Long-Term Tissue Preservation

Comparative study of slide quality across decades in formalin-fixed tissues, paraffin blocks, and stained-glass slides

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Introduction

Formalin-fixed tissues, formalin-fixed paraffin-embedded (FFPE) blocks, and glass slides are widely used for long-term sample preservation in pathology. Regulatory authorities and Good Laboratory Practice (GLP) guidelines require that such materials be archived for extended periods, to allow retrospective evaluation if needed. Despite their routine use, there is limited published evidence on the effects of prolonged storage on the histological quality of tissues in preclinical studies. Scientific literature on tissue preservation in formalin, paraffin blocks or slides beyond five years is particularly scarce (Likhithaswamy et al., 2022; Ono et al., 2018).

The aim of this study was to microscopically assess tissue quality and morphological changes in samples stored for different durations (1.5 to over 30 years), comparing three preservation formats: **formalin-fixed tissues, FFPE blocks, and H&E-stained slides**, to determine their suitability for histopathological evaluation.

Material and Methods

Formalin-stored tissues, FFPE blocks, and glass slides from **rodents** (Wistar and Sprague Dawley rats) and **non-rodents** (Cynomolgus macaques and Beagle dogs) were retrieved from the AnaPath Research S.A.U. archive. The samples had been stored at room temperature under controlled conditions from **1.5 to >30 years.** Studies were anonymized as shown in Table 1. One control male and one control female per group were selected.

Formalin-stored tissues were trimmed and embedded in paraffin. Newly prepared and archived paraffin blocks were sectioned (2–4 µm) and stained with hematoxylin and eosin (H&E). See Table 2. Evaluated organs included **liver, spleen, heart, lung, stomach, ileum, brain, and spinal cord**. Two pathologists examined both newly stained and original slides, and findings were recorded using consensus terminology and severity grading.

Table 1. Tissues retrieved from the archive

Animal Species	Group ID	Years post-sampling
Rat	Rod-A	1.5-2
	Rod-B	6-7
	Rod-C	10-11
	Rod-D	12-13
	Rod-E	>30
NHP	NonR-A	1.5-2
	NonR-B	6-7
	NonR-C	10-11
	NonR-D	15-16
Dog	NonR-E	>30

Rod: Rodents, Non-R: Non-rodents

Table 2. Workflow of tissue processing and evaluation

Source of Tissues	Trimming, Embedding & Blocks	Cutting & Staining	Evaluation
Archived FF tissues (Remnants after trimming)	✓	✓	✓
Archived FFPE blocks (prepared after necropsy)	-	✓	✓
Archived glass slides (prepared after necropsy)	-	-	✓

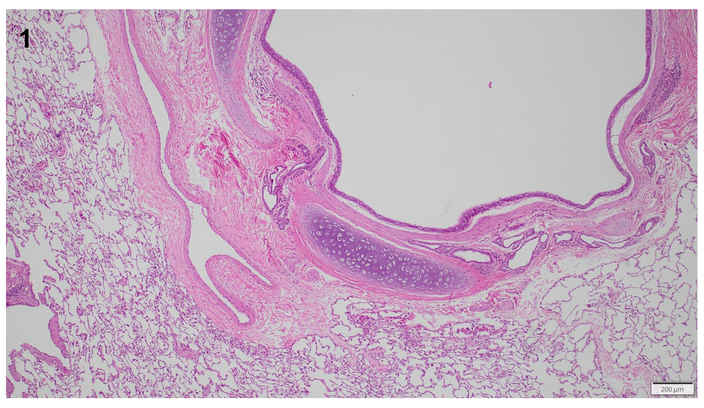
Results

1) Macroscopically, formalin-stored samples presented a **pale-yellow** discoloration, most evident in parenchymal organs (e.g., liver) and intensifying with longer storage. Prolonged exposure also made tissues **harder** in consistency and/or more **friable.** Despite these changes, all formalin-stored tissues remained **suitable for histological processing**. No differences were noted during embedding, block formation or microtome cutting in any of the samples evaluated.

2) Histologically, tissue integrity was preserved across all storage methods; however, several artifacts were observed, increasing with storage duration, particularly in **formalin-fixed tissues beyond 10–13 years**, with organ-dependent differences.

