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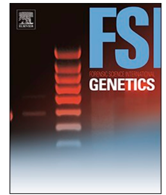

Evaluation of five preservation methods for recovery of DNA from bone

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ABSTRACT

The effectiveness of five methods for the preservation of bone were assessed: cell lysis solution (with 1% sodium azide), dehydration/freeze-drying, ethanol (96%), freezing, and room temperature storage. Preserved bone samples were extracted using five optimised extraction methods. These preservation methods were tested for their efficiency for storage of fresh and degraded bone samples for 6 weeks, 6 months and 1 year. Freezing was found to be the best preservation method for longer-term storage of bone samples; this was followed by ethanol (96%), dehydration/freeze-drying, and room temperature storage. Full profiles were obtained from bone samples using all these preservation methods.

1. Introduction

Effective preservation of biological evidences allows samples to be stored until they can be analysed. In the case of mass disasters, the identification of human remains may be prolonged, thus preservation of those remains to prevent DNA degradation is very important [1].

2. Materials and methods

2.1. Sample preparation

Fresh pig bone samples were purchased from a butcher. Prior to preservation, any soft tissue on the bone was removed. The bones were soaked in commercial bleach (5% sodium hypochlorite) for 15 min, rinsed with water and then dried. Small pieces of approximately 1 cm^3 were cut using a bone saw. A total of 90 bone pieces were prepared, and each piece was placed into a labelled 15 ml polypropylene tube. Each sample was prepared in triplicate.

2.2. Preservation

A total of 5 preservation methods were tested; cell lysis solution (with 1% sodium azide), dehydration/freeze-drying, ethanol (96%), freezing and room temperature storage. Bone samples for cell lysis solution (with 1% sodium azide) and ethanol (96%) methods were covered with approximately 3 ml of each solution respectively. For the dehydration/freeze-drying method, bone samples were freeze-dried using Micro Modulyo® Freeze Dryer (Thermo Electron). Freezing method bone samples were stored in the freezer at −20 °C while room temperate bone samples were stored in the incubator at 25 °C.

2.3. DNA extraction

Samples were extracted after 6 weeks, 6 months and 1 year of preservation. The DNA extraction was carried out using ChargeSwitch® gDNA Plant Kit, DNA IQ™ System Kit, DNeasy® Blood & Tissue Kit, and PrepFiler® BTA Forensic DNA Extraction Kit and a conventional phenol-chloroform method, according to the manufacturers' instructions and published methods [2]. Approximately 50 mg of pulverised bone samples were used in each extraction without decalcification [3]. Extractions were carried out in triplicate and the final elution volume was set at 100 µl in each extraction.

2.4. DNA quantification and amplification

The extracted DNA samples were quantified with GoTaq® qPCR Master Mix amplifying a 70 bp target using the Applied Biosystems® 7500 Real-Time PCR System. Reactions comprised 6.25 µl 2x GoTaq® qPCR Master Mix, 4.75 µl H_2O, 0.50 µl primers and 1.00 µl template DNA. The thermal cycler conditions were: 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The DNA concentration for each sample was estimated in ng/µl. All DNA extracts were amplified using an in-house multiplex (4-plex with Internal Amplification Controls) with 1 µl of each extract [4].

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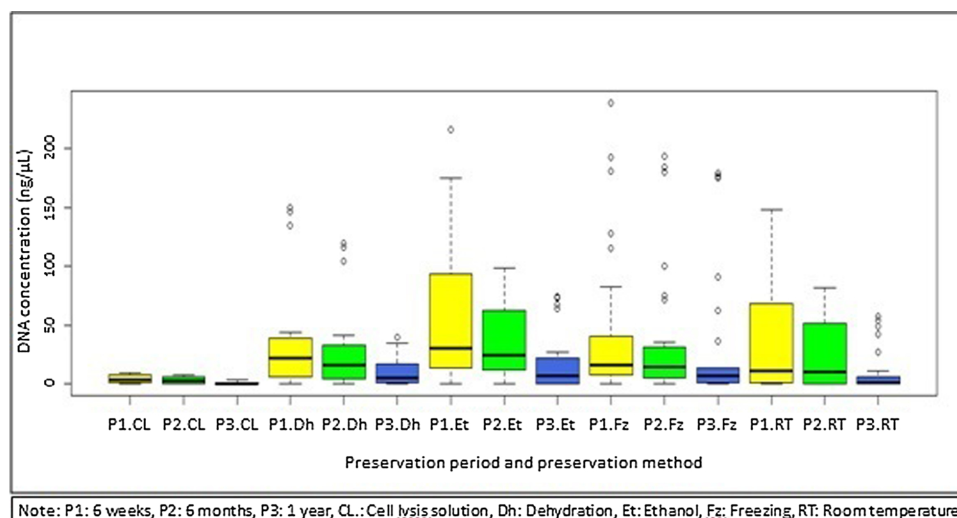

Fig. 1. Boxplots generated from the concentrations of DNA extracted after 6 weeks, 6 months, and 1 year preserved bone samples. Each boxplot represents 30 samples which were extracted using 5 different extraction methods.

