# 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 2009





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### **FOREWORD**

Armed conflict, whether war or widespread political violence, often results in the disappearance of large numbers of people. They may be forcibly displaced; they may be arrested and denied contact with friends or families; they may be military personnel "missing in action"; or they may be victims of massacres. Whatever the reason for their disappearance, the combination of lack of news and uncertainty about their whereabouts can be unbearable for the families affected.

More often than not in these situations, those who are missing are dead. The only relief for their families is receiving credible confirmation of the death and knowing that the remains of their relative have been or can be treated with dignity, in keeping with their culture and religious beliefs. The proper recovery and identification of human remains is thus a fundamental part of the healing process for families and even whole communities.<sup>1</sup>

The development of forensic science, including DNA analysis, has led to the possibility that families of missing people will not only be able to establish the fate of their missing relative, but also that any remains will be identified and returned to the families. Before the advent of DNA analysis, forensic haemogenetics had been used as an element of human identification programmes, most notably in Argentina in the 1980s, but the scope of such analysis was limited. In recent years, the ability to recover and analyse minute amounts of deoxyribonucleic acid (DNA) from biological material has revolutionized forensic science. Since the first DNA profile was produced in 1984, the development of DNA analysis has been dramatic: it has become more sensitive, more discriminating, less costly and faster. The same technology that allows samples

<sup>1</sup> This fact was highlighted in a study launched by the ICRC on missing persons and their families. During 2002 and 2003, a series of meetings was held, convening international experts with experience in many different contexts. Two of the meetings focused on the role of forensic science. This document builds on the recommendations from these meetings and the experience gained since. See: ICRC, *The Missing and their families: Documents of Reference*, ICRC, Geneva 2004. (Available via: www.icrc.org [Accessed 10 July 2009]).

recovered from a crime scene to be matched to a suspect can be used to match human remains to the biological relatives of missing individuals.

Initially, in the early 1990s, DNA analysis was used to identify one or a few individuals, usually following presumptive identifications using other methods. It is now routinely used to assist in the identification of tens or hundreds of individuals, often following transport accidents, and is increasingly being applied to help identify victims of armed conflicts and other situations of armed violence.

Guidance for managing human remains in post-conflict and post-disaster environments can be found in other publications produced by or with assistance from the ICRC.<sup>2,3</sup> This guide offers an overview of forensic human identification and the use of DNA analysis in both small and large-scale identification programmes. In addition, the guide offers some practical advice on the selection, collection and storage of biological material for the purpose of DNA identification work. It also highlights ethical and legal concerns that should be considered when employing DNA analysis.

This new and expanded edition of *Missing People, DNA Analysis* and Identification of Human Remains: A Guide to Best Practice in Armed Conflicts and Other Situations of Armed Violence incorporates the lessons learnt by the forensic community over the past few years.<sup>4</sup> It was produced in response to a recommendation by an experts' panel, convened by the ICRC in May 2008, to discuss the use of DNA in the identification of human remains, and in order to respond more effectively to operational needs identified by the ICRC.

<sup>2</sup> ICRC, Operational Best Practices Regarding the Management of Human Remains and Information on the Dead by Non-specialists, ICRC, Geneva, 2004 (Available via: www.icrc.org [Accessed 10 July 2009]).

<sup>3</sup> Morgan, O., Tidball-Binz, M., and van Alphen, D., eds., Management of Dead Bodies after Disasters: A Field Manual for First Responders, Pan American Health Organization, Washington D.C., 2006. (Available at: http://www.paho.org/english/dd/ped/deadbodiesfieldmanual.htm [Accessed 10 July 2009]).

<sup>4</sup> Prinz, M., et al., DNA Commission of the International Society for Forensic Genetics, "Recommendations regarding the role of forensic genetics for disaster victim identification," *Forensic Science International: Genetics*, no. 1, 2007, pp. 3-12.

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Dr Robin Coupland, from the ICRC, who helped compile the previous edition of the guide, also contributed to this publication.

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## 1 INTRODUCTION TO THE FORENSIC IDENTIFICATION OF HUMAN REMAINS

The forensic investigation of human remains following armed conflict and other situations of armed violence<sup>1</sup> has two objectives. The first is to recover and examine the remains for criminal investigations, including establishing the cause and manner of death; the second is to identify the remains and, if possible, return them to the family of the dead person.<sup>2</sup> This second objective helps family members by ascertaining the fate of their relative and allowing the remains to be handled in a culturally appropriate manner, thus enabling the families of the missing to mourn their loss. Neither objective has priority over the other, and forensic specialists have a duty to try to fulfil both. Indeed, there should be no tension between these objectives.

Identification is defined as "individualization by attribution of birth name or other appropriate name to human remains." Identification is a process that involves one or more of the following means:

<sup>1 &</sup>quot;Armed conflict and other situations of armed violence" covers events during or after:

International armed conflict and non-international armed conflict as defined in the 1949 Geneva Conventions and their 1977 Additional Protocols; and

Internal violence meaning internal disturbances (internal strife) and situations requiring a
specifically neutral and independent institution and intermediary, in conformity with the Statutes
of the International Red Cross and Red Crescent Movement, article 5(2)(d) and 5(3), adopted by the
25th International Conference of the Red Cross in Geneva in October 1986 and amended by the 26th
International Conference of the Red Cross and Red Crescent in Geneva in December 1995.

