Improved Preservation and Purification Methods for DNA in Decomposing Human Tissue Samples; A DVI Application

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INTRODUCTION

One of the most important considerations after a mass disaster is victim identification. In these circumstances forensic personnel may be faced with the task of identifying hundreds or even thousands of human remains.

Adequate freezer facilities to house the victims may not exist and therefore bodies decompose rapidly in hot and humid conditions. Harsh environmental conditions degrade DNA in tissues, making identification more difficult.

To minimize DNA degradation without refrigeration, one possible solution may be tissues preservatives. An ideal liquid preservative would leach DNA into the surrounding solution whilst also protecting that DNA from degradation. By maximizing the quantity and quality of 'free' DNA in solution, not only could samples be stored adequately at ambient temperatures, but the time consuming steps of tissue digestion and DNA extraction may be eliminated, allowing for higher throughput of samples for identification.

Several studies [1-3] have investigated the utility of room temperature storage of tissues for DNA analysis, but few have focused on assessing the quantity and quality of the DNA which leaches into solution in decomposing tissues.

The objectives of this study were to:

1. Determine which preservatives will release the most good quality DNA into solution for rapid purification.

2. Assess which preservatives would best protect that 'free' DNA from further degradation.

MATERIALS AND METHODS

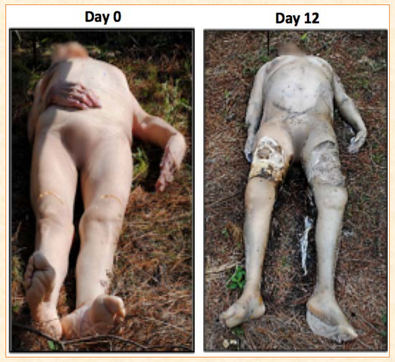


Fig 1 – Cadaver at day 0 and day 12 of decomposition

Muscle and skin samples (N=21 each) were taken from the thigh of a human cadaver placed outdoors in October, 2013 at the Southeast Texas Applied Forensic Science (STAFS) Facility at Sam Houston State University, Huntsville, TX (Fig 1). Samples were taken every second day for up to two weeks.

Tissues samples (30mg) were placed in 300µL of one of five liquid preservatives:

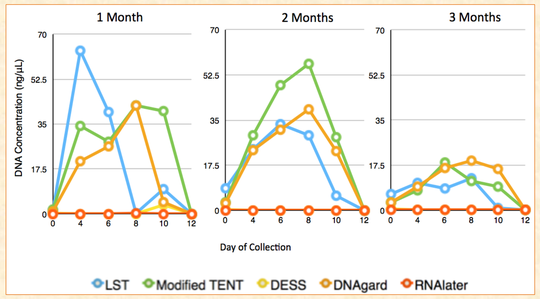
Preservatives	Constituents
DNAgard® Tissue	Proprietary (Biomatrica®)
RNA*later*	Proprietary (Qiagen/Life Technologies/Sigma-Aldrich)
LST Buffer	0.1M Tris-HCl, 0.5M KCl, 4.5% IGEPAL CA-630, 4.5% Tween 20, 1% NaN_3 (pH 7.5)
Modified TENT	10mM Tris, 10mM EDTA, 2M NaCl, 2% Tween 20
DESS Buffer	20% DMSO, 0.25M EDTA, saturated NaCl (pH 8.0)

All samples were incubated at 35°C with relative humidity ≈70% for 1, 2 and 3 months.

RESULTS

The preservatives were evaluated based on the quantity and quality of DNA isolated directly from the liquid preservative surrounding the skin and muscle samples.

A. Skin



B. Muscle

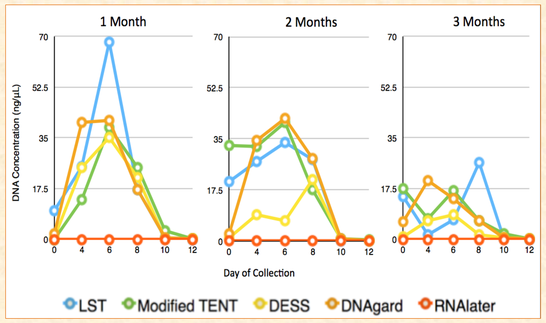


Fig 2 – Amount of amplifiable DNA yielded from 100µL of liquid preservative surrounding 30mg of A) skin and B) muscle tissue over 12 days of decomposition and stored for 1, 2 and 3 months at 35°C with relative humidity ≈70% .

