Microautoradiography Techniques: A Comparison of Frozen and Wax-Embedded Tissue Procedures and Outcomes

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Introduction
Covance Laboratories Ltd. has conducted micro-autoradiography (MARG) investigations to study the distribution of radioactivity at a cellular level for many years. Based on experience, MARG images of fixed and wax-embedded tissues seldom turn out to be comparable with regards to location and density of silver grains. Covance has recently conducted two investigations to determine if fixed and wax-embedded tissue samples are appropriate for use in MARG by processing and comparing results from frozen and wax-embedded tissue samples obtained from the same subject. The test compounds used for these comparisons were two different tritium labelled molecules and tissue-images with clear differences have been selected and presented in this poster.

Objectives
The objectives of this work were:

➢ to compare the distribution of radioactivity in selected frozen and wax-embedded tissues using qualitative MARG;

➢ to determine the extent of radioactivity migration from tissue samples into solvents used during the wax-embedding process.

Methods
Frozen tissue samples, collected from dosed and control animals, sectioned at 5µm, under darkroom conditions, were transferred to microscope slides pre-coated with nuclear emulsion (maintained at room temperature). Slides were placed in light tight cassettes and stored at -20°C to expose. Wax-embedded tissues, collected from dosed and control animals, sectioned at 5µm, under normal laboratory lighting were mounted on glass slides and de-waxed. Slides were placed in nuclear emulsion, under darkroom conditions, dried, placed in light tight cassettes and stored at -20°C to expose.

Following exposure, all slides were photographically processed and the tissue sections histologically stained with haematoxylin and eosin. Slides were examined under a light microscope, with objectives up to x100, to assess the distribution of silver grains (radioactivity). The distribution in frozen and wax-embedded liver, kidney, testis, cerebellum and spleen from the rat, and brain and thymus from the marmoset were compared.

Samples of rat liver, cerebellum, spleen, kidney and testis were placed in solvents used during the wax embedding processes as detailed in Figure 1.

Microautoradiography Radioanalysis of Wax-Embedded Tissue: (1) portal tract / (2) Kuffer-cells

Wax-embedded Tissue: (1) portal tract / (2) Kuffer-cells

All solvents of each subject were subjected to liquid scintillation counting to determine if radioactivity had migrated from the tissue in to the solvents.

Table 1. Radioactive Content of Investigated Solvents

<table>
<thead>
<tr>
<th>Sample</th>
<th>Liver (µCi)</th>
<th>Ethanol (µCi)</th>
<th>Toluene (µCi)</th>
<th>Xylene (µCi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18% neutral</td>
<td>778902</td>
<td>149716</td>
<td>10926</td>
<td>4843</td>
</tr>
<tr>
<td>10% neutral</td>
<td>157945</td>
<td>2532</td>
<td>26845</td>
<td>25</td>
</tr>
<tr>
<td>Ethanol (72 hours)</td>
<td>50577</td>
<td>5630</td>
<td>2913</td>
<td>60</td>
</tr>
<tr>
<td>Toluene (72 hours)</td>
<td>7353</td>
<td>12421</td>
<td>84</td>
<td>99</td>
</tr>
<tr>
<td>Xylene (72 hours)</td>
<td>1183205</td>
<td>127750</td>
<td>778</td>
<td>99</td>
</tr>
</tbody>
</table>

Advantages and Disadvantage of the Two Processing Techniques

<table>
<thead>
<tr>
<th>Wax-Embedded Tissue</th>
<th>Frozen Tissue</th>
<th>Wax-Embedded Tissue</th>
<th>Frozen Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of radioactivity during embedding</td>
<td>++++</td>
<td>++</td>
<td>++++</td>
</tr>
<tr>
<td>Ease of sectioning and mounting</td>
<td>++</td>
<td>++++</td>
<td>++</td>
</tr>
<tr>
<td>Morphology</td>
<td>++</td>
<td>++++</td>
<td>++</td>
</tr>
</tbody>
</table>

A rating of + (poor) to ++++ (excellent) was used.

Conclusions

➢ Administered radioactivity was distributed into, and was observed, at the cellular level in frozen tissue samples.

➢ There was specific uptake of radioactivity noted in frozen tissues. This included elevated levels of radioactivity in the Kuffer cells of the liver, in the tubules of the medullary rays in the kidney and in connective tissue in interstitial spaces of the testis. A differential pattern of distribution in the spleen was also observed.

➢ In comparison, in wax-embedded tissues there was very little radioactivity associated with these tissues. Low levels of radioactivity were associated with the lumen in the testis and the renal papillae and outer renal cortex.

➢ Radioanalysis confirmed that radioactivity migrated from the tissue and into all investigated solvents used during the wax-embedding process. Significant levels of radioactivity were recovered in the 10% neutral buffered formalin used to fix the tissue samples.

➢ Frozen tissue MARG provides an idea of total radioactivity distribution. Whereas wax-embedded tissue MARG supports distribution investigations but may be limited unless radioactive material is highly bound.

As demonstrated, the recovery of radioactivity, analyses of the images and the conclusions drawn are affected by the choice of preparation method. The amount of radioactivity recovered in the different solvents may be related to compound type. It is therefore recommended to evaluate how much is lost from wax-embedding and if this will influence the study evaluation prior to selecting sample preparation technique.

Acknowledgments

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Figure 1. Investigated solvents.

Figure 2. Example of rat liver depicting frozen and wax-embedded sample differences (pelvic objective).

Figure 3. Example of rat testis depicting frozen and wax-embedded sample differences.

Figure 4. Example of marmoset brain (choroid plexus) depicting frozen and wax-embedded sample differences (pelvic microscope objective).