THE WINSTON CHURCHILL MEMORIAL TRUST OF AUSTRALIA

Dr Jodie Ward

2015 Churchill Fellow

To Investigate Specialist DNA Techniques for the Identification of Compromised Human Remains

Italy, Bosnia & Herzegovina, France, The Netherlands, United States of America, Argentina

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Signed: Dated: 24 October 2016

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Executive Summary

Name: Dr Jodie Ward

Position: Team Leader – Specialist DNA Laboratory, NSW Forensic & Analytical Science Service

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Project Description:

This Fellowship investigated: 1) Specialist DNA techniques and massively parallel sequencing (MPS) applications for the identification of compromised human remains; and 2) Exemplar DNA-led identification programs for criminal, coronial and disaster victim identification (DVI) casework to inform recommendations for the establishment of an Australian DNA identification program.

Fellowship Highlights:

1) Attending the IALM Symposium and hearing of the future of forensic genetics by leaders in this field, and participating in workshops on identifying victims from mass disasters, terrorist attacks and armed conflicts.

2) Spending a week at the ICMP in Bosnia and Herzegovina to experience the standard operating procedures that have enabled them to develop the world's largest missing persons (MP) DNA identification program.

3) Learning of INTERPOL's proposed international MP DNA database and their recommended international standards for the establishment of a DNA-assisted MP program.

4) Hearing of NFI's experience conducting the large-scale DNA-led identification of victims of the Malaysia Airlines flight MH17 crash and the new sampling procedures which evolved during this effort.

5) Discussing how Prof. de Knijff has pioneered the application of MPS to forensic casework in Europe.

6) Sharing research ideas on improving the DNA recovery from compromised human remains at SHSU.

7) Attending the FBI-hosted SWGDAM meeting, which focussed on MPS applications for forensic casework.

8) Visiting DNA laboratories in the USA and Argentina which are dedicated to the DNA-led identification of MP and unidentified human remains (UHR) resulting from criminal matters, war, migration or human rights abuses; and observing the specialist DNA technologies they have implemented to achieve this.

Recommendations:

1) The establishment of an Australian DNA identification program should be made a national priority, be coordinated by the National Missing Persons Coordination Centre (NMPCC), and will require support, funding and convening of expertise from relevant policing, government, legal and forensic agencies.

2) A Missing Persons Centre of Specialisation should be established for dedicated processing of UHR and MP casework in Australia; but in the short-term, DNA testing of backlogged UHR cases could be outsourced to an international DNA laboratory that specialises in MP casework.

3) Government funding will need to be secured to support the DNA identification program.

4) Forensic Pathologist/s, Anthropologist/s and/or Odontologist/s should examine all UHR prior to DNA testing.

5) All metadata for existing UHR and MP cases should be catalogued on the National Missing Persons and Victim System.

6) The NMPCC should facilitate a campaign to obtain reference samples from all long-term MP or their relatives for comparisons with DNA profiles from all UHR.

7) All genetic data (new and existing) should be submitted to the National Criminal Investigation DNA Database (NCIDD) for national DNA matching; and INTERPOL for international DNA matching as appropriate.

8) Direct and kinship matching should be performed using the Bonaparte software integrated in the NCIDD capability and any DNA match reports issued to relevant authorities.

9) Relevant legislation should be reviewed and/or amended to permit testing of UHR, MP or MP relative samples with new DNA technologies to aid identifications.

10) MPS has the potential to decrease DNA testing costs and labour; and increase throughput and the genetic information obtained.

Conclusions:

These recommendations will be disseminated to relevant agencies to inform them of international best practice for utilising DNA to identify missing and unidentified persons. The prioritisation of a national DNA identification program will assist in solving Australia's UHR and MP cases and bringing closure to hundreds of Australian families whom have never had confirmation of the fate of their missing loved ones. Finally, the longer we deliberate about the path forward for these samples, the more degraded they become.

Introduction

"Countries need to acknowledge the importance of identifying the missing and deceased, and provide adequate resources to do this" (INTERPOL, 2015b)

It is estimated that there are 500+ cases of unidentified human remains (UHR) and 1600 long-term missing persons (MP) in Australia (ACIC, 2016; NMPCC, 2016). Currently the cost, labour and success rate of the DNA identification of compromised human remains is prohibitive, resulting in the backlog of identification casework now facing the forensic and law enforcement communities in Australia. However, DNA testing has been used worldwide to successfully identify large numbers of UHR and MP resulting from armed conflicts, human rights abuses, migration, terrorist attacks or mass disasters; and best practice guidelines on using DNA for this purpose have been published by various authorities in the field (AABB, 2010; ICRC, 2009; ICRC, 2013; INTERPOL, 2014; INTERPOL, 2015b; NIJ, 2005; Prinz *et al.*, 2007; SWGDAM, 2014a).

The condition of these skeletal remains vary and some are up to 50 years old, so the age and state of degradation will have an impact on DNA testing success. DNA profiling of compromised human remains will require validation and use of specialised methods; and not all forensic DNA laboratories have the infrastructure, equipment or validated SOPs to perform this type of casework. Alternative DNA technologies may also need to be explored such as massively parallel sequencing (MPS), to both improve and expand upon the specialist DNA testing services for highly compromised remains when routine DNA profiling is unsuccessful. Furthermore, for the majority of these UHR, there is a lack of intelligence information surrounding their possible identity, so exploiting new DNA markers that have been designed to predict a person's ancestry or physical appearance (e.g. skin, eye and hair colour), will aid the identification effort in the absence of other investigative leads. This genetic information could also be used to support or complement the anthropological findings (e.g. sex, ancestry etc.). Per sample, MPS has the potential to decrease the costs and labour involved in large-scale DNA testing efforts, coupled with an increase in throughput, genetic information and discriminatory power, to more efficiently and effectively process hundreds of UHR and the thousands of associated reference samples.

When a person is reported missing or when human remains are found, the centralisation and comparison of this information at a national level is paramount (INTERPOL, 2015a; INTERPOL, 2015b). To date, there has not been a concerted effort to ensure all MP or relatives of Australia's long-term MP have relevant DNA profiles on a national DNA database for searching and comparison with UHR DNA profiles as the genetic data is obtained. The Australian Criminal Intelligence Commission (ACIC) has recently launched the National Missing Persons and Victim System (NMPVS) and forthcoming is an enhanced National Criminal Identification DNA Database (NCIDD) capability, which will facilitate the sharing, comparing and matching of all types of genetic data from the UHR and MP (or their relatives), as well as non-genetic data associated with the MP (e.g. date or geographic location last seen). A campaign designed to increase the number of MP or family reference DNA profiles on this database will increase the likelihood of identifications being made.

A number of countries have implemented successful DNA identification programs for the purposes of resolving large numbers of unaccounted for MP and UHR. The coordination of a national DNA identification program will be essential for the cost effective and time efficient processing of Australia's outstanding UHR and MP cases; and it is essential that Australia acknowledges the importance of identifying their missing and deceased, and provide adequate resources to do so. Once the infrastructure, SOPs and expertise has been compiled for this immediate task, it will ensure this specialised testing is readily available for the future identification of human remains as they are recovered or any Australian victims resulting from natural or man-made atrocities. Finally, in recognition of our humanitarian obligations, all countries, including Australia, should be taking all practicable steps to identify their unknown and missing citizens and bring closure to families whom have never had confirmation of the fate of their missing loved ones.

Fellowship Program

I was awarded a 2015 Winston Churchill Fellowship to travel to Europe, United States of America (USA), and South America to visit experts, laboratories, and academic institutions that: 1) Specialise in the nuclear (nDNA) and mitochondrial DNA (mtDNA) identification of human remains; 2) Have applied new technologies to DNA-based identification casework, including the MPS of whole mtDNA genomes and predictive DNA markers (e.g. ancestry and phenotype panels); and/or 3) Have established successful DNA-led MP and UHR identification programs for criminal, coronial and disaster victim identification (DVI) casework. This opportunity has armed me with current knowledge, skills and tools in DNA-based identification. In this report, I will present key points from my Fellowship and translate these findings into recommendations for the establishment of an Australian DNA identification program based on international best practice.

Venice, ITALY

20 June – 24 June 2016
International Academy of Legal Medicine (IALM) Intersocietal Symposium
Euroforgen Network of Excellence International Dissemination Conference
The Application of Forensic Science to Identifying Victims of Armed Conflicts and Humanitarian Operations Workshop
Multi-disciplinary Approaches to the Management of the Dead Associated with Mass Disasters and Terrorist Attacks Workshop

Sarajevo and Tuzla, BOSNIA AND HERZEGOVINA

27 June – 1 July 2016
International Commission on Missing Persons (ICMP)
- Dr Tom PARSONS, Director – Science and Technology
- Rene HUEL, Head – DNA Laboratories Division
- Dr Sylvain AMORY, Coordinator – DNA Validation and Development
- Sabina TASLAMAN, Training Coordinator – Science and Technology
- Ana BILIC, Deputy Head – DNA Laboratories Division
- Jasmina KURBASIC, Legal Officer
- Samira KREHIC, Deputy Head – Western Balkans Program
- Ian HANSON, Deputy Director – Archaeology and Anthropology Division
- Adnan RIZVIC, Director – Data Systems
- Dragana VUCETIC, Senior Forensic Anthropologist
- Edin JASARAGIC, Head – Data Analysis Division
- Zlatan BAJUNOVIC, Manager – DNA Matching Unit
- Dragan IVETIC, Manager – Post Mortem Unit
- Dijana TANKOVIC, Manager – Extraction Unit
- Arijana SELMANOVIC, Manager – Amplification Unit
- Dr Lejla SMAJLOVIC SKENDERAGIC, Manager – Genetic Analysis Unit

Lyon, FRANCE

4 July 2016
INTERPOL
- Dr Rebecca HOILE, Coordinator – Bioterrorism Prevention Unit

- Dr Susan HITCHIN, Coordinator – DNA Unit
- Andrea FISCHER, Assistant Analyst – DNA Unit

The Hague, THE NETHERLANDS

5 July 2016
Netherlands Forensic Institute (NFI)
- Dr Arnoud KAL, Senior Forensic Scientist – Biological Traces, DNA, Kinship Analysis
- Drs Reza GERRETSEN, Senior Forensic Anthropologist
- Gerco KRAMER, Forensic Biologist – Trace Recovery
- Dr Titia SIJEN, R&D Team Leader
- Saskia REURTS, Forensic Biologist – DNA Profiling
- Dr Harald TEPPER, Head of the Division – Biological Traces
- Kris VAN DER GAAG, Forensic scientist – MPS
- Jerry HOOGENBOOM, Forensic scientist – Bioinformatics
- Sander KNEPPERS, Program Manager – Lab Automation
- Prof Dr Klaas SLOOTEN, Forensic scientist – Statistics, Kinship Analysis

Leiden, THE NETHERLANDS

6 July 2016
Forensic Laboratory for DNA Research, Leiden University Medical Centre (LUMC)
- Prof Dr Peter de KNIJFF – Director

Quantico, VIRGINIA

7 July – 8 July 2016
Federal Bureau of Investigation (FBI) DNA Laboratory
- Anthony ONORATO, Chief – DNA Support Unit
- Dr Jodi IRWIN, Research Biologist
- Dr Odile LOREILLE, Research Biologist
- Dr Michael BRANDHAGEN, Forensic Biologist
- Dr Brandon LETTS, Research Biologist
- Dr Lilly MORENO, Research Biologist
- Dr Rebecca JUST, Research Biologist
- Erica AMES, Forensic Biologist
- Tim ZOLANDZ, Supervisory Biologist

Dover, DELAWARE

11 July & 15 July 2016
Armed Forces DNA Identification Laboratory (AFDIL)
- Dr Timothy McMAHON, Chief – Forensic Science Services
- Mark WADHAMS, Quality Manager
- Dr Charla MARSHALL, Chief – Emerging Technologies Section
- Suzanne BARRITT-ROSS, Technical Leader – Past Accounting Section
- Suni EDSON, Assistant Technical Leader – Past Accounting Section

- Kimberley ANDREAGGI, Research Scientist – Emerging Technologies Section
- Kerriann MEYERS, Assistant Technical Leader – Past Accounting Section
- Sean PATTERSON, DNA Analyst – Quality Management Section
- Colleen DUNN, DNA Analyst – Past Accounting Section
- Susan BELOTE, DNA Analyst – Past Accounting Section

Fredericksburg, VIRGINIA

12 July – 14 July 2016
Scientific Working Group on DNA Analysis Methods (SWGDAM) Meeting

Huntsville, TEXAS

18 July 2016
Department of Forensic Science, Sam Houston State University (SHSU)
- Prof Sarah KERRIGAN, Department Chair – Forensic Science
- Dr Sheree HUGHES-STAMM, Assistant Professor & Director of Graduate Programs – Forensic Science
- Dr Bobbie LARUE, Associate Professor – Forensic Science
- Dr David GANGITANO, Associate Professor – Forensic Science
- Amy SORENSON, PhD student – Forensic Science

Fort Worth, TEXAS

19 July – 22 July 2016
University of North Texas Health Science Centre (UNTHSC)
- Prof Bruce BUDOWLE, Director
- Dr Angie AMBERS, Postdoctoral Research Associate
- Jonathon KING, Research and Development Laboratory Manager
- Dr Jennifer CHURCHILL, Postdoctoral Research Associate
- Dixie PETERS, Technical Leader – Missing Persons Unit
- Melody JOSSERAND, CODIS Administrator
- BJ SPAMER, Director – Forensic and Analytical Services (NamUs)

