

Direct-to-PCR Tissue Preservation for DNA Profiling

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ABSTRACT

Tissue preservation offers the ability to stabilize 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 collection¹. 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 analysis².

In previous work, we have demonstrated the ability to obtain full AmpF[®]STR[®] Identifier[®] (Life Technologies) STR profiles from DNA extracted from fresh muscle tissue preserved in TENT buffer (Tris, EDTA, NaCl, Tween 20), salt-saturated DMSO-EDTA solution (DESS) and two proprietary preservatives: DNAGard[®] (Biomatrix) and one from DNA Genotek, Inc³. Three of the preservatives (DESS, DNAGard and DNA Genotek) also yielded full profiles from DNA extracted from aliquots of the preservative solution surrounding the muscle tissues.

In this study, we explore 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. We obtained full PowerPlex[®] 21 (Promega) and GlobalFiler[®] (Life Technologies) DNA profiles from fresh and decomposed tissue preserved at 35 °C for up to 28 days as well as from fresh tissue which had been stored at 35 °C for up to 28 days, and then at -80 °C for four years.

INTRODUCTION

Tissue preservation offers the ability to stabilize 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 collection¹. 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 analysis².

In previous work, we have demonstrated the ability to obtain full AmpF[®]STR[®] Identifier[®] (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 Biomatrix)
DNA Genotek	DNA Genotek	Not disclosed (manufactured by DNA Genotek)

MATERIALS AND METHODS

Fresh human muscle tissue samples were preserved in each of the four preservatives at 35°C (Table 1). After 3, 7, 14 and 28 days, an aliquot of each preservative surrounding each tissue was archived at -80°C 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°C 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°C 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.