DNA Quantity

- Adequate amounts of DNA (2-68 ng/µL) for STR typing leached from both tissues into solution with all preservatives tested except the RNA*later* and DESS.
- However, it should be noted that the highest DNA yields from the skin and muscle tissues themselves (not the liquid preservative) were obtained when the samples were preserved with RNAlater *(data not shown).*
- DESS leached DNA into solution from muscle tissue only.
- The amount of DNA leaching from both tissues was consistent for two months of storage, with a substantial decrease observed after three months (Fig 2).

DNA Quality

A. Skin

Percentage of alleles correct (%)	LST			Modified TENT			DNAgard		
	1	2	3	1	2	3	1	2	3
Day 0									
Day 4									
Day 6									
Day 8									
Day 10									
Day 12									

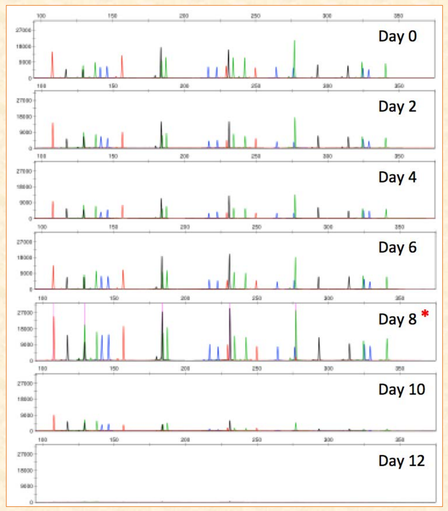
B. Muscle

100% 70-99% 50-69% 0-50%

Percentage of alleles correct (%)	LST			Modified TENT			DNAgard			DESS		
	1	2	3	1	2	3	1	2	3	1	2	3
Day 0												
Day 4												
Day 6												
Day 8												
Day 10												
Day 12												

Fig 3 – Number of alleles obtained using the Identifiler® Plus STR amplification kit with 0.8 ng DNA extracted from 100µL of liquid preservative surrounding 30mg of A) skin and B) muscle tissue over 12 days of decomposition and stored for 1, 2 and 3 months at 35°C with relative humidity ≈70%. C. STR Profiles from skin over 12 days of decomposition showing a rapid decrease in peak heights and increase in allele and locus dropout after the body bloated (*) at day 8.

C.



- Complete STR profiles were obtained from the DNAgard® and modified TENT solutions only for skin up to day 10 and muscle up to day 8, suggesting that these preservatives leach and protect the 'free' DNA in solution from further damage and/or degradation better than the other preservatives tested (Fig 3A & B).
- The LST buffer consistently produced the least complete STR profiles from all samples. The quantity and quality of the DNA also decreased with time, suggesting that this solution is not a good DNA preservative.

MATERIALS AND METHODS CONT.

DNA was isolated from skin and muscle tissue using the full QIAamp DNA Investigator kit protocol, whilst the 'free' DNA in solution was purified from the liquid preservative using the Investigator kit protocol Part B (purification only) (Fig 4).

DNA quantification was performed on a StepOne™ Real-Time PCR System (Life Technologies) using SYBR® Green Master Mix (Life Technologies). DNA profiles were generated using the AmpFlSTR® Identifiler® Plus STR amplification kit (Life Technologies) and analyzed on a 3500 Genetic Analyzer (Life Technologies) using GeneMapper™ software v4.1 (Life Technologies).

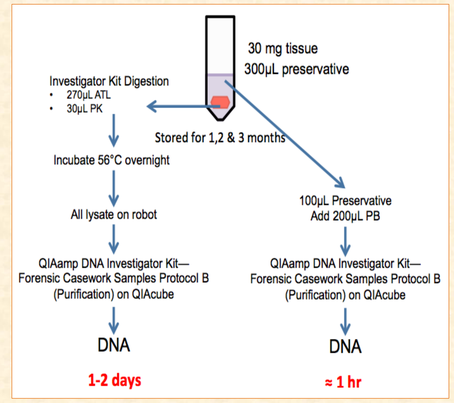


Fig 4 – DNA Extraction process.

CONCLUSIONS

- DNA in skin is more resistant to degradation than muscle tissue during decomposition, and therefore would be a better tissue for sampling from victims following a mass disaster.
- Both DNAgard® and TENT buffer leached DNA of high quantity and quality into the surrounding solution, making it 'free' for direct purification, and the overall genotyping process much faster.
- DNAgard® Tissue (Biomatrica®) and the modified TENT buffer both present as possible solutions for rapid purification and preservation of DNA in decomposing tissues at room temperature for up to three months.

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