Quality assessment of long-term stored formalin-fixed paraffin embedded tissues for histopathological evaluation

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Abstract: Histopathological examination of formalin-fixed paraffin-embedded (FFPE) tissues that had been stored for 30 years was conducted, and resectivity of the results was verified. These FFPE tissues, which were from all organs of male and female rats, were re-sectioned and histopathologically examined using hematoxylin and eosin (HE) staining. In particular, the stainability and morphology of HE sections and reproducibility of microscopic findings in the liver and kidney demonstrated in the original final reports were evaluated. Although the stainability of hematoxylin was slightly weaker and some morphological artifacts were observed in tissues in re-prepared slides, these deteriorations in the quality of HE sections were considered to be permissible for histopathological examination so long as control sections were also prepared. Most microscopic findings recorded in the original final reports were confirmed using re-prepared HE sections in the present study. While some focal findings, which were judged to be either incidental or spontaneous in nature, were not observed in the sections as expected, this was not considered to be a problem in reconstructing the results of the original histopathological examination because most findings related to the test articles were generally observed diffusely or multifocally in each organ. We concluded that results of the original histopathological examinations could be reconstructed using paraffin blocks that had been stored for up to 30 years. (DOI: 10.1293/tox.2017-0046; J Toxicol Pathol 2018; 31: 61–64)

Key words: FFPE tissue, long-term storage, hematoxylin and eosin, histopathological examination, quality assessment, reconstruction

Appropriate archiving of records and materials generated in good laboratory practice (GLP) studies is required to comply with the principles of GLP. This is because the maintenance of raw data associated with a specific study and the specimens generated from that study are the only means that can be used to reconstruct the study. In several countries, the retention period for specimens of a GLP study is set such that it expires when they can no longer be evaluated. Consequently, formalin-fixed organs, paraffin-embedded blocks, and hematoxylin and eosin (HE)-stained slides have been stored for long periods in each test facility.

The quality of long-term stored formalin-fixed wet tissues has been histopathologically evaluated by producing HE sections. To the best of our knowledge, no studies have reported the quality evaluation of long-term stored formalin-fixed paraffin-embedded (FFPE) tissues. Here we re-prepared HE sections from FFPE tissues that had been previously stored for approximately 30 years and histopathologically evaluated the quality of these tissues and reproducibility of microscopic findings. We then investigated the possibility of reconstructing the results of the original histopathological examination using re-prepared HE sections from long-term stored paraffin blocks.

This study evaluated FFPE tissues that had been stored for approximately 30 years. FFPE blocks evaluated in this study contained all the organs of 4 males and 4 females, i.e., 1 or 2 males and females each from three GLP toxicity studies in rats (9- to 10-week-old Crj:CD(SD) rats, 57-week-old Slc:Wistar rats, and 9- to 10-week-old Slc:Wistar rats; age at the time of sacrifice). They were stored with paraffin coating in one GLP study and without paraffin coating in two GLP studies. These FFPE blocks were sliced into 3- to 5-µm-thick sections and stained with HE. The following organs and tissues were histopathologically examined: liver, kidneys, heart, lung, spleen, adrenals, thymus, stomach, duodenum, jejunum, ileum, colon, rectum, skeletal muscle, brain, femur, sternum, bone marrow, eyes, optic nerves, trachea, tongue, esophagus, pancreas, mesenteric and submandibular lymph nodes, submandibular glands, sublingual glands, parotid glands, urinary bladder, thyroid, parathyroids, pituitary, aorta, skin, mammary gland, spinal cord, Harderian glands, testes, epididymides, prostate, seminal vesicles, ovary, uterus, vagina, administration site, sciatic nerve, and larynx.
Quality assessment of HE sections was conducted in all of the organs mentioned above for stainability (hematoxylin, eosin, integrated color, and unevenness) and morphology (thickness, deteriorations, and wrinkles). In addition, the reproducibility of microscopic findings that were described in the original final reports was evaluated, for which liver and kidney tissues were used because they are major target organs in toxicity studies. Morphology and reproducibility were evaluated by comparing with the original slides (HE sections prepared 30 years before).

No significant problems were encountered during tissue processing; however, there were a number of small difficulties. For example, paraffin sections were easily damaged by the microtome blade during tissue processing. It was particularly difficult to section the lens from the eye because the slice easily broke down.

Stainability and morphology of HE sections were evaluated in all organs. Stainability of hematoxylin was slightly weaker in some tissues (Fig. 1A), the cause of which is unknown. Weaker stainability of hematoxylin is also reported in HE sections prepared from long-term stored formalin-fixed wet tissues; the cause in this case is presumed to be...
the occurrence of hydrolysis of hematoxylin-positive materials such as DNA with the decrease in pH of the formalin solution, which resulted from oxidation of formaldehyde to formic acid

Morphological artifacts observed in organs and tissues are shown in Table 1. Cracks were observed in several organ sections and were somewhat severe in brain sections (Fig. 1B). This phenomenon in the brain was improved by changing the temperature during the section drying process; while a temperature of 40°C caused several cracks, room temperature caused fewer cracks. Dilatation of spaces (Fig. 1C, 1D) was also observed in the heart, femoral muscle, testis, and epididymis. Spaces among the bundle of muscle fibers or ductal component were expanded in the re-prepared sections compared with the original ones, but the degree was slight in all the above organs. A vacuole-like artifact was frequently seen in the renal tubular epithelium (Fig. 1E, 1F). The causes of these morphological artifacts were unidentified. None of these artifacts were observed in HE sections of formalin-fixed tissues stored over a long period

Overall, we concluded that some types of deterioration in the quality of HE sections mentioned above were considered to be permissible because histopathological examination was performed in comparison with controls. Most of the original microscopic findings were confirmed in re-prepared HE sections, while some focal findings that were judged to be either incidental or spontaneous in nature were not observed in these sections. Therefore, we concluded that the results of the original histopathological examinations could be reconstructed using paraffin blocks that had been stored for up to 30 years.

Table 1. Organs and Tissues with Morphological Artifacts

| Morphological artifacts | Organs and tissues                                      |
|-------------------------|--------------------------------------------------------|
| Crack                   | Brain, liver, thymus, lymph node, eye (lens), prostate |
| Dilatation of space     | Heart, skeletal muscle, testis, epididymis             |
| Vacuole-like artifact   | Kidney                                                 |

In summary, we evaluated the quality of long-term stored FFPE tissues and reproducibility of microscopic findings using re-prepared HE sections. Some types of deterioration in the quality of HE sections were considered to be permissible because histopathological examination was performed in comparison with controls. Most of the original microscopic findings were confirmed in re-prepared HE sections, while some focal findings that were judged to be either incidental or spontaneous in nature were not observed in these sections. Therefore, we concluded that the results of the original histopathological examinations could be reconstructed using paraffin blocks that had been stored for up to 30 years.

Disclosure of Potential Conflicts of Interest: All authors are employees of Mitsubishi Tanabe Pharma Corporation. The authors declare that they have no conflicts of interest.
Acknowledgment: The authors thank Mr. Yoshifumi Uno for helpful discussions.

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Fig. 2. Reproduced microscopic findings in the kidney. Chronic nephropathy in the original section (A) and in a re-prepared section (B). Eosinophilic inclusion bodies in the proximal tubular epithelium in the original section (C) and in a re-prepared section (D). Hematoxylin and eosin staining. Bar = 100 μm (A, B) and 25 μm (C, D).