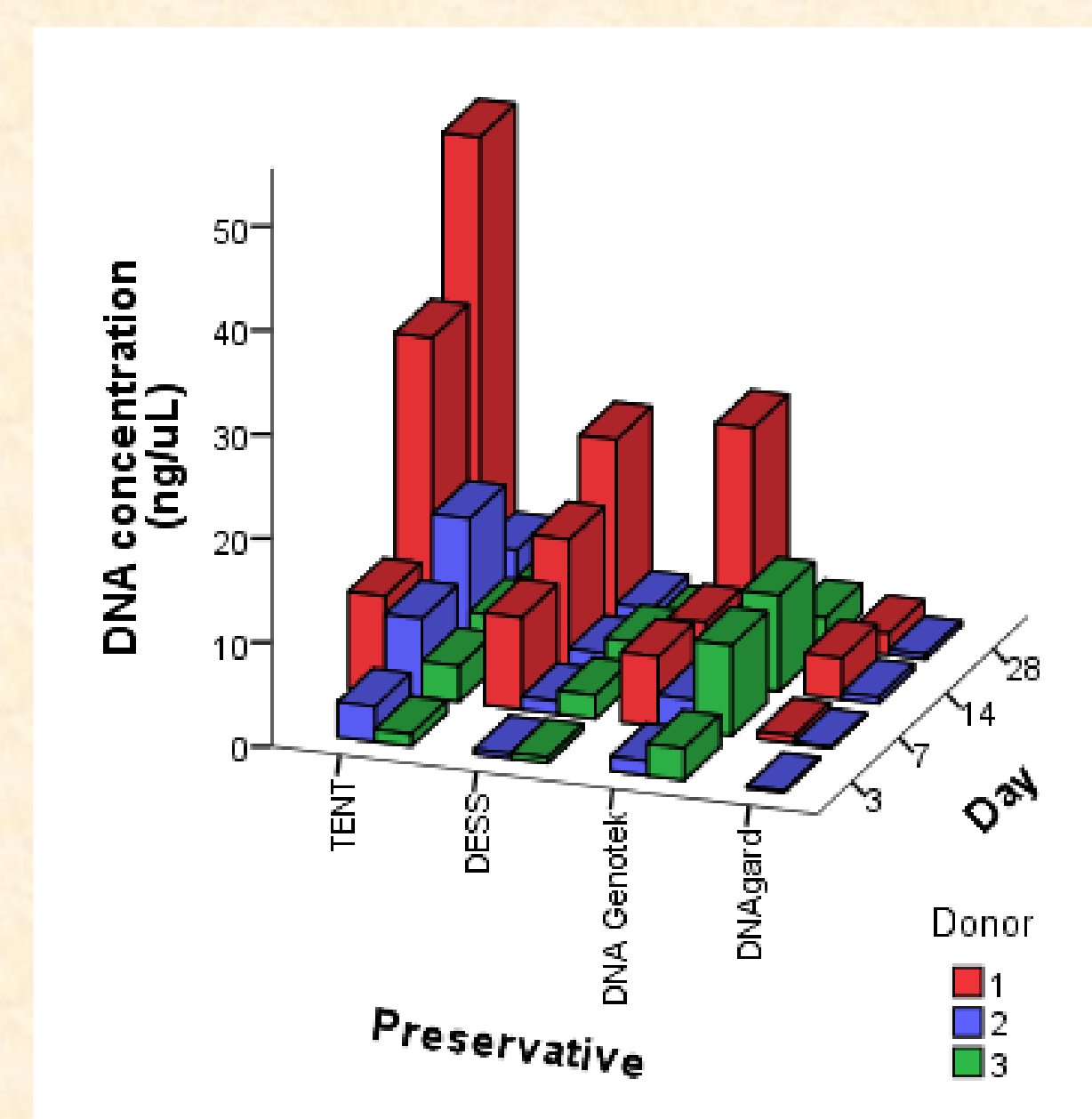
CONCLUSIONS

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.

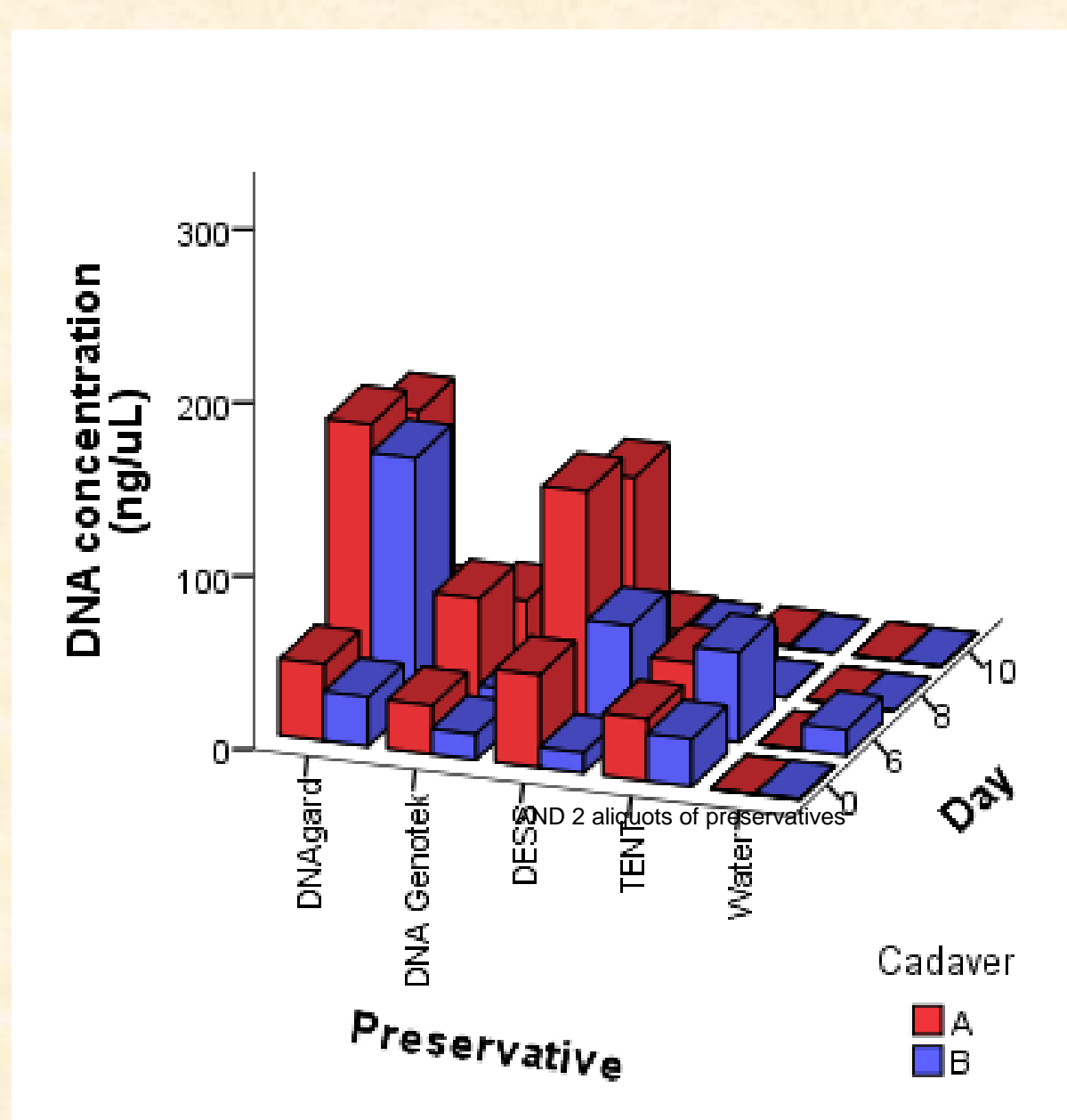
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- [1] Montelius K, and Lindblom, B: DNA analysis in disaster victim identification. *Forensic Science, Medicine, and Pathology*. 2012; 8(2): 140-147.
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Muscle tissue stored at -80 °C for four years