<sup>2</sup> See: Human remains and forensic sciences, Electronic Workshop, 02.2002 - 03.2002; Human remains: Law, politics and ethics, 23.05.2002 - 24.05.2002 and Human remains: Management of remains and of information on the dead, 10.07.2002 - 12.07.2002, Workshops: Final report and outcome. (Available via www.icrc.org [accessed 10 July 2009]).

## 1.1 Visual and other customary means of identification

This usually involves relatives or acquaintances of the missing person(s) viewing the remains. Presumptive identifications can also be made through associated personal documents or identification discs and through event documentation, such as eyewitness reports. There are a number of important points relating to visual and customary identification:

- it may be the only pragmatic option;
- it carries a significant risk of misidentification;
- the risk of misidentification is substantially greater as the number of deceased grows;
- the risk of misidentification is substantially greater as increasing numbers of bodies are collected together in one place and are exposed to relatives, who will inevitably be in various states of shock;
- visual/customary methods should be used as the sole means of identification only when the bodies are not decomposed or mutilated and there is a well-founded idea of the victim's identity, such as when the killing and burial of an individual has been witnessed;
- before using visual identification, consideration should be given to the traumatic effects it can have on the families, and how the impact of a viewing can adversely affect a relative's judgement when trying to make an identification; and
- it may be possible to collect biological samples from both relatives and the body for subsequent DNA analysis. These can be used to either confirm or refute an identification at a later date, if DNA analysis becomes available. While desirable, this may be very difficult to implement in the field (see Section 4).

#### 1.2 Systematic comparison of ante-mortem and post-mortem data

Visual recognition, associated evidence, such as personal belongings, and event documentation, such as eyewitness reports, may lead to a single-case presumptive identification. Such identifications are often unreliable and, wherever possible, should be supported by matching ante-mortem data with information collected during the post-mortem examination. An identification may then be confirmed by additional "hard features," such as previous medical conditions and fractures. Such an identification may be equal to one by "scientific" means (see below) in terms of degree of certainty. However, it is a matter of subjective judgement as to how many softer findings, such as gender, height, or age, are required to conclude an identification; thus it is difficult to arrive at a standardized practice.

- There are standard forms for collecting ante-mortem and post-mortem data. The most commonly used system for mass disasters is the INTERPOL Disaster Victim Identification (DVI) system,<sup>3</sup> which is supported by the Plass Data software.<sup>4</sup> INTERPOL forms have been developed for disaster-victim identification and are appropriate for human remains that are not highly decomposed. However, they are not necessarily suitable for recording details of human remains from a post-conflict environment, which are usually highly decomposed or skeletal.
- The ICRC has developed forms that are designed for use in post-conflict environments. Supporting software has been developed that allows for comparing many ante- and post-mortem records.
- Without "hard" scientific identifiers there is a significant risk of false identification, that is, a presumptive identification is incorrectly declared to be an identification.

<sup>3</sup> INTERPOL DVI Form set (Available at: http://www.interpol.int/Public/DisasterVictim/Forms/Default.asp [Accessed 10 July 2009]).

<sup>4</sup> See http://www.plass.dk/index.php?option=com\_content&task=view&id=44&ltemid=78 [Accessed 10 July 2009].

- As with visual identification, the risk of misidentification is substantially greater as the number of deceased grows, unless scientific/objective means are used.
- The "softer" findings serve as an important check of scientific/objective identifications (see below).

#### 1.3 Scientific/objective means

Each of the following, which are part of ante-mortem and postmortem data collection, can conclude an identification with a high degree of confidence that would be considered beyond reasonable doubt in most legal contexts:

- matching post-mortem and ante-mortem dental radiographs;
- matching post-mortem and ante-mortem fingerprints;
- matching DNA samples from the human remains with reference samples (see Section 4); and
- matching other unique identifiers, such as unique physical or medical traits, including skeletal radiographs, and numbered surgical implants/prostheses.

The above categories of identification are not necessarily sequential, but usually as identification becomes more difficult, the emphasis moves from 1.1 through to 1.3 (Figure 1).

In practice, there may be multiple constraints hampering the identification of human remains in conflict situations, including a lack of security for personnel, an inability to establish systems that ensure that there is robust continuity of evidence, lack of will, and lack of resources. These constraints may limit or even preclude more sophisticated technologies that can be brought to bear.

