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The role of DNA technology in identification of skeletal remains discovered in mass graves

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The identification of human remains found in mass graves employs different methods: identification of remains by a living person who knew the decedent by direct facial recognition, fingerprint analysis, dentition analysis, identification of special features such as individual scars or tattoos, recognition of clothing and belongings, autopsy findings, the analysis of skeletal remains by forensic anthropologists to estimate the species of the remains, sex, age, race, stature and length of time since death, reconstruction of facial features from skulls by forensic anthropologists or sculptors, hair comparisons and DNA analysis. Successful identification, which depends on human factors, mostly relies on the source of available information and the experience of the forensic team members.

For more than 10 years Croatia has been dealing with laborious identification process of human remains found in mass graves during and after the war [1]. Though the majority of identifications had been achieved by standard forensic methods, there were still many cases that needed to undergo DNA analysis, due to lack of ante mortem data and body decomposition. Degradation and contamination of DNA extracted from bone and teeth samples were often obstacles for PCR and that made the entire process more difficult [2]. DNA laboratories experimented with different techniques in an attempt to overcome the problems encountered. Techniques included decalcification, repurification, testing new DNA extraction, DNA quantitation procedures and validation of different amplification systems. DNA technology used in Croatia for identification purposes so far include different systems such as: AmpliType HLA DQA1 and PM PCR amplification and typing kit, AmpliFLP D1S80 PCR amplification kit, AmpFlSTR Profiler PCR amplification kit, AmpFlSTR Profiler Plus PCR amplification kit, Power Plex 16 system (European STR's validation study), AmpFLSTR® Identifiler™ PCR amplification kit, Y-PLEX™5, immobilized sequence-specific oligonucleotide probe analysis of mtDNA hypervariable regions I and II and sequencing of mtDNA [3–5].

While working with bones forensic scientists are confronted with many problems. If amplification fails, scientist must determine: is there enough DNA, what is the level of DNA degradation and what is the amount of potential PCR inhibitors. Amplification of genomic DNA is often affected by the influence of a heavy metals and humic acids. The most common heavy metals found with the bone samples from mass graves are iron, copper, cadmium, lead. For nuclear human DNA quantification in casework slot–blot hybridization/chemiluminescent detection (Quantiblot™) and single-tube hybridization/luciferase detection (AluQuant™) can be used. Non-detectable human DNA as revealed by Quantiblot™ does not mean absence of human DNA. High amounts of non-human DNA can interfere with the specific hybridization of human sequences in a slot–blot format. In practice the main limitation of hybridization methods is the lack of sensitivity to detect LCN samples (above the limit of the PCR profiling methods).

In some cases NaOH repurification treatment is used to overcome potential inhibitors of Taq Polymerase when DNA fails to amplify. However, the NaOH protocol is not advised when the quantity of DNA is limited, since the treatment results in significant loss of DNA. DNA IQ™ is the intelligent way to purify DNA and this system employs a novel technology with magnetic particles designed to easily and efficiently eliminate PCR inhibitors.

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AluQuant™ Human DNA quantitation system is used to determine the precise amount of DNA. It has a solution-based hybridization format that is unaffected by degraded DNA. This system uses a luciferase reaction to give a light output that is read by a luminometer, giving a numeric readout that allows the human DNA in a sample to be easily calculated.

Real-time PCR is relatively new technology that provides more accurate quantitation. Real-time PCR monitors the accumulation of PCR product while amplification is occurring. It analyzes cycle to cycle changes in a fluorescent signal generated during the three phases of PCR. The fewer cycles needed to detect the fluorescence signal, the greater amount of DNA is present.

With the 10-year experience of the identification process, it is concluded that the success of amplification of DNA extracted from skeletal remains depends on:

(1) DNA extraction methods (modified phenol/chloroform extraction procedure is highly recommended as well as modified DNA IQ procedure);
(2) DNA degradation level (length of time since death, location of mass graves, sample storage procedures, high temperatures, salt, water or soil conditions (heavy metals, acids, bacteria));
(3) the type of skeletal remain analyzed (DNA is better preserved in teeth and long bones than in other bone types such as ribs, skull);
(4) the percentage of enzyme inhibitors per reaction (adding more Taq, reducing the amount of input DNA (effectively diluting the inhibitor) or adding the chemically inert protein BSA, and/or NaOH treatment);
(5) size of STR products (stronger amplification with the smaller size STR products);
(6) DNA concentration (most of the bone samples were amplified from approximately 10–15 pg of DNA);
(7) Appropriate database.

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