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The importance of Guthrie cards and other medical samples for the direct matching of disaster victims using DNA profiling☆

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ABSTRACT

The identification of disaster victims through the use of DNA analysis is an integral part of any Disaster Victim Identification (DVI) response, regardless of the scale and nature of the disaster. As part of the DVI response to the 2009 Victorian Bushfires Disaster, DNA analysis was performed to assist in the identification of victims through kinship (familial matching to relatives) or direct (self source sample) matching of DNA profiles. Although most of the DNA identifications achieved were to reference samples from relatives, there were a number of DNA identifications (12) made through direct matching. Guthrie cards, which have been collected in Australia over the past 30 years, were used to provide direct reference samples. Of the 236 ante-mortem (AM) samples received, 21 were Guthrie cards and one was a biopsy specimen; all yielding complete DNA profiles when genotyped. This publication describes the use of such Biobanks and medical specimens as a sample source for the recovery of good quality DNA for comparisons to post-mortem (PM) samples.

1. Introduction

On Saturday the 7th of February 2009, Victoria experienced a destructive firestorm that devastated 4500 km^2 of the state including bushland and townships [1]. Over the next few of days, it was evident that the fires had destroyed several thousand structures (homes and businesses) and affected many different localities. The 2009 Victorian Bushfires Disaster was Australia's worst natural disaster; as a consequence 173 individuals died, with thousands injured or left homeless.

In August 2008, the Biology Specialist Advisory Group (BSAG) of the Senior Managers of Australia and New Zealand Forensic Science Laboratories (SMANZFL) supported the recommendations made by the DNA Commission of the International Society for Forensic Genetics (ISFG) for DNA laboratories supporting Disaster Victim Identification (DVI) operations [2,3]. As part of the recommendations for ante-mortem (AM) sample collection, it was advised that direct reference samples should be sought in addition to family reference samples. These commonly include items such as toothbrushes, razors, or hairbrushes. However, the use of such items in the DNA identification process can lead to misidentifications [4], as some of these items are often shared – thus resulting in the recovery of DNA profiles from contaminating sources (other users) [5]. Other types of direct reference samples that are less likely to result in misidentification include Guthrie cards – blood cards from phenylketonuria (PKU) newborn screening programs – or medical specimens such as paraffin embedded biopsy specimens.

Australia has conducted PKU screening of newborns for the last 30 years, with the blood sample obtained by a heel prick, and placed on special pre-printed filter paper (Guthrie card). In Victoria, Guthrie cards are medical records that are securely stored in compliance with government regulations at Genetic Health Services Victoria. In the event of a disaster, the State Coroners can request the Guthrie cards be made available for the purposes of identification using DNA testing. As part of Phase 3 of the DVI response for the 2009 Victorian Bushfires Disaster, AM data was used to identify victims who, based on their age, should have Guthrie cards available for a direct reference sample. Of the 163 victims, 34 were identified as being of the appropriate age for having undergone PKU screening as infants; Guthrie cards were therefore sought for these individuals under a Coroner's directive, with 21 Guthrie cards received for analysis.

DNA analysis undertaken as part of the DVI response assisted in the identification of 67 DVI cases. This communication details those cases for which Guthrie cards were used to assist in the identification of human remains. In addition, the importance of medical samples, such as biopsy specimens, as direct reference samples is also presented.

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2. Materials and methods

2.1. Samples

The details of all samples received into the laboratory were recorded and cross referenced with PM and AM information available for this DVI. PM samples (blood stains, tissues, or bones) and AM samples (buccal swabs, Guthrie cards, or biopsy specimen) were received into the DNA laboratory and stored at the appropriate temperature (bones and tissue samples stored at −20 °C; with all other sample types stored at 4 °C) until DNA extraction. Guthrie cards and the biopsy specimen were obtained under a Coroner's order for the purpose of DNA testing for the identification of victims of the 2009 Victorian Bushfires Disaster. Details of the relationship between the Victorian Institute of Forensic Medicine (VIFM) and Victoria Police Forensic Services Department (VPFSD) for the processing of DNA samples in DVI events have been described elsewhere ([6] in this issue).

2.2. DNA extractions

For bone and tissue specimens, approximately 100 mg of bone powder or 100 mg of tissue slices were placed into 1.6 mL Eppendorf tubes (Pathtech). For blood stains or Guthrie cards, two or three pieces (5 mm × 3 mm) from each specimen were placed into 1.6 mL Eppendorf tubes. All samples were extracted using the QIAamp DNA Investigator Kit (Qiagen) under conditions specified by the manufacturer. In brief, samples were incubated with Buffer ATL (tissue lysis buffer – 360 μL for bones, or 300 μL for other specimen types) and 20 μL 20 mg/mL Proteinase K (Qiagen) at 56 °C in a rotating oven, or Eppendorf thermomixer (9000 rpm), for at least 1 h or until lysis had occurred (tissue samples). Following this incubation, DNA was extracted using the QIAamp kit on the QIAcube (Qiagen) liquid handling robot under conditions specified by the manufacturer. DNA was eluted in 100 μL Buffer ATE (elution buffer) – and samples stored at 4 °C until analysis.

