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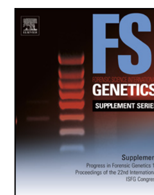

Factors influencing the reliability of DNA typing results for bone samples

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ABSTRACT

The quality and reliability of DNA typing results produced by research and forensic laboratories are limited by the amount and condition of the bone samples processed and are influenced by the applied laboratory practice(s). The chance of false-negative or false-positive identification results increases with longer post-mortem intervals and is due to the different environmental factors and common laboratory errors.

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1. Introduction

DNA-based identification of skeletal remains can be used in different cases involving forensic identification of bone samples from plane crashes [1], terrorist attacks [2], archeological artefacts, war victims [3–5], ship wrecks [6], or slave burial grounds [7] or involving identification efforts for royal family members [8], war criminals [9] or patron saints [10]. DNA analysis of bone samples is frequently used in standard casework of forensic DNA laboratories [11,12]. We have to remember that bone samples are considered to be among the most difficult forensic samples used for DNA identification; therefore, these samples have a relatively high risk of false-positive or false-negative results. Some of the root causes of unreliable results are discussed below.

2. Discussion

Excavated skeletal remains are very often analyzed first by the archaeologists and anthropologists followed by DNA analysis at the very end of the examination process. However, excavated bones have been demonstrated to be the best source of DNA immediately after excavation [13], and any additional handling of the specimen has been shown to increase contamination with recent DNA.

Wrong sample selection can be the root cause of false-negative results. Femur and teeth are the best sources for DNA typing [14,15], and recent findings suggest using petrous bone as a source of relatively intact DNA [16]. However, Mundorff and Davoren reported that small cancellous bones have much higher amounts of DNA per unit mass on average than do dense cortical bones [17].

DNA extracted from bone specimen should contain the DNA profile of one person only. Improper cleaning of the specimen before extraction [18] can cause the detection of a mixed DNA profile [19]. A frequently used cleaning method for the removal of contaminating DNA employs bleach (sodium hypochlorite). The practice of using bleach differs from protocol to protocol. For example, Kemp and Smith reported that the elimination of surface contamination from bone requires immersion in at least 3.0% (w/v) sodium hypochlorite for at least 15 min. Endogenous DNA has been shown to be quite stable even under extreme sodium hypochlorite treatments (6% for 21 h), suggesting that DNA adsorbs to hydroxyapatite in the bone; this process facilitates the preservation of DNA in ancient skeletal remains [18]. Other protocols suggest much shorter bleach exposure periods [20]. However, the use of bleach has also been evaluated as the worst maceration technique in terms of both bone quality and subsequent DNA purity, as too little nuclear DNA was extracted for amplification [21]; thus, the appropriate bleach exposure period should be tested on a particular bone set (excavation site) to prevent false-negative results caused by total DNA degradation.

False-negative results can also be obtained if the laboratory uses a DNA extraction protocol that does not provide the expected yield of usable (amplifiable) DNA. The optimization of a standard bone protocol [22] can provide higher DNA yield for short DNA fragments [23]. Another frequent problem is associated with PCR inhibitors that are co-extracted together with DNA. Specifically, the

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standard procedures used for PCR inhibitor removal are not often efficient. Chemically defining organic or inorganic inhibitors to be able to find an appropriate method for their removal is not a standard procedure [24].

Extracted DNA must be processed using an appropriate amplification protocol that reflects the size of the DNA fragments [20,25,26]. Massive parallel sequencing enables the processing of DNA samples that are degraded to a level that does not allow standard amplification [27].

DNA extracted from decomposed human remains frequently contains fragmented human DNA and microbial DNA. Human DNA-specific extraction techniques are not available, particularly for very low quantities of DNA; thus, the presence of microbial DNA in extracts is unavoidable. Some widely used human forensic multiplexes have the ability to amplify various microbial DNAs, thus generating non-specific PCR products [11] that appear in true locus positions.

Other problems arise when the laboratory needs to statistically evaluate the results of the DNA analysis for a person from a population whose population data have not been published and are not accessible by other means. This problem is common for all older burial sites where proper population data do not exist [28] but where kinship analysis is required. Such results can be biased due to the kinship index variation between populations [29] or the use of improper population data.

3. Conclusion

The evaluation of Y-haplotypes, mtDNA sequences and autosomal STR profiles derived from aged bone samples must be performed very carefully to avoid false-positive or false-negative results.

Conflict of interest

The authors of this manuscript have no conflict of interest. The work on this project was supported by the Czech Science Foundation (grant no. 14-36938G).

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