Preservation of human muscle in conditions commonly associated with mass disasters

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Bachelor of Applied Science (Forensic Investigation)

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Faculty of Applied Science
ABSTRACT

Muscle tissue is sampled during Disaster Victim Identification (DVI) operations to identify victims by DNA analysis. The tissue is preserved to prevent DNA degradation and improve the chances of obtaining good short tandem repeat (STR) profiles. Biological samples are normally preserved at low temperatures. However, refrigeration may not always be readily available in the field. Therefore, alternative preservation methods that do not require refrigeration would be useful. Because DVI operations often occur in remote locations under adverse conditions, these alternative methods should ideally make use of substances that are inexpensive, non-toxic and readily available in the field (or easily transportable).

Human muscle tissue was stored in a number of preservatives at 35°C to simulate the conditions that may be encountered during DVI operations in warm conditions. The tissue was stored for up to one month, to represent the amount of time that may be required to transport the samples back to the laboratory. DNA was quantified using Quantifiler™ Human DNA Quantitation Kit (Applied Biosystems) and profiled using AmpF(STR® Identifiler® PCR Amplification Kit (Applied Biosystems) to determine the success of each method.

The following preservatives were successful under the conditions of the study when sampling DNA directly from the tissue and full STR profiles were obtained after one month’s storage: 1) DMSO-EDTA-NaCl, 2) 70 % ethanol, 3) DNA Genotek Tissue Stabilising Kit, 4) Biomatrica® DNAgard™, and 5) oven drying. DNA could also be extracted directly from aliquots of the DNA Genotek Tissue Stabilising Kit and Biomatrica® DNAgard™ preservatives, giving them an advantage over other methods because the tissue did not require handling. Sodium chloride was shown to be less successful than these methods but still produced full DNA profiles after storage for one month.
ACKNOWLEDGEMENTS

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Secondly, thank you to Ms Mojca Keglovic and Dr Cindy Lim from the Australian Federal Police. Thanks for organising funding for the expensive DNA consumables used during the research. It has been an incredibly busy year and we haven’t seen much of each other, but I look forward to working with the two of you in the future.

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### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ABI</td>
<td>Applied Biosystems™</td>
</tr>
<tr>
<td>A</td>
<td>adenine</td>
</tr>
<tr>
<td>bp</td>
<td>base pairs</td>
</tr>
<tr>
<td>CE</td>
<td>capillary electrophoresis</td>
</tr>
<tr>
<td>C</td>
<td>cytosine</td>
</tr>
<tr>
<td>Dgard</td>
<td>Dgard™ preservative (Biomatrica®)</td>
</tr>
<tr>
<td>ddH₂O</td>
<td>distilled deionised water</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide preservative (20 % DMSO, 0.25 M EDTA, saturated with NaCl, pH 8.0)</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DNA-G</td>
<td>DNA Genotek Tissue Stabilising Kit</td>
</tr>
<tr>
<td>DTT</td>
<td>dithiothreitol</td>
</tr>
<tr>
<td>DVI</td>
<td>disaster victim identification</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EIOH</td>
<td>ethanol preservative</td>
</tr>
<tr>
<td>EIOH-E</td>
<td>ethanol-EDTA preservative</td>
</tr>
<tr>
<td>G</td>
<td>guanine</td>
</tr>
<tr>
<td>INTERPOL</td>
<td>International Criminal Police Organisation</td>
</tr>
<tr>
<td>LST</td>
<td>lysis, storage and transportation buffer</td>
</tr>
<tr>
<td>NaCl</td>
<td>solid sodium chloride preservative</td>
</tr>
<tr>
<td>OvnD</td>
<td>oven drying preservation</td>
</tr>
<tr>
<td>PPE</td>
<td>personal protective equipment</td>
</tr>
<tr>
<td>rfu</td>
<td>relative fluorescence unit</td>
</tr>
<tr>
<td>RFLP</td>
<td>restriction fragment length polymorphism</td>
</tr>
<tr>
<td>RNA/later</td>
<td>RNA/later®</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulphate</td>
</tr>
<tr>
<td>STR</td>
<td>short tandem repeats</td>
</tr>
<tr>
<td>TENT</td>
<td>TENT preservative (10 mM Tris base, 10 mM EDTA, 100 mM NaCl, 2% Tween 20)</td>
</tr>
<tr>
<td>T</td>
<td>thymine</td>
</tr>
<tr>
<td>TORU</td>
<td>Trauma and Orthopaedic Research Unit (Canberra Hospital)</td>
</tr>
<tr>
<td>UC</td>
<td>University of Canberra</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>VNTR</td>
<td>variable number tandem repeats</td>
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