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Short communication

Human tissue preservation for disaster victim identification (DVI) in tropical climates

A. Allen-Hall [1], D. McNevin *

National Centre for Forensic Studies, Faculty of Applied Science, University of Canberra, ACT 2601, Australia

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ABSTRACT

Disaster victim identification (DVI) poses unique challenges for forensic personnel. Typical scenarios may involve many bodies or body parts to identify in remote locations with limited access to laboratory facilities and in extreme temperatures. Transportation of tissue samples to a forensic laboratory for DNA profiling can take weeks without refrigeration. As well as protecting DNA for subsequent analysis, tissue preservation methods ideally should be safe, readily available and easy to transport to the scene at relatively low cost. We examined eight tissue preservatives (salt, DMSO, ethanol, ethanol with EDTA, TENT buffer, RNA*later*®, DNA Genotek Tissue Stabilising Kit and DNAgard®) and compared the quantity and quality of DNA recovered from human tissue and preservative solution stored at 35 °C. Salt, DMSO, ethanol solutions, DNA Genotek and DNAgard® produced full Identifiler® genotypes up to one month from DNA extracts. In addition, DMSO, DNA Genotek and DNAgard® produced full profiles from aliquots of the liquid preservative.

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1. Introduction

Disaster victim identification (DVI) is part of the response to a mass disaster with the intention of identifying and repatriating all victims and body parts. Forensic DNA analysis is one of the three primary methods of identification recommended by the International Criminal Police Organisation (INTERPOL), together with fingerprint and dental analysis [1], and can therefore play a crucial role for identifying victims. Mass disasters that occur in remote locations pose special problems. For example, the 2004 Asian tsunamis occurred in countries that lacked the resources needed to effectively deal with the disaster on their own [2,3]. The 1979 Mount Erebus air disaster that occurred in Antarctica posed problems for recovery and DVI operations because of its remote location and extreme weather conditions [4].

Mass disasters in tropical climates require samples to be processed quickly as degradation of bodies is accelerated. Analysis should be as easy and efficient as possible and repeat analyses due to problems like low template DNA and PCR inhibition should be avoided. Preservation methods should be able to generate a profile using commercial multiplex PCR chemistries.

Skeletal muscle tissue is typically sampled during DVI operations for DNA analysis [1,5] and requires preservation, from the time of collection to the point at which it can be transported to a laboratory refrigerator/freezer. Portable refrigeration can be employed; however, mass disasters can occur in locations where field-based refrigeration may not be feasible. INTERPOL guidelines state that preservatives can be used to conserve soft tissue at room temperature but that formaldehyde or formalin should not be used because it degrades DNA [1]. The International Society for Forensic Genetics also advises against the use of formalin [5]. One alcohol-based tissue preservative (GenoFix™) has been shown to preserve DNA for extended periods of time and allow full STR profiling [6]. There is a lack of detail on whether other preservatives may be suitable, especially in tropical climates.

The constituents of DNA preservatives should play a role in arresting DNA degradation. NaCl is a common preservative that has been used for centuries. In solid form, it desiccates the sample which inactivates nucleases and slows microbial growth [7]. Other desiccants such as silica beads have a similar effect [7,8]. When in an aqueous solution the NaCl also denatures proteins including nucleases [7]. Chelating agents like EDTA bind to metal ions such as those required by nucleases for normal function (*e.g.* Mg^{2+} and Ca^{2+}) and therefore will slow or stop the nuclease activity on DNA [9,10]. Detergents such as Tween 20 are also thought to lyse cells and inactivate nucleases, although the precise mechanism has not been described [11]. Ethanol removes water from the sample and denatures proteins and nucleases [9,12]. Ethanol is also an antimicrobial agent and will protect against bacterial degradation

* Corresponding author. Tel.: +61 2 6201 2634; fax: +61 2 6201 2461.
E-mail addresses: aidan.allenhall@gmail.com (A. Allen-Hall), dennis.mcnevin@canberra.edu.au (D. McNevin).
[1] Tel.: +61 424 640 684.

