DNA Persistence in Soft Tissue Comparing Vodka and Absolute Ethanol

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ABSTRACT

Successful DNA analysis of human remains relies on the collection and preservation of biological material. Low temperatures are typically used to preserve soft tissues; however, this is not always available, for example, after mass disasters and conflict, where the infrastructure has been damaged. Lysis buffers and absolute ethanol have also been shown to be effective at preserving material, but again are not always readily available. This study assess the use of drinking alcohol as an alternative preservative solution for muscle tissue storage. Pig muscle was incubated for up to 42 days at different temperatures (-20 °C, 25 °C, and 37 °C) with 95% ethanol, 37.5% ethanol, vodka (37.5%), and no preservative. Samples were collected weekly and analysis was based on agarose gel electrophoresis, DNA quantitation and amplification success of a multiplex with amplicons between 70 bp and 384 bp. Samples incubated with 37.5% ethanol and vodka had high molecular weight DNA and all samples incubated with preservative solutions generated complete profiles until the last collection point, while samples left untreated had drop-outs after 21 days at 25 °C and 37 °C.

KEYWORDS

DNA degradation; DNA preservation, drinking alcohol

1. INTRODUCTION

In situations where human remains are fragmented, burnt and/or decomposed, DNA profiling is a valuable tool to contribute to identification; however, successful DNA analysis relies on the appropriate collection and preservation of biological material [1]. Inefficient preservation methods can lead to DNA degradation, which may prevent successful profiling.

Whilst low temperature, typically below -15 °C, is widely used to preserve soft tissue samples, it is not available in all contexts, especially following mass disasters and conflict [2,3]. The DNA Commission of the International Society for Forensic Genetics says that storing soft muscle tissue samples in preservative solutions at room temperature can be an alternative to cold storage [4]. The INTERPOL Disaster Victim Identification Guide also recommends preserving soft tissue in ethanol [5].

Previous work has shown that absolute ethanol is effective at preserving DNA from fresh and partially degraded soft tissues [6,7]. This study intended to assess the use of drinking alcohol as an alternative preservative solution for muscle tissue storage. The rationale being that drinking alcohol is available in many contexts, whereas absolute ethanol is more restricted.

2. MATERIALS AND METHODS

Approximately 0.25 g of pig muscle was incubated in 1.5 ml tubes for up to 42 days at different temperatures (-20 °C, 25 °C, and 37 °C) with 95% ethanol, 37.5% ethanol, vodka (37.5%), or no preservative. Samples were collected weekly and extraction started immediately; DNA extraction was performed using DNeasy® Blood and Tissue kit and quantification using Qubit. Samples were amplified and the PCR products were analysed using an ABI 3500. Analysis was based on agarose gel electrophoresis (AGE), DNA quantitation and amplification success of a multiplex with amplicons of 70 bp, 194 bp, 305 bp, and 384 bp [8].

3. RESULTS

As expected, incubation at -20 °C generated clear HMW DNA bands in AGE for all samples up to 42 days of incubation. All samples stored at 25 °C and 37 °C displayed some degradation. Samples incubated with 37.5% ethanol or vodka still had HMW bands after 42 days, similar to samples incubated with 95% ethanol. Untreated samples did not present HMW after 14 days of incubation at 25 °C and 37 °C (data not shown).

Samples stored in 37.5% ethanol and vodka had higher DNA concentration than samples stored in absolute ethanol. Samples incubated at -20 °C resulted in the less variation in DNA concentration than other temperatures. Samples incubated at 37 °C and left untreated had a decreasing average in DNA concentration through the collection points (Figure 1).