#### CHAPTER '



Figure 1 Identifications obtained using visual recognition or other customary means should, where possible, be supported by comparing ante-mortem and post-mortem data to allow biological profiling and collection of additional evidence to support the identification. Ideally, identifications should be supported by at least one form of scientific identification, which greatly reduces the possibility of misidentification. \* SNP-based DNA analysis could also provide scientific identification but is not widely used. See Section 2 for an overview of different DNA analysis methods. \*\*Scientific identification using odontology normally requires comparison and matching of unique dental features. A key element to consider, even by non-forensic personnel, is the need for proper recovery and management of human remains and associated evidence.<sup>5</sup>

In some situations, forensic standards may be sub-optimal. This does not render the practice unethical;<sup>6</sup> however, if standards of presumptive identification are not sufficiently high, at a certain point, the potential harm of attempting identifications, or the risk of misidentifications, exceeds the potential benefits. Most important, there are certain principles relating to standards of laboratory practice, including the protection of personal and genetic data, that should be adhered to under all circumstances.

In recent years, the techniques used to identify remains have been expanded, improved, and rendered more complex by the emergence of technologies based on DNA analysis. In situations where DNA can be analysed, compared and eventually matched with that of the relatives of missing people, the identity of the human remains can be proved beyond reasonable scientific and legal doubt. Conversely, DNA analysis can also prove that no familial relationship exists.

<sup>5</sup> UNODC, Crime Scene and Physical Evidence Awareness for Non-forensic Personnel, UNODC, New York, 2009 (available via www.unodc.org).

<sup>6</sup> See: Human remains and forensic sciences, Electronic Workshop, 02.2002 - 03.2002; Human remains: Law, politics and ethics, 23.05.2002 - 24.05.2002 and Human remains: Management of remains and of information on the dead, 10.07.2002 - 12.07.2002, Workshops: Final report and outcome (Available via www.icrc.org [accessed 10 July 2009]).

## 2 DNA AND IDENTIFYING HUMAN REMAINS

DNA is useful in identifying human remains and in criminal investigations for several reasons: DNA is unique to an individual and remains constant through life; it follows the laws of Mendelian inheritance, with a child's DNA composed of equal parts of its parents' DNA; DNA can be analysed to produce a profile that can be reliably compared with other profiles; it can be recovered and analysed from minute biological samples, such as bloodstains or even a single hair; and compared to proteins, it is a resilient molecule, and degrades slowly in hard tissues, such as bones and teeth, allowing it to be recovered from old biological samples when the environmental conditions are favourable.

#### 2.1 Standard forensic DNA analysis

The human genome, which contains 3.2 billion base pairs, is physically arranged on 23 pairs of chromosomes (22 pairs of autosomal chromosomes and a pair of X/Y sex chromosomes). These chromosomes are located within the nucleus of the cell, hence the term nuclear DNA. Two copies of each chromosome can be found in each of a person's cells except the sperm and ova, which contain only one copy. Red blood cells, which are an exception, contain no nuclear DNA.

Using DNA analysis to identify human remains is a five-step process that involves:

- retrieval (collection, storage and extraction) of DNA from the human remains;
- retrieval of DNA, for comparison, from either the relatives of the missing person or from sources such as hair, saliva stains or other biological material known to be from the missing person and predating his or her disappearance;
- generating a DNA profile from both the human remains and reference samples(s);

- comparing the DNA profiles; and
- deciding on the degree of matching that is compatible with the claimed relationship between the deceased and the family member (or other reference material), given other evidence.

Nuclear DNA extracted from fresh blood, buccal (cheek) swabs or tissue can be analysed easily and quickly – as long as the storage conditions prior to analysis are adequate. In the past, it was difficult to extract suitable nuclear DNA from skeletal material. However, as a result of rapid advances in technology in recent years, it is now possible to recover DNA from fresh skeletal material and, in cases where preservation conditions are suitable, from material that is several years old.

The most powerful matching is done either when quality nuclear DNA can be harvested from biological material such as hair or saliva left by the missing person prior to death, which permits a direct comparison with a set of remains, or when several close relatives are available for testing. Nuclear DNA cannot easily be used for matching with relatives other than close family members; ideally, children and parents would be used for comparison.

#### 2.1.1 Short tandem repeats (STRs)

For most forensic work, only a tiny portion of the total DNA is analysed. The genome contains regions that vary widely between individuals, called short tandem repeats (STRs). After analysing 15 or more of these hyper-variable regions of DNA, which are located on the autosomal (non-sex) chromosomes, the resulting profile can be used to ascertain family relationships with a high degree of confidence. STR analysis will not always be successful when analysing degraded human remains. Mini STRs, which can provide a result with degraded DNA, have been developed to increase the success rate when working with degraded human remains.

In some cases it will not be possible to generate an STR profile from human remains; in other cases it may be possible to generate a profile, but there may be no suitable reference sample with which to compare and potentially match the profile. Alternative technologies of mitochondrial DNA profiling, single nucleotide polymorphisms (SNP) profiling, and sex chromosome (the X and Y chromosomes) STR profiling can be employed in an attempt to overcome some of these problems.

#### 2.2 Mitochondrial DNA

Mitochondrial DNA (mtDNA) is a small circular chain of DNA containing only 16,569 base pairs. It resides within the cell's energy-producing organelles called mitochondria. The advantage of using mitochondrial DNA is that it is present in multiple copies within the cell, and therefore is easier to recover from remains that are not well preserved.