Cordoba, ARGENTINA

25 July – 28 July 2016
Argentine Forensic Anthropology Team (EAAF) Genetics Laboratory
- Dr Carlos VULLO, Laboratory Director
- Magdalena ROMERO, Forensic Biologist
- Laura CATELLI, Forensic Biologist
- Carola ROMANINI, Forensic Biologist
- Micaela LONGARAY, Forensic Biologist
- Andrea ROCHA, Forensic Biologist
- Martina ROTONDO, Forensic Biologist
- Marcela VILLAR, Administration Support

International Academy of Legal Medicine (IALM) Intersocietal Symposium

The theme of this conference was focussed on the present and future evolution of the bio-medicolegal sciences, including forensic genetics and genomics. Highlights from the forensic genetics session included presentations by leaders in this discipline such as Angel Carracedo (Institute of Legal Medicine, University of Santiago de Compostela, Spain), Manfred Kayser (Department of Forensic Molecular Biology, Erasmus MC University Medical Centre Rotterdam, The Netherlands), Walther Parson (Institute for legal Medicine, Innsbruck Medical University, Austria) and Peter Schneider (Institute of Legal Medicine, University of Cologne, Germany), who discussed new and emerging technologies such as:

1. Single nucleotide polymorphism (SNP) marker panels
    *   Identity SNPs
    *   Lineage SNPs
    *   Ancestry informative SNPs
    *   Phenotype SNPs
    *   X-chromosome SNPs
2. Whole mtDNA genome sequencing
3. Forensic DNA phenotyping
    *   Hair, eye, and skin colour (e.g. HIrisPlex-S DNA Test)
    *   Additional markers include hair morphology, male pattern baldness, hair greying, facial (i.e. eyebrow and beard) hair morphology, ear morphology, face morphology and body height but these markers are still in early development
4. DNA methylation profiling
    *   Age estimation
    *   Body fluid/tissue identification
5. mRNA/miRNA profiling
    *   Body fluid/tissue identification
    *   Combined with genomic DNA profiling for donor identification
6. Third generation sequencing of single DNA molecules (e.g. Oxford Nanopore MinION or PacBio SMRT® sequencing)

Besides the main scientific program, this conference also hosted the Euroforgen Network of Excellence International Dissemination Conference titled '*Forensic DNA analysis in the light of new security needs*'. John Butler (National Institute of Standards & Technology, Maryland, USA) and Peter Gill (Department of Forensic Medicine, Oslo University, Norway) gave presentations on some of the challenges facing forensic genetics today, including backlogs, greater detection sensitivity, privacy concerns and miscarriages of justice. One session was dedicated to forensic genetic intelligence, focussing on predictive DNA analysis of externally visible traits and their application to forensic casework, with speakers such as Chris Phillips (Institute of Legal Medicine, University of Santiago de Compostela, Spain) providing examples. In addition to scientific developments in forensic genetics, the legal and societal developments of this field were considered by speakers such as Erin Murphy (New York University, USA; author of '*Inside the Cell: The Darker Side of Forensic DNA*') and Kristiina Reed (King's College, London), who detailed the differing legislation across Europe concerning the permission or prohibition of the use of coding (or informative or functional) DNA, phenotypic analysis and familial testing.

Of particular relevance was the presentation by Lourdes Prieto (University Institute of Research Police Sciences, Spain) on the contribution of DNA typing to the resolution of crimes against humanity. She detailed some of the lessons learnt from the identification process of the victims of the Madrid Bombings and Presidential Palace 'La Moneda' attacks, including:

1. Ensuring biological samples are collected from every sample recovered regardless of level of fragmentation

2. Using standard forms for the collection of samples for DNA analysis
3. Using established DNA sampling criteria
4. Considering anthropological analysis and DNA analysis as complementary identification processes
5. Importance of using appropriate (and multiple) reference samples for comparison
6. Exercising caution when using personal items as direct reference samples
7. Consideration of genetic mutations when searching DNA databases
8. Producing a single expert report containing the conclusions of all discipline areas (e.g. anthropology, archaeology, odontology and genetics), with an individual detailed report for each aspect of the identification process attached as Appendices

Peter Schneider presented a SWOT analysis for MPS in his talk titled '*Future Evidence in Forensic Genomics – European Perspectives for an Integrated Approach to the Use of Genetic Evidence in Criminal Investigations*' which I believe summarises the current global view on this technology:

Strengths:
- Processing and analysis of large numbers of genetic markers simultaneously
- Maximum amount of data for minimal amount of sample input

Weaknesses:
- Increased sample-to-data period (compared to CE-based STR analysis)
- Currently expensive
- Lack of standardisation
- New resources required for bioinformatics and data storage

Opportunities:
- May provide new intelligence leads for cold cases
- All genetic data can be generated in advance, irrespective of available information at the time of analysis

Threats:
- All-inclusive typing kits may collect more information than needed
- Entering the 'slippery slope' of accumulating sensible personal data
- The large amounts of genetic data generated need to be secured and filtered appropriately

I also participated in two workshops titled '*The Application of Forensic Science to Identifying Victims of Armed Conflicts and Humanitarian Operations'* presented by the International Committee of the Red Cross (ICRC) and '*Multi-disciplinary Approaches to the Management of the Dead Associated with Mass Disasters and Terrorist Attacks'* presented by the International Association of Forensic Sciences (IAFS) and the Asia Pacific Medico-Legal Agencies (APMLA). Representatives from different countries presented their experiences with DVI. It became evident that there was variation in each countries standard operating procedures (SOPs) for the collection of biological samples for DNA testing. This is partly dictated by the condition of the remains and the environmental insults suffered, but this does highlight the need for continuing research to investigate optimal sample selection given certain DVI scenarios. In humanitarian cases, DNA testing can be utilised to identify remains and establish biological relationships among families (e.g. cases of non-biological children being used to seek asylum as a family group), however DNA testing can also be of limited value for crises where close relatives are missing. Across both workshops, it was emphasised that data management was crucial to the forensic identification process and a relevant or custom-built Database Management System (DMS) should be utilised for storing, comparing and protecting quality data. As with biological sample collection, the databases utilised for DVI varies between countries.

1. The forensic identification of UHR resulting from a criminal act, serves to: 1) Establish cause and manner of death; and 2) Establish the identity of the UHR and bring closure to living relatives
2. The use of visual identifications, personal documentation or eyewitness reports to identify remains can result in misidentifications
3. Matching of ante-mortem (AM) and post-mortem (PM) data, including DNA samples, is considered a more scientific means of identification
4. If a DNA sample taken from the MP prior to going missing (e.g. medical sample) can not be obtained for comparison, reference samples from multiple relatives should be tested for comparison to the DNA profiles from the UHR
5. A DNA-led identification program will require the use of a computer software program which searches DNA profiles from the UHR against a population-level database of relevant DNA profiles, such as:
    *   DVI System International (Plass Data)
    *   iDMS (ICMP)
    *   AMPM database (ICRC)
6. A DNA match from the database can be confirmed by testing of additional relatives and should be supported by other non-genetic evidence, such as consistent AM and PM data (e.g. age, sex, healed injuries, tattoos), personal belongings or eyewitness reports/event information (e.g. time since last seen) to reduce the risks of misidentification
7. DNA and anthropological analysis can be used to reassociate commingled or fragmented remains and determine the minimum number of individuals (MNI)
    *   Decisions will need to be made as to what will be the minimum size of a UHR sample to undergo DNA testing or the number of skeletal elements that should be tested
8. There is inconsistency across countries and/or events as to the most optimal samples to collect for DNA testing; and this seems to be dictated by which samples have been considered gold standard historically
9. Soft tissues (e.g. deep muscle tissue) can be collected for DNA analysis soon after death
    *   Sample collection should follow SOPs and be performed by suitably trained personnel
    *   Approximately 1 g of tissue should be collected
    *   Samples should be collected from deep tissues not surface muscle/skin
    *   Duplicate samples should be taken from other parts of the body if possible
    *   Samples should be stored at sub-zero temperatures ideally, refrigerated or in a storage buffer (e.g. 95% ethanol or commercial preservative solution)
    *   Samples should be taken in controlled conditions to minimise contamination if possible
10. If soft tissues cannot be obtained, plucked hairs, fingernail cuttings, buccal swabs or rib cartilage may be suitable alternatives
    *   Post-mortem blood samples were targeted in some mass disasters, however it has been suggested these are generally a poor source of DNA
    *   Skin samples are considered a poor source of DNA
11. Hard tissues can be collected for DNA analysis in the absence of soft tissues or when tissues show signs of putrefaction or decomposition
    *   Archaeologists and anthropologists should be involved in the recovery of skeletal remains to document the remains and artefacts appropriately
    *   Intact molar teeth are an ideal sample for DNA testing if available
    *   If teeth are not available, femurs are considered the ideal skeletal sample for DNA testing
    *   Up to 10 g of bone should be collected and knowledge of the DNA extraction method used will guide sample quantity needed
    *   A window section of bone should be taken from the mid-shaft of the femur (up to 5 mm^3) and the bone should not be sawed in half

- Samples should be stored at sub-zero temperatures ideally or refrigerated and kept dry (NB: samples taken from old dry bones should also be stored at sub-zero temperatures if possible to prevent further DNA degradation)
- Samples should be taken in controlled conditions to minimise contamination if possible

12. Other bones suggested as being suitable alternatives to femur (shaft) did include femoral head, humerus, radius, ulna, sternum and rib, however other research suggests these are not ideal samples for DNA testing (refer to the ICMP section on page 15)
13. Direct reference samples may be able to be collected from the MP and include medical samples (e.g. biopsies or blood), umbilical cords, baby or extracted teeth, hairbrushes, toothbrushes and razors
14. The preferred method for the collection of family reference samples is blood or buccal cell collection on FTA® (or similar) cards
    - Cards are air dried, then stored at room temperature
    - Suitable for automated processing of reference samples in the DNA laboratory
15. The use of direct reference samples collected from the MP can provide simple and powerful DNA match results, however it is also advisable to use these personal artefacts in conjunction with family reference samples to verify the identity of the biological material presumed to be from the MP
16. A reference sample from at least two close relatives of the MP should be collected and tested
17. Additional reference samples should be collected to reduce the possibility of coincidental matches and to improve the certainty of identity
18. A graphical representation of a family tree should be used to identify the exact biological relationship of an individual to the MP
19. Relatives must provide informed consent prior to the collection of reference samples for DNA testing
20. Individuals collecting reference samples must identify and record the biological relationship accurately, be familiar with collecting and handling of both biological and forensic samples (e.g. health and safety, and chain-of-custody), have knowledge of the suitability of a particular relative as a reference, and have appropriate psychological support if required as a result of dealing with the MP family
21. Psychological support should also be extended to the MP families as part of the collection process
22. DNA samples should be collected at the same time as the other AM data to minimise trauma to the family

Summary of Key Points – Conference:

A number of points for consideration regarding emerging technologies were raised during this conference (and reiterated during my Fellowship) and will be pertinent to the field of UHR and MP casework, including:

1. Should (or could) these emerging technologies be first introduced into forensics for coronial casework applications such as identifying human remains, to demonstrate the utility of these markers/technologies (e.g. whole mtDNA genomes, predictive DNA panels) whilst buying time for all stakeholders (e.g. legal community, public) to become educated, engaged and accepting of applying the use of MPS, coding DNA markers or whole genomes to criminal casework?
2. Why is predictive DNA information such as hair, eye or skin colour (which is externally visible to anyone in the community) currently considered more sensitive than using DNA to determine an individual's sex at birth or biological parentage?
3. Should the use of predictive DNA markers to generate investigate leads be considered any different to other historically accepted discipline areas which have been used to generate investigative leads, such as hair examination (to predict an individual's hair colour, length and racial origin)?
4. Whole mtDNA genome sequencing will be one of the first MPS panels validated and accepted for routine forensic applications
5. MPS marker panels should be modular so coding, non-coding, ancestry or phenotype markers can be used alone or in combination depending on the depth of the investigation or the countries legal framework but at what point is it more sensible to just sequence an individual's whole genome?
6. Predictive DNA data should be kept separate to genetic data on national criminal DNA databases

7. A 'privacy by design' framework and bioinformatics filters could be utilised to protect (or mask) certain personal data and not disclose any DNA data when communicating predictive DNA testing results to the end-user
8. Ensure that DNA-based investigations are performed by informed and trained scientists; the forensic community needs to offer these specialist DNA services to law enforcement or else they will use commercially available services

To conclude, in the near future will it be acceptable (and expected) that when skeletal remains are located, that specialist DNA testing is performed to predict that individual's sex, age, ancestry, skin colour, hair colour and eye colour in order to narrow down the pool of possible MP that it could be and/or the MP family reference samples that should be tested for comparison?

International Commission on Missing Persons (ICMP)

The ICMP manages a high throughput DNA identification program dedicated to identifying persons missing as a result of armed conflict, human rights abuses, migration, crime, trafficking and disasters around the world. This is the world's largest accredited missing persons DNA laboratory, having successfully tested more than 50 000 bone samples and established a database of almost 100 000 family reference DNA profiles to support the identification of approximately 20 000 missing persons globally. Developments in the field of forensic genetics and the creation of dedicated databases have made it possible to locate and identify missing persons with a level of efficiency and certainty that was not possible before (ICMP, 2016).