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[9]. Dimethyl sulphoxide (DMSO) is known to promote the dermal absorption of chemicals with which it is mixed [13,14] so, whilst it does not directly preserve DNA, it enhances the absorption of other preservatives across biological membranes and into the cell [15]. Finally, buffering the pH stabilises DNA by decreasing the rate of acid-catalysed depurination [16,17].

Preservatives that have been used for human tissue and forensic STR profiling include salts, lysis storage and transportation (LST) buffer, GenoFix® (DNA Genotek Inc.: not currently available) and Oragene® (DNA Genotek Inc.). Other preservatives have been used in non-forensic contexts and for non-human samples [for a review, see 7]. They are nonetheless likely to be useful for forensic STR profiling. They include DMSO, ethanol, lysis buffers and proprietary preservatives.

Storage conditions for this study have a forensic context. Much of the published research examines tissue preservation methods for medium to long time periods, *i.e.* for one year or more. There is more of an archival focus with long-term tissue storage. Forensic situations, such as mass disasters, are mostly field-based and the focus is on short-term storage because the samples will be analysed or placed in refrigeration following transportation to the laboratory. This may take a few weeks or a month at most.

Most of the published experimental data shows preservation methods that are successful for storing tissue at ambient or room temperature (~20–25 °C). However, mass disasters often occur in tropical climates where tissue may have to withstand higher temperatures and a typical scenario is 35 °C. Oven drying has been shown to be a successful preservation strategy [18,19] and so dehydration at 35 °C has been used as a control in this study.

Few tissue preservation methods have been shown to be effective for forensic STR profiling although many have been demonstrated to perform well with non-human tissue. As such, we compared the preservatives summarised in Table 1. This includes drying at 35 °C (used as a control), a non-liquid salt (for ease of use), DMSO solution with standard constituents as used by most researchers, 70% ethanol (considered to be the ideal concentration), a lysis buffer with typical constituents (and readily available in most forensic laboratories) and three proprietary solutions.

2. Materials and methods

Three volunteers consented to donate muscle tissue retrieved from surgical procedures. Approval was provided by ACT Health Human Research Ethics Committee (ETH 9.07.865) and the University of Canberra Committee for Ethics in Human Research (Project number 09-01). A buccal swab was collected from each patient for a DNA reference. The samples were transported on ice to the University of Canberra, dissected into ~300 mg slices on a sterilised tile inside a laminar flow cabinet and weighed. The tissue slices from each of the three samples were placed in sterile 10 mL screw top test tubes in 2 mL of each of the preservatives in Table 1 with the following exceptions: tissue slices from each of the three samples were placed in 4 g NaCl; tissue slices from two samples were placed in 1 mL DNAgard (due to limited availability of the preservative); tissue slices from two samples were placed in each of two empty, open 10 mL tubes (as controls). The samples were stored at 35 °C in a Digital Series oven (Contherm, Wellington, NZ). The temperature and humidity of the oven was monitored using an EasyLog USB Data Logger (Lascar Electronics, Salisbury, UK). At 0, 4, 7, 14 and 28 days, approximately 10% by volume of each tissue slice was excised and placed in sterile 1.5 mL microcentrifuge tubes and at 4, 7, 14 and 28 days, 50 μL of each preservative solution (20 μL for DNAgard) was pipetted into sterile 1.5 mL microcentrifuge tubes. Buccal swab gauzes were removed from their stalks and similarly placed in sterile 1.5 mL microcentrifuge tubes. Negative controls consisted of empty microcentrifuge tubes and positive controls consisted of 5 μL human female genomic DNA (Promega, G1521). All equipment used to directly handle tissue samples was either autoclaved or sterilised using 10% bleach, 70% ethanol and UV light irradiation.