3. Results

The results showed that freezing is the best method for bone preservation prior to DNA analysis. Even though ethanol (96%) preservation method performs marginally better than freezing on shorter preservation time (6 weeks), the freezing preservation method is the best for longer preservation (1 year) (Fig. 1).

All the extracted samples were amplified using the multiplex (4-plex with Internal Amplification Controls) [3]. 1 µl of each extract was used for amplification. The electropherograms showed that good quality DNA profiles were obtained from 6 weeks preservation but DNA degradation occurred as the time of preservation increased. Cell lysis solution (with 1% sodium azide) extracts produced electropherograms with low peak height, concordant with their DNA concentration. No PCR inhibitors indicated with both the Internal Amplification Controls peaks balanced in all the profiles (Fig. 2).

4. Discussion and conclusion

This study aimed to access the effectiveness of five preservation methods on bone samples. Full profiles were obtained from bone samples from all the preservation methods. However, freezing was found to be the best preservation method for longer-term storage. In this study, freezing was at −20 °C, thus highly specialised equipment is not required. Another advantage of freezing is that no other substances need to be added to the samples. However, in some contexts, stable freezing may not be an option.

Ethanol (96%) was found to be the best preservation method for shorter-term storage (6 weeks) but its performance dropped slightly with longer-term storage (6 months and 1 year) compared to the freezing preservation method. Potentially this situation could be alleviated by changing the ethanol for continuous freshness and effect of the ethanol or by adding more ethanol [5].

Cell lysis solution (with 1% sodium azide) preservation method tended to be good for shorter-term storage but with the longer-term preservation, less DNA yield was obtained and also the electropherograms showed DNA degradation.

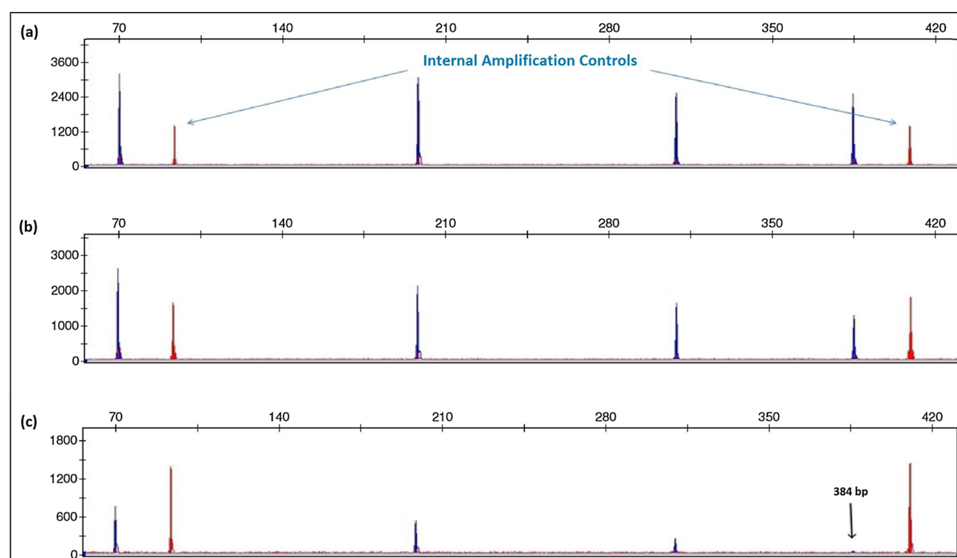

Fig. 2. Example of electropherograms generated from DNA extracts of fresh bone samples preserved using cell lysis solution (with 1% sodium azide) for (a) 6 weeks, (b) 6 months, and (c) 1 year. The bone samples were extracted using the phenol-chloroform-isoamyl alcohol extraction method. 1 µl of each extract was used.

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Declaration of Competing Interest

None.

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