The ICMP was established in 1996 to help account for the approximately 40 000 MP resulting from the conflict in the former Yugoslavia from 1991-1995. The ICMP's DNA identification program was initiated in 2001 to support the identification effort, which had previously relied on traditional identification methods such as visual and anthropological examinations. To date, over 70% of these MP have been accounted for. Since 2004, the ICMP has been instrumental in providing technical assistance and capacity building internationally to assist with the location, recovery and identification of MP resulting from armed conflicts, human rights abuses and natural or man-made disasters. The ICMP was officially established as a treaty-based international organisation in 2014.

The Science and Technology department of the ICMP consists of the Archaeology and Anthropology Division based in Sarajevo (and the field), and the DNA Laboratory Division based in both Sarajevo and Tuzla. The Archaeology and Anthropology Division is tasked with locating and recovering UHR in mass graves spread across the region. The DNA Laboratory Division coordinates the receipt of the PM and AM samples, cut and sand the bones, punches blood reference sample cards, washes and grinds the bones, performs the analytical processing (DNA extraction to DNA interpretation) of the bones and family reference samples, and performs kinship matching and reporting.

The Data Systems department is tasked with developing a suite of software applications that span the entire forensic program, called the Information Database Management System (iDMS). The iDMS also has an embedded Laboratory Information Management System (LIMS).

The interactions with families of the missing and the collection of AM samples is program based. Each program, such as that of the Western Balkans or Iraq, interacts with each department in a synergistic manner whereby training can be provided, or protocols can be disseminated, to governments or ministries to be able to carry out certain tasks such as AM sample collection or PM sampling for DNA testing.

A number of interrelated mechanisms have contributed to the success of the ICMP's large-scale identification efforts:

1. Standing capacity to conduct high-throughput DNA identifications
   - Routine and low copy number (LCN) short tandem repeat (STR) testing methods used for bone samples
   - Automation of blood card punching, direct amplification of punches and processing of up to 96 samples per batch
2. Simple yet efficient instrumentation e.g. Waring® blenders for grinding bones
3. Redundancy of processes e.g. ability to perform manual and automated DNA extraction
4. Archaeology and Anthropology expertise in locating, recovering and examining UHR; and also the selection of bone samples for DNA testing
5. Modular streamlined workflow which ensures high throughput and high quality sample processing; divided into the following functions:
   - AM or family reference sample receipt and processing

- PM sample receipt and processing
- DNA extraction
- PCR amplification
- Genetic analysis
- DNA matching and reporting

6. The in-house development and utilisation of the iDMS, which is a modular system that houses all of the archaeology, anthropology, AM references, sample receipt, sample examination, DNA testing, DNA matching and reporting information

I commenced my visit at the ICMP DNA Laboratory and offices based in Sarajevo. This facility is central to the ICMP operations in Bosnia and Herzegovina. I received an overview of the ICMP, its legal framework, the Western Balkans/Srebrenica Program, the Archaeology and Anthropology Division and the iDMS. I then visited the Podrinje Identification Project (PIP) and the Data Systems Division in Tuzla. The PIP is the largest mortuary in Bosnia and Herzegovina and conducts the anthropological analysis and DNA sampling for remains recovered from mass graves associated with the 1995 Srebrenica event. At the Tuzla ICMP facility, I received an overview of sample receipt, AM sample processing, PM sample examination and mechanical cleaning process and DNA matching and reporting. On return to Sarajevo, I was allowed access to the DNA laboratory and observed the complete DNA testing process from PM sample washing, grinding and digestion, DNA extraction, DNA quantification, polymerase chain reaction (PCR) amplification and STR analysis and interpretation. I also met with the DNA Validation and Development Coordinator to discuss the research being conducted to complement or enhance the routine identification of compromised remains.

Summary of Key Points:

1. The establishment of a National Missing Persons Institute is fundamental to Bosnia and Herzegovina's on-going DNA-led identification program success
2. Archaeology and anthropology examinations are important to assist identifications in scenarios where close relatives are missing and DNA testing may not be that useful (e.g. artefacts or personal effects in graves, determination of height or age at death)
3. Misidentifications can occur if identification tools are used in isolation (e.g. anthropological analysis only)
4. Bones exposed to acidic soils, bacteria and fluctuating water tables are particularly susceptible to DNA degradation
5. DNA testing is extremely useful for reassociating commingled and fragmented remains
6. DNA testing success declines when testing compromised bones from very young or old people
7. The DNA laboratory is ISO/IEC 17025 accredited and has been designed to minimise contamination events (e.g. separate AM and PM sample processing laboratories; post-amplification laboratory is on a separate floor to the pre-amplification laboratories; separate pre-amplification laboratories are dedicated to sample preparation, DNA extraction and PCR set-up; use of procedure-specific and DNA-free hoods; strict PPE requirements; and use of UV sterilisation)
8. A modular workflow with staff specialising in a limited number of processes increases sample throughput
9. All mortuary staff refer to the ICMP Sampling Guide (ICMP, 2015) for guidance on selection of optimal bone samples for DNA testing
10. Sample selection is based on the skeletal elements available for the case (e.g. full or partial skeleton), anthropological criteria (e.g. pathology or trauma) and DNA testing success rates
11. Besides teeth and long weight bearing bones (e.g. femur or tibia), other skeletal elements that should be targeted to improve the success rate of recovering a useable quantity and quality of DNA include the temporal/petrous bone, metatarsal, talus and pelvis, followed by the mandible, vertebra and fibula
12. The selection of the most optimal sample for DNA testing up front will result in more efficient processing times and costs as further work or re-work of samples will not be required
13. Bones are cut with an autopsy saw and the external surface sanded with a sanding bit attached to a rotary tool

14. Whole teeth are cleaned of adhering tissue or dirt (no removal of crown)
15. Samples are washed in a series of 10% bleach, water and ethanol washes
16. Samples are ground using a commercial blender (Waring®)
17. Samples are batched (up to 11 samples and an extraction reagent blank) for nDNA testing
18. A demineralisation/silica column DNA extraction method is used; the silica column purification steps have been automated on the QIAcube (Qiagen)
19. A target amount of 1 g of bone/tooth powder is extracted initially; if a sample is inhibited 500 mg of powder can be extracted subsequently
20. Bone DNA extracts are quantified to estimate DNA yield, optimise the quantity of DNA template for PCR and detect samples that contain PCR inhibitors so they can be re-purified using a silica column purification system prior to PCR
    - DNA extracts with a Ct IPC of 31+ are purified again, followed by re-quantification
    - DNA extracts can be purified up to two additional times
21. Extraction reagent blanks are not quantified; 5 uL is amplified per PCR reaction
22. Depending on the DNA concentration result, different DNA extract volumes can be added to PCR reactions for each sample
23. Multiple autosomal STR kits are validated, in addition to a mini-STR kit for compromised samples
24. A Y-chromosome STR (Y-STR) kit and mtDNA SNP assay (mtHV+ HaploArray) are also validated if additional genetic data is required for identification or if distant maternal or paternal relatives are only available for comparison
25. LCN approaches have been validated for autosomal STR kits, using 34 cycles, additional Taq polymerase, and modified capillary electrophoresis (CE) injection parameters
26. Bone DNA extracts are amplified in duplicate (as a minimum), with consensus designation of alleles to determine profile
27. Thresholds are applied to profiles to ensure only profiles of a requisite quality are uploaded onto the database for matching
28. All profiles are compared to staff elimination databases and previously obtained results prior to database submission
29. Typically three reference samples are collected from relevant relatives of the MP to provide samples for comparison to UHR profiles
30. Informed consent is obtained from each family member, including consent of families to allow ICMP to grant access by courts to their information (or not)
31. Direct and kinship matching of DNA profiles is performed using the iDMS through pairwise comparison of bones against bones, and bones against references
32. A Paternity Index (PI) and Sibling Index (SI) is used to indicate possible associations between the DNA profile obtained from the bone/s and a paternal or sibling reference sample respectively
33. Once candidate matches are found via a pairwise search, a Likelihood Ratio (LR) is calculated based on the full pedigree of references and the unknown profile, which considers the probability of genotypes if individuals are biologically related as claimed vs. the probability of the genotypes if individuals are unrelated
34. A Prior Odds value based on the estimated or reported number of victims in some specific event or geographic area is necessary to combine the non-genetic information with the DNA results to produce the Posterior Probability used in the final DNA match report; this value can be adjusted as identifications are made or more information is known
35. ICMP uses a Prior Odds of 1/N where N = number of missing people for a given event
36. The Posterior Odds provides a numerical weight to the inference of the identification and the Posterior Probability presents the probability of the relationship as a percentage
    - Posterior Odds = LR x Prior Odds
    - Posterior Probability = [Posterior Odds/(1 + Posterior Odds)] x 100
37. A Posterior Probability of >99.95% is used for identification

38. The LR values should be provided on the DNA match report so that additional statistical analysis can be performed in light of a new Prior Odds value
39. LR values should be presented separately for each genetic marker (autosomal STRs, Y-STRs, mtDNA) and can also be multiplied together to determine the cumulative LR value
40. If a Posterior Probability of >99.95% cannot be reached using a standard autosomal STR kit, testing of additional loci (different kit, larger multiplex, smaller amplicons), additional genetic markers (Y-STRs, mtDNA, SNPs) or the collection and testing of additional family reference samples may be strategies to exceed the reporting threshold for identification
41. MPS technologies are being evaluated for MP casework applications
42. A new method being evaluated for the biological sampling of victims in mass disasters involves making an incision in the tissue, swabbing inside the incision, and transferring the biological material onto an FTA® card for direct amplification
43. The ICMP, in collaboration with the ENSFI DNA Working Group, has developed a: 1) Searchable Manufacturers' DNA Exclusion Database, which will contain STR profiles provided by manufacturers of DNA kits and consumables; and 2) An Unsourced Contaminant DNA Database, which will contain unknown (yet known not to be crime-related) profiles seen on multiple occasions across different laboratories, often in controls (e.g. extraction reagent blank or negative controls)
    - Currently, there are 600 contaminant profiles in the Unsourced Contaminant DNA Database but no profiles in the Manufacturers' DNA Exclusion Database
44. Countries undertaking large-scale DNA testing of human remains for humanitarian purposes can request access to the ICMP's iDMS to facilitate the identification effort

INTERPOL

INTERPOL is the world's largest international police organisation, with 190 member countries. INTERPOL maintains an international DNA database for its member countries to search and/or store DNA profiles from known offenders, crime scenes, MP and UHR. INTERPOL's DNA Unit is currently exploring the possibility of a more facilitated international approach for the searching of DNA profiles from MP and UHR. This service would allow member countries to submit DNA profiles to INTERPOL from MP relatives for kinship searching against UHR DNA profiles submitted by other countries. The DNA profiles from the UHR can be searched against profiles from relatives as well as profiles in all other indices of the DNA database (e.g. crime scene, offender or direct MP DNA profiles). However, MP and family reference sample DNA profiles can only be searched against profiles of UHR. Bonaparte software will be used by INTERPOL for kinship matching. This capability offers the international law enforcement community an exciting new avenue for bringing closure to MP cases that may have remained unsolved to date because the remains were located outside of Australia.

INTERPOL has also published recommendations for the establishment of a national DNA database and on the use of DNA for the identification of MP and UHR (INTERPOL, 2015a; INTERPOL, 2015b); some of which have been summarised below. These documents are relevant for any country wanting to establish a national DNA-assisted MP program in accordance with best practice principles and for the effective comparison of DNA profiles at an international level. The INTERPOL DNA Monitoring Expert Group recommends that every one of its member countries should implement a MP identification program according to the international recommended standards presented in this report.

Summary of Key Points:

1. It is important that DNA profiles from UHR and MP are searched at the international level given:
   - Ease of international travel
   - Increased global migration
   - Growing international crime (e.g. human trafficking, people smuggling)
2. AM samples should be collected from the MP directly (prior to going missing) and at least two closely related biological relatives (e.g. parents, children and siblings)
3. Direct AM samples can include:
   - Baby or extracted teeth
   - Dried umbilical cord
   - Clinical samples (e.g. biopsy samples, neonatal screening cards, or donated bone marrow, blood or sperm samples)
   - Criminal databases, paternity testing laboratories, military reference samples
   - Personal objects (e.g. toothbrushes, razors, hairbrushes, lipstick dispensers, roll-on deodorant, watches, headphones, glasses)
4. Care should be taken when using certain personal objects (e.g. toothbrush) as an AM sample for comparison and it is recommended that relative reference samples be tested to support the authenticity of the direct reference samples
5. It is important to establish and document the biological relationship of the relative providing the reference sample to the MP
6. Collection of reference samples should only be performed after obtaining informed and written consent from the donor
7. Consent forms used for the collection of family reference samples should request permission for DNA profiles to be submitted to INTERPOL for international data sharing
8. The most appropriate PM samples to take from the UHR will be dependent on the condition of the body
   - Complete, non-decomposed corpse: blood or buccal swabs/FTA® cards
   - Mutilated, non-decomposed corpse: blood or 1 g of deep seated red muscle tissue

- Complete, decomposed corpse or mutilated corpse: 5 cm window section from long, compact bone, healthy molar teeth or 10 g of any other dense, cortical bone
- Severely burned corpse: teeth/bones if available (as above) or bladder smears

9. More than one PM sample should be collected if possible
10. Chain-of-custody is an important consideration when handling, transporting or storing samples
11. Laboratories will need to have SOPs for the DNA testing of compromised samples
12. STR typing using kits with markers commonly used in different regions of the world is beneficial for international searching (e.g. European Standard Set, CODIS markers and D6S1043)
13. Samples should be retained to allow for additional testing when STR typing fails (e.g. lineage markers)
14. STR profiles from UHR and MP relatives can be submitted to INTERPOL for inclusion on the INTERPOL Missing Persons DNA Database
15. Y-STR and mtDNA profiles will provide supplementary genetic data for searching/matching at INTERPOL (but will not be included in the kinship calculations)
16. DNA data must be exported in a format that is compatible with international DNA data exchange formats
17. Sample retention will also facilitate confirmative analysis if required
18. Laboratories performing MP casework should be ISO/IEC 17025 accredited
19. Minimum requirements to report an identification should be established

Netherlands Forensic Institute (NFI)

The NFI is a purpose built, world leading laboratory that specialises in 40+ forensic disciplines. The NFI forensic biology laboratory was responsible for the DNA identification of 296/298 mostly severely fragmented victims of the Malaysia Airlines flight MH17 crash. An outcome of this identification effort, was new or revised protocols for processing DNA samples for DVI. The NFI co-developed the Bonaparte software (www.bonaparte-dvi.com), which was the kinship analysis tool used for the MH17 identification effort. In addition, this software is used on a daily basis for immigration and MP cases.

