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Ambient temperature storage of tissue samples (bovine) in readily available media during mass fatality incidents

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ABSTRACT

The possibility of using readily available media such as salt and alcohol to preserve tissue samples in non-tropical climates has been previously reported. This present study is a proof of concept trial to investigate the efficacy of salt, alcohol and FTA card for the preservation of soft tissue samples in a tropical hot and humid environment. Using bovine flesh as proxy, salt and commercial liquors such as whiskey and vodka were found to be effective in preserving the tissue samples at ambient temperatures over a 3 month period.

1. Introduction

Forensic deoxyribonucleic acid (DNA) profiling is often the only primary identifier available for re-association and identification of fragmented human remains. In hot and humid tropical climates, effective tissue preservation is crucial for obtaining a DNA profile from the remains. The storage conditions recommended in the 2018 INTERPOL DVI Guide are 4 °C to 6 °C (short term) and -14 °C (long-term) [1]. However, in mass fatality incidents, there may be insufficient refrigerated storage, electrical outages and even prolonged delays prior to laboratory analysis. Such conditions may be exacerbated in situations involving military conflicts. The present proof of concept study seeks to identify alternative media for quick and easy tissue preservation at ambient temperatures using bovine samples as proxies.

2. Materials and methods

2.1. Experimental design

Seven preservation media were examined in this study:

1) Control condition of 10 g bovine flesh at 4 °C;
2) 10 g bovine flesh in 500 g of kitchen salt at ambient temperature (~24 °C);
3) 10 g bovine flesh in 50 mL of 70% alcohol (diluted from 100% Ethanol, Sigma Aldrich, USA) at ambient temperature;
4) 10 g bovine flesh in 50 mL of 40% alcohol (diluted from 100% Ethanol, Sigma Aldrich) at ambient temperature;
5) 10 g bovine flesh in 50 mL of vodka (40% alcohol, Absolut, Sweden) at ambient temperature;
6) 10 g bovine flesh in 50 mL of whiskey (40% alcohol, Chivas Regal 12 Years, Scotland) at ambient temperature; and
7) Bovine flesh smeared onto Whatman® FTA Classic Cards (GE Healthcare Life Sciences, UK).

The effects on preservation in various media, with the exception of vodka and whiskey, were investigated across a total of six time points: 2 days, 5 days, 9 days, 2 weeks, 1 month, and 3 months. The effects on preservation in vodka and whiskey were investigated across two time points: 1 month and 3 months.

2.2. DNA processing

At each time point, five tissue samples (0.3 g each) were cut from each piece of flesh. For the bovine flesh smeared onto FTA card, five excisions of 2.5 mm diameter were made. DNA extraction was performed using the DNA IQ™ Casework Extraction Kit and DNA IQ™ Casework Pro Kit on the Maxwell® FSC instrument (Promega, USA). As the tissue samples preserved in salt and alcohol appeared dehydrated, an additional 300 µL extraction buffer and 350 µL lysis buffer was required from time point “5 days” onwards. DNA was eluted in a final volume of 50 µL. STR-PCR amplification (29 cycles) was performed using the StockMarks® Kit for Cattle - Bovine Genotyping Kit (Applied Biosystems, USA) with 1 µL of DNA template. Amplicon separation and detection were performed using the 50-cm capillary on the ABI PRISM® 3500 Genetic Analyzer (Applied Biosystems, USA) with an injection

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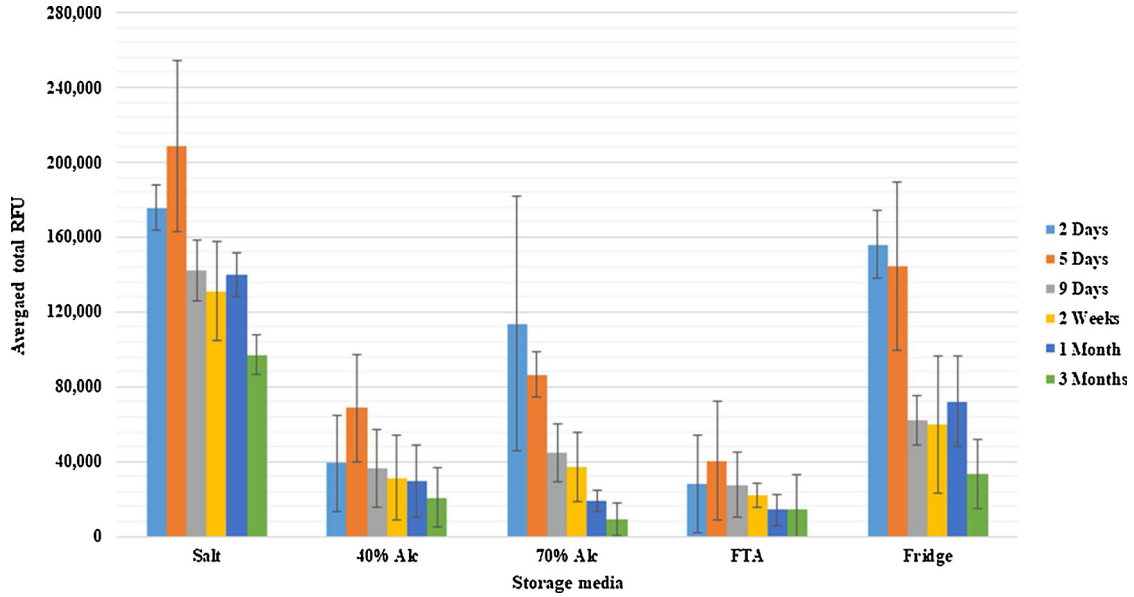