Mitochondorial DNA is inherited solely from the mother. This means that a person's remains can be matched with the mother or the maternal grandmother, a sibling, maternal aunts or uncles, or with even more distant relatives as long as they belong to the maternal line of inheritance. While this feature makes it easier to find reference sample(s), it means that special care should be taken in evaluating this type of evidence, as the strength of a match can be difficult to assess.

#### 2.3 Sex chromosomes

Human beings have two kinds of sex chromosome, X and Y. Normal males possess one X and one Y chromosome and normal females two X chromosomes.

It is possible to analyse a panel of STRs that are located on the Y chromosome in order to match the remains of the deceased with their male relatives. This can be useful when close relatives are not available for comparison; any member of the paternal line, including brothers, paternal uncles, and paternal male cousins, may be used for matching. As with mitochondrial DNA, a match made using Y chromosome markers is not as powerful as standard DNA profiling because the Y chromosome profile is not unique and may be shared by people who are only distantly related. X chromosome STRs can also be useful in some special cases.

#### 2.4 Single nucleotide polymorphisms (SNPs)

SNPs have been shown to be valuable genetic markers for forensic analysis. There may be circumstances, such as when the DNA to be analysed is highly degraded, where SNPs are the only DNA polymorphism that can be successfully analysed.

### 3 USING DNA ANALYSIS TO IDENTIFY MULTIPLE HUMAN REMAINS RESULTING FROM ARMED CONFLICT OR OTHER SITUATIONS OF ARMED VIOLENCE

Although the authorities and the parties to an armed conflict are responsible for informing families about the disposal of their loved ones' bodies, it is often the case that one or both are without the will to do so. When human remains are recovered, forensic science can be used to identify them, thereby establishing the fate of individuals, and allowing their remains to be returned to their families. The effectiveness, and therefore advisability, of using DNA analysis as a part of an identification programme will vary depending on the specific circumstances.

## 3.1 DNA analysis to support presumptive identifications

Following the presumptive identification of a set of human remains, attempts should be made to provide additional proof of their identity, ideally using at least one form of scientific identification (see Figure 1). DNA analysis is one such method of identification. In order to undertake DNA analysis, it is necessary to:

- obtain DNA from the human remains;
- obtain DNA samples taken from the missing person prior to his or her disappearance or from biological relatives

(parents or children and in some cases siblings). Collecting samples from more than one relative is advisable because it will make matches more powerful (statistically significant). It is advisable to collect the DNA reference sample when the ante-mortem data interview is conducted; and

 compare and evaluate the significance of a match between the DNA from the remains and that from the reference sample(s).

If there is a forensic DNA laboratory available within the country that is working to acceptable standards (see Section 5), it should be capable of undertaking DNA analysis of relatives' reference samples, as this will use the same or similar processes as its routine caseload. Extraction of DNA from human remains, especially skeletal elements, can be more complex and may require different methods.<sup>1</sup> Comparing DNA profiles from human remains with those of reference samples should be within the scope of most laboratories, as the process is based on the same principles as paternity testing. However, comparing many remains to many reference samples is far more complex than simple paternity testing.

When newer techniques are required to analyse samples, either they can be introduced into the laboratory, possibly with more advanced equipment, or the DNA analysis can be outsourced to a laboratory that specializes in such techniques. If this cannot be done within the country, the samples may have to be transported across international borders for analysis abroad. The latter scenario may require a special legal, procedural and logistical framework, including for the samples' transportation, preservation and chain of custody.

1 See Annex A.

#### 3.2 Database-led identification programmes

Database-led identification programmes compare identifiers, from individual human remains, such as fingerprints, antemortem/post-mortem data, and DNA, with a populationlevel database of the identifiers. Special computer software programmes used for searching population-level databases will provide possible matches.<sup>2</sup> These matches are presumptive and require further analysis before they can be considered to be a scientific identification. An identification strategy that uses a database of DNA profiles to achieve matches would be termed "DNA-led."

When identifications are difficult to obtain, particularly in the aftermath of armed conflict, it may be technically feasible to initiate a DNA-led identification programme. The scale of DNA-led programmes can range from a local incident, involving only a few individuals, to programmes attempting to identify tens of thousands of individuals. The two largest DNA-led programmes to date have helped to identify thousands of individuals killed in the Balkans between 1991 and 2000, and approximately 1,700 individuals killed in the September 2001 attack on the World Trade Center in New York, USA.

Direct reference samples, that is, DNA profiles generated from missing persons, provide statistically powerful matches when compared with the DNA profiles of human remains, and should be used when they are available. However, this direct reference material is not available in many cases. In those cases, reference samples from relatives are analysed and compared to DNA profiles from the human remains in order to generate matches. In most cases, these matches require further evaluation, involving additional family members, to assess their statistical significance. To reduce the possibility of false matches, these

<sup>2</sup> Examples of such software include: the Automated Fingerprint Identification System (AFIS), for fingerprint database searches; the Combined DNA Index System (CODIS), M-FISys, MDKAP and DNA-View for DNA database searches; and INTERPOL DVI's Plass Data and the ICRC AMD/PMD Database for ante-mortem and post-mortem database searches.

matches should be supported by other evidence, such as comparison of ante-mortem and post-mortem data, event information, and personal belongings (Figure 2).