The NFI is also conducting cutting-edge research in areas such as MPS of whole mtDNA genomes and commercial marker panels, biological fluid and organ identification, DNA/RNA profiling of excavated human remains, SNP typing of the mtDNA control region and have developed a custom-built data analysis software program for MPS called FDS Tools.

Summary of Key Points:

1. Targeted sampling of soft tissues such as muscle or a swab of the bone marrow (from inside an intact bone) for DNA testing proved to be an efficient and effective procedure for DNA identification of human remains in a mass disaster scenario
   - However for commingled remains, the use of soft tissue is discouraged because of contamination risks from other remains
2. Bones are cleaned of adhering tissue using a scalpel, then scrubbed with water; bleach is only used if contamination is likely or when a mixed profile was obtained previously
3. Teeth are washed in a series of bleach and water washes
4. A demineralisation/silica column DNA extraction method is used for bones
5. A target amount of 1 g of bone/tooth powder is extracted but a new method is being validated that only requires 50-100 mg bone powder
6. The Bonaparte software is suitable for a large-scale victim identification process
   - The Bonaparte matching process calculates the LR for autosomal STR profiles
   - The Bonaparte matching process calculates the number of (mis)matches for Y-STR and mtDNA profiles but not a LR for each
   - One-to-one or pedigree matching can be selected for all profiles
7. The FDS Tools software is open source and a manuscript detailing the software will be published soon
8. The InnoTyper® 21 Human DNA Analysis Kit (InnoGenomics), which targets insertions and null alleles of retrotransposable elements, shows promise for profiling of degraded tissue samples
9. Brain and heart tissues provide the best profiling results compared to lung, liver, skeletal muscle, kidney or skin samples obtained from cadavers exhumed from graves 4-42 years post-mortem
10. The development of a SNaPshot™ assay targeting 18 SNPs in the mtDNA control region is an excellent screening tool to select or exclude samples for Sanger sequencing or MPS of mtDNA
11. The ForenSeq™ DNA Signature Prep Kit (Illumina) is being evaluated for forensic applications

Forensic Laboratory for DNA Research, Leiden University Medical Centre (LUMC)

The Forensic Laboratory for DNA Research at LUMC, directed by Prof Peter de Knijff, is the first forensic laboratory to be accredited to conduct STR and SNP typing using MPS technology for casework in the world.

Summary of Key Points:

1. The PowerSeq™ Auto System (Promega) is validated for casework
2. Custom-designed mtDNA and Y-chromosome SNP panels are validated for casework
3. A phenotype predictive SNP panel is validated for casework
4. Currently evaluating a 4-allelic SNP panel for forensic applications
5. It is important to have full reads in both directions when sequencing using MPS platforms
6. FDS Tools is used for MPS data analysis
7. MPS technology can be integrated into an existing laboratory with minimal modifications to the existing infrastructure of the laboratory
8. It is important to have legal support (explicitly defined in legislation) prior to investigating new technologies or DNA markers for determining the ancestry or externally visible characteristics of a cell donor
9. Relevant reference population databases need to be developed prior to implementation of new genetic markers in order to provide statistical support for results

Federal Bureau of Investigation (FBI) DNA Laboratory

The FBI DNA Laboratory conducts both nDNA and mtDNA testing for criminal, intelligence and MP cases. The FBI established and now maintains the Combined DNA Index System (CODIS), a software program that databases and permits searching of DNA profiles submitted by local, state and federal forensic laboratories. The latest version of CODIS introduced the capability to build and search pedigree trees, and utilise metadata or non-genetic data (e.g. sex, age, date of last sighting etc.) and additional technologies (e.g. Y-STR and mtDNA testing) to increase the efficiency of searches and reduce the number of adventitious associations. The National DNA Index System (NDIS) has established a National Missing Persons DNA Database to assist with MP and UHR identifications. This database has indices for MP DNA profiles, UHR DNA profiles and relatives of MP DNA profiles. The DNA profiles from UHR without a presumed identity can be compared to DNA profiles from MP, relatives of MP and other indices on CODIS such as offenders.

The FBI DNA Laboratory is also currently evaluating a number of MPS platforms and kits. Dr Jodi Irwin is the co-chair of the Scientific Working Group on DNA Analysis Methods (SWGDAM) Next Generation Sequencing (NGS) Working Group.

Summary of Key Points:

1. A mtDNA specific quantitation assay is used to accurately determine the quantity and quality of mtDNA extracted from compromised samples
2. The laboratory has automated many manual steps of the mtDNA analysis process for high quality samples including PCR set-up, ExoSAP-IT® purification, cycle sequencing set-up and Centri-sep™ purification
3. The PowerSeq™ Auto and Mito Systems (Promega) are being evaluated for forensic applications
4. The ForenSeq™ DNA Signature Prep Kit (Illumina) is being evaluated for forensic applications
5. The Applied Biosystems™ Precision ID mtDNA Whole Genome Panel (Thermo Fisher) is being evaluated for forensic applications
6. A custom shotgun and hybridisation capture method has been developed to sequence whole mtDNA genomes from historical case samples
7. The CLC Genomics Workbench software is used for the analysis of MPS data
8. CODIS is a suitable DNA database and kinship analysis tool for a national MP program
    - UHR, MP relatives and direct references of MP categories
    - Many identifications have been made matching the DNA profiles of UHR to DNA profiles in the Offender index of the database
    - DNA profiles of relatives of MP are only compared to UHR DNA profiles
    - UHR samples should be sent to an accredited forensic DNA laboratory for analysis and submission to CODIS
    - All reference samples should be sent to the same forensic laboratory to ensure a comprehensive pedigree tree is constructed for each MP
    - A manual comparison of DNA profiles from nominated reference samples and UHR can be performed without the need for a database search if requested
9. UHR should not be buried, cremated or returned until a DNA sample has been taken; even if a positive identification has been made in case a sample is needed in the future
10. Long bones and unrestored teeth are suitable biological samples for DNA testing if skeletonised remains are located
11. Muscle tissue and nails are suitable biological samples for DNA testing if mildly decomposed remains are located
12. Blood is the preferred biological sample for DNA testing if available

13. Direct reference samples from the MP should be used for comparison if available, as simplified statistics (e.g. Random Match Probabilities) can be used, which provide strong statistical significance and a high probability of identity
14. The comparison of UHR to family reference samples requires more complex statistics (e.g. Kinship Indices and LRs) and the ability to determine identity will be dependent on the degree of relatedness to the references
15. When a direct reference sample from the MP is collected, family reference samples should also be collected to confirm that the DNA profile from the direct reference sample is that of the MP
16. At least one maternal and one paternal family reference sample should be collected to construct a pedigree tree
17. A consent form must be signed by each relative donating a reference sample
18. Law enforcement must collect the reference samples
19. The relationship of the reference sample donor to the MP must be verified and documented
20. Never refuse to collect a reference sample from a relative; the laboratory can determine which reference samples are most appropriate for DNA testing
21. A range of DNA technologies are often needed in a national MP identification program to ensure the most robust searches possible, including STR, Y-STR and mtDNA genetic data
22. Pairwise direct matches can be performed (e.g. MP against UHR, UHR against UHR, MP and UHR against Offender and Forensic indices)
23. Kinship matches can be performed (e.g. relatives of MP against UHR)
24. Pedigree tree ranking can be performed
    - Uses all of the genetic data from all available relatives for searching
    - LR determines the results
    - LR is a combination of the individual STR, Y-STR and mtDNA LRs
    - More discriminatory than pairwise kinship matching
    - Makes use of data from second and third degree relatives
    - Allows for mutations
25. Lineage marker matching can be performed
    - Compares UHR without an STR profile to reference samples which have lineage marker profiles
    - Search occurs automatically every 3 months
    - Requires either a Y-STR or mtDNA profile
    - A LR threshold of 1000 eliminates matching to common haplotypes in the population
26. STR profiles from UHR must have at least one STR locus to be uploaded to CODIS and searched against pedigree trees, but eight or more loci are required to return a match in a direct comparison search (corresponds to the number of core loci in mini-STR kits)
27. STR profiles generated using LCN techniques can be uploaded onto CODIS for MP applications
28. Metadata should be collected and entered into CODIS as soon as possible and should be evaluated during a match or rank confirmation to strengthen or refute a DNA match

Scientific Working Group on DNA Analysis Methods (SWGDAM) Meeting

SWGDAM serves as a forum to discuss, share, and evaluate forensic biology methods, protocols, training, and research to enhance forensic biology services. In addition, SWGDAM provides recommendations to the FBI Director on quality assurance standards for forensic DNA analysis. I was invited to attend the FBI-hosted SWGDAM July meeting and was privileged to participate in the Next Generation Sequencing (NGS) Working Group activities (NB: NGS and MPS are interchangeable terms). An overview of the meeting is summarised below:

Day 1: Presentations by NGS vendors to all SWGDAM representatives
- Life Technologies
- Illumina
- Promega

Day 2: Vendors addressed questions prepared by the NGS Working Group in one-on-one sessions covering:
- Quality assurance and quality control (QA/QC) recommendations
- Bioinformatics, data storage and data security
- Reference population databases
- Kit validation documentation
- Automation of analytical processes (e.g. library preparation)
- Future releases of instruments, kits, software

Day 3: NGS Working Group update
- Reviewed Quality Assurance Standards (QAS) and validation guidelines
- Will now focus on development of interpretation guidelines

Summary of Key Points:

1. A confidentiality agreement does not allow me to publish key points from this meeting
2. A synopsis of the meeting is available on the SWGDAM website (http://www.swgdam.org/)

Armed Forces DNA Identification Laboratory (AFDIL)

AFDIL is a world leader in the DNA identification of compromised human remains from casualties of war. They primarily conduct mtDNA testing and are the first laboratory in the world to be accredited to perform whole mtDNA genome sequencing for casework. They have developed an in-house LIMS, with DNA database and matching software.

Summary of Key Points:

1. The DNA laboratory is ISO/IEC 17025 accredited and has been designed to minimise contamination events (e.g. separate high and low yield sample processing laboratories; separate pre-amplification and post-amplification laboratories; separate nDNA and mtDNA laboratories; separate pre-amplification laboratories are dedicated to sample preparation, DNA extraction and PCR set-up; use of procedure-specific and DNA-free hoods; strict PPE requirements; use of UV sterilisation; and dedicated reagent preparation/QC laboratory and staff)
2. Bones are cut with a cutting wheel attached to a rotary tool and the external surface sanded with a sanding bit attached to a rotary tool
3. The crown of the tooth is removed using a dental bur attached to a dental drill and dentine is removed from the crown of the tooth and the root cavity using a dental bur attached to a dental drill
4. Bone samples are washed in water and ethanol
5. Teeth are washed in 8.5% bleach (with sonication) and ethanol and then UV sterilised
6. Samples are ground using a commercial blender (Waring®)
7. Bone samples are processed individually or up to 4 samples (and an extraction reagent blank) from within the same case can be batched for nDNA and/or mtDNA testing (NB: samples could be from multiple individuals)
8. A demineralisation/silica column DNA extraction method is generally used
9. For samples that appear to be inhibited (e.g. from fuel or chemicals), an organic solvent (PCIA) purification can be used instead of a silica column purification
10. A target amount of 200-500 mg of bone powder is extracted
11. Bone DNA extracts can be amplified with an autosomal STR kit or a mini-STR kit as per manufacturer's instructions
12. A LCN Y-STR method is validated using 36 cycles and additional Taq polymerase for degraded bone samples
13. Bone DNA extracts are amplified in triplicate using the LCN Y-STR method, with each replicate having a different amount of starting template, with consensus designation of alleles to determine profile
14. Bone DNA extracts are amplified in duplicate with 2 x large, 4 x moderate or 8 x mini mtDNA primer sets using 36-42 cycles to sequence the HVI and HVII regions
15. A hybridisation capture-based whole mtDNA genome method was developed and validated for sequencing mtDNA from severely compromised samples

Department of Forensic Science, Sam Houston State University (SHSU)

The Department of Forensic Science at the SHSU delivers a postgraduate forensic program consisting of the first Forensic Science Education Programs Accreditation Commission (FEPAC) accredited Master of Science in Forensic Science in Texas, and the first PhD in Forensic Science in the USA. The Department consists of eight full-time faculty with expertise across all major forensic disciplines (e.g. forensic biology, toxicology, controlled substances, pattern and physical evidence, trace and microscopy, CSI and forensic anthropology). The forensic biology faculty members (Dr Sheree Hughes-Stamm, Dr David Gangitano and Dr Bobby LaRue) conduct research covering topics such as miRNA body fluid identification, marijuana identification using STR analysis, forensic botany, the genetic basis of anti-social and criminal behaviour, recovery of genetic information from "touch" samples and post-blast explosive fragments, and alternate genetic markers for identification and ancestry determination.

