WHITEPAPER

Troubleshooting: Tissue Processing

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In the preparation of paraffin sections for staining, fixation and processing go hand in hand. Proper tissue fixation ensures tissue processing will be effective, and a lab's chosen processing method may also influence their choice of fixative.[1] Errors in the processing stage have the potential to seriously affect staining quality. However, because tissue specimens vary widely in size, type, volume, and fixation method, there can be no universal processing protocol. Laboratory technicians will need to take all individual factors into account when programming processing protocols and should be familiar with the signs of common processing errors.

Tissue processing can be summarized by three steps: dehydration, clearing, and infiltration.[2] Automated tissue processors will also include additional fixation steps prior to the dehydration phase. While the fixation step serves to preserve cellular structures, the ultimate purpose of tissue processing is to strengthen and reinforce those structures so they can be thinly cut into 4 to 5 µm sections while still maintaining their cellular integrity.[3]

After fixation, a dehydration solution will remove unbound water molecules and any residual fixative.[4] At all stages, times and temperatures must be monitored and controlled. If specimens are subjected to excessive heat, or are left in solution for too long, it will cause both bound and unbound water molecules to be removed from the tissue.[4] This over-dehydration causes the tissue to overharden and shrink, creating a shriveled appearance in the section commonly referred to as "parched earth" artefact. This will negatively affect cellular morphology and antigenicity.[4] To avoid this distortion, technicians should consult recommended guidelines for processing times and temperatures, which may vary by tissue size and type.

Conversely, incomplete dehydration will leave the specimen too soft, preventing complete clearing and infiltration with the paraffin wax.[4] In this case, sections may be difficult to obtain or may disintegrate before they can be transferred to the slide. Soft specimens will also be vulnerable to indentation by the tissue cassette or sponge, which will appear as artefact during staining.[2] To remedy this, solutions should be changed regularly to prevent the dehydration solutions from becoming diluted with the removed fixative solution and water.

Following dehydration, clearing solutions replace the dehydration solutions and prep the tissue for infiltration with a support medium, typically paraffin wax.[4] Inadequate clearing will leave tissue too soft to effectively section. However, excessive clearing can cause protein denaturation, creating adverse effects similar to over dehydration.[2] Once again, exposure times must be optimized so that antigenicity is preserved and the paraffin can successfully infiltrate the tissue. Once again, the temperature must be closely monitored and controlled during infiltration, as overheated paraffin can also cause shrinkage and overhardening.

Overprocessed tissue can be very difficult to remedy, and specimens may not be able to be salvaged. However, if sections show signs of insufficient processing, the paraffin blocks may be melted down so that the specimen can be reprocessed, starting from the automated fixation step.[4]

Once tissue has been successfully processed, labs must "unfix" the sections to retrieve the antigens that would have been obscured by proper fixation and processing.

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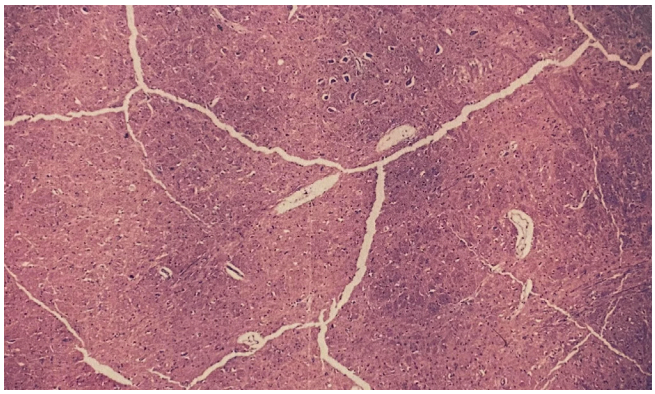

Overprocessing has created a "parched earth" effect in this tissue section, giving it an appearance similar to dry, cracked earth as pictured on the right.
From Histotechnology: A Self-Instructional Text (p. 69), by Carson, F. L., & Cappellano, C. H., 2009, United States: American Society for Clinical Pathology Press. Copyright 2009

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WP_0073