Fig. 1. Comparison of the average total RFU recovered from the bovine samples after 2 days to 3 months storage in various preservation medium.

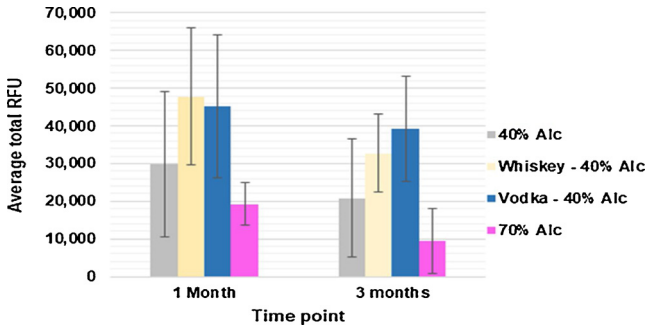

Fig. 2. Comparison of the average total RFU recovered from the bovine samples after 1 month and 3 months storage in various alcohol media.

parameter of 3 µL at 15 kV 180 s. Results were analyzed using GeneMapper® ID-X v1.2 software.

3. Results and discussion

3.1. Comparison between different preservation media

The average total relative fluorescence units (RFU) recovered from the five technical replicates of the bovine tissue (Fig. 1) preserved in the different preservation media appeared to be on a decreasing trend. Interestingly, the average total RFU recovered for the second time point "5 Days" was generally higher than the first time point of "2 Days". This phenomenon was likely due to the dehydration of the bovine flesh leading to more cells being present in the same sample mass being used for DNA extraction. In the absence of a bovine DNA quantitation kit, the subsequent PCR amplification would likely have had more template DNA present (based on a constant template volume of 1 µL) in the "5 Days" samples as compared to the "2 Days" samples.

The tissue samples preserved in salt had, on average, the highest total RFU as compared to all other preservation media, including storage at 4 °C, across the time points. Additionally, only tissue samples preserved in salt yielded full DNA profiles across all the time points. Contrary to the study by Connell et al., who reported that 70% alcohol conferred comparable preservation effect on human samples to refrigeration [2], our study showed that samples preserved in both 40% and 70% alcohol and on FTA yielded lower total average RFU indicating a weaker preservation effect as compared to storage at 4°C. This difference may have been due to the smaller sample size and different sample type used in this proof of concept study. We also observed that for samples preserved in 40% alcohol and FTA, there was little change in the average total RFU over time, suggesting that there was only minimal DNA degradation beyond that which occurred at the start of the experiment.

3.2. Comparison between commercial liquors and laboratory grade alcohol

The average total RFU recovered from the tissue samples (Fig. 2) preserved for 1 month and 3 months in commercial liquors, as compared to laboratory grade alcohol, were generally higher. It was also observed that there was little change in average total RFU for the tissue samples preserved with whiskey and vodka, similar to that of samples preserved in 40% laboratory alcohol. As commercial liquors may be more readily available than laboratory grade alcohol, the results from this study suggest that commercial liquors may be a viable alternative for the preservation of tissue samples at ambient temperatures.

4. Conclusion

This proof of concept study has demonstrated that salt, which is a readily available household item, and commercial liquors may be viable alternatives to refrigeration for effective, cheap and quick preservation of tissue samples at ambient temperatures. Additional studies incorporating bovine DNA quantitation with a larger sample size would be performed to elucidate the long-term preservation capacity of these alternative media.

Declaration of Competing Interest

None.

References

[1] 2018 INTERPOL DVI Guide Annexure 5: Phase 2 - Post-Mortem.
[2] Connell, et al., Tissue preservation in extreme temperatures for rapid response to military deaths, Forensic Sci. Int. Genet. 36 (2018) 86–94.