Talk Abstract

qPCR Symposium 2011
Prague, Czech Republic, June 13-17, 2011

The Impact of Tissue Pre-Analytics on Molecular Analyses

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Molecular characterisation of human disease requires analysis of multiple parameters ranging from classical histopathological features to a broad spectrum of molecular biomarkers. The morphological characterisation is routinely based on the analysis of formaldehyde-fixed and paraffin-embedded (FFPE) tissues, but it is known that formalin fixation impairs molecular analyses which typically require frozen tissue samples. Within the EU FP7 project SPIDIA, we evaluated a new technology for combined tissue diagnostics as well as the impact of several pre-analytical variables on tissue sample quality and subsequent molecular analyses. Therefore, FFPE samples were compared to formalin-free fixatives including a novel fixative for simultaneous preservation of morphology and biomolecules, the PAXgene Tissue System, and corresponding snap-frozen tissue samples served as reference. Comparative studies of nucleic acids preservation were performed with a focus on RNA quality using electropherograms, spectroscopy and qPCR based assays.
Results demonstrated that established methods for quality control of RNA (e.g. 28s:18s ratio, RIN value) are well-suited for frozen tissue but do not readily correlate with PCR amplification efficacy of RNA isolated from paraffin-embedded tissues. In these samples a more detailed analysis, like a qPCR assay based on different amplicon length we have developed, is needed to estimate suitability of RNA for downstream applications. Furthermore, we could demonstrate that results of qPCR were sensitive to degradation of RNA introduced by pre-analytical procedures such as time or type of fixation and storage. Gene signature arrays for 92 cancer-pathway associated genes revealed major gene-to-gene variations between FFPE and freshly frozen tissues whereas PAXgene-fixed and paraffin-embedded (PFPE) tissues showed a strong correlation.
In conclusion, pre-analytical parameters have a major impact on molecular tissue diagnostics and assay results. Consequently, the quality of tissue-based molecular analyses can only be defined in the context of the pre-analytical procedures. The excellent preservation of biomolecules in PFPE samples provides new opportunities for comprehensive tissue diagnostics, biomarker discovery and reliable molecular analyses whenever a collection of snap-frozen material is impossible.