Figure 2 DNA-led identification relies on matches between the DNA profiles generated from human remains and the relatives of the victims. Matches generated by comparing the databases need to be evaluated using all of the available familial reference profiles, to allow the statistical significance of a match to be assessed. The possibility of misidentification will be reduced if the results of the DNA matching can be supported by biological profiling and other forms of identification. In small-scale DNA-led identification programmes involving only a few individuals, the process is very similar to when using DNA to confirm presumptive identifications. However, when the identification programme involves hundreds or even thousands of unidentified individuals, there are additional considerations:

- for a DNA-led programme for identifying human remains, the following should be assessed with regard to the size and cost of the programme: the proportion of individuals who are likely to be recovered; the proportion of remains from which a DNA profile can be generated; and the proportion of missing individuals for whom a sufficient number of reference samples from relatives can be obtained;
- the additional cost and complexity of a strategy using DNA analysis should be outweighed by the anticipated additional benefit of the programme, i.e. the realistic possibility of obtaining identifications;
- the task of processing hundreds or thousands of human remains samples, which are often challenging, will overwhelm most laboratories' capabilities. Therefore, it may be necessary to expand existing facilities or outsource the work;
- in relation to the above point, prior consideration must be given to the unintended impact on pre-existing legal and forensic services. For example, implementing such a programme may drain existing forensic expertise from the local forensic service and render it unable to deal with everyday criminal matters;
- the acceptable levels of quality assurance and control required are considerably higher when dealing with largescale programmes because of the compounding problems caused by any errors in managing and processing the samples, including the chain of custody, or in generating, interpreting and comparing DNA profiles;
- large-scale identification programmes in the aftermath of conflict or other contexts of violence will take several years to complete; it is likely that not all missing people will be

identified. This fact should be made clear to the families of the missing people, the authorities undertaking the work, and the authorities/organizations that are funding the work;

- introducing DNA analysis into an identification programme that has used non-scientific means of identification could reveal earlier misidentifications, so it is advisable to consider how to handle any misidentifications that may be discovered;
- the framework for evaluating matches has to consider the very large numbers of comparisons and thus the possibility of coincidental matches;
- software will be required to compare the profiles from relatives and human remains (see 3.2). This matching process is beyond the scope of most forensic laboratories' normal casework. As with all other processes, the matching technique should be validated to ensure that it is robust;
- when misidentifications have or are believed to have occurred, requests by governments, organizations or individuals to re-examine previously identified remains for the purpose of DNA analysis should be decided on a case-by-case basis; and
- there should be an "exit strategy" through which the process can be brought to a close when the cost and complexity outweigh the social benefit.

#### 3.3 Reassociation of human remains

When skeletal remains are highly fragmented/disarticulated and commingled (mixed with the skeletal elements of other individuals), it is possible to combine the use of DNA analysis, when DNA can be successfully extracted and profiled, with other forensic methods, such as skeletal morphological analysis, to help reassociate elements. Reassociation of human remains:

- increases the number of physical remains that can be returned to the family, which may have great cultural significance; and
- along with anthropological analysis, can help to determine the minimum number of individuals whose remains are commingled.

Strategies are required to deal with partial and commingled remains. For example, will all recovered human remains be identified, or just those that are recognizable body parts or tissue of a certain size?

#### 3.4 Cooperation among multiple parties

When the identification process is likely to involve different parties, possibly working in different countries and under different legal frameworks, prior agreement should be sought regarding:

- the logistic implications of handling samples, including collection, storage, transport and a chain of custody;
- an overall coordinating body that would be in charge of collecting and labelling samples, and transporting and analysing them. In the absence of such a coordinating body, how those steps are taken and by whom should be stipulated in advance according to competencies;
- protocols for analysing material and mechanisms for comparing results;
- the criteria used to determine an identification;
- any issues relating to the ownership, transport, and final distribution of the remains (repatriation); and
- data generated on human remains and samples. These should be handled in accordance with rules governing the protection of personal data and human remains, which include the protection of ante-mortem data and DNA samples and results (see Section 6).

## 4 TECHNICAL ASPECTS OF COLLECTING AND STORING BIOLOGICAL MATERIAL

A prerequisite for any identification using DNA analysis is that biological material must be collected and analysed from:

- the human remains; and either from
- relatives of the missing person; or from
- biological samples left by the missing person, in the form of medical samples or other biological artefacts.

#### 4.1 Chain of custody

A fundamental principle of all forensic work is that there must be procedures to ensure a secure and robust chain of custody for any evidence collected. The same principle applies to programmes in which identification of human remains is for the benefit of the families and not for use by courts. Failure to maintain a chain of custody could result in the wrong set of remains being returned to grieving family members. The chain of custody should include systematic labelling of all evidence and appropriate documentation to show "the order of places where, and the persons with whom, physical evidence was located from the time it was collected to its submission at trial."<sup>1</sup> In human identification, the evidence may be presented in a legal process other than a trial; however, in both cases, the admissibility of the evidence may be similarly affected if the chain of custody is not secure and robust.

