus classical embedding

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Diagnostic electron microscopy, dealing with viruses usually uses negative staining for analysis. The breakout of avipox in 2006/2009 showed that ultrathin sections are necessary for the early diagnosis of fowlpox besides the standard negative staining technique. However the period from the start of the preparation to the analysis took at least five days. This span is too long for a quick diagnosis. In early infection, begin of an appropriate therapy, immunization or even, in the case of veterinary, elimination of infected animals are of main importance to stop dispersal of disease. Viruses are occasionally, although suspected as estimative cause of infection, not the main reason for a disease – bacteria, fungi or parasites can often induce symptoms similar to virus diseases. Sometimes, also the sudden death of many individuals in an epidemic situation can pose problems if the epidemic agent is not the main initiator. In both incidences, ultrathin sectioning with its open view and its independence from special agents like primers or sera is the solution for diagnostics. The main emphasis of this study is to compare special embedding resins and embedding receipts for pathological purposes in regard of the time needed and the resulting quality.

Epoxy resins, Epon, Spurr and Agar Low Viscosity Kit, and acrylic resins, LR-White and Unicryl, were used for embedding. For technical comparison, microwave technique, classical embedding by hand and embedding with the embedding machine was performed. Embedding receipts with long time schedules were compared with embedding receipts with short time span. An avipox infected skin of a cockscomb was taken as sample, as veterinary diagnosis is dealing mostly with vertebrates. The skin is perfect for this experiment because of its high tissue divergence of a hardly permeable, keratinized Stratum corneum, the Stratum transitivum and Stratum intermedium with their cells attached to one another by desmosomes, the epithelial layer of the Stratum basale and the dermis consisting of collagen and elastic fibers in a glycosaminoglycan ground substance rich in fat vacuoles and nerves.

Epon was the resin of choice. It showed the best results in microwave-, in classical- and in machine-embedding. In contrary to all embedding experiments, microwave technique [1] was the only technique where embedding artifacts in the Stratum corneum were minimized. The sections, approximately 2 mm² large, were uniformly even; tissue cracks, found between the sheets of flat keratinocytes were rare (Figure 1). Virus assembly in Stratum transitivum and intermedium, characterized by virus factories full of viroplasma, crescents and immature viruses, was easily identifiable. A-type inclusions with mature virions presented their typical structure. With microwave embedding including polymerization in the microwave, a one-day-analysis is possible. Polymerization in- and outside of microwave showed the same results. Classical embedding and machine embedding

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were of exactly the same quality – the only difference was shorter process (four days instead of five days with analysis). Like in fast epon embedding technique including vaccuum resin infiltration [2] virus assembly in highly keratinized cells was difficult to analyze, sections were uneven and cracky in Stratum corneum. The quality of fast epon embedding (a 2 day process with analysis) was a good deal worse than the long lasting epon embedding. LR-White after Laue et al. [3] was much better than fast epon embedding. The time schedule was not as mentioned in the literature a one day diagnosis – diagnosis was ready soonest after one day and a half, and the samples have to be smaller (< 1 mm^3). For diagnosis this means that you have to look to the double number of sections. LR-White for immunology (4-6 days process) was of better quality than Unicryl, which gave unacceptable results in both cold UV-polymerization and heat polymerization. Spurr and Agar LV with vacuum resin infiltration were compareable: Both embedding techniques lasted three days with section analysis. Agar LV, although propagated showed not the same viscosity as Spurr.

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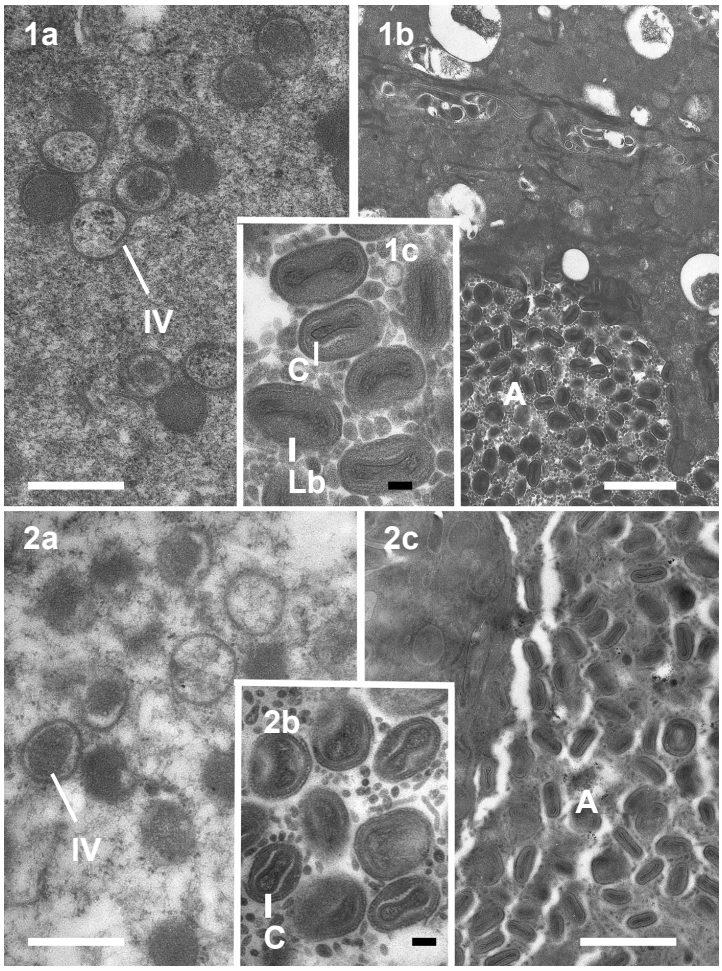

Figure 1. Chicken – Fowlpox infection, Epon embedding, microwave technique with resin polymerization in microwave

Figure 2. Chicken – Fowlpox infection, LR-White embedding after Laue et al. [3]

a) Virus factory with immature virus particles (IV) with more or less condensed viroplasma, bar = 0,5 µm b) mature virus particles with core and lateral bodies, bare = 100 nm c) A-type inclusion (A) with mature virions, bare = 1 µm

Both figures represent optimal results of fast embedding techniques: Note that section in figure 2c is uneven and that there are cracks. Cytoplasma of 2a shows less opaque granulations and virus structures are not as clearly defined as in 1a. Structure of mature viruses is nearly identical except of larger artificial spaces around the core in 2b.

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