A12 DNA Extraction From Putrefied and/or Skeletonized Human Remains

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After attending this presentation, attendees will be provided with guidelines for accurate, reproducible, and efficient DNA extraction from either putrefied or skeletonized human remains.

This presentation will impact the forensic science community by discussing how DNA extraction from either putrefied or skeletonized human remains recovered in open spaces or in cemetery areas where the period of inhumation, exhumation, and subsequent tumulation in stone niches is regulated by local laws.

Compact bone represents a suitable tissue for DNA extraction even if the applied methodology is complex and requires a correct procedure, including cleansing of the obtained fragments, pulverization, demineralization, phenol-chloroform extraction, and subsequent purification of the DNA on silica columns. The quality of the STR genetic profiles is acceptable and they can be used for forensic purposes without regard to both quality and quantity of the extracted DNA, which as can easily be foreseen, are often low.

The personal identification test and the following comparison between the profile obtained and that of close relatives is requested by the Judicial Authority in the case where the mortal remains found are compatible with a missing person record filed or in the case of a request of parent attribution regarding a deceased person. Though DNA extraction can be carried out in any body region, it is recommended to perform it from compact bone tissue when in the presence of postmortem degenerative phenomena.

In this study, the human remains made available from the Judicial Authority were found in a wade range of conditions, from the conservation point of view, due to the different kind and time of exposition to biotic and abiotic factors. They were recovered in open spaces or in cemetery areas where the period of inhumation, exhumation, and subsequent tumulation in stone niches is regulated by local laws. Some of them were also recovered in cemeteries where the dead are buried in zinc coffins. The operations which made their exposition possible were the following: extumulation from a zinc coffin; removal from cemetery niche following exhumation; exhumation after inhumation in a wooden coffin; and recovery in open spaces after death.

DNA extraction from bone fragments can be obtained with several methods, depending on the conditions of the human remains. In the case under examination, the extraction methodology was complex, due to the fact that DNA had to be extracted from corpses which were undergoing putrefaction and/or were reaching the stage of skeletonization. A fragment of femoral diaphysis of approximately 4.0 cm was fixed in alcohol and subsequently deprived of the muscles and the inner trabecular structure (where present) before being rinsed with water – alcohol – ether and later pulverized and demineralized.

Pulverization was carried out with steel balls. Demineralization of 0.5 g of bone powder was obtained by means of a 0.5 M EDTA solution with a pH of 8.0. Purification after phenol-chloroform extraction was achieved with silica gel columns.

The quality of the DNA extracted was assessed via 2% P/V agarose gel electrophoresis run, in the presence of ethidium bromide.

The quantity of the DNA contained in the extracts was determined with the REAL-TIME PCR technique.

The individual profiles were obtained with STRs multiplex amplification followed by separation using capillary electrophoresis.

Statistical processing of the results obtained has shown that, without regard to the state of degradation of the specimen, it is possible to extract an individual profile. The methodology proposed here is also useful for personal and criminological identification on human remains in a bad state of preservation.

DNA, Extraction, Human Remains

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