A50 DNA Preservation in Partially Decomposed Soft Muscle Tissue Samples Using Different Preservative Solutions

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After attending this presentation, attendees will better understand how to choose different preservative solutions for better preservation of DNA in partially decomposed soft muscle tissues under field conditions where no cold storage is available.

This presentation will impact the forensic science community by providing new ideas to preserve DNA under field conditions for successful profiling. The solutions mentioned in presentation are easy to handle under field conditions, and DNA can be preserved for a period of six months successfully at room temperature using these preservatives.

Following mass fatality incidents, DNA profiling is essential for identification and reassociation of fragmented, burnt, or decomposed corpses that would be very difficult or impossible by traditional means such as fingerprinting and odontology. However, successful DNA recovery depends on the collection and preservation of biological material obtained from deceased individuals and the availability of reference samples (Graham et al., 2008).[1] Inefficient preservation methods can cause destruction of intact DNA to such an extent that data is not always available for victim identification (Bing and Bieber, 2001).[2]

In order to assess the efficiency of different preservative solutions (10% buffered formalin, 96% ethanol and cell lysis solution - with and without sodium azide), partially decomposed soft muscle tissue samples were collected at different accumulated degree-days (ADD) from pig (0, 70 and 150 ADD) and rabbit (0 and 70 ADD) carcasses and were placed in preservative solutions.

DNA extraction was performed using DNeasy® Blood and Tissue kit according to the manufacturer's instructions at different time points (1 month and 6 months). DNA quantification was performed using agarose gel electrophoresis (2%) and Quant-iT™ PicoGreen® dsDNA Reagent from Invitrogen™, UK. DNA fragment analysis was performed using Applied Biosystems™ 310 and 3500 genetic analyzers.

In order to perform DNA fragment analysis from postmortem soft muscle tissue samples of the model organisms chosen, two nuclear genes, Connexin 43 and RAG-1, were aligned to identify conserved regions. Primers were designed to amplify 70 bp, 194 bp, 305 bp, and 384 bp amplicons. The primers were also designed to amplify human DNA which allowed the use of commercially purchased DNA standards to be used as controls. Following DNA extraction, PCR analysis was performed using the four primers sets in a multiplex (4-plex) and was optimized so that it worked over a wide range of template amounts (0.1ng to 75.83ng). The multiplex (4-plex) PCR was found to work efficiently in triplicate samples of all three species down to 0.3ng of DNA template.

The results showed that the 96% ethanol and cell lysis solution, with and without 1% sodium azide, are better solutions for DNA preservation in both fresh (0 ADD) and partially decomposed tissues (0, 70 and 150 ADD) for a period of six months, whereas 10% buffered formalin is a poor source of DNA preservation causing high DNA degradation.

Future work will include: DNA extraction from preserved samples after one year, development of real-time PCR quantification assays, DNA fragment analysis using ABI 310 and 3500 genetic analysers, and analysis of results using appropriate methods such as ANOVA, ANCOVA, and regression analysis.

References:

1. Graham, EAM, Turk, EE and Rutty GN. Room temperature DNA preservation of soft tissue for rapid DNA extraction: An addition to the disaster victim identification investigators toolkit? *Forensic Science International: Genetics,* 2**,** 29-34.
2. Bing, DH and Bieber, FR. (2001). Collecting and Handling Samples for Parentage and Forensics DNA-Based Genetic Testing, *John Wiley & Sons*, Inc.

ADD, RAG-1, Multiplex (4-plex)

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