# Direct-to-PCR tissue preservation for DNA profiling

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## Introduction

Tissue preservation offers the ability to stabilise and isolate DNA from tissues in the field, far from a laboratory setting, where refrigeration may not be available. This has potential application to disaster victim identification (DVI) as well as to any form of field based forensic biological evidence or intelligence collection[1]. Forensic DNA analysis is one of the three primary methods of identification recommended by the International Criminal Police Organization (INTERPOL), together with fingerprint and dental analysis[2]. In previous work, we have demonstrated the ability to obtain full AmpFℓSTR® Identifiler® (Life Technologies) STR profiles from DNA extracted from fresh muscle tissue preserved in four preservatives (Table 1)[3]. In this study, we explored the possibility of obtaining DNA profiles without DNA extraction, by adding aliquots of preservative solutions surrounding fresh and decomposing human tissue samples directly to PCR.

***Table 1*** *Tissue preservatives compared in this study (percentage concentrations are v/v).*

| Preservative | Abbreviation | Constituents |
|---|---|---|
| Salt-saturated DMSO/EDTA | DESS | 20 % DMSO, 0.25 M EDTA, saturated with NaCl, pH 8.0 |
| TENT buffer | TENT | 10 mM Tris, 10 mM EDTA, 100 mM NaCl, 2% Tween 20 |
| DNAgard | DNAgard | Not disclosed (manufactured by Biomatrica) |
| DNA Genotek | DNA Genotek | Not disclosed (manufactured by DNA Genotek) |

## Methods

Fresh human muscle tissue samples were preserved in each of the four preservatives at 35 ºC (Table 1). After 3, 7, 14 and 28 days, an aliquot of each preservative surrounding each tissue was archived at -80 ºC for four years. Skin and muscle tissue samples from two decomposing cadavers were collected at 0, 6, 8 and 10 days and stored in each of the four preservatives at 35 ºC for one month. DNA in the preservatives was quantified using Quantifiler® (Life Technologies) and genotyped using either PowerPlex® 21 (Promega) or GlobalFiler® (Life Technologies) without DNA extraction.

## Results

While no PCR inhibition was observed for preservatives from fresh human muscles samples stored at -80 ºC for four years, those from cadavers required either no dilution (TENT), a 1:10 dilution (DESS, DNAgard) or a 1:20 dilution (DNA Genotek) before both quantitation and genotyping (Figures 1 and 2). All four preservatives generated full DNA profiles from fresh muscle tissues and skin and muscle tissues from decomposing cadavers up to six days (before the appearance of bloat). The quantity of DNA retrieved varied widely and bore no relation to the subsequent success of DNA profiling and the resultant allele peak heights.

