G12 Usability of RNA Purified From Forensic Pathology Tissue Samples for Molecular Investigations: The Effect of Tissue Decay on RNA Quality

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After attending this presentation, attendees will learn more about the quality and integrity of RNA extracted from human tissue samples with variable degree of tissue decomposition, and its usability for molecular research. Knowledge about the postmortem stability of RNA and usability of RNA, purified from forensic samples with variable degree of tissue decomposition, can contribute to resolve numerous causes of unexpected deaths. The molecular RNA investigations, such as gene expression analyses, can be a usable supplement to routinely perform histopathological and toxicological investigations.

This presentation will impact the forensic science community by improving the knowledge about the influence of postmortem degradation of RNA due to decomposition of tissue on the results of molecular analyses based on Polymerase Chain Reaction (PCR) in order to support the growing interest in applying molecular analysis in forensic pathology.

RNA is generally considered to be more unstable than DNA, and its usability in PCR-based analysis is more challenged. The goal of this study is to perform a systematic investigation of how body decomposition affects the quality and usability of RNA extracted from muscle tissue samples taken at forensic autopsies and from the corresponding samples of muscle undergoing the standard procedure of fixation in formaldehyde and embedding in paraffin (FFPE).

The degree of decomposition was based on postmortem interval in bodies stored under similar conditions. In order to study the influence of storing condition at low temperature, a group of bodies which were found in water, which is normally cold in Denmark, were identified.

Using the internal autopsy database; random samples of 40 bodies of adults who were found inside at room temperature and had different degrees of body decomposition ranging from no sign of body decomposition to severe body decomposition with mummification and sceletation prior to autopsy, were identified. Moreover, nine bodies were identified that had been missing at least two weeks and were found in the water. The cases were routinely autopsied at the Department of Forensic Medicine University of Aarhus between 2009 – 2011. The study samples were stratified into five groups according to postmortem interval and storing condition.

RNA from unfixed muscle samples was isolated by organic extraction and isopropanol precipitation. RNA from FFPE muscle was isolated using the commercially available kit (Roch). Complementary DNA (cDNA) was synthesized by using the RT-for-PCR kit cDNA synthesis kit with the use of random hexamer primers. The real time PCR assay targeted an 86 basepair amplicon of the human ACADM gene. The thermal cycling consisted of 15 min at 95°C followed by 50 cycles of 30 s at 95°C and 1 min at 60°C with collection of fluorescent data at 60°C.

It was found that the RNA from unfixed samples yield, quality, and ability to support RT-PCR amplification though decreasing with increasing decay of the source tissue. Interestingly, it was found that the ACADM transcript was detectable in almost all samples with the least decay (until one week after death). In contrast, RT-PCR amplification of the ACADM transcript was only possible for one sample with the high degree of decay with a quantification cycle of 38 (indicating low abundance). Furthermore, the ACADM transcript was amplifiable in all RNA samples from tissue derived from bodies found in water, thus, supporting that RNA molecules are well-preserved within water-immersed bodies. The only RNA, purified from FFPE muscle samples taken from bodies with no decay (one to three days after death), were able to support RT-PCR amplification of the ACADM transcript.

The data supports that the standard FFPE procedure increases degradation of RNA. RNA transcripts from unfixed muscle samples often were detectable in muscle tissue up to one week postmortem that may stimulate further RNA-based molecular analysis in forensic science.

Forensic Pathology, RNA Recovery, Autopsy

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