Embedding Brain Tissue for Routine Histopathology: A Processing Step Worthy of Consideration in the Digital Pathology Era

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Abstract: The importance of technical quality for histopathologic examination has only increased in recent years with the expanding use of digital pathology. The University of Kentucky Alzheimer’s Disease Center (UK-ADC) Neuropathology Core has decades of experience with brain histopathology and has emphasized the importance of quantitative assessments of histopathologic hallmarks. Technical artifacts and nonuniform samples are challenging for high-throughput digital analyses after the slides have been scanned, so that methodological optimization may be helpful. We do not know of published literature that systematically reviews how different procedures at the various stages of tissue processing can impact the quality of the histopathologic preparations in human brain samples. We wanted to pass along our experience in the hope that it will help others to improve their results. Here we describe the UK-ADC method of embedding for neuropathologic evaluation and provide specific examples (with a comparison to another processing workflow) that help support the idea that the methods and tools used in the embedding process can alter the quality of the formalin-fixed paraffin-embedded histopathologic results. The process used at the UK-ADC has been successful for us, but results may vary in relation to each embedding machine and with other factors.

Key Words: neuropathology, neurohistology, embedding, processing, protocol, FFPE, brain, ScanScope

The purpose of this Technical Article is to highlight the potential importance of tissue embedding methods for neuropathologic studies. Common preparation methods used for neuropathologic evaluation often involve the study of formalin-fixed and paraffin-embedded (FFPE) tissue, cut and mounted onto glass slides. This basic tissue preparation technique has been used for over a century, but differing specific methods, reagents, and machines are used.

The importance of technical quality for architectural and cytopathologic examination has only increased in recent years. The University of Kentucky Alzheimer’s Disease Center (UK-ADC) Neuropathology Core has decades of experience with brain histopathology and has emphasized the importance of quantitative assessments of histopathologic hallmarks. Recently, at the UK-ADC and elsewhere, a strong focus has been on digital neuropathology. Whole slide digital pathologic methods provide rigorous and quantitative histopathologic measurements, but these investigations require high-quality, standardized tissue preparations. Technical artifacts and nonuniform samples are challenging for high-throughput digital analyses after the slides have been scanned, so, methodological optimization may be helpful.

Within the University of Kentucky, both the UK-ADC and the University of Kentucky Department of Pathology and Laboratory Medicine (UK-DP) perform work involving histopathologic preparation of human brain tissue. However, the UK-ADC and the UK-DP are in separate locations and use different biosample processing workflow. Whereas the UK-ADC processes exclusively human brain samples, the UK-DP also processes a heavy clinical load with many other tissue types. Although both facilities utilize FFPE, the preparation methods differ.

We recently had the opportunity to assess the processing of the same tissue specimens in parallel, in both facilities. The UK-ADC protocol differs from the UK-PD protocol in several ways. For example, the UK-ADC protocol used Richard-Allan Scientific paraffin Type 9, whereas the UK-PD used Leica EM-400 Embedding Medium paraffin (notably, both of these have low melting points in the 55 to 57°C range). In the UK-ADC protocol, the tissue is placed into 50/50 Alcoholic formalin before pure alcohol, while in the UK-PD protocol it is not. Also, the UK-ADC uses Xylol 50/50 before emerging the tissue in Xylene, whereas the UK-DP does not. The times spent in each chemical throughout each protocol differ as well. For embedding, the UK-DP uses Thermo Shandon Excelsior ES Processor while the UK-ADC uses the Sakura Tissue-Tek Vacuum Infiltration Processor. Full protocols from both the UK-ADC and UK-DP are attached as
FIGURE 1. Human neocortical brain tissue embedded in paraffin blocks using different methods. A and B, Human frontal cortex tissue processed at the University of Kentucky Alzheimer's Disease Center (UK-ADC). C, the same brain sample as (B), at higher magnification to show the edge of the tissue and paraffin. D and E, adjacent tissue from the same cases as in A and B, but processed at the University of Kentucky Department of Pathology and Laboratory Medicine (UK Path.Dept.). Higher magnification of tissue (F) shows a crack between the tissue and the surrounding paraffin (red triangles). A, B, D, and E, are at the same magnification (× 1). G–J, Photomicrographs of frontal cortex tissue from the same case, stained for hematoxylin and eosin (H&E). G and I, Photomicrographs were taken at the same magnification. G, H&E stained brain tissue that was embedded at the UK-ADC. H, Higher magnification of tissue in (G). I, H&E stained brain tissue that was embedded at the UK Path.Dept. J, Higher magnification of tissue in (I). Methods for cutting and H&E staining were the same for all the samples. Scale bars: G and I: 6 mm; H: 2 mm; I: 1 mm.
Supplementary Materials (Supplemental Digital Content 1, http://links.lww.com/AIMM/A252).

To evaluate the results of the different embedding protocols, we processed formalin-fixed brain portions (mid-frontal gyrus, Brodmann area 9) from the same 2 brains, on the same day, using the 2 different embedding protocols. More specifically, we performed 2 different runs for each of 2 different cases (both cases were run on both UK-ADC and UK-DP workflows), with a total of 12 different tissue blocks produced. Each case was fixed in 10% neutral buffered formalin for 2 days. The specimens’ processing differed in only the embedding methods because the goal was to elucidate the impact of embedding methods on final slide quality. Thus, after being embedded in FFPE blocks at the different locations, the tissues were cut and stained with hematoxylin and eosin in the same batch by the same histotechnologist who was blinded to the study design and the derivation of the tissue blocks.

When the tissues were embedded using the routine UK-DP workflow, the edges of the tissue in several of the FFPE blocks showed cracks with a clear separation between the tissue and the paraffin (Fig. 1). By contrast, the FFPE blocks processed using the UK-ADC protocol showed no gaps where the tissue ended. We hypothesize that such cracking and drying could increase over time if the blocks were archived for future work. The surface of the UK-ADC paraffin block was overall smoother with fewer air bubbles. This appeared to affect the tissue after it was stained. The UK-DP prepared slides had more air bubbles and small tears in the tissue. The tissue on the slide appeared more ragged, with more rarefaction in white matter, in comparison to the UK-ADC samples. We performed immunohistochemical stains for phospho-TDP-43 (1D3 clone) on slides cut from each block and did not see an appreciable difference related to the embedding methods used (data not shown).

We are not implying that all tissue processed through the UK-ADC show near-perfect results, nor that the UK-DP blocks are always marred by artifacts. However, we have noticed a consistently high quality in the UK-ADC preparations. For tissue embedding, there are many variables (processing machines, reagents, and many different protocol parameters including timing and temperatures), so, for researchers and clinical pathologists alike, we cannot make specific recommendations. However, it may be worthwhile to ascertain if workflows can be optimized for human brain samples. The process used at the UK-ADC has been successful for us, but results may vary in relation to each embedding machine and with other factors. We do not know of published literature that systematically reviews how different procedures at the various stages of tissue processing can impact the quality of the histopathologic preparations in human brain samples. We wanted to pass along our experience in the hope that it will help others to improve their results.

REFERENCES