



# Tsunami-disaster: DNA typing of Sri Lanka victim samples and related AM matching procedures

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**Abstract.** More than 400 samples related to 101 tsunami victims from Sri Lanka were sent to our laboratory. Bar-coded sampling kits were used to collect two swabs from intact inner surfaces, one 4-cm piece of long bone and two teeth, which were all shipped on ice. A high throughput DNA extraction and STR analysis procedure for the swabs were developed in order to provide STR profiles within 24 h. For the skeletal remains and teeth a highly sensitive DNA extraction procedure was applied. Both processes included electronic accessioning to maintain the numbering system of the DVI team in Sri Lanka and the data exchange via Interpol. Swab profiling was successful in 42% of the cases. For the remaining cases skeletal remains had to be typed and produced useful results in 65% for teeth and in 94% for bone. A sufficient profile was obtained from each victim, so the overall success rate was 100%. Until now DNA matching procedures in Innsbruck facilitated by using DNAVIEW software enabled 24 DNA-based identifications of victims from nine countries. © 2005 Elsevier B.V. All rights reserved.

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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. 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 aid the identification process by providing bar-coded sampling kits, by performing DNA typing of the human remains and ante mortem/kinship samples and by accomplishing DNA matching services to support DNA based identifications.

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## **2. DNA sampling**

We developed a sampling kit [1] which can be used for the collection of both post mortem (PM) samples from victims and ante mortem (AM) samples (from direct reference material or from relatives). The kit contained two sterile swabs devices, two bar-coded sterile 1.5 ml reaction tubes filled with 1.4 ml absolute 2-propanol in which the swab devices are transported and stored, two extra barcodes and a pair of gloves. 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 and was printed using the 2 of 5 interleaved barcode technology.

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, urinary bladder or other intact organs, one 4-cm piece of long bone and two teeth per examined victim if available. More than 400 samples related to 101 victims were sent to our laboratory were all shipped on ice.

## **3. STR analysis procedures**

A high throughput DNA extraction and STR analysis procedure were developed in order to provide STR profiles from the tissue swabs within 24 h.

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® Identifier® 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 electronic transfer of the STR profiles to the database of the Austrian Ministry 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

Table 1  
Success rates of PM DNA analyses

Types of samples	<i>n</i>	16 loci	7–15 loci	<7 loci
Swabs	185	12%	20%	68%
Teeth	42	10%	55%	35%
Bone	34	59%	35%	6%

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, obtaining 34% full profiles (16 loci) and 66% partial profiles ( $\geq 7$  STR loci). In ten cases the DNA investigations were stopped due to successful dental identifications.

#### 5. DNA matching procedures

AM DNA profiles related to missing persons from Sri Lanka and Austria were generated in our laboratory; AM DNA profiles related to missing persons 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 Brenner's DNAVIEW software [7].

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

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