<sup>1</sup> Wild, S.E., ed., Webster's New World Law Dictionary, John Wiley & Sons, Inc., Hoboken, NJ, 2006.

#### 4.2 Collection of human remains for DNA analysis

After an individual dies, his or her DNA will start to degrade, breaking down into small pieces. If the degradation of the DNA is extensive, then analysis becomes very difficult, and at times impossible. Degradation is largely dependent on what happens to the body after death, as certain environments, such as those that are warm and humid, are particularly destructive to DNA, whereas others that are cold and dry help to preserve it.

#### 4.2.1 Collection of soft tissue

In most conditions, DNA in soft tissues will degrade very quickly. However, when human remains are recovered shortly after death, it may be possible to take a sample of soft tissue for DNA analysis.

- Soft tissues must be collected soon after death, if they are to be used for DNA analysis. Authorities likely to be tasked with this role should have clearly defined procedures for collecting the samples, and forensic/medical personnel should be trained to undertake the sample collection.
- DNA can be preserved in muscle tissue. The time period during which DNA will be present depends on the environmental conditions: in hot climates, putrefaction, and the associated breakdown of the DNA, can start within hours, whereas in cooler conditions, DNA can be recovered from muscle tissue several days post-mortem and in some cases much longer.
- Only small amounts of muscle are required to generate a DNA profile. Published guidelines recommend that 1 gram of muscle be taken.<sup>2</sup> In most cases, 100 mg of tissue (a 3-4 mm cube) will provide ample DNA for analysis.

<sup>2</sup> The INTERPOL Disaster Victim Identification Guidelines and the International Society for Forensic Genetics' DNA Commission recommend that 1 gram of deep muscle tissue be taken (see technical publications in Annex A).

- Whenever possible, samples should be collected from deep tissues, as surface muscle may be contaminated through contact with DNA from other bodies.
- Where possible, duplicate samples should be taken from other parts of the body that show no visible signs of putrefaction or decomposition.
- The muscle samples should be stored in conditions that will limit any further degradation of the DNA. The simplest method of storing tissue is freezing at -20 °C (if the facilities are available, tissue stored at -80 °C will be more stable). If continual storage at sub-zero temperatures cannot be assured, then storage for short periods at 4 °C is preferable, as freeze-thaw cycles accelerate the breakdown of the DNA.
- An alternative and simple form of preservation is storing under 95% ethanol; commercial storage buffers are also available. The use of both alcohol and storage buffers reduces the need for refrigeration.<sup>3</sup>
- Samples should be taken in controlled conditions, where possible, to avoid possible contamination.
- In some circumstances it will not be appropriate to take muscle tissue, owing to practical or cultural reasons. Other, non-invasive sources of DNA include: plucked hairs, including roots; fingernail cuttings; and swabs taken from the mouth (buccal surface). These samples can be stored in the same way as muscle tissue. In most cases, however, these samples will be more difficult to analyse and the analysis more likely to fail than when using muscle samples.
- Skin samples and post-mortem blood samples tend to be poor sources of DNA.

If there is doubt as to whether DNA can be recovered from muscle samples it is advisable to take a sample of hard tissue (see below).

<sup>3</sup> See technical publications in Annex A.

#### 4.2.2 Collection and storage of skeletal material

The cells within the hard tissues (bones and teeth) are embedded within a dense bio-mineral matrix and are largely protected from the effects of putrefaction and decomposition. The hard tissues can therefore act as a source of DNA.<sup>4</sup> It is advisable to take hard tissue samples from human remains to maximize the possibility of obtaining a DNA profile. In many cases, where there has been a delay in recovering the human remains, skeletal elements are often all that is available for sampling. There are a number of technical points to bear in mind when collecting hard tissues:

- recovery of skeletal remains should take place using appropriate archaeological and anthropological techniques. Incomplete recovery and commingling of remains will, in many cases, lead to complications with DNA analysis and possibly lost opportunities to identify individuals, as well as to the misidentification of some human remains;
- in most cases, teeth are the best source of DNA. Ideally two teeth, without any evidence of dental work or decay, such as cavities, should be taken for analysis in the following order of preference: molar, pre-molar, canine, incisor;
- teeth that have characteristics that could assist with an identification, for example, front teeth that could be compared to a photograph of the missing person, should not be taken. If there are no alternatives, the characteristics of the teeth should be fully documented, including with photographs, prior to extraction;
- most methods of DNA extraction from hard tissues use approximately 100 mg of material; however, some published methods use up to 10 grams.<sup>5</sup> Some knowledge of the extraction process to be employed by the laboratory would help to direct the sampling procedure;

<sup>4</sup> See technical publications in Annex A.

<sup>5</sup> See Davoren et al., 2007, in Annex A.



Figure 3 Window section taken from a femur.