Muscle tissue from decomposed cadavers



Skin tissue from decomposed cadavers

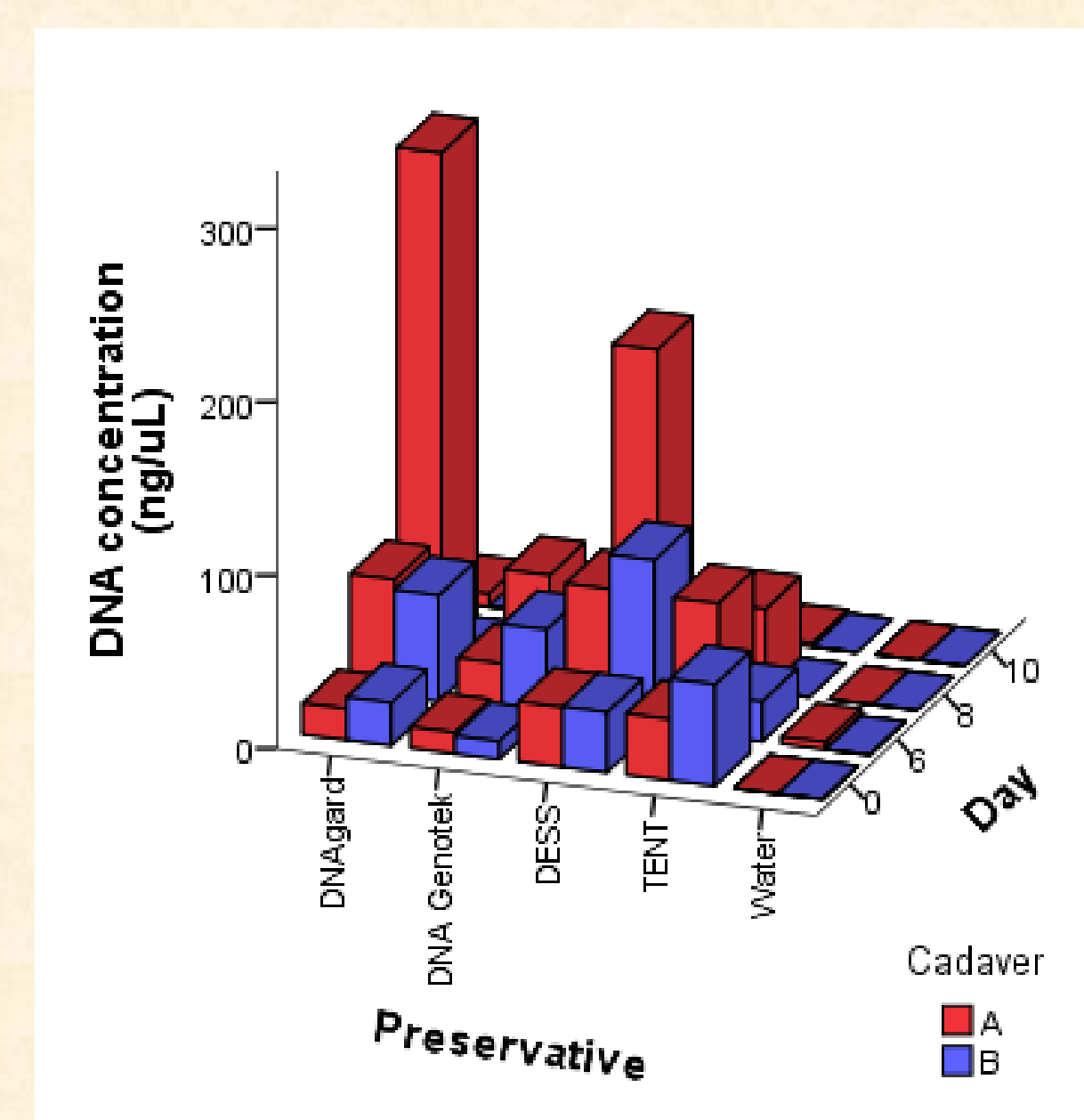
