G64 Simple Tissue Preservation Methods That Result in Reliable DNA Analyses

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The goal of this presentation is to inform attendees of several simple tissue preservation methods that are conducive to obtaining a quality DNA sample. The procedures outlined can be applied to many situations, since the methods examined were developed for use in situations where time, materials, and facilities are limited.

This presentation will impact the forensic community and/or humanity by demonstrating providing valuable information on how to successfully preserve tissue samples for subsequent DNA analysis. The methods examined for this study required minimal materials, storage space, and temperature considerations. For these reasons, the results of this research can be useful to many factions of the forensic community, from mass disaster response teams, to conservation officers, to crime scene technicians. By having a simple tissue preservation method available in the field, samples can be preserved immediately, which increases the potential for a successful DNA analysis in the laboratory.

This presentation provides an evaluation of on-site tissue preservation methods, examining the success of each in yielding high quality DNA. The research examined factors such as availability and portability of materials, tissue storage life at room temperature, ease of use, ease of subsequent DNA extraction, and the quantity and quality of DNA obtained from preserved samples. Attendees will gain an understanding of the range of tissue preservation methods available, as well as the efficacy of each method in preserving DNA. The goal of this study was to develop a rapid, reliable method for storing tissue samples that can be easily employed in the field.

In the event of a mass disaster, where a large number of victims must be located and identified, it becomes difficult to process the site in a timely and orderly manner. Due to extensive injury or decomposition, many victims may only be identified through DNA analysis; therefore, obtaining viable tissue samples is of great importance. Amidst the rush of locating survivors, making anthropological identifications, and gathering other information about the victims of the disaster, tissue collection for subsequent DNA testing is often delayed. Likewise, tissue preservation of remains discovered in very remote areas can also be hindered. The goal of this study was to examine protocols for on-site tissue preservation that would undergo later DNA analysis. Through development of simple, portable, and readily available methods for preserving tissues in the field, robust DNA results are more likely to be obtained.

Testing was conducted on tissues taken from recently killed pig carcasses that had been placed in a field during the summer; samples were collected regularly over a one-month period. Six preservation methods were evaluated: storage of tissue in ethanol, isopropanol, RNAlater (Ambion, Inc.), and silica desiccant, as well as hot air drying and freezer storage. Muscle, skin, and brain samples were collected in triplicate from each animal, and ca. 0.25 g placed in each storage medium. DNA extractions were performed after two weeks and after three months for each storage method. DNA quality and quantity were assessed using quantitative PCR of three species-specific single-copy nuclear genes. Results were analyzed in order to determine which preservation methods were the most successful in yielding a viable DNA sample after a period of storage.

Although DNA quantity and quality were the most significant factors in the evaluation, many other issues were addressed. Tissue type and level of decomposition, portability of materials, toxicity of the preservative, shelf life of preserved samples, and ease of subsequent DNA extraction were also factored into the analysis. By considering all of these interdependent variables, an optimized, reliable procedure for preserving tissue samples when adequate storage and DNA processing facilities are not readily available can be developed and implemented.

DNA Extraction, Mass Disaster, Tissue Preservation

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