



### **B93 Identification of Skeletal Remains From Mass Graves: Ten-Year Experience of Our Work**

*Simun Andjelinovic, MD, PhD, Davorka Sutlovic, BS, Ivana Erceg, MD, Vedrana Skaro, BS, Ante Ivkovic, MD, Boja Rezic, Marija*

*Definis-Gojanovic, MD, PhD, and Dragan Primorac, MD, PhD\*, Split University Hospital, Laboratory for Clinical and Forensic Genetics, Spinciceva 1, Split, Croatia*

Significant efforts are currently underway to identify missing individuals discovered in mass graves situated throughout Croatia and southern Bosnia and Herzegovina.

By the end of 1992, more than 15,000 persons were missing in the Republic of Croatia as a result of the war. According to the authors' records, 9,000 persons were killed. To date, 3,353 bodies have been exhumed and 2,745 bodies identified. However, another 608 bodies have to be identified.

During the last ten years, more than 900 bodies found in several mass graves in Croatia, Bosnia, and Herzegovina have been identified by the Laboratory for Clinical and Forensic Genetics using standard forensic methods. Unfortunately, common methods for human identification, like direct facial recognition or recognition of special features, such as scars or marks, matching of fingerprints, dentition, and detailed examination of the clothing and belongings of the dead, were not sufficient in approximately 30-35% of all cases and DNA identification was requested. The ability to analyse trace amounts of human DNA from old teeth and bone samples offers the opportunity to identify unknown skeletal remains by a comparative genetic analysis with their presumptive relatives. However, DNA degradation and DNA contamination are encountered frequently with DNA extracted from bone and teeth samples recovered from mass graves. Furthermore, the quality of DNA obtained from femur and teeth was higher than that obtained from other types of bone samples. DNA isolation was performed, in addition to some advanced methods, using standard phenol/chloroform/isoamyl alcohol procedure. Some samples that failed to give results after a second phenol/chloroform/isoamyl alcohol extraction were subjected to additional procedures such as decalcification method with EDTA (ethylenediamine-tetraacetic acid) prior to extraction of DNA and a NaOH repurification method. Recently, new procedures for DNA extraction (DNA IQ System) and DNA quantitation (AluQuant Human DNA Quantitation System) were successfully tested in the laboratory.

During the last ten years, the following DNA identification systems were used: AmpliType<sup>®</sup> PM+DQA1 PCR Amplification and Typing Kit, AmpFISTR Profiler<sup>®</sup> PCR Amplification Kit, AmpFISTR Profiler Plus<sup>®</sup> PCR Amplification Kit, PowerPlex<sup>®</sup> 16 System, AmpFISTR Identifier<sup>®</sup> PCR Amplification Kit, immobilized SSO (sequence-specific oligonucleotide) probes for the mitochondrial DNA control region, and Y-Plex<sup>®</sup> 6.

At the beginning of the identification process, AmpliType<sup>®</sup> PM+DQA1 PCR Amplification and Typing Kit was used; however, it proved unsuccessful in 75% of all cases. Common problems with this kit were either amplification difficulties or nonspecific hybridization that caused ubiquitous data. At the current time, two multiplex short tandem repeats systems (PowerPlex<sup>®</sup> 16 System and AmpFISTR Identifier<sup>®</sup> PCR Amplification Kit) are being used which amplify 16 loci including all CODIS core loci in a single reaction with great success. Up to date, 406 samples have been analyzed by DNA technology and obtained full genotypes in 355 samples (87%) with DNA matches confirmed in 55 cases.

#### **Identification, Mass Grave, STR**