However, the major area of research focusses on improving DNA profiling from compromised human remains including the collection and room temperature preservation of decomposing tissues for identification purposes. This research is led by Dr Sheree Hughes-Stamm and is primarily conducted at the Southeast Texas Applied Forensic Science Facility, or the "body farm", which is an outdoor research facility with a predominant focus on the study of applications of forensic science to the human body.

Summary of Key Points:

1. DNA quality and quantity obtained from tissue samples decreases significantly after cadavers enter the bloat stage of decomposition
2. Buccal swabs are unlikely to be a useful sample to target for DVI after a post mortem interval of 2 days due to maggot mass in oral cavity; however alternate strategies are being explored such as various swabbing devices for swabbing skin, swabbing inside incisions and tissue biopsies
3. Muscle tissue produces slightly better STR results than skin samples preserved at room temperature
4. Collaborative research conducted at "body farms" across continents will be invaluable in determining optimal sample types to target for DVI or the identification of compromised remains in general and SHSU welcomes any collaborative research projects with the Australian Facility for Taphonomic Experimental Research (AFTER)
5. Thermo Fisher MPS panels are being evaluated in postgraduate student research projects, particularly with respect to various skeletal, highly inhibited, and low template samples
6. Research is currently being conducted in collaboration with the University of North Texas Health Science Center (UNTHSC) on the effects of different PCR inhibitors (e.g. heme, collagen, calcium, melanin and humic acid) on sequencing success rates with different MPS platforms and marker kits
7. Concentrating DNA prior to PCR amplification with MPS kits may improve sequencing quality
8. The InnoTyper® 21 Human DNA Analysis Kit (InnoGenomics) shows promise for profiling of degraded tissue samples as a complement to STR typing

University of North Texas Health Science Centre (UNTHSC)

The UNTHSC Center for Human Identification (UNTCHI) is an accredited forensic laboratory dedicated to the DNA-based identification of missing and unidentified persons. The Center is comprised of a DNA Laboratory and a Forensic Anthropology Laboratory. The UNTHSC also has a Research and Development Laboratory lead by Prof Bruce Budowle. This laboratory is central to the research, development and validation of a number of MPS platforms and kits.

The UNTCHI also manages the National Missing and Unidentified Persons System (NamUs). NamUs is a national centralised online repository of physical information for both missing and unidentified persons. It is a publicly accessible and searchable database, with restricted access to law enforcement and medical examiners/coroners for case information entry.

Summary of Key Points:

1. Only a small number of laboratories in the USA conduct MP casework and the UNTCHI is the only laboratory dedicated to performing MP casework
2. A Missing Persons Committee was established by SWGDAM to develop best practice guidelines for MP casework (published 2014) so that MP laboratories use standardised protocols
3. The UNTCHI has received funding from the National Institute of Justice (NIJ) and the state of Texas to perform DNA testing for MP casework and upload DNA profiles to CODIS for searching and comparison
4. CODIS houses both metadata and DNA data for UHR, MP and relatives of MP
5. The DNA laboratory is ISO/IEC 17025 and FBI QAS accredited and has been designed to minimise contamination events (e.g. separate AM and PM sample processing laboratories; separate pre-amplification and post-amplification laboratories; some mtDNA-specific procedures separated from main DNA laboratory e.g. cycle sequencing set up in the post-amplification laboratory; separate pre-amplification laboratories are dedicated to sample preparation, DNA extraction and PCR set-up; use of procedure-specific and DNA-free hoods; strict PPE requirements; and use of UV sterilisation)
6. The DNA laboratory works collaboratively with the Forensic Anthropologists on staff
7. Modular workflow such that specific days are dedicated to sample preparation of bones, DNA extractions etc. to enhance sample throughput
8. Whole bones are preferred for submission to the laboratory so sampling can be performed under controlled conditions and an optimal sample is selected
9. Long bones and unrestored teeth are preferred samples for DNA testing
10. A sample of bone should also be archived for future testing if needed
11. Bones are cut with an autopsy saw and the external surface sanded with a sanding bit attached to a rotary tool
12. Whole teeth are cleaned of adhering tissue or dirt (no removal of crown)
13. Samples are washed in a series of 50% bleach, water and ethanol washes
14. Samples are ground in a freezer mill
15. Samples are batched (up to 7 samples and an extraction reagent blank) for nDNA and mtDNA testing
16. A demineralisation/organic solvent/silica column DNA extraction method is used
17. A target amount of 1 g of bone/tooth powder is extracted
18. Bone DNA extracts are quantified
19. Extraction reagent blanks are not quantified; maximum template volume (10 uL) is amplified once using most sensitive conditions employed for each kit used
20. Multiple STR kits are validated, in addition to a mini-STR kit for compromised samples
21. A standard method and an LCN method using 32 cycles has been validated for the main autosomal STR kit used
22. Bone DNA extracts are amplified once if using the standard method, or in triplicate if using the LCN method, with consensus designation of alleles to determine profile

23. A Y-STR kit is validated and used as per manufacturers instructions
24. Bone DNA extracts are amplified once with 2 x large or 4 x smaller primer sets using 36-38 cycles to sequence the HVI and HVII regions
25. The reference sample workflow is streamlined as much as possible
    - Automated extraction of buccal swabs
    - No quantification
    - Processed with autosomal STR and Y-STR and/or mtDNA markers as appropriate
    - The whole mtDNA control region is amplified as one fragment, and sequenced as 4 x fragments (targeting the HVI/II region only)
26. A maximum of three family reference samples are generally tested for comparison to DNA profiles from UHR
    - A sample from the mother of the MP is preferred as one of those samples
27. Most bone samples are typed with an autosomal STR kit and mtDNA markers
28. Y-STR testing is generally only performed for samples where only a paternal relative is available for comparison or to provide additional information for a genetic association
29. Autosomal STR testing is performed for up to three relatives who donate a family reference sample for a particular MP
30. mtDNA testing is only performed for one of the maternal relatives who donate a family reference sample for a particular MP
31. Y-STR testing is only performed for one of the paternal relatives who donate a family reference sample for a particular MP
32. The relationship of relatives providing reference samples to the MP should be confirmed and documented
33. CODIS is used for comparing AM and PM data, including DNA data
34. No Prior Odds value is used in statistical calculations
35. LR values are presented separately for each genetic marker (autosomal STRs, Y-STRs, mtDNA) and can also be multiplied together to determine the cumulative LR value
36. The InnoTyper® 21 Human DNA Analysis Kit (InnoGenomics) is being evaluated for forensic applications
37. The MiSeq FGx™ System (Illumina) and ForenSeq™ DNA Signature Prep Kit (Illumina) is being evaluated for forensic applications
38. The Ion Chef™ System and Ion S5™ System (Thermo Fisher) and the following panels are being evaluated for forensic applications:
    - Applied Biosystems™ Precision ID mtDNA Whole Genome Panel (Thermo Fisher)
    - Applied Biosystems™ Precision ID Identity and Ancestry Panels (Thermo Fisher)
    - Applied Biosystems™ Precision ID GlobalFiler™ NGS STR Panel (Thermo Fisher)
    - Applied Biosystems™ Externally Visible Characteristics Prototype Panel (Thermo Fisher)
39. NamUs is comprised of three databases:
    - The Missing Persons Database contains information about the MP that can be entered by the family, the public or law enforcement (e.g. physical and circumstantial details, photographs, dental contacts etc.), however before it appears as a case the information is verified by NamUs staff; can be searched by anyone
    - The Unidentified Persons Database contains information about the UHR recovered that can only be entered by a medical examiner/coroner; anyone can search this database using characteristics such as sex, race etc.
    - The Unclaimed Persons Database contains information about deceased persons who have been identified by name, but for whom no next of kin has been identified or located to claim the body; information can only be entered by a medical examiner/coroner but the database can be searched by the public using a MP name or year of birth
40. When a new MP or UHR is entered into NamUs, the system automatically performs cross-matching comparisons between databases, searching for matches or similarities between cases

41. NamUs Regional Specialists are located throughout the USA and provide training on the use of NamUs to law enforcement and medical examiners/coroners, publicise the benefits of populating the database, and assist with confirming matches made between UHR and relatives
42. The DNA section of NamUs is populated by the DNA laboratory that has completed the DNA testing and only a record that DNA testing has been completed, the type of DNA testing performed (e.g. STR or mtDNA testing) and a DNA association report has been issued is recorded (i.e. no genetic information)
43. Images and documents (e.g. Anthropologist's reports) can be uploaded
44. NamUs analysts are responsible for examining records to assist with locating MP ("proof of life") or locating families of long-term MP to gather information and collect reference samples
45. NamUs provides free DNA testing and specialists (e.g. forensic anthropology and odontology services) to perform analysis of UHR as required
46. Law enforcement or medical examiners/coroners can request a DNA sample collection kit from NamUs for collecting family reference samples
47. UHR and MP family reference samples should be sent to a DNA laboratory that can perform both nDNA and mtDNA testing
48. Any individual believed to be missing in the USA can be entered as a MP on NamUs regardless of their nationality
49. There is some repetition of metadata between CODIS and NamUs
50. A copy of the NamUs software may be available for use by other countries in the near future

Argentine Forensic Anthropology Team (EAAF) Genetics Laboratory

EAAF is a non-government, not-for-profit, scientific organisation that applies forensic sciences to the investigations of human rights violations in Argentina and worldwide. The EAAF Genetics Laboratory uses both nDNA and mtDNA techniques to identify human remains, including up to 10 000 people who disappeared under the Argentinian military government that ruled from 1976-1983. The laboratory also conducts DNA testing for other countries in Latin America, Africa, Asia and Europe, and was pivotal to the DNA-based identification of the Ned Kelly remains on behalf of Australia.

Summary of Key Points:

1. The DNA laboratory is ISO/IEC 17025 accredited and has been designed to minimise contamination events (e.g. separate AM and PM sample processing laboratories; post-amplification laboratory is on a separate floor to the pre-amplification laboratories; separate laboratories are dedicated to sample preparation and DNA extraction/PCR set-up; use of procedure-specific and DNA-free hoods; strict PPE requirements; and use of UV sterilisation)
2. Bones are cut with a cutting wheel attached to a rotary tool and the external surface of the bone is sanded with a sanding bit attached to a rotary tool
3. Whole teeth are cleaned of adhering tissue or dirt (no removal of crown)
4. Samples are washed in a series of water, 10% bleach, water and ethanol washes and UV sterilised
5. Samples are ground in a freezer mill
6. Samples are batched (up to 6 samples and an extraction reagent blank) for nDNA testing
7. Samples are batched (up to 4 samples and an extraction reagent blank) for mtDNA testing
8. A demineralisation/magnetic bead DNA extraction method is used
9. A target amount of 1 g of bone/tooth powder is extracted
10. Bone DNA extracts can be re-purified using a silica column purification system if inhibited
11. Bone DNA extracts are quantified
12. Bone DNA extracts are amplified with two different autosomal STR kits using 32 cycles
13. A mini-STR kit can be used for compromised samples as per manufacturer's instructions
14. Y-STR and/or mtDNA testing can be performed if additional genetic data is required for identification or if distant maternal or paternal relatives are only available for comparison
15. An LCN Y-STR method is validated for compromised samples using 34 cycles and additional Taq polymerase
16. Bone DNA extracts are amplified in duplicate using the LCN Y-STR method, with consensus designation of alleles to determine profile
17. Bone DNA extracts are amplified with 2 x large, 4-6 x moderate or 8 x mini mtDNA primer sets using 36-38 cycles to sequence the HVI and HVII regions
18. Bone DNA extracts are amplified once with large or moderate mtDNA primers sets but duplicate amplifications are performed when using mini mtDNA primer sets
19. The reference sample workflow is streamlined as much as possible
    - Automated extraction of FTA® blood cards or buccal swabs
    - No quantification
    - Processed with autosomal STR, Y-STR or mtDNA markers as appropriate
    - Amplification of autosomal STR kits is performed using half strength PCR reactions
    - The whole mtDNA control region is amplified as one fragment, and sequenced as 6-8 fragments
20. More than one family reference sample is preferred for comparison to DNA profiles from UHR
21. The relationship of relatives providing reference samples to the MP should be confirmed and documented
22. DNA-View or Familias is used for large-scale kinship matching
23. A Prior Odds value is used for calculating the Posterior Probability and is stipulated by the lead Forensic Anthropologist; it can be based on the estimated or reported number of victims in a specific event, the

geographic location where the remains were found, whether the remains were male or female, age at death etc.