2.A: Staining quality:

- ✓ Minimal to slight reduction in H&E staining affinity, resulting in pale nuclei and decreased cytoplasmic contrast, became apparent in formalin-fixed and FFPE tissues after 10 to 13 years.
- ✓ The loss of basophilia, characterized by diminished nuclear chromatin detail and weaker staining of RNA-rich cytoplasm, was evaluated separately and was reported from 6 years onward in formalin-stored tissues, with milder intensity in FFPE blocks.
- ✓ Changes in staining quality are likely due to acid-induced DNA and RNA degradation.



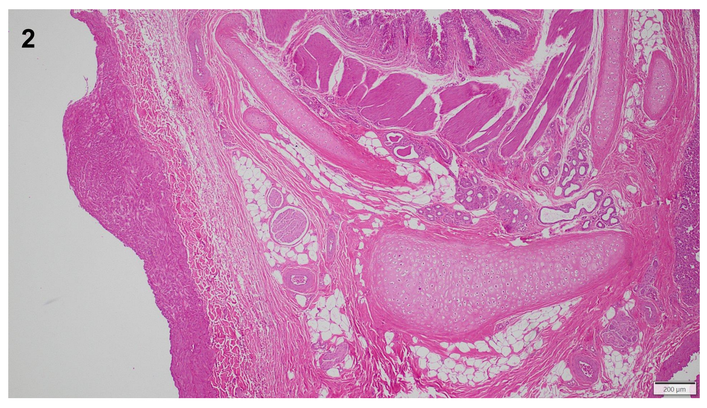
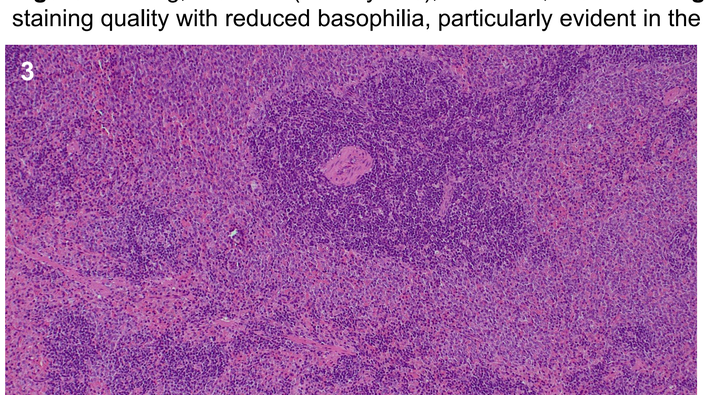


Figure 1. Lung, NonR-A (1.5-2 years), Formalin, H&E. 4x. **Figure 2**. Lung, NonR-E (>30 years), Formalin, H&E. 4x. Loss of staining quality with reduced basophilia, particularly evident in the bronchial cartilage.



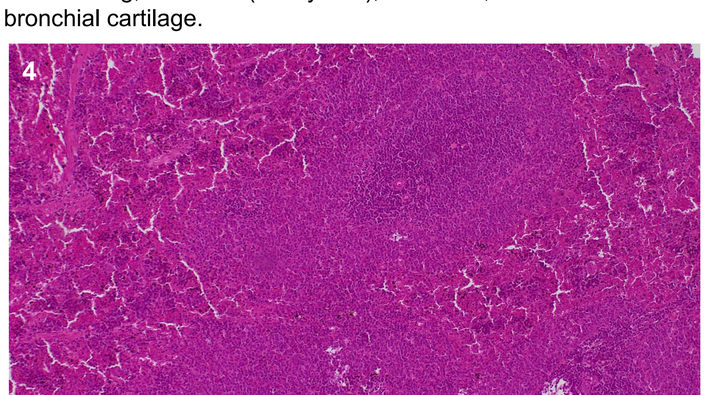


Figure 3. Spleen, Rod-A (1.5-2 years), Formalin, H&E. 10x. **Figure 4**. Spleen, Rod-E (>30 years), Formalin, H&E. 10x. Loss of staining quality and notably decreased basophilia accompanied by deterioration of tissue morphology.