- all bone contains DNA, but some bones are better at preserving DNA than others. Long bones, in particular femora, are the best source of DNA, after teeth. A "window" section should be taken out of the mid-shaft of the long bone (Figure 3). A section of approximately 2-5 cm will be adequate, using most extraction protocols, to allow multiple extractions. A medical oscillating saw (Stryker<sup>®</sup> saw) is recommended, but other saws will suffice if a medical saw is not available. (Note: Bones should not be sawed through, as this will impede anthropological assessment of the material, such as stature estimates);
- in many circumstances, it will not be possible to sample a femur. In these cases the order of preference for sampling is: tibia and fibula, humerus, radius and ulna;
- in human remains that are not highly decomposed, sections of rib provide a good source of DNA that is relatively easy to sample as part of a post-mortem examination;
- following the sampling of either bone or teeth from human remains, appropriate storage is important to prevent the further degradation of DNA. Samples from relatively intact human remains require storage at low temperatures, ideally -20 °C, to prevent microbial growth;

- samples taken from older "dry" bones should also ideally be stored at -20 °C. This may not be practicable in many contexts and samples can be stored at room temperature, but the breakdown of DNA will continue; and
- if freezing is not possible, samples should be kept as cool and as dry as possible. If samples become damp, microbial activity will accelerate the breakdown of DNA.

#### 4.3 Collection of reference samples for DNA analysis

When human remains are recovered and can be analysed using DNA profiling techniques, reference samples are needed for comparison in order to provide matches. The most common type of reference sample is from a biological relative. In some circumstances, biological trace evidence belonging to the missing person can be recovered and analysed.

#### 4.3.1 Biological relatives

Biological relatives share a proportion of their DNA; the degree of relatedness determines how much of their genetic make-up two individuals will have in common. Parents and children will share half of their DNA; an individual will also, on average, share a quarter of their DNA with their grandparents and grandchildren. Unless mitochondrial or Y-chromosome DNA analysis is being undertaken (see Section 2), the most powerful DNA comparisons can be carried out using samples from parents and children of missing persons.<sup>6</sup>

Collection of samples from relatives raises a number of ethical and legal issues that are addressed in Section 6. When planning a collection programme, consideration should be given to the following:

• the reference samples that should be collected depend on the circumstances surrounding the missing persons. If

<sup>6</sup> See Annex B.

the number of missing people is relatively low, then one reference sample from a parent or child may be sufficient, although it is always advisable to collect reference samples from at least two close relatives (parents or children) when possible. When the number of missing is in the hundreds or the thousands, then the possibility of coincidental matches becomes significant (see Section 3.2) and, ideally, additional reference samples from close relatives should be collected;

- the language used to describe biological relationships can be confusing, for collectors and for the families of the missing. It is therefore recommended that a pictorial representation of a family tree be used to identify the exact biological relationship of an individual to a missing person.<sup>7</sup> Individuals collecting the reference samples should be trained, and competent, in interviewing techniques to identify and record the exact nature of biological relationships. Access to a geneticist, who would be able to clarify the suitability of a particular relative as a reference, is desirable;
- the personnel involved in collecting samples do not need to be forensic specialists, but familiarity with taking and handling biological samples, and an accompanying knowledge of health and safety issues, is desirable;
- regardless of background, all personnel involved with sample collection should be trained in collection procedures and in the importance of the chain of custody. It is also important that collectors are taught methods to cope with the psychological pressures they will inevitably encounter when dealing directly with the families of the missing;
- psychological support for the families or individuals must be systematically planned and provided as an integral part of the collection process in order to help avoid further trauma; and
- the fact that the identification process does not include DNA analysis at a given time does not mean that samples

<sup>7</sup> See Annex C.

should not be collected. Samples can be collected and stored, and it may be possible or necessary to analyse them at a later date. This should be explained to the family members, as well as the possibility that the samples may not be used. The decision to proceed with collection will depend on whether or not there is a satisfactory legal framework, including for ensuring a chain of custody throughout the process, on whether the samples can be safely stored and catalogued, and on whether there is a realistic possibility that the samples will be analysed in the future. In principle, the aim should be to avoid multiple interviews and uncoordinated requests for DNA samples. Thus, the ante-mortem data and DNA samples should ideally be collected as part of the same interview.

Different methods can be used to collect reference samples. Some technical issues are noted below:

- the most common form of sample collection involves pricking the finger and collecting drops of blood on absorbent paper. A commercial product, FTA® paper,<sup>8</sup> has been extensively used for archiving biological material. If FTA® paper, or a similar commercial product, is not available, then collection can be undertaken using any clean absorbent paper, such as blotting or filter paper;
- after collection, the blood spot(s) MUST be allowed to dry completely and MUST be kept dry. After air drying, storage in a sealed plastic/foil packet along with a sachet of silica gel is recommended. As long as the blood sample and paper remain dry, the DNA will be relatively stable. Samples collected on FTA® are stable for extended periods (years) at room temperature;
- collection can also be made using biological material other than blood. Swabs taken from the oral cavity (buccal swabs) are commonly used in forensic science. The swab should be rubbed on the buccal (inner cheek) surface for

<sup>8</sup> FTA® paper is supplied in various formats by Whatman®. Details of products can be found at: http://www.whatman.com [Accessed 10 July 2009].

approximately 30 seconds to collect cellular material. As with blood samples, to preserve the sample, the swabs should be air dried. Once dry, they can be stored in paper/ foil envelopes containing a sachet of silica gel until they reach the laboratory, where, ideally, the swabs will be stored at -20 °C. Buccal samples can also be collected using FTA® paper, which removes the requirement for low-temperature storage prior to analysis; and

if air drying is not practicable during collection or if freezers are not available for storage, then commercially available buffers<sup>9</sup> offer an alternative for collection. Using these methods, the swab is placed in a plastic tube and a liquid buffer that retards the breakdown of DNA is added.