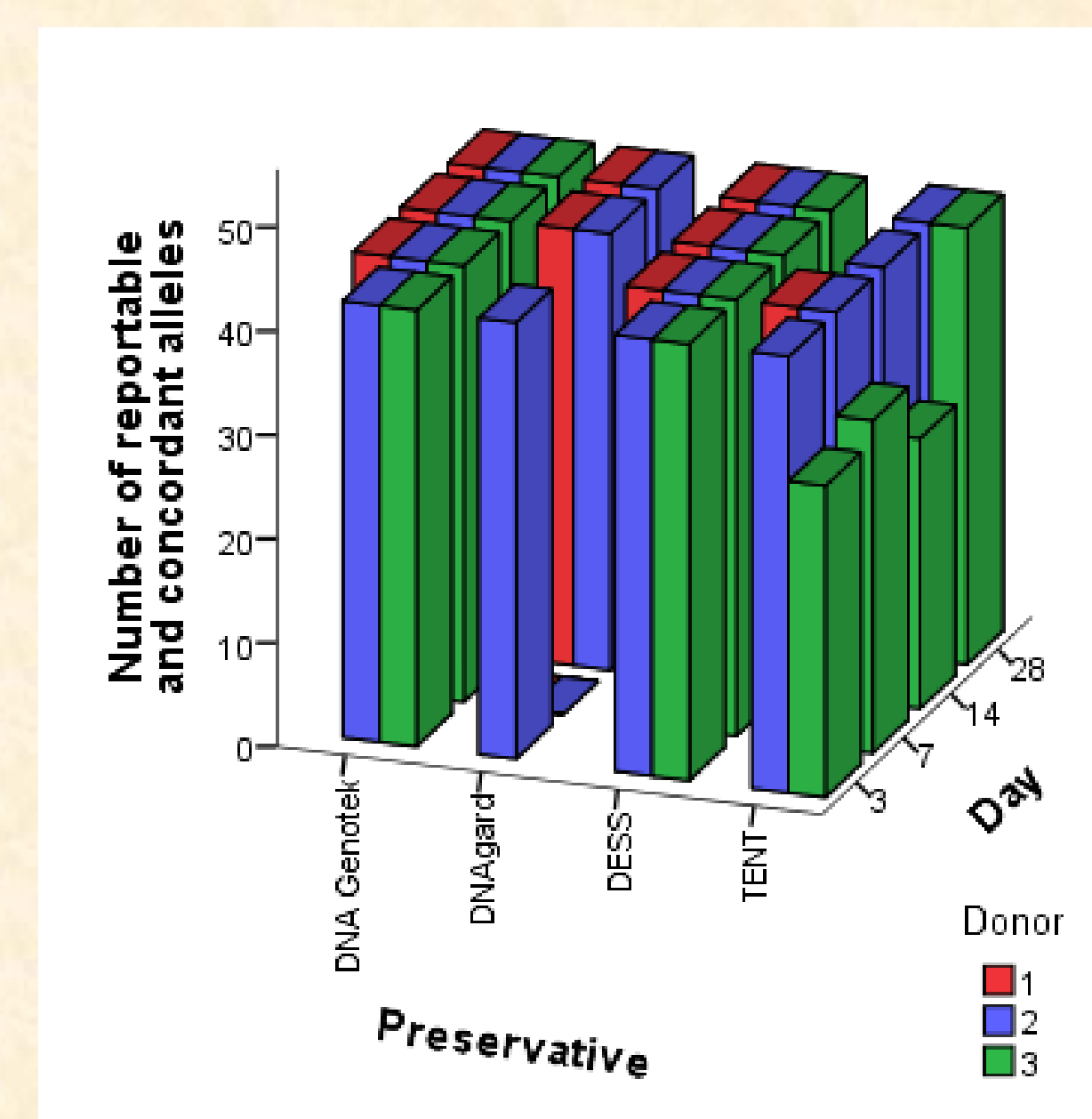
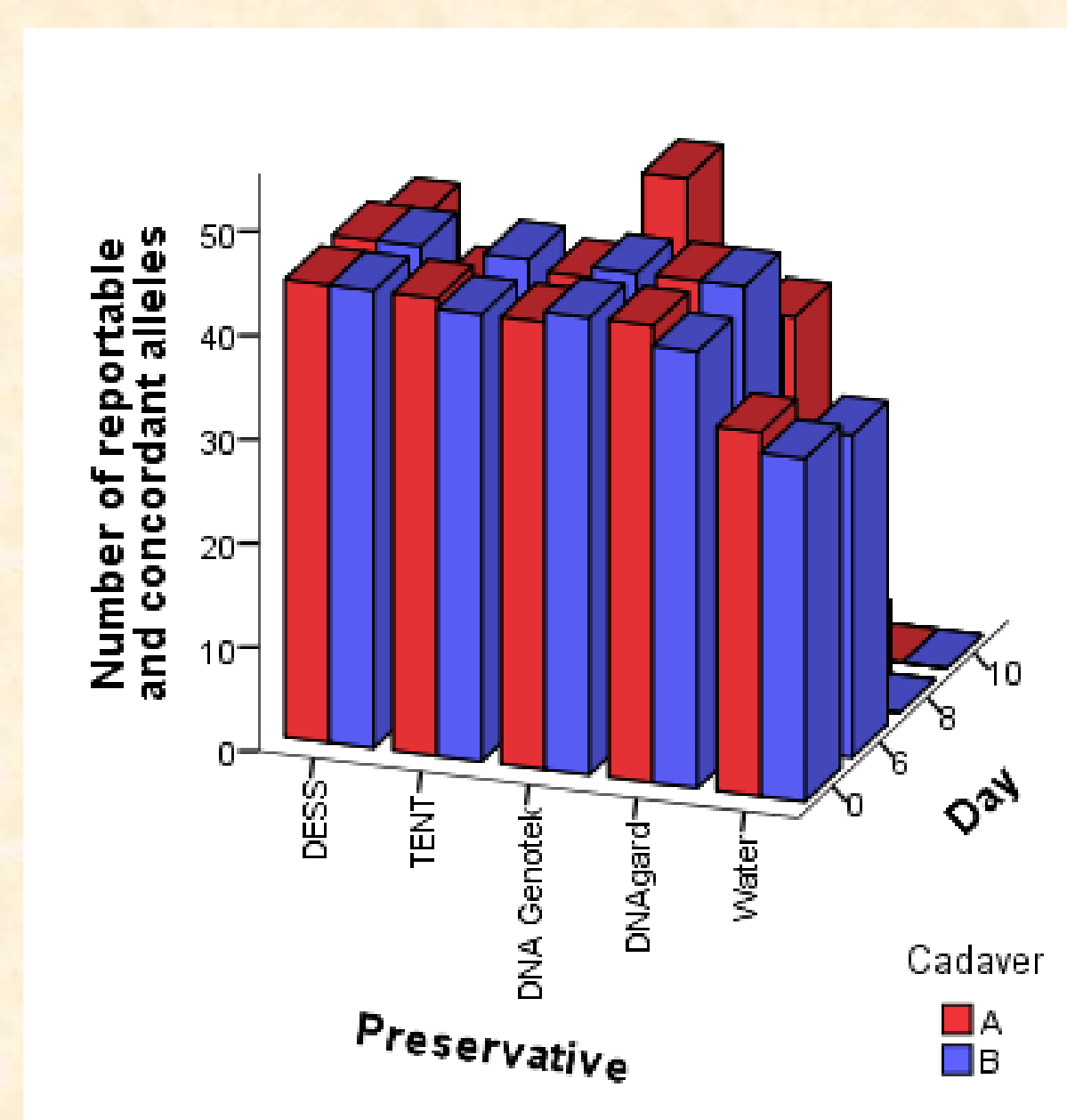


