TSUNAMI-Disaster:
DNA typing of Sri Lanka victim samples and related AM matching procedures

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1. Introduction

In Sri Lanka more than 38.000 individuals of varied racial and ethnic background lost their lives when the island was affected by the South Asian tsunami in December 2004. Among the victims were 80 foreigners from 17 countries. Disaster victim identification (DVI) teams from many countries were involved in examining victims and taking DNA samples from victims for identification procedures. The principal role of our laboratory in this process was to provide bar-coded sampling kits, to perform DNA typing of human remains and ante mortem/kinship samples and to accomplish DNA matching services to support DNA based identifications.

2. DNA Sampling

We developed a sampling kit [1] which contained two sterile swabs devices, two bar-coded sterile 1.5 ml reaction tubes, two extra barcodes and a pair of gloves (**Fig. 1**). The two extra barcodes were used for labeling the PM identification form and additional sample containers. The numbering system [2] used on the barcodes had a unique 8-digit number.

300 bar-coded sampling kits were dispatched from us to Colombo before the DVI teams started examining the victims. One of the advantages of our sampling kit was that the prefabricated barcode numbering system could be used on the identification form to introduce safe sample tracking from the very beginning to the final evaluation of the DNA results, as we were using the identifier as sample information in the entire laboratory process.

According to our recommendations the DVI teams collected two swabs from intact inner surfaces like muscle (**Fig. 2**), urinary bladder etc., one 4 cm piece of long bone and two teeth per examined victim if possible. More than 400 samples related to 101 victims were sent to our laboratory, which were all shipped on ice (**Fig. 3**).

Fig. 1 Bar-coded sampling kit

Fig. 2 Swab collection muscle

Fig. 3 Set of PM victim samples

3. STR analysis procedures

Both swabs collected in each case were analysed in parallel. After Proteinase K digestion the robotic system BioRobot M48 Workstation (Qiagen, Germany) was used for automated DNA extraction [3]. DNA was further quantified by using a real time PCR based method [4]. These data were transferred to a Freedom EVO® 100 (TECAN, Switzerland) robotic instrument to dilute each sample to the appropriate concentration. Amplification was carried out using the AmpFLSTR® Identiler® PCR Amplification Kit (Applied Biosystems, CA, USA) in a DNA Engine Tetrad 2 (Bio-Rad Laboratories, Inc., MA, USA) thermal cycler, according to the manufacturers recommendations and products were loaded on the 3100 Genetic Analyzer (Applied Biosystems, CA, USA). GeneScan® analysis was performed on the raw data, and alleles were designated using the GenoTyper® Software v3.7 (Applied Biosystems, CA, USA) by two independent qualified DNA analysts. The analysed data were imported into our custom-designed laboratory information management system (LIMS) [2], which after comparison and quality checking allowed for electronical transfer of the STR profiles to the database of the Austrian Ministery of the Interior in Vienna. The electronic version of the Interpol PM forms, which were sent to Vienna from Colombo in the meantime, were automatically completed with the STR profiles and then transmitted back to Colombo.

We want to point out that the application of our numbering system enabled a fast and consistent automated data exchange and minimized the risk of sample mix-up and transcription errors.

For the skeletal remains and teeth a highly sensitive DNA extraction procedure was used based on previously published protocols [5, 6] in order to maximise DNA recovery. After removing adhering tissue the samples were cleaned and washed with bleach and ethanol at least twice. A variable speed drill was used to sample the specimens. The bone/tooth powder was decalcified with an initial soak of 0.5 mol/L EDTA solution and digestion achieved by a Proteinase K digestion step. The samples were purified with phenol-chloroform and filtrated with Centricon 100 devices. The following steps were performed as described above.

4. STR analysis results

The established DNA typing procedures proved to be highly efficient (**Table 1**). The swab typing procedure produced successful results in 42% of the completed cases (n=91). For the remaining cases skeletal remains had to be typed via the sensitive DNA extraction procedure and produced useful results in all investigated cases (**Fig. 4**), obtaining 34% full profiles (16 loci) and 66% partial profiles (>=7 STR loci). In ten cases the DNA investigations were stopped due to successful dental identifications.

Type of samples	n	16 Loci	7-15 Loci	< 7 Loci
Swabs	185	12 %	20 %	68 %
Teeth	42	10 %	55 %	35 %
Bone	34	59 %	35 %	6 %

Table 1 Success rates of PM DNA analyses

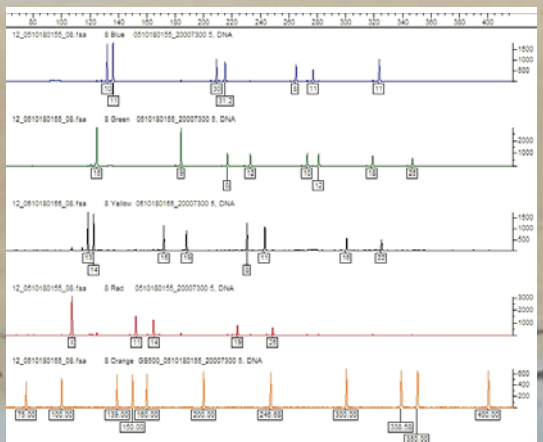

Fig. 4 STR profile bone (16 loci)

5. DNA matching procedures

AM DNA profiles related to missing persons from Sri Lanka and Austria were generated in our laboratory, AM DNA profiles from other countries were transmitted via Interpol or directly sent to our laboratory for matching purposes. DNA matching procedures were facilitated by using the Mass Disaster Matches option of Charles Brenners DNAVIEW software [7].

Until now, the DNA matching procedures enabled 24 DNA based identifications of victims from nine countries.

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