B73 Room Temperature DNA Preservation and Rapid Purification of Decomposing Human Tissue Samples: An Alternative DVI Approach

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After attending this presentation, attendees will be provided with information regarding the efficiency of various solutions to preserve DNA in decomposing skin and muscle samples when stored in tropical ambient temperatures for up to three months. In addition to the benefits of storing samples at room temperature, this presentation will also describe the results of combining these preservatives with various purification methods in order to more rapidly process a high volume of tissue samples for DNA identification.

This presentation will impact the forensic science community by examining how these technologies may have an important impact in the field of forensic genetics and for Disaster Victim Identification (DVI) protocols regarding DNA identification in particular. The application of enhanced liquid tissue preservatives coupled with a reduced DNA extraction procedure may improve the sample processing throughput during a mass fatality incident. Therefore, the implementation of these techniques in future mass disaster responses may help preserve the quality of DNA and improve Short Tandem Repeat (STR) results from decomposing human tissues by improving sample preservation methods and providing a more direct and streamlined high-throughput strategy for processing those samples.

After a mass fatality incident, adequate freezer facilities to house victims may not be available and, therefore, rapid decomposition of bodies will be seen in hostile climates. In order to minimize the DNA degradation, and maximize throughput of samples for identification, an ideal preservative would protect the DNA within tissues while also leaching DNA into the surrounding solution. By maximizing the quantity and quality of "free" DNA in the preservative solution, the time-consuming steps of tissue digestion and DNA extraction may be eliminated.

Skin and muscle samples were harvested over a two-week time period in early fall from three human cadavers placed in an open field at the Southeast Texas Applied Forensic Science facility in Huntsville, TX. Five liquid preservatives (LST, DESS, modified TENT, DNAgard® Tissue, and RNA*later*®) were evaluated at 35°C and 60% to 70% humidity for up to three months of storage. DNA was extracted from tissues using the QIAamp® DNA Investigator kit on the QIAcube® platform. The efficiency of isolating the "free" DNA directly from the liquid preservative was tested using the QIAamp® DNA Investigator kit, QIAquick® PCR purification kit, modified ethanol precipitation followed by Microcon® filtration, Agencourt® AMPure® XP PCR Purification system, and the Fingerprint DNA Finder (FDF) kit.

All solutions except the LST buffer adequately preserved the DNA in fresh and decomposed skin and muscle. RNA*later*® consistently generated the highest DNA yields (up to 250ng/µL); however, DNAgard® and the modified TENT buffer were the only two preservatives which consistently leached high amounts (0.06-40ng/µL) of good quality DNA into solution for DNA isolation and successful genotyping using the AmpFlSTR® IdentiFiler® Plus STR Amplification kit.

The QIAquick® PCR purification kit was the best method tested. It isolated adequate amounts of DNA (0.5-4ng/µL) from all tissues to produce complete profiles in less than 25 minutes. Results suggest that DNAgard® and the modified TENT buffer are better at leaching and simultaneously protecting the "free" DNA in solution than the other methods tested. Therefore, extracting DNA directly from the DNAgard® or the modified TENT buffer preservative using the QIAquick® purification kit may be the best options for room-temperature storage and rapid sample processing in cases of mass disasters.

DNA Preservation, DNA Extraction, DVI