Figure 1 DNA concentrations (adjusted for dilution) in aliquots of each of four preservative solutions and water surrounding muscle tissue stored at -80 °C for four years (left) and tissue from decomposing cadavers stored for one month (middle and right). The preservative solutions were added directly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

Muscle tissue stored at -80 °C for four years



Muscle tissue from decomposed cadavers



Skin tissue from decomposed cadavers

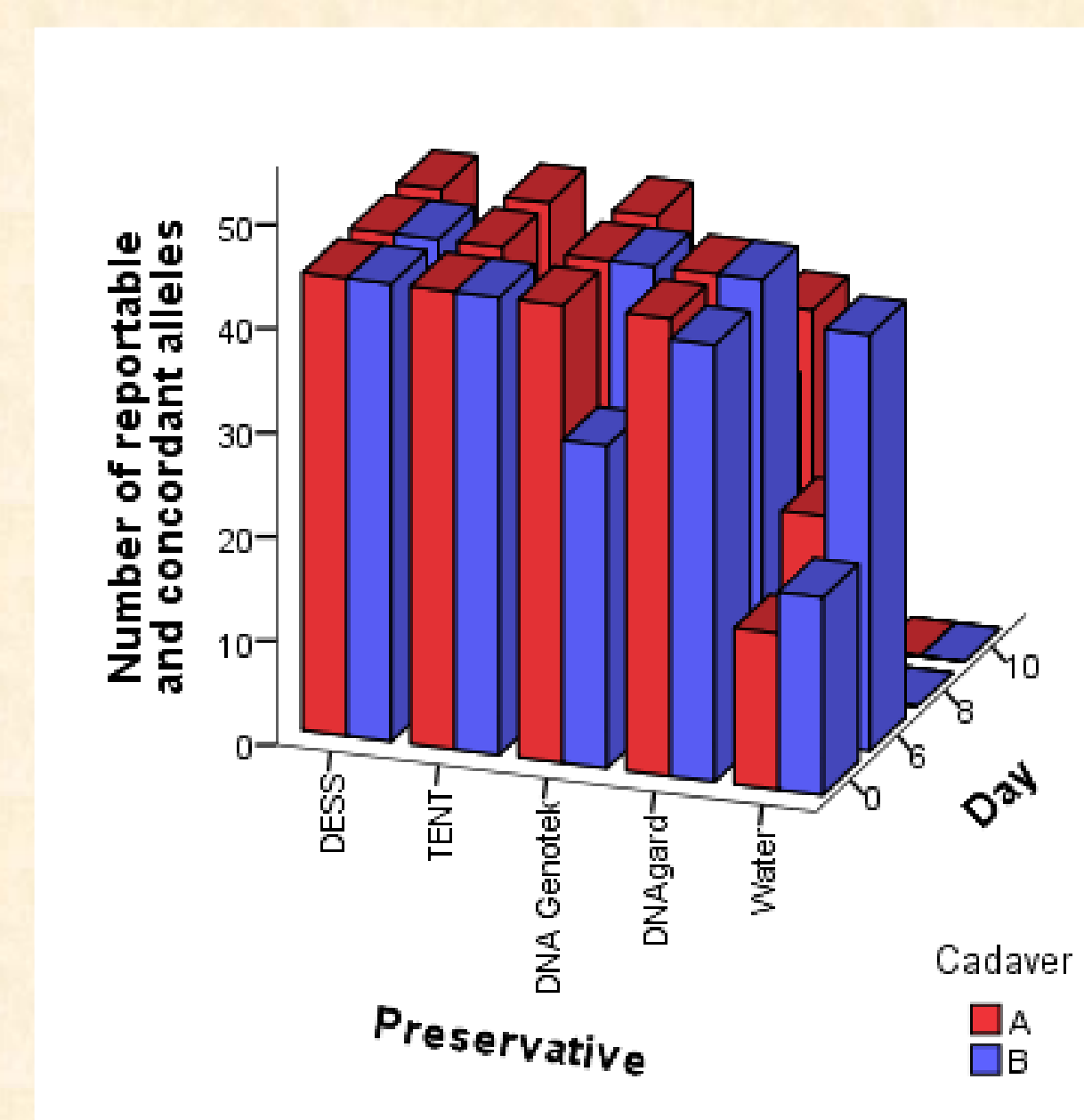


Figure 2 Number of reportable alleles in STR genotypes from aliquots of each of four preservative solutions and water surrounding muscle tissue stored at -80 °C for four years (left) and tissue from decomposing cadavers stored for one month (middle and right). The preservative solutions were added directly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