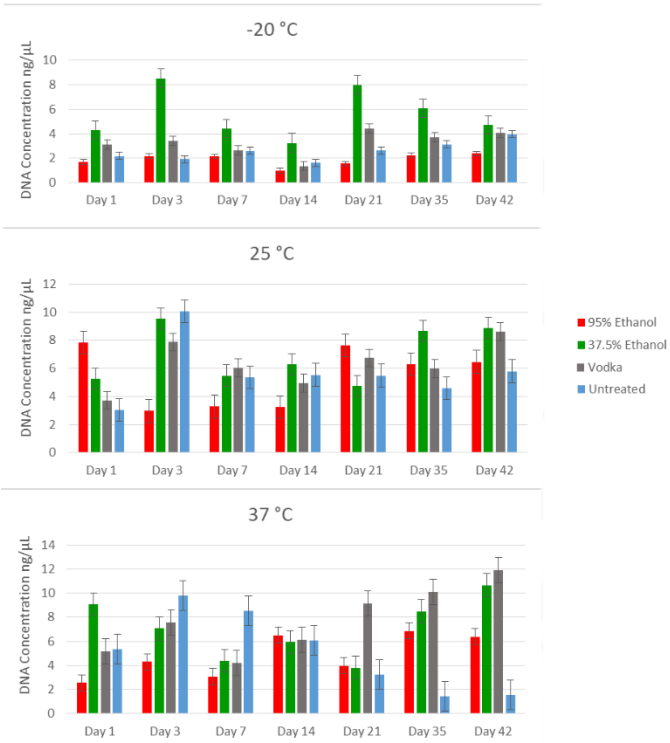

Figure 1 DNA quantification results for muscle tissue stored in 95% ethanol, 37.5% ethanol, vodka, and untreated for up to 42 days (n=3).

In all samples incubated at -20 °C, all alleles were amplified in all time points (Table 1). Samples incubated at 25 °C and 37 °C with any preserving agent also had complete amplification at all time points. Samples incubated untreated showed drop-outs after 14 days. After 21 days at 37 °C, there was a drop-out of the 384 bp allele in all untreated samples and after 35 and 42 days of incubation only the 70 bp allele was present.

Table 1 Amplification results. Samples were amplified with a multiplex with amplicons of 70 bp, 194 bp, 305 bp, and 384 bp.

Time	Preservation Agent	4 Plex amplification		
		-20 °C	25 °C	37 °C
Day 1	95% ethanol	++++	++++	++++
	37.5% ethanol	++++	++++	++++
	Vodka	++++	++++	++++
	Untreated	++++	++++	++++
Day 3	95% ethanol	++++	++++	++++
	37.5% ethanol	++++	++++	++++
	Vodka	++++	++++	++++
	Untreated	++++	++++	++++
Day 7	95% ethanol	++++	++++	++++
	37.5% ethanol	++++	++++	++++
	Vodka	++++	++++	++++
	Untreated	++++	++++	++++
Day 14	95% ethanol	++++	++++	++++
	37.5% ethanol	++++	++++	++++
	Vodka	++++	++++	++++
	Untreated	++++	++--	+++-
Day 21	95% ethanol	++++	++++	++++
	37.5% ethanol	++++	++++	++++
	Vodka	++++	++++	++++
	Untreated	++++	++--	+---
Day 35	95% ethanol	++++	++++	++++
	37.5% ethanol	++++	++++	++++
	Vodka	++++	++++	++++
	Untreated	++++	+---	+---
Day 42	95% ethanol	++++	++++	++++
	37.5% ethanol	++++	++++	++++
	Vodka	++++	++++	++++
	Untreated	++++	+---	+---

The “+” denotes the presence and “-“ the absence of PCR amplicons in samples. Triplicates were grouped and the data presented are the result of at least two of the three samples.

4. DISCUSSION

Storing samples in 95% ethanol and/or freezing are established procedures for preserving muscle tissue. In this experiment, these samples were used as controls to compare the efficiency of the tested solutions.

All the preservation solutions used improved DNA preservation. Vodka has been used before to preserve zebra liver over several days in the African bush and the DNA extracted amplified a 1.4 kb gene [9]. This is similar to results in this study where samples incubated for up to 42 days amplified fragments in the range of most STR kits.

5. CONCLUSION

The use of drinking alcohol or ethanol diluted to lower concentrations can be considered for preserving samples. In a situation where the stock of absolute ethanol is low or it is not available and it is also not possible to freeze samples, these alternatives can be considered in a way to preserve soft tissue samples within the timeframe studied.

6. ACKNOWLEDGMENTS

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7. CONFLICT OF INTEREST

None.

8. REFERENCES

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