24. The Prior Odds value can be adjusted as identifications are made or more information is known
25. A Posterior Probability of >99.99% is used for identification
26. When haplotype frequencies are reported, a frequency is provided for the Argentinian population represented on EMPOP/YHRD and for the entire EMPOP/YHRD database
27. Autosomal STR and mtDNA LRs cannot be multiplied together if multiple maternal relatives are missing

Recommendations for an Australian DNA identification program

1. ***Missing Persons Casework:***
    a. Short-term:
        i. One Australian DNA laboratory could be dedicated to MP casework in Australia for a finite period in order to resolve the backlog of UHR (and this laboratory should ideally be separate from a criminal casework laboratory)
        OR
        ii. The DNA testing of Australia's UHR and associated reference samples could be outsourced to a dedicated MP laboratory outside of Australia
    b. Long-term:
        i. A Missing Person Centre of Specialisation could be established to provide a specialist, cost-effective MP casework service to all jurisdictions in Australia; and be comprised of:
            - DNA laboratories which can offer a range of specialist DNA services including:
                - Sample preparation of skeletal or compromised samples
                - nDNA, Y-DNA and mtDNA testing
                - LCN testing
                - Whole mtGenome sequencing
                - Forensic DNA phenotyping
                - Automated workflow for reference samples
            - Other specialists that may be required for the forensic examination of UHR including:
                - Forensic Pathologist
                - Forensic Anthropologist
                - Forensic Odontologist
                - Coroner
    c. An outsourcing workflow could involve:
        i. An Anthropologist examines the skeletal remains and an optimal sample is selected for DNA testing (ensuring some sample remains in country for further work as necessary)
        ii. Skeletal samples are sent to the overseas DNA laboratory for processing (NB: depending on the laboratory selected, this could be for nDNA testing only or for nDNA, Y-DNA and mtDNA testing as appropriate)
        iii. Reference samples are sent to the overseas DNA laboratory for processing (as above)
        iv. The overseas laboratory performs direct and kinship searching of all DNA profiles using in-house software and issues DNA match reports to Australian authorities
        v. Unmatched nDNA profiles should be submitted to the NCIDD for comparison to DNA profiles in all indices of the database
        vi. Unmatched nDNA profiles should be sent to INTERPOL for international searching
        vii. If only nDNA testing was conducted overseas, any skeletal samples that were unsuccessful in producing a nDNA profile or require supplementary genetic data for an identification, should be submitted to a suitable Australian laboratory for mtDNA and Y-DNA testing (if required)
        viii. Relevant reference samples should be submitted to a suitable Australian laboratory for mtDNA or Y-DNA testing (if required)
        ix. mtDNA and Y-DNA profiles should be submitted to the NCIDD capability for comparison to UHR and MP mtDNA and Y-DNA profiles in the database
        x. Alternative DNA testing methods (e.g. SNPs) could be considered if both nDNA and mtDNA testing is unsuccessful, if additional genetic data is still required to reach the reporting threshold for identification or if supplementary information is required to provide an investigative lead for unmatched samples (e.g. forensic DNA phenotyping markers)
    d. If more than one laboratory is responsible for MP casework nationally, the laboratories should use common SOPs

e. It is essential that DNA testing of multiple reference samples from all long-term MP is performed and these profiles are housed in the NCIDD for comparison to UHR DNA profiles as they are submitted

f. A DNA-led (not intelligence-led) approach needs to be implemented because:
    i. Majority of cases have minimal or no investigative leads
    ii. Sheer numbers of UHR and MP in this country
    iii. Potential reassociation of remains located in different areas

2. *Database Management System:*

a. A specialised 'grave-to-grave' DMS is required that is capable of managing a large-scale identification program

b. Modules should include:
    i. MP registry
    ii. Forensic archaeology
    iii. Forensic anthropology
    iv. LIMS for DNA testing
    v. DNA database/s
    vi. DNA matching, calculation and reporting software

c. A LIMS is essential for sample tracking and the documentation of sample receipt, sample examination, sample preparation and all DNA testing processes

d. Request access to ICMP's iDMS to centrally manage the DNA identification effort, especially if more than one laboratory or country is performing the DNA testing

3. *Metadata*

a. All UHR should be catalogued in the NMPVS

b. A Forensic Anthropologist and Odontologist (or multiple) should be employed short-term to examine all of the backlogged UHR prior to DNA testing commencing

c. If this is not possible, an Anthropologist or Odontologist should be consulted prior to sampling a bone or tooth for DNA testing to ensure the samples taken will not interfere with their examinations in the future

d. An anthropological examination can estimate sex, age at death, potential ancestry, stature and time since death, detect AM and PM skeletal damage, and determine the MNI represented by the remains

e. An odontological examination can chart the teeth recovered, the position and condition of the teeth, any dental restorations, and take PM dental x-rays

f. Relevant metadata obtained from pathology, anthropology or odontology reports for the UHR should be entered in the NMPVS by the police and relevant specialists or specialist reports uploaded

g. Metadata obtained from law enforcement and family members for the MP should be entered in the NMPVS by the police (e.g. date of birth, date of last contact and last geographic location)

4. *PM Compromised Sample Selection:*

a. Education and training is provided to all relevant parties involved in the collection, selection and sampling of UHR (e.g. forensic anthropologists, pathologists and DNA analysts) to ensure all have an awareness of optimal samples for DNA testing

b. A best practice sampling guide should be prepared and distributed to all relevant parties involved in the collection, selection and sampling of UHR

c. Pathologists/anthropologists and DNA laboratory staff should collaboratively examine, assess and select samples for DNA testing, to ensure an optimum sample is selected whilst avoiding anatomically significant or individualising characteristics that may be used for identification purposes, areas of damage/trauma, degradation and/or contamination

d. Entire or intact samples are submitted to the laboratory so sub-sampling is performed by laboratory staff under controlled conditions, including using a number of methods to decontaminate laboratory spaces, equipment and the exterior surface of the sample prior to sampling

e. Any personnel handling human remains should be wearing appropriate PPE and a DNA sample be provided to the laboratory for inclusion on a DNA elimination database
f. Standard forms should be used for the collection of PM data (e.g. INTERPOL or ICRC forms)
g. Samples should be transported to the DNA laboratory in a manner that will minimise or avoid further degradation or contamination of the sample
h. On receipt of the samples in the DNA laboratory, sample information could be recorded using electronic means if this technology is available (e.g. sample description worksheets, packaging description and photo documentation)
i. For remains that consist of multiple skeletal elements (where the assumption is these are from a single individual), the size and number of elements that are submitted for DNA testing should be determined early on

5. *AM Reference Sample Selection:*
a. Direct or family reference samples should be collected for all known MP
b. Direct reference samples can be categorised as either a MP sample (e.g. clinical samples and bio bank samples) or a 'deduced' MP sample (e.g. toothbrush)
c. DNA profiles from 'deduced' MP samples should be compared to DNA profiles from known family reference samples to ensure the DNA profile obtained is attributable to the MP
d. More than one direct reference sample should be submitted for direct matching, in addition to relevant family elimination samples as appropriate
e. More than one family reference sample should be submitted for kinship matching (1 x maternal and 1 x paternal relative if possible) and first degree biological relatives are preferred, specifically (in order of preference):
    i. 2 x parents
    ii. 1 x parent, child, spouse
    iii. 1 x child, spouse
    iv. 1 x parent, sibling
    v. 2+ siblings
f. Second (e.g. grandparent, aunty and half-sibling) or third (e.g. great-grandparent, great-aunt and first cousin) degree relatives can be useful if lineage markers are used for kinship calculations
g. A reference sample should be collected from all relatives willing to donate a reference sample and the laboratory can decide which samples are most appropriate for analysis and pedigree tree construction
h. Devise a maximum number of family reference samples that will be processed (e.g. only one maternal relative would be processed for mtDNA testing)
i. Reference samples should be collected by law enforcement
j. A signed consent form should accompany all reference samples
k. A consent form should have a graphical representation of a pedigree tree so the relationship of the donor to the MP can be clearly documented
l. A consent form should request permission for DNA profiles to be submitted to INTERPOL for international data sharing
m. Standard forms should be used for the collection of AM data
n. The exhumation of remains may need to be considered to obtain relevant reference samples as appropriate

6. *Laboratory Design & Workflow:*
a. Dedicated reagent preparation laboratory
b. Dedicated skeletal sample preparation laboratory with appropriate bone sanding hoods and equipment
c. Separation of casework and reference sample processing areas
d. Separation of nDNA and mtDNA laboratories or the SOPs (e.g. workflows, batching sizes, decontamination procedures, PPE requirements etc.) reflect best practice for mtDNA laboratories
e. Separation of sample preparation, DNA extraction and PCR set-up pre-amplification laboratories

f. Separation of pre-amplification and post-amplification laboratories

g. Space for integration of MPS in the post-amplification laboratory as this technology matures

h. Separation of the MPS pre- and post-indexing processes in the post-amplification laboratory

i. Modular laboratory workflow with dedicated personnel for each analytical process (e.g. sample preparation, DNA extraction/PCR set up, post-amplification processes)

j. Regular dedicated days for performing certain analytical processes (e.g. bone sanding, cutting and milling X number of samples for 1 day of the week)

7. *DNA Testing SOPs:*

a. Sample preparation of bones
   i. Removal of any dirt, adhering tissue etc. with a detergent solution or scalpel
   ii. Sanding the surface of the bone using a sanding attachment fitted to a rotary tool in an appropriate bone hood, preferably with negative air pressure
   iii. Excising a section of the bone using a saw blade attachment fitted to an autopsy saw or rotary tool in an appropriate bone hood (a window section should be excised for long bones to avoid cutting completely through the bone)
   iv. Excising an additional section of bone to be archived
   v. Washing the bone section in a 10-50% bleach wash, multiple water washes and an ethanol wash
   vi. Powdering the bone section using a freezer mill or commercial blender

b. Sample preparation of teeth
   i. Removal of any dirt, adhering tissue etc. with a detergent solution or scalpel
   ii. Washing the tooth in a 10-50% bleach wash, multiple water washes and an ethanol wash
   iii. Powdering the tooth using a freezer mill or commercial blender

c. DNA extraction
   i. Total demineralisation of bone/tooth powder, proceeded by an organic or inorganic DNA extraction method
   ii. Organic or inorganic extraction method for all other sample types (e.g. tissue, nail and hair)
   iii. Samples can be batched in small numbers (recommend no more than 6 including an extraction reagent blank) if required (and this batch of samples can proceed as a batch for the entire analytical process)
   iv. Automated punching of blood/buccal reference sample FTA® (or similar) cards (if only nDNA testing is required); no extraction procedure
   v. Automated extraction of blood/buccal reference sample FTA® (or similar) cards or swabs (if nDNA, Y-DNA and/or mtDNA testing is required)

d. DNA quantitation
   i. Commercial or custom quantitation assays
   ii. Use of a Degradation Index is beneficial
   iii. A mtDNA specific target or mtDNA specific quantitation assay is beneficial
   iv. Quantitation of reference sample DNA extracts is not necessary

e. DNA testing capabilities
   i. Multiple autosomal STR kits with a large number of markers (noting that highly mutating loci are poor markers for kinship analysis), including mini-STR markers or a separate mini-STR kit
   ii. Y-STR kit
   iii. X-STR kit
   iv. mtDNA sequencing (targeting minimum of control region), including mini-primer sets
   v. Other CE-based markers (e.g. SNP or indel kits)
   vi. LCN methods for STR kits

      vii.   Direct amplification of FTA® punches

     viii.   Access to emerging technologies, such as MPS panels (e.g. whole mtDNA genomes, ancestry and phenotype predictive SNP markers), to provide additional or complementary genetic information

8. *DNA Testing Approach:*

a. UHR should be typed with all standard DNA testing methods if relevant (i.e. nDNA, mtDNA and Y-DNA if DNA quantitation/profiling indicates male)

b. Replicate testing should be a QA/QC consideration for each PM sample

c. nDNA testing should be performed with 2 x different autosomal STR kits (ideally from two different manufacturers), one of which could be a mini-STR kit

d. LCN methods should be applied when routine nDNA and Y-DNA testing fails; LCN testing should be duplicated at a minimum and a consensus profile generated

e. A tiered amplification approach (inclusive of mini-primer sets) should be available for mtDNA testing of the HVI/HVII region at a minimum; mtDNA sequencing using MPS would be a time and cost efficient option for a large-scale identification effort of compromised samples

f. Reference samples should be profiled once using 1 x autosomal STR kit and the relevant lineage marker, depending on relationship to the MP

g. Only perform mtDNA and Y-DNA testing on 1 x relevant maternal and 1 x paternal relative respectively

h. Reference sample validated workflow could include direct to amplification (i.e. no DNA extraction or quantitation) for appropriate samples and the use of half strength amplification reactions

i. Reagent blanks do not need to be quantified and could be amplified once per batch using the maximum amount of template with each relevant kit (using the most sensitive conditions employed)

9. *MPS Considerations:*

a. MPS Scientific Working Group (SWG) need to develop QA/QC, validation and interpretation guidelines for MPS applications

b. Australian population databases for marker panels (e.g. whole mtDNA genomes, ancestry SNP panels etc.) need to be established

c. Target enrichment by hybridisation approaches are likely to be more appropriate for severely degraded DNA than PCR-based multiplex marker panel approaches to recover short (<100 bp) DNA fragments

d. More research is needed to optimise the use of commercial off-the-shelf MPS marker panels for compromised samples

e. Per sample, MPS has the future potential to decrease costs and labour; and increase throughput and genetic information obtained

f. A laboratory that has validated an MPS platform and DNA marker panel/s should be a member laboratory of the Centre of Specialisation