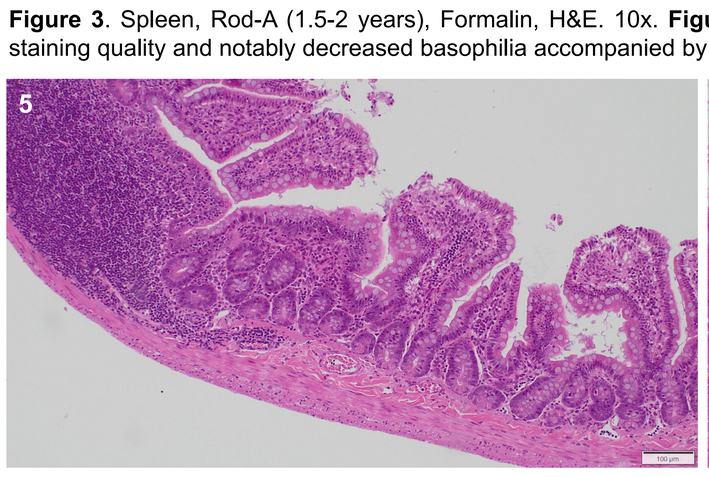


Figure 5. Small intestine, Rod-A (1.5-2 years), Formalin, H&E. 10x. **Figure 6**. Small intestine, Rod-E (>30 years), Formalin, H&E. 10x. Loss of staining quality and notably decreased basophilia accompanied by deterioration of tissue morphology.

2.B: Morphological integrity:

- ✓ Tissue architecture was generally well preserved across all formats. In formalin-fixed samples stored for more than 30 years, minimal to slight shrinkage, fragmentation, or distortion of cellular structures were observed.
- ✓ This change was less evident in the central nervous system compared to other organs.

2.C: Crystal-like structures:

- ✓ Refractile, vacuole-like deposits were observed in the brain, spinal cord, liver, spleen, and heart, with the greatest severity noted in the white matter of the spinal cord.
- ✓ These artifacts emerged as early as 1.5 to 2 years in formalin-fixed samples and became more pronounced after 10 years. They were absent in FFPE blocks and slide stored tissues.

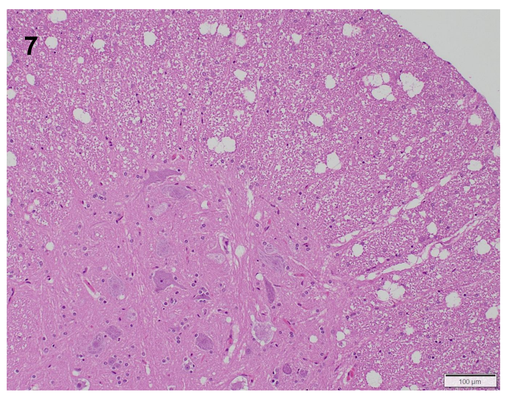


Figure 7. Spinal cord, Rod-A (1.5-2 years), Formalin, H&E. 10x. Multifocal crystal-like structures in the white matter.

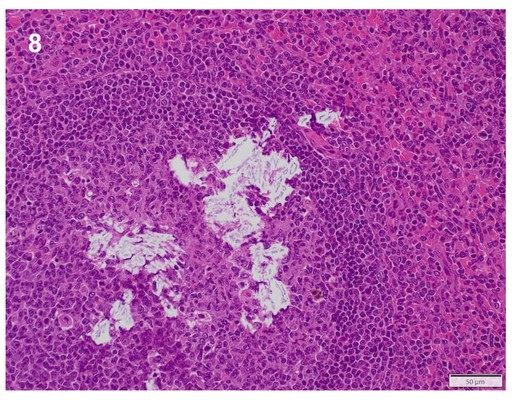


Figure 8. Spleen, NonR-C (10-11 years), Formalin, H&E. 20x. Multifocal crystal-like structures in the germinal center.

2.D: Formalin pigment:

- ✓ Low amounts of brown-black birefringent deposits were primarily observed in blood-rich organs, notably the liver and spleen, and were more pronounced in formalin-fixed samples stored for more than 30 years (minimal to moderate). Minimal pigment was occasionally observed in the spleen after 6 to 7 years.
- ✓ These deposits were typically located in the vascular endothelium or lumen.
- ✓ They resulted from an acid-mediated reaction of heme with formaldehyde.