Table 1
Preservation methods compared in this study (percentage concentrations are v/v).

Preservation	Abbreviation	Constituents
Dehydration	Dehydration	Oven drying at 35 °C
Solid NaCl	NaCl	Laboratory grade NaCl
Dimethyl sulphoxide	DMSO	20% DMSO, 0.25 M EDTA, saturated with NaCl, pH 8.0
Ethanol	EtOH	70% ethanol, 30% ddH_2O
Ethanol + EDTA	EtOH–EDTA	70% ethanol, 30% ddH_2O, 0.1 mM EDTA
TENT buffer	TENT	10 mM Tris, 10 mM EDTA, 100 mM NaCl, 2% Tween 20
RNA*later*®	RNAlater	Not disclosed
DNA Genotek Tissue Stabilising Kit	DNA-Gen	Not disclosed
DNAgard®	DNAgard	Not disclosed

A phenol–chloroform/ethanol precipitation method was used to extract DNA from all samples. Initially, a lysis buffer was prepared with 60 μL of 10 mg mL^{-1} proteinase K (Sigma–Aldrich) and 6 mg of solid dithiothreitol (DTT: Sigma–Aldrich) per mL of TENT buffer. A 350 μL aliquot of the lysis buffer was placed in each microcentrifuge tube and the sample was mixed by inverting. The samples were incubated at 56 °C overnight with intermittent inversion. The next day, 30 μL of 10 mg mL^{-1} proteinase K and 2 mg of solid DTT were added to each tube. Incubation at 56 °C was continued for 1 h. A 350 μL aliquot of phenol/chloroform/isoamyl alcohol (24:25:1) (Sigma–Aldrich) was added to each tube. After vortexing, the tubes were centrifuged at 12,000 rpm for 15 min to separate the aqueous and solvent phases. A 220 μL aliquot from the upper (aqueous) layer was carefully removed and placed in a new microcentrifuge tube. The following reagents were then added: 22 μL EDTA (125 mM), 22 μL sodium acetate (3 M, pH 5.2) and 550 μL of absolute ethanol (−20 °C). Each tube was mixed by inverting and incubated at −20 °C for 15 min then centrifuged at 2000 × *g* for 45 min. The liquid was decanted immediately and 60 μL of 70% (v/v) ethanol (−20 °C) was added. The tubes were then incubated at −20 °C for 15 min and centrifuged at 1700 × *g* for 15 min. The liquid was decanted immediately and the DNA pellet was air dried. A 50 μL aliquot of TE buffer (10 mM, 0.1 mM EDTA, pH 8.0) was added to resuspend each pellet. All samples were stored at −20 °C.

Quantifiler™ Human DNA Quantitation Kit (Applied Biosystems) was used to quantify the DNA according to the manufacturer's instructions [20]. The Quantifiler™ Human DNA standard was used to prepare a dilution series to establish a standard curve. A quantitation negative control was prepared with 2 μL of TE buffer in place of the DNA extracts. Thermal cycling was conducted in a 7300 Real-Time PCR System (Applied Biosystems). The criteria for accepting the standard curve were an r^2 value greater than 0.98 and a slope of <-3.0.

Only extracts that contained more than 0.1 ng μL^{-1} DNA were genotyped using AmpFℓSTR® Identifiler® PCR Amplification Kit (Applied Biosystems) according to the manufacturer's instructions [21]. This meant that extracts from liquid aliquots of EtOH, EtOH–EDTA and RNAlater were not genotyped (in most cases, DNA was not detected at all in these extracts). All other extracts were genotyped. DNA extracts were diluted to 0.1 ng μL^{-1} with TE buffer and 10 μL of the diluted extract was added to the Identifiler® master mix to deliver a total of 1 ng of template DNA. A negative control was prepared with 10 μL of TE buffer in place of DNA and a positive control was prepared using the AmpFℓSTR® Identifiler® DNA Standard. Thermal cycling was

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