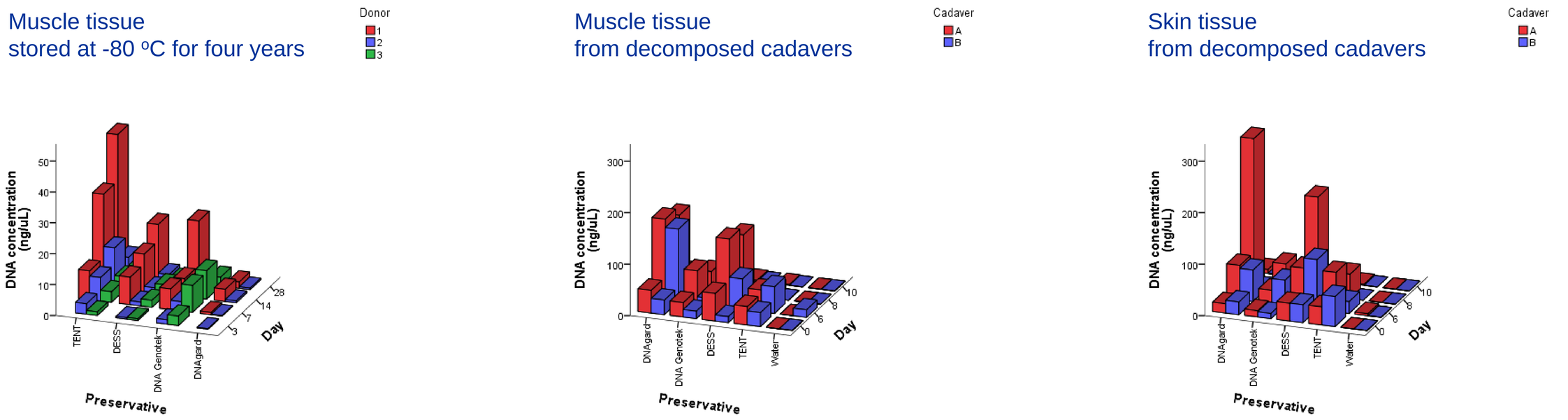


***Figure 1*** *DNA concentrations (adjusted for dilution) in aliquots of each of four preservative solutions and water surrounding muscle tissue stored at -80 ºC for four years (left) and tissue from decomposing cadavers stored for one month (middle and right). The preservative solutions were added directly to Quantifiler real time PCR assays (without DNA extraction).*

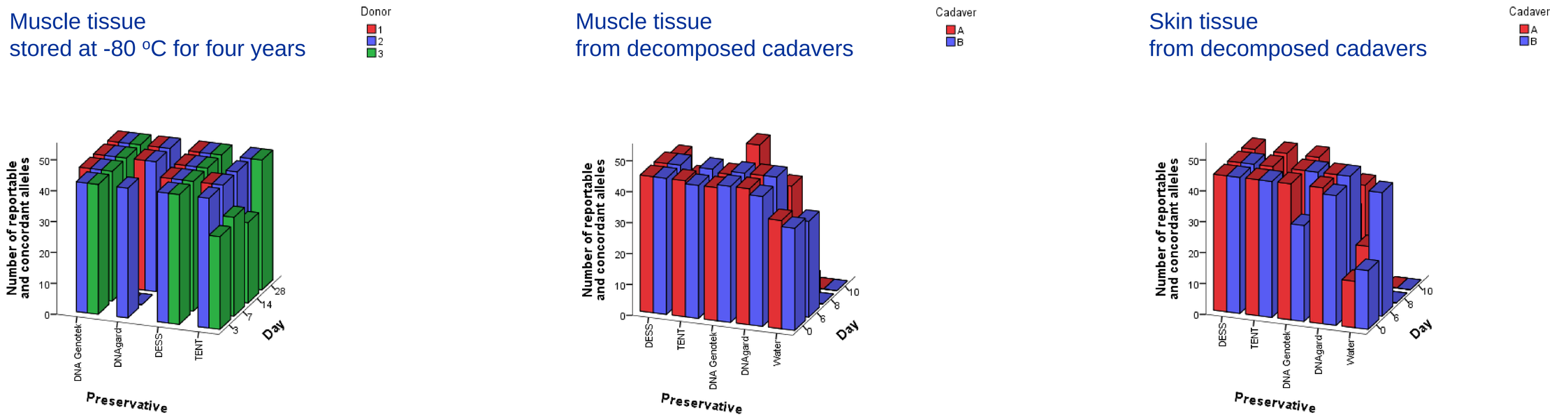


***Figure 2*** *Number of reportable alleles in STR genotypes from aliquots of each of four preservative solutions and water surrounding muscle tissue stored at -80 ºC for four years (left) and tissue from decomposing cadavers stored for one month (middle and right). The preservative solutions were added directly to PowerPlex® 21 (left) and GlobalFiler® (middle and right) PCR assays (without DNA extraction).*

## Conclusions

A direct-to-PCR approach for identifying fresh and decomposing tissue samples preserved at room temperature or higher is possible. By directly amplifying DNA in solution (with dilution in some cases), DNA extraction from the dense tissues can be avoided, and successful STR profiles can be obtained in a more timely manner.

## References

[1] Montelius K, and Lindblom, B: DNA analysis in disaster victim identification. *Forensic Science, Medicine, and Pathology*. 2012; **8**(2): 140-147.

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