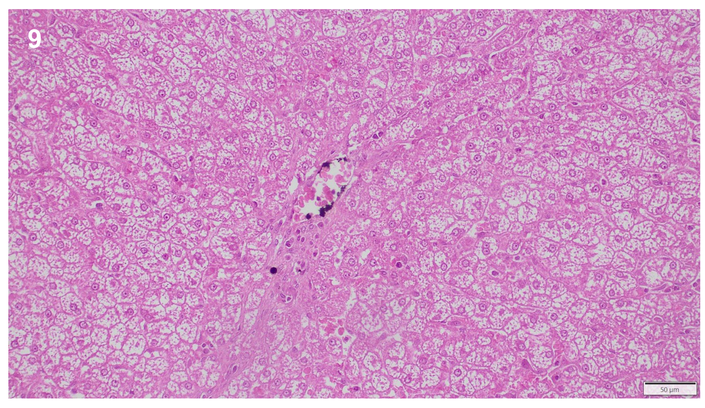


Figure 9. Liver, NonR-E (>30 years), Formalin, H&E. 20x. Loss of staining quality, reduced basophilic staining, and decreased morphological detail. Note the presence of formalin pigment localized within the vascular endothelium.

2.E: Organ-specific changes:

- ✓ Keratin lamination and perinuclear vacuolation in rodent non-glandular stomach (Formalin >12 years, FFPE >30 years).
- ✓ Cardiac fiber separation in both formalin and FFPE stored tissues.
- ✓ Background findings (e.g., inflammatory infiltrates) were used as quality controls to confirm that accurate diagnoses can still be established long after tissue sampling. These findings were well preserved across all formats.

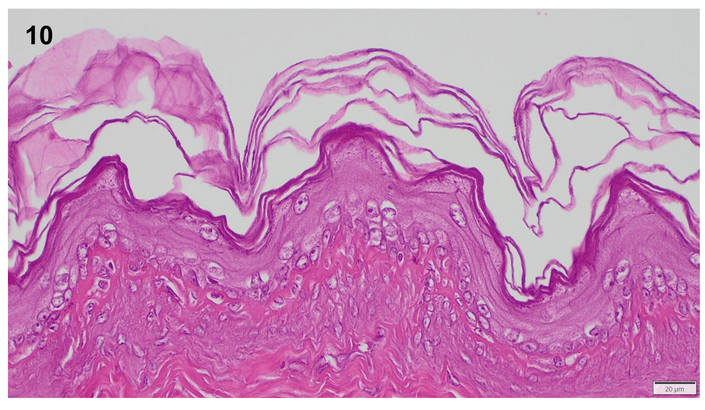


Figure 10. Non-glandular stomach, Rod-E (>30 years), formalin-fixed, H&E. 40x. Marked loss of basophilia with pale nuclear staining and reduced chromatin detail. Keratinocytes display perinuclear vacuolation, and the keratin layer shows artifactual separation.

Conclusion

- This study provides evidence that, under appropriate archival conditions, tissues from preclinical studies can be reliably **evaluated even after more than 30 years** of storage as formalin-fixed samples, FFPE blocks, or mounted slides, without loss of essential histological information.
- Preservation quality **depends on method, storage time, and organ type**.
- Mounted slides showed the best preservation, followed by FFPE blocks.
- **Formalin-fixed tissues were the most prone to artifacts** (crystal formation, pigment deposition, and staining loss), primarily due to fixative acidification over time.
- **Well-maintained archives** remain a valuable resource for research and regulatory studies.
- Pathologists should be aware of storage-induced artifacts and consider them during the evaluation of long-stored material to minimize the risk of **misinterpretation** or missed diagnoses.

Likhithaswamy HR, Madhushankari GS, Selvamani M, Mohan Kumar KP, Kokila G, Mahalakshmi S. Assessing the quality of long-term stored tissues in formalin and in paraffin-embedded blocks for histopathological analysis. J Microsc Ultrastruct. 2022;10(1):23–29. doi:10.4103/JMAU.JMAU_53_20

Okazaki K, Onozawa M, Itō T.長期保存したホルマリン固定標本の品質に関する検討 “A study on the quality of long-term preserved formalin-fixed specimens” [in Japanese]. Jpn J Histotech. 2002;11:25–31

Ono Y, Sato H, Miyazaki T, Fujiki K, Kume E, Tanaka M. Quality assessment of long-term stored formalin-fixed paraffin-embedded tissues for histopathological evaluation. J Toxicol Pathol. 2018;31(1):61–64. doi:10.1293/tox.2017-0046