



## Publication

### Residual Humidity in Paraffin-Embedded Tissue Reduces Nucleic Acid Stability

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Researchers from BBMRI.at partner Med Uni Graz, who also have contributed the development of pre-analytical quality standards for FFPE tissue for DNA analyses, investigated the effect of residual humidity in paraffin-embedded tissue on RNA and DNA quality. Residual water had a negative effects of residual water on nucleic acid stability and contributed to reduced qRT-PCR quality.

Besides the duration of ischemia and fixation type, nucleic acid quality depends on a variety of other pre-analytical parameters, such as storage conditions and duration. water content within tissue samples is also among the potential pre-analytical variable.

The amount of residual water in paraffin-embedded tissue depended on the fixative type and the dehydration protocol and to some extend on the storage condition and duration.

Residual water negatively affected nucleic acid quality due to hydrolysis of nucleic acids and became visible as reduced qRT-PCR performance.

The experts conclude that proper dehydration and dry storage are essential to minimize degradative influence of residual water on nucleic acids and to improving the shelf life of fixed paraffin-embedded tissue.

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Article  
**Residual Humidity in Paraffin-Embedded Tissue Reduces Nucleic Acid Stability**

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**Abstract:** Molecular diagnostics in healthcare relies increasingly on genomic and transcriptomic methodologies and requires appropriate tissue specimens from which nucleic acids (NA) of sufficiently high quality can be obtained. Besides the duration of ischemia and fixation type, NA quality depends on a variety of other pre-analytical parameters, such as storage conditions and duration. It has been discussed that the improper dehydration of tissue during processing influences the quality of NAs and the shelf life of fixed tissue. Here, we report on establishing a method for determining the amount of residual water in fixed, paraffin-embedded tissue (fixed by neutral buffered formalin or a non-crosslinking fixative) and its correlation to the performance of NAs in quantitative real-time polymerase chain reaction (qRT-PCR) analyses. The amount of residual water depended primarily on the fixative type and the dehydration protocol and, to a lesser extent, on storage conditions and time. Moreover, we found that these parameters were associated with the qRT-PCR performance of extracted NAs. Besides the cross-linking of NAs and the modification of nucleobases by formalin, the hydrolysis of NAs by residual water was found to contribute to reduced qRT-PCR performance. The negative effects of residual water on NA stability are not only important for the design and interpretation of research but must also be taken into account in clinical diagnostics where the re-analysis of archived tissue from a primary tumor may be required (e.g., after disease recurrence). We conclude that improving the shelf life of fixed tissue requires meticulous dehydration and dry storage to minimize the degradative influence of residual water on NAs.

**Keywords:** fixed tissue; nucleic acid quality; next-generation sequencing

**1. Introduction**  
Diagnostics in healthcare increasingly relies on the detailed molecular analyses of alterations of the genome and transcriptome using tissue specimens harvested during surgery or biopsy. Such analyses have become invaluable for diagnosis, therapy selection, and eventually personalized patient treatment. Quantitative real-time polymerase chain reaction (qRT-PCR)-based molecular diagnostics and gene-panel-based Next Generation sequencing (NGS) are readily established in cancer diagnostics, and due to the lowering of costs, whole-genome and whole-exome sequencing are increasingly used not only for research but also for diagnostics, which, however, places increasing demands on sample quality. Tissue used for diagnostic purposes is usually formalin-fixed, paraffin-embedded (FFPE), where the fixative is 10% neutral buffered formalin (NBF, 4% formaldehyde) [1], in an aqueous phosphate-buffered solution. This material is widely available and has been

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