For the biopsy specimen received, there were four 2 μm sections cut from the tissue blocks, and excess paraffin was removed from the sections prior to DNA extraction under conditions specified by the manufacturer. In brief, sections were incubated with 150 μL Buffer ATL and 100 μL 20 mg/mL Proteinase K at 56 °C in an Eppendorf thermomixer (9000 rpm), or rotation oven, overnight for 16 h. Following this incubation, DNA was extracted using the QIAamp kit (manual extraction) and DNA was eluted in 100 μL Buffer ATE and sample stored at 4 °C until analysis.

2.3. DNA quantification

DNA quantification was undertaken on 2 μL of each extracted sample using the human DNA quantification kit Quantifiler™ (Applied Biosystems) under conditions specified by the manufacturer. Amplification was performed on a 7500 Real-Time PCR system (Applied Biosystems) under conditions specified by the manufacturer.

2.4. DNA profiling

DNA profiling was performed using the AmpFlSTR Profiler Plus™ (Applied Biosystems) in a final reaction volume of 12.5 μL with approximately 1 ng of DNA template added to each reaction (where possible). Amplification was performed on a 9600 or 9700 PCR Thermocycler (Applied Biosystems) under cycle conditions specified by the manufacturer.

Amplification products were separated and visualised on a 3100 or 3130xl Genetic Analyser (Applied Biosystems), with allele designation performed using GeneMapper ID software version 3.2 (Applied Biosystems).

2.5. Matching of DNA profiles

Comparisons of DNA profiles obtained for Guthrie cards and the biopsy specimen to DNA profiles obtained for PM samples were undertaken to look for direct (self-to-self) and/or kinship (victim-to-relative) matches.

Direct matching of DNA profiles was performed using the NCIDD (National Criminal Investigation DNA Database) software version 4.4.0 (CrimTrac, Australia), whilst kinship matching of DNA profiles was carried out using CODIS 6.0 software (Federal Bureau of Investigation (FBI) supplied by CrimTrac, Australia) using pedigree trees, and shared allele search options. Both pieces of software were made available to VIFM on stand-alone PCs to ensure the integrity and privacy of the data.

All matches (direct or kinship) were cross referenced with PM and AM information available for this DVI, as well as PlassData (DVI information management system) matches provided by the DVI Reconciliation Centre.

2.6. Statistical analysis

The statistical weightings of the matches were obtained using software compiled by John West, formerly of the Division of Analytical Laboratories, NSW, for the Northern Territory, using population statistics for an Australian Caucasian sub-population [7]. The program provides conditional probability calculations, which account for population substructure by means of using theta parameters that measure population differentiation/substructure, as recommended by the second National Research Council report on forensic DNA evidence.

3. Results

3.1. DNA profiling

DNA was successfully extracted from the 21 Guthrie cards and the biopsy specimen received for analysis (Table 1). DNA extracts were analysed using Profiler Plus™ generating full DNA profiles for all the AM samples (Table 1).

PM samples corresponding to 120 DVI cases were received for DNA analysis. Of these, 7 were not extracted as identification was achieved using another primary identifier. For the remaining 113 DVI cases, DNA was successfully extracted (data not shown). These were subsequently analysed using Profiler Plus™, with close to 90% of the 113 DVI cases producing full DNA profiles (Table 2).

Table 1
Quantification and DNA profiling results obtained for the AM samples.

Sample	Quantifiler results			Profiler Plus™ results	
	HUM	IPC	DNA concentration (ng/μL)	Autosomal STRs	Amelogenin
Guthrie card 1	31.92	27.03	0.09	✓	Male
Guthrie card 2	29.42	27.06	0.47	✓	Male
Guthrie card 3	27.34	26.91	1.74	✓	Female
Guthrie card 4	28.65	27.07	0.69	✓	Female
Guthrie card 5	28.80	27.10	0.62	✓	Male
Guthrie card 6	28.53	26.93	0.94	✓	Male
Guthrie card 7	27.49	26.84	1.88	✓	Female
Guthrie card 8	28.27	26.96	1.06	✓	Female
Guthrie card 9	29.42	27.12	0.40	✓	Female
Guthrie card 10	28.48	26.92	0.92	✓	Female
Guthrie card 11	28.38	26.83	0.83	✓	Male
Guthrie card 12	27.61	26.76	1.69	✓	Male
Guthrie card 13	29.14	27.01	0.48	✓	Male
Guthrie card 14	29.29	27.06	0.43	✓	Male
Guthrie card 15	28.84	27.12	0.60	✓	Female
Guthrie card 16	29.10	26.95	0.59	✓	Female
Guthrie card 17	27.31	26.73	2.10	✓	Male
Guthrie card 18	27.58	26.92	1.67	✓	Female
Guthrie card 19	29.29	27.14	0.43	✓	Female
Guthrie card 20	29.22	27.06	0.46	✓	Female
Guthrie card 21	27.88	26.84	1.19	✓	Male
Biopsy specimen	28.48	26.90	0.80	✓	Female

✓ denotes complete DNA profile 9 autosomal loci.