10. *Contamination Minimisation:*

a. 10% bleach and ethanol decontamination of work surfaces and equipment

b. UV sterilisation of all appropriate reagents, consumables and equipment

c. If commercial kits are used, ensure they are manufactured according to the ISO/DIS 18385 standard (Minimizing the risk of human DNA contamination in products used to collect and analyze biological material for forensic purposes) and are suitable for all possible downstream processes

d. Adherence to strict contamination minimisation SOPs

e. Laboratory workflow prevents personnel re-entering low-yield areas after being in high-yield areas or pre-amplification areas after being in post-amplification areas

f. A representative of the Centre of Specialisation should register for access to the ICMP Exclusion Databases so unknown DNA profiles can be searched on the databases to investigate if that profile has originated from a contamination in the reagent or consumable supply chain

11. DNA Databases:

a. All genetic data (i.e. autosomal STR, Y-STR and mtDNA) that has been obtained from any UHR or MP reference sample previously (e.g. mtDNA reports from overseas service providers) should be back captured and submitted to the NCIDD

b. All genetic data obtained for UHR and reference samples as part of the proposed identification program should be submitted to the NCIDD (including UHR DNA profiles produced using LCN methods)

c. Devise minimum guidelines for uploading to the NCIDD
   - i. Minimum number of alleles/loci
   - ii. Minimum mtDNA coverage
   - iii. Duplicate analysis and consensus profiles

d. UHR DNA profiles should be searched against DNA profiles in all indices of the NCIDD

e. Reference sample DNA profiles should only be searched against UHR DNA profiles

f. Pedigree trees should ideally have at least one first degree relative of the MP and both STR and lineage marker data

g. UHR and family reference DNA profiles that do not match at the national level should be submitted to INTERPOL for international searching via Australia's National Central Bureau (AFP)

h. The DNA testing status of each sample could be recorded in the NMPVS by the DNA laboratories (e.g. nuclear and mitochondrial DNA testing completed, DNA profile uploaded to the NCIDD, DNA match report issued)

12. Reporting:

a. Direct (pairwise) and kinship (pedigree) searches of STR, Y-STR and mtDNA profiles are performed using integrated Bonaparte software in the NCIDD capability (all these functions will be operational in 2017)

b. UHR should be compared in a pairwise manner to all profiles in the NCIDD to identify a match to the source of the unidentified profile, or an association to profiles of closely related relatives

c. UHR should be compared to all known family members at once in a kinship search to generate a ranked list of pedigree trees and UHR associations based on LRs
   - i. The true biological relationship may not be represented by the highest LR, since that statistic is dependent on the number and type of relatives used to construct the pedigree tree

d. LRs should be used to evaluate genetic associations

e. LRs should be reported for each genetic typing systems used (i.e. autosomal STRs, Y-STRs and/or mtDNA)

f. All LRs can be multiplied together to generate the cumulative/combined LR, and this value can also be presented in the report
   - i. Prior to reporting combined statistics for autosomal STR, mtDNA and Y-STR results, it should be determined that each population used demonstrates independence between the genetic data results

g. Prior Odds can be used for MP casework; this can change as the identification process progresses

h. If an accurate Prior Odds value cannot be determined, a high Probability of Identity can be set which also must be supported by a lineage marker match to avoid false positives

i. Posterior Probability of >99.95%

j. Relevant population databases should be used to determine allele or haplotype frequencies when calculating LR's

k. Report frequencies using local, national and/or international databases (e.g. mtDNA population data representative of both the Australian and international population)

l. A genetic association should be compared to the metadata to validate or refute the likelihood of relatedness provided by the DNA results and the metadata for the UHR and MP should be consistent
   - i. NB: there may be discrepancies between anthropological and genetic determination of sex, ancestry etc.
   - ii. NB: metadata can be incorrect and the sources and limitations of the metadata should be reviewed before an association is confirmed

m. If an association cannot be confirmed using a standard autosomal STR kit, testing of additional loci (e.g. different kit, larger multiplex or smaller amplicon sizes), additional genetic markers (e.g. Y-STRs, mtDNA or SNPs) or the collection and testing of additional family reference samples may be strategies to exceed the reporting threshold for identification

n. Different hypotheses may need to be considered to determine the best fit of the UHR data with a pedigree
   i. Genetic mutations between UHR and relatives
   ii. Incorrect relationships between UHR and relatives (e.g. full-sibling is actually a half-sibling)
   iii. The UHR represents a different missing family member

o. A DNA match report should be issued to the relevant authorities once an identification or biological relationship has been established (inclusion of the genetic information, i.e. DNA profile, is not necessary)

p. The DNA match report could be uploaded onto the NMPVS by the DNA laboratories

13. *Governance, Legislation and Policy:*

a. The AFP's National Missing Persons Coordination Centre (NMPCC) should develop and document an Action Plan for the establishment of a national UHR and MP program in Australia, which details:
   i. The size of the current problem (i.e. the numbers of UHR and long-term MP in this country)
   ii. Best practice recommendations for establishing a national UHR and MP program (as outlined in this report and other publications mentioned herein)
   iii. The importance of a DNA-led identification program which will utilise new DNA capabilities in Australia (e.g. ACIC's NCIDD capability)
   iv. Funding sources for the program
   v. Case examples to demonstrate the effectiveness of DNA matching at the national level for identifying UHR and MP (e.g. the case of Karlie Pearce-Stevenson and Khandalyce Pearce)

b. This Action Plan should be prepared for the AFP Police Commissioner to table for consideration at the Australia and New Zealand Police Commissioners' Forum (ANZPCF)
   i. If a national program is mandated at this level, other relevant agencies such as the Australia New Zealand Policing Advisory Agency (ANZPAA), National Institute of Forensic Sciences (NIFS) and ACIC could assist the NMPCC establish such a program

c. Discipline experts (e.g. Anthropologists and DNA Biologists) should be consulted during the development of the Action Plan
   i. The Missing Persons Centre of Specialisation (if/when established) would be a valuable pool of expertise
   ii. A relevant expert could be seconded to the NMPCC to assist with the development of the Action Plan

d. The NMPCC should steer a campaign for reference sample collection from relatives of all long-term MP and these should be submitted to a DNA laboratory for testing (if not completed already)

e. Workflows for submission of large numbers of UHR and MP DNA profiles to the NCIDD and INTERPOL should be developed

f. Reference sample donor consent forms should be revised to include a graphical pedigree tree so the sample donor's relationship to the MP can be clearly documented, the wording used should encompass all DNA testing possibilities, and it should specifically request consent for searching of their DNA profile internationally

g. NMPCC should coordinate the entry of all of the UHR and MP metadata into the NMPVS by State/Territory police

h. Relevant policy and legislation should be reviewed and/or amended to permit testing of UHR and MP family reference samples with new technologies or DNA markers (e.g. whole mtDNA genomes, coding region markers and phenotype predictive markers)

14. *Funding*

a. Funding needs to be secured to support the DNA identification program

b. At a minimum, funding will need to cover all DNA testing costs

c. Funding may be required for new laboratory infrastructure, equipment purchases or method validation depending on the approach taken

d. Cost savings could be achieved by:
   i. Outsourcing
   ii. Concentrating the DNA testing effort within one laboratory or as a Centre of Specialisation to take advantage of economies of scale
   iii. Use of new technologies (e.g. whole mtDNA genome sequencing using MPS vs. CE-based Sanger sequencing of multiple mini-primer sets)

Conclusions

Australia needs to take affirmative action in identifying the 500+ sets of UHR in this country, in order to bring closure to potentially hundreds of missing person and criminal cold cases; and especially for the living relatives who suffer each day not being able to lay to rest their loved one. Furthermore, the longer we deliberate about the path forward for these samples, the more degraded they become; making an already difficult task more challenging. For these reasons, I will endeavour to be an advocate for Australia's 'no name' citizens and campaign for their humanitarian right to be identified and named; as well as having the privilege of a full forensic examination in order to establish the cause and manner of their death.

In this report, I have highlighted the need for the establishment of an Australian DNA identification program as the only mechanism for potentially identifying these remains; however, the program will only be successful if the relevant reference samples are also available for DNA comparisons. Through the dissemination of my report and accompanying recommendations for a DNA-led identification program in this country, I hope to bring awareness of similar programs elsewhere in the world so that we can learn from the successes and mistakes of other countries.

ANZPAA published a Missing Persons Policy in 2015 ('*Missing Persons: A Policy for Australian Policing - 2015*'), with the aim of providing national consistency in the management and investigation of MP cases; including the DNA testing of UHR and MP reference samples. Furthermore, the ICRC (2009) and INTERPOL (2015b) have published a number of considerations for the establishment of large-scale DNA-led identification programs. Many of these are pertinent for an Australian DNA identification program and have been summarised here for review:

a. The routine processing of DNA samples for MP investigations does not occur or is deemed low priority in most countries; however countries need to acknowledge the importance of identifying the missing and deceased, and provide adequate resources to do this (in addition to standard forensic casework)

b. A DNA identification program should adhere to internationally accepted standards to enable the effective and efficient searching of DNA profiles internationally

c. The size and cost of the DNA identification program will be dependent upon the number of UHR, the proportion of these UHR from which a DNA profile can be generated, and the number of MP for whom a sufficient number of relevant family reference samples can be obtained

d. The cost and complexity of a DNA identification program should be outweighed by the realistic possibility of obtaining identifications in the absence of any intelligence information

e. The labour and expertise involved in processing large numbers of compromised UHR samples will overwhelm most forensic laboratories capabilities and will impact the day-to-day processing of routine forensic samples for criminal casework

f. Introducing DNA testing into an identification program that has previously used non-scientific means to identify UHR could reveal misidentifications so procedures for dealing with this scenario must be devised

g. It is realistic that not all MP will be identified as a result of a DNA-led program and all stakeholders should be aware of this (i.e. families, laboratory staff and the authorities funding the effort)

h. An exit-strategy should be devised to cease the identification effort when the cost and complexity of the program outweighs the social benefit

i. If the identification program spans different laboratories, countries or is outsourced entirely:
   i. An overall coordinating body should facilitate the collection, labelling, transport and chain-of-custody of the samples
   ii. Common SOPs should be used for analysing samples, comparing results and reporting matches
   iii. The ownership of the UHR and responsibility for the repatriation of the UHR should be determined at the outset
   iv. The genetic data generated should be handled in accordance with domestic legislation and international human rights law governing the protection of personal and genetic data

v. The DNA testing should be performed in laboratories that can demonstrate adherence to strict QA/QC procedures to minimise the possibility of misidentifications and be accredited to ISO/IEC 17025 standards

My suggestions for a path forward for Australia involves taking the following steps:

a. The NMPCC should prepare a national UHR and MP program Action Plan for consideration by the Police Commissioners, specifically addressing:
  i. The current status of UHR and MP casework
  ii. All three options for DNA testing, focussing on turn-around-times, costs, resources and quality:
    - Outsource the DNA testing of these backlogged UHR cases (and all relevant reference samples) to a laboratory overseas that specialises in this type of casework (this process could be coordinated by a relevant expert through a secondment arrangement)
    - Dedicate one DNA laboratory in Australia to the DNA testing of these backlogged UHR cases (and all relevant reference samples) for a finite period with the goal of having these DNA profiles on the NCIDD capability for national searching/matching as soon as possible (routine forensic casework could be outsourced to another state laboratory if necessary)
    - Establish a Missing Persons Centre of Specialisation so the UHR and MP casework is distributed between Australian laboratories with the required equipment and expertise (this testing could be performed concurrently with each laboratory's routine forensic casework)
  iii. Sources of funding
b. In collaboration with NIFS, establish a Missing Persons Centre of Specialisation for the specialist processing of UHR and MP casework (for the processing of any new UHR and MP cases and for the immediate processing of the backlogged UHR and MP cases if selected as the preferred option)
c. Police should enter all metadata associated with UHR cases into the NMPVS so the number of UHR cases in Australia can be established
d. Police should enter all metadata associated with MP cases into the NMPVS so the number of long-term MPs in Australia can be established
e. The NMPCC should steer a campaign (aiming for Missing Persons Week in 2017) designed for relatives of long-term MP to contact police and provide a reference sample so their DNA profile/s can be submitted to the NCIDD for searching/matching against DNA profiles of all UHR
f. Police should ensure that all genetic data obtained from any UHR, MP or MP relatives previously (including Y-DNA and mtDNA profiles) is submitted to the NCIDD when this capability becomes available in 2017
g. DNA laboratories should ensure that all new genetic data obtained from any UHR, MP or MP relatives (including Y-DNA and mtDNA profiles) is submitted to the NCIDD for the direct or kinship searching/matching of DNA profiles nationally
h. Police should recruit a dedicated Forensic Anthropologist to examine all UHR cases in their jurisdiction so DNA testing can commence for these cases, and the Anthropologist report should be uploaded to the NMPVS
i. Request access to ICMP's iDMS to facilitate the large-scale DNA testing effort across different laboratories, states or countries

The knowledge, skills and tools I have acquired during my Fellowship, and the recommendations described here for the establishment of an Australian DNA identification program, have been or will be disseminated as follows:

   a. Publication of my Fellowship Report on the Winston Churchill Memorial Trust website
   b. Presentation at the Australia and New Zealand Forensic Science Society (ANZFSS) NSW Branch meeting in Aug 2016
   c. Presentation to colleagues at the NSW FASS in Sep 2016
   d. Presentation at the ANZFSS 23rd International Symposium on the Forensic Sciences in Sep 2016
   e. Provision of my Fellowship Report to NIFS and a summary published in their newsletter
   f. Presentation at the Police Consultative Group on Missing Persons meeting in Nov 2016
   g. Incorporation into the SOPs of the Specialist DNA Laboratory at NSW FASS
   h. Publication of a manuscript in a relevant Australian peer reviewed journal
   i. Embedded into my role as a forensic biology lecturer and research supervisor at various Australian academic institutions

Bibliography

AABB, 2010, *Guidelines for mass fatality DNA identification operations*, Bethesda, Maryland, viewed 1 August 2016, <http://www.aabb.org/programs/disasterresponse/Documents/aabbdnamassfatalityguidelines.pdf>.

