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DNA analysis of degraded materials using different extraction methods

A thesis

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Abstract

This project was designed and developed in response to the need to improve the methodology employed in the DNA extraction of the Kuwaiti victims of the First Gulf War (1990-1991). The main challenges have involved developing the methodology in an attempt to increase the DNA recovery from the skeletal remains and also assess the preservation of DNA.

In order to assess the methodology for DNA extraction and prediction of DNA preservation, 25 samples from femur and humerus of individuals who were killed during the Gulf War, which had not been analysed, were taken for analysis. These were exhumed from five gravesites, three in Iraq and two in Kuwait. All previous attempts to generate DNA profiles from the samples had failed. Therefore, some samples have been sent to three international laboratories; FSS (forensic Science Service), Bode Technology group (an international private DNA analysis laboratories) and ICMP (International Community OF Missing People). Unfortunately, results from those laboratories showed failed and partial profiles.

In the present study, DNA from bone samples were again extracted using five different extraction methods. PCR amplification of the extracts and the real-time quantification results showed that the modification of a silica-based method, using the Qiagen DNeasy® kit, was successful in removing inhibitors that were present in the extracts while other extraction methods failed.

The power of different methods to allow an effective system of triage (sorting of samples based on the likelihood of successful analysis) was examined. Two parameters were assessed: gross morphology and histology with the amount and quality of DNA that was recovered from different samples. Samples examined have displayed varying degrees of change. The samples from Iraqi site generally displayed good morphological and histological preservation. In contrast, the samples from the two sites within Kuwait displayed an almost complete lack of histological features and changes (pitting/cracks) to the surface. The morphological and histological preservation correlated closely with the success rate when extracting DNA from casework samples that were buried in Iraq and Kuwait.

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LIST OF ABBREVIATIONS

aDNA	Ancient DNA
AE	Qiagen elusion buffer
AL	Qiagen lysis buffer
ATL	Qiagen tissue lysis buffer
AW	Qiagen washing buffer
BTA	AB Bone, Tooth, and Adhesives buffer
CE	**C**apillary Electrophoresis
CODIS	Combined DNA Index System
DNA	Deoxyribonucleic acid
DTT	Dithiothretol
EDTA	Ethylene diamine tetra acetic acid
FSS	Forensic Science Service
H&E	Haemotoxlin and Eosin
HVS-1	Hypervariable sequence region 1
HVS-2	Hypervariable sequence region 2

IPC	Internal positive control
mtDNA	Mitochondrial DNA
PCR	Polymerase chain reaction
PK	Proteinase K
RFLP	Restriction fragment length polymorphism
SDS	sodium dodecyl sulphate
SNP	Single nucleotide polymorphism
STR	Short tandem repeats
VNTRs	Variable number tandem repeats

Chapter 1

Introduction and aim of the work