



G30 DNA Extraction From Paraffin Blocks: Organ Selection and Pre-Embedding Fixation Times – Practical Implications for Forensic Pathologists

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After attending this presentation, attendees will gain an understanding of which organs or tissues are optimal for paraffin archival for the purposes of subsequent DNA extraction; attendees will recognize the importance of the length of formalin fixation prior to paraffin embedding.

This presentation will impact the forensic science community by helping attendees to be able to direct histologic sampling to one or two tissue types (in decomposed and non-decomposed bodies) for the purposes of DNA sampling. Attendees will understand the critical time parameter for pre-embedding formalin fixation.

Synopsis: In recent years re-examination of old death investigation cases in the form of prosecutorial post-conviction review, defense review (prompted by the Innocence Project and others), law enforcement cold case initiatives (often with federal grant support), and ancestry inquiries is increasingly frequent. These reviews are aided and sometimes initiated by the existence of advanced DNA technology that may not have existed until after conclusion of the original investigation. Therefore the ability to obtain usable DNA evidence from items stored for extended periods is increasingly important. While standard forensic practices vary with regard to storage times for various pathology specimens (formalin fixed wet tissues in particular), forensic centers and hospital pathology services often retain paraffin embedded tissue blocks indefinitely; current National Association of Medical Examiners (NAME) accreditation guidelines require a retention time of at least 10 years. Consequently, paraffin blocks are an available resource for stored DNA evidence. However, forensic pathology practice varies regarding number of organs sampled for paraffin preservation, and even whether or not sections are taken in all types of cases; NAME accreditation guidelines allow discretion as to types of cases in which slides are produced but do recommend a one-year retention of paraffin embedded tissues when slides are not produced. To address the question of which tissue would be optimal for DNA recovery using modern methods, these experiments were designed to determine whether decomposition changes that answer, and whether the length of pre-embedding fixation is a critical variable.

Tissue obtained at autopsy was divided into three groups based on formalin fixation times of 1, 5, and 12 days prior to dehydration and paraffin embedding. Sections of each organ were deparaffinized with heat and DNA was purified from the residual tissue via organic extraction. Subsequent purification was accomplished by one of several different methods. Extracted DNA was quantified then amplified using a PCR amplification kit and separated by 3130xl capillary electrophoresis. The electropherograms were analyzed for DNA profiles.

Tissues compared were heart muscle, skeletal muscle (psoas), liver, spleen, and brain. Cases included both non-decomposed and decomposed bodies. Preliminary results for quantitative DNA recovery indicate that spleen is superior to liver, heart or skeletal muscle. A striking loss of recoverable DNA is observed between pre-embedding fixation times of 5 to 12 days. The loss of DNA with increasing fixation times is maximum in spleen and skeletal muscle followed by liver and heart, but the differences between organs are small. Studies are ongoing, but preliminary data suggest that although quantitative DNA recovery is greatest from spleen, the degree of degradation may also be greatest with spleen. Studies comparing brain to the other tissue types and the effects of decomposition are ongoing.

Impact: This project was undertaken to better define which tissue types are the best for extraction of DNA from paraffin blocks using modern DNA technology. With this knowledge forensic pathologists will be able to selectively sample organs in order to efficiently preserve DNA evidence while minimizing the expense of embedding multiple tissues and organs from all cases.

DNA Extraction, Paraffin, Formalin Fixation Time