Alonso, A., Martin, P., Albarran, C., Garcia, P., Fernandez de Simon, L., Jesus Iturralde, M., Fernandez-Rodriguez, A., Atienza, I., Capilla, J., Garcia-Hirschfeld, J., Martinez, P., Vallejo, G., Garcia, O., Garcia, E., Real, P., Alvarez, D., Leon, A. & Sancho, M., 2005, Challenges of DNA profiling in mass disaster investigations, *Croatian Medical Journal*, 46(4): 540-548.

Australia New Zealand Policing Advisory Agency (ANZPAA), 2015, viewed 1 October 2016, <http://www.anzpaa.org.au/>.

Australian Criminal Intelligence Commission (ACIC), 2016, *Missing Persons*, viewed 1 October 2016, <https://www.acic.gov.au/our-services/missing-persons>.

Bennett, C., 2014, 'Who knows who we are? Questioning DNA analysis in Disaster Victim Identification', *New Genetics and Society*, 33(3): 239-256.

Bikker, J., 2014, Identification of missing persons and unidentified remains in disaster victim identification: recommendations and best practice, in: X. Mallett, T. Blythe & R. Berry (eds.), *Advances in Forensic Human Identification*, CRC Press, Boca Raton, pp. 37-58.

Budowle, B., Beiber, F. R. & Eisenberg, A. J., 2005, Forensic aspects of mass disasters: strategic considerations for DNA-based human identification, *Legal Medicine*, 7(4): 230-243.

Budowle, B., Ge, J., Chakraborty, R. & Gill-King, H., 2011, Use of prior odds for missing persons identifications, *Investigative Genetics*, 2: 15-20.

Families and Friends of Missing Persons Unit, 2016, viewed 1 October 2016, <http://www.missingpersons.justice.nsw.gov.au/>.

Foy, S., 2006, *Profiling missing persons within New South Wales*, PhD thesis, Charles Sturt University, Sydney NSW.

Ge, J., Eisenberg, A. & Budowle, B., 2012, Developing criteria and data to determine best options for expanding the core CODIS loci, *Investigative Genetics*, 3(1): 1.

Gusmao, L., Butler, J. M., Carracedo, A., Gill, P., Kayser, M., Mayr, W. R., Morling, N., Prinz, M., Roewer, L., Tyler-Smith, C. & Schneider, P. M., 2006, DNA Commission of the International Society for Forensic Genetics (ISFG): An update of the recommendations on the use of Y-STRs in forensic analysis, *Forensic Science International*, 157(2-3): 187-197.

Hartman, D., Drummer, O., Eckhoff, C., Scheffer, J. W. & Stringer, P., 2011, The contribution of DNA to the disaster victim identification (DVI) effort, *Forensic Science International*, 205(1-3): 52-58.

International Commission of Missing Persons (ICMP), 2015, *Standard operating procedure for sampling bone and tooth specimens from human remains for DNA testing at the ICMP*, viewed 2 July 2016, <http://www.icmp.int/wp-content/uploads/2015/04/icmp-sop-aa-136-2-doc.pdf>.

International Committee of the Red Cross (ICRC), 2009, *Missing people, DNA analysis and identification of human remains: A guide to best practice in armed conflicts and other situations of armed violence (Second Edition)*, ICRC, Geneva, viewed 25 June 2016, <http://www.icrc.org/web/eng/siteeng0.nsf/html/p0871>.

International Committee of the Red Cross (ICRC), 2013, *Forensic identification of human remains*, ICRC, Geneva, viewed 25 June 2016, <https://www.icrc.org/eng/assets/files/publications/icrc-002-4154.pdf>.

International Organization for Migration (IOM), 2016, *Fatal Journeys Volume 2: Identification and tracing of dead and missing migrants*, IOM, Geneva, viewed 4 June 2016, <https://publications.iom.int/system/files/fataljourneys_vol2.pdf>.

INTERPOL, 2014, *Disaster victim identification guide*, viewed 4 June 2016, <https://www.interpol.int/content/download/9158/68001/version/18/file/INTERPOL%20DVI%20GUIDE.pdf>.

INTERPOL (DNA Monitoring Expert Group), 2015a, *Recommendations for the establishment of a national DNA database,* viewed 4 June 2016, <http://www.google.com.au/url?sa=t&rct=j&q=&esrc=s&source=web&cd=3&ved=0ahUKEwijt5WL5efPAhVKlFQ KHRzWAPcQFggkMAI&url=http%3A%2F%2Fwww.interpol.int%2Fcontent%2Fdownload%2F27993%2F375175 %2Fversion%2F5%2Ffile%2FMEG_Recommendation_Establishing_DNA_Database.pdf&usg=AFQjCNG4xyDx-ONxXIIVNhnIb6NN3uKaBg>.

INTERPOL (DNA Monitoring Expert Group), 2015b, *Recommendations on the use of DNA for the identification of missing persons and unidentified human remains*, viewed 4 June 2016, <http://www.google.com.au/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0ahUKEwiqjbCA5OfPAhVChlQ KHenfDdMQFggdMAA&url=http%3A%2F%2Fwww.interpol.int%2FMedia%2FFiles%2FINTERPOL-Expertise%2FDNA%2FINTERPOL-Best-practice-principles-Recommendations-on-the-Use-of-DNA-for-the-Identification-of-Missing-Persons-and-Unidentified-Human-Remains&usg=AFQjCNEt26VOWCP95yH1gIXMaC4 ZG1D7-Q>.

James, M., Anderson, J. & Putt, J., 2008, Missing persons in Australia, *Trends & Issues in Crime and Criminal Justice no. 353*, Australian Institute of Criminology, Canberra ACT, viewed 1 August 2016, <https://www.google.com.au/url?sa=t&rct=j&q=&esrc=s&source=web&cd=2&cad=rja&uact=8&ved=0ahUKEwiF1 7qq-vHPAhVCKpQKHQ10AnQQFggkMAE&url=https%3A%2F%2Fwww.missingpersons.gov.au%2Fsites%2Fdefault %2Ffiles%2FPDF%2520-%2520Publications%2FTrends%2520%2520Issues.pdf&usg=AFQjCNHiO3ignyoeiLwsjXt8mbkZUO8DNw>.

Lee, J., Scott, P., Carroll, D., Eckhoff, C., Harbison, S., Ientile, V., Goetz, R., Scheffer, J. W., Stringer, P. & Turbett, G., 2008, Letter to the editor: Recommendations for DNA laboratories supporting Disaster Victim Identification (DVI) Operations – Australian and New Zealand consensus on ISFG recommendations, *Forensic Science International: Genetics*, 3(1): 54-56.

Lessig, R., Edelmann, J., Aspinall, L., Krumm, P., Bastisch, I., Wiegand, P., Hohoff, C., Steinlechner, M. & Roewer, L., 2011, Letter to the editor: German standards for forensic molecular genetics investigations in cases of mass disaster victim identification (DVI), *Forensic Science International: Genetics*, 5(3): 247-248.

Montelius, K. & Lindblom, B., 2012, DNA analysis in disaster victim identification, *Forensic Science, Medicine & Pathology*, 8(2): 140-147.

Mundorff, A. & Davoren, J., 2014, Examination of DNA yield rates for different skeletal elements at increasing post mortem intervals, *Forensic Science International: Genetics*, 8(1): 55-63.

National Institute of Forensic Science (NIFS), 2016, viewed 1 October 2016, <https://www.anzpaa.org.au/nifs>.

National Institute of Forensic Science (NIFS), n. d., *Centre of Specialisation Framework: A concept paper*, viewed 1 October 2016,
<http://www.anzpaa.org.au/ArticleDocuments/676/Centres%20of%20Specialisation%20Framework.pdf.aspx>

National Institute of Justice (NIJ), 2005, *Mass fatality incidents: A guide for human forensic identification*, viewed 1 August 2016, <https://www.ncjrs.gov/pdffiles1/nij/199758.pdf>.

National Institute of Justice (NIJ), 2006, *Lessons learned from 9/11: DNA identification in mass fatality incidents*, viewed 1 August 2016, <https://www.ncjrs.gov/pdffiles1/nij/214781.pdf>.

National Missing and Unidentified Persons System (NamUs), 2008, viewed 1 October 2016, <http://namus.gov>.

National Missing Persons Coordination Centre (NMPCC), 2016, viewed 1 October 2016,
<https://www.missingpersons.gov.au>.

Parson, W., Gusmao, L., Hares, D. R., Irwin, J. A., Mayr, W. R., Morling, N., Pokorak, E., Prinz, M., Salas, A., Schneider, P. M. & Parsons, T. J., 2014, DNA Commission of the International Society for Forensic Genetics (ISFG): Revised and extended guidelines for mitochondrial DNA typing, *Forensic Science International: Genetics*, 13: 134-142.

Prinz, M, Carracedo, A., Mayr, W. R., Morling, N., Parsons, T. J., Sajantila, A., Scheithauer, R., Schmitter, H. & Schneider, P. M., 2007, DNA Commission of the International Society for Forensic Genetics (ISFG): Recommendations regarding the role of forensic genetics for disaster victim identification, *Forensic Science International: Genetics*, 1(1): 3-12.

Scientific Working Group on DNA Analysis Methods (SWGDAM), 2016, viewed 1 October 2016,
<http://www.swgdam.org/>.

Scientific Working Group on DNA Analysis Methods (SWGDAM), 2013, *Interpretation Guidelines for mitochondrial DNA analysis by forensic DNA testing laboratories*, viewed 1 August 2016,
<http://media.wix.com/ugd/4344b0_c5e20877c02f403c9ba16770e8d41937.pdf>.

Scientific Working Group on DNA Analysis Methods (SWGDAM), 2014a, *Guidelines for missing persons casework*, viewed 1 August 2016,
<http://media.wix.com/ugd/4344b0_2ba78a46a2664b29948c60bc0aebc902.pdf>.

Scientific Working Group on DNA Analysis Methods (SWGDAM), 2014b, *Interpretation Guidelines for Y-chromosome STR typing,* viewed 1 August 2016,
<http://media.wix.com/ugd/4344b0_da25419ba2dd4363bc4e5e8fe7025882.pdf>.

Scientific Working Group on DNA Analysis Methods (SWGDAM), 2016, *Contamination prevention and detection guidelines for forensic DNA laboratories_DRAFT,* viewed 1 August 2016.

Sorensen, A., Berry, C., Bruce, D., Gahan, M., Hughes-Stamm, S. & McNevin, D., 2016, Direct-to-PCR tissue preservation for DNA profiling, *International Journal of Legal Medicine*, 130(3): 607-613.

Sozer, A. C., 2014, *DNA analysis for missing person identification in mass fatalities*, CRC Press, Boca Raton.

Sweet, D., 2010, INTERPOL DVI best-practice standards – An overview, *Forensic Science International*, 201(1-3): 18-21.

Tidball-Binz, M., 2007, Managing the dead in catastrophes: guiding principles and practical recommendations for first responders, *International Review of the Red Cross*, 89(866): 421-442.

US Department of State, 2016, *Trafficking in Persons Report (June 2016),* viewed 4 June 2016, <http://www.state.gov/documents/organization/258876.pdf>.

van den Berge, M., Wiskerke, D., Gerretsen, R. R. R., Tabak, J. & Sijen, T., 2016, DNA and RNA profiling of excavated human remains with varying post-mortem intervals, *International Journal of Legal Medicine*, 130(6): 1471-1480.

van Dongen, C. J., Slooten, K., Slagter, M., Burgers, W. & Wiegerinck, W., 2011, Bonaparte: Application of new software for missing persons program, *Forensic Science International: Genetics Supplement Series,* 3(1): e119-e120.

Vullo, C. M., Romero, M., Catelli, L., Sakic, M., Saragoni, V. G., Jose Jimenez Pleguezuelos, M., Romanini, C., Joao Anjos Porto, M., Puente Prieto, J., Bofarull Castro, A., Hernandez, A., Jose Farfan, M., Prieto, V., Alvarez, D., Penacino, G., Zabalza, S., Hernandez Bolanos, A., Miguel Manterola, I., Prieto, L. & Parsons, T., 2016, GHEP-ISFG collaborative simulated exercise for DVI/MPI: Lessons learned about large-scale profile database comparisons, *Forensic Science International: Genetics*, 21: 45-53.

Weiler, N. E. C., de Vries, G. & Sijen, T., 2016, Development of a control region-based mtDNA SNaPshot™ selection tool, integrated into a mini amplicon sequencing method, *Science & Justice*, 56(2): 96-103.

Zietkiewicz, E., Witt, M., Daca, P., Zebracka-Gala, J., Goniewicz, M., Jarzab, B. & Witt, M., 2012, Current genetic methodologies in the identification of disaster victims and in forensic analysis, *Journal of Applied Genetics*, 53(1): 41-60.