## 4.3.2 Biological artefacts from missing persons

In some cases it may be possible to match, with a high level of certainty, medical samples or personal objects with a missing person. These may be in the form of:

- medical samples, such as biopsies and blood samples;
- umbilical cords, teeth and other parts of the body, which are commonly retained as mementoes in some cultures; and
- personal belongings, such as hairbrushes, toothbrushes, and razors.

Storage conditions for artefacts will vary, depending on the type of material; however, low temperatures and dry conditions help to preserve all types of biological sample.

The major advantage of using personal artefacts is that it allows simple and very powerful DNA matching. The DNA profile obtained from the artefact and the human remains will

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<sup>9</sup> See publications in Annex A.

be identical, provided that DNA in the remains is sufficiently intact to obtain a full DNA profile. It is advisable, however, unless a secure chain of custody exists, to use personal artefacts along with samples from relatives of the missing person. This allows the identity of the artefact to be tested. For example, if a razor from a missing person is analysed, it could be compared with the DNA profile from the mother, father or children of the missing person to confirm the identity of the biological material, presumed to be from the missing person.

### 5 QUALITY ASSURANCE AND CONTROL IN DNA ANALYSIS

The power of DNA analysis has led to the development of stringent quality assurance and control measures to minimize the possibility of laboratories providing misleading or incorrect results. Quality assurance and control measures consist of several elements, including documentation and validation of methodologies, internal and external proficiency testing, and periodic case review. Laboratories can demonstrate that they are adhering to international standards through third-party accreditation. The most common third-party accreditation system adopted by DNA testing laboratories is ISO/IEC 17025.<sup>1</sup>

 When choosing a testing laboratory to undertake DNAbased human identification, careful consideration should be given as to whether the testing work should be performed by an accredited laboratory. There are several established and respected laboratories that do not have third-party accreditation, and the lack of accreditation should not necessarily exclude the participation of a laboratory, unless it is stipulated by the relevant legal system. However, in cases where laboratories do not have third-party accreditation, details of the quality assurance and control procedures should be sought. There is an increasing trend for laboratories to obtain third-party accreditation to the ISO/IEC 17025 standard. It is now commonly requested by organizations that are commissioning forensic DNA analysis.

<sup>1</sup> International Organization for Standardization, ISO/IEC 17025:2005(E), " General requirements for the competence of testing and calibration laboratories," Geneva, 2005.

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- The DNA collection and analysis techniques must be reliable and scientifically valid. Validation is a process by which a procedure is evaluated to determine its efficacy and reliability for forensic casework. It includes strict testing of the technique against known variables and determining the conditions and limitations of its use on forensic samples.
- Laboratories should establish a set of protocols to deal with all aspects of the process of identifying human remains, including: collection, storage and, ultimately, disposal of human remains; collection, storage and disposal of reference samples; processing of both human remains and reference samples; comparison and statistical analysis of data; and the reporting of matches and non-matches.

## 6 ETHICAL AND LEGAL ISSUES RELATED TO THE USE OF DNA FOR THE IDENTIFICATION OF HUMAN REMAINS

Information contained in a person's DNA is sensitive because it is a unique identifier and may contain information about a person's family and intimate associations. Therefore, it must be rigorously protected to ensure the right to privacy. Information derived from DNA can also contain details about a person's genetic predisposition to certain diseases, which, in turn, can bias insurers against him or her. For these reasons, wherever domestic legislation permits the use of DNA analysis for law-enforcement purposes, in almost every case, laws have also been adopted for the protection of genetic data that is collected. However, standard STR testing examines nondescript locations in human DNA and so is not useful for geneticpredisposition analysis.

International law does not have any specific provisions for protecting genetic data. International humanitarian law and international human rights law recognize the need to provide special protection for persons affected by armed conflict.<sup>1</sup> However, these bodies of law contain only general principles relating to confidentiality, privacy, non-discrimination, and human dignity that can be applied to the protection of genetic data.

In October 2003, UNESCO finalized the text of the International Declaration on Human Genetic Data.<sup>2</sup> As of 2009, this declaration and UNESCO's earlier Universal Declaration on the Human Genome and Human Rights (1997) were the only international declarations that addressed the issue of

<sup>1</sup> This refers in particular to persons deprived of their liberty, the wounded, displaced persons, refugees, and persons who have no news of their relatives.

<sup>2</sup> UNESCO, The International Declaration on Human Genetic Data, 2003: http://portal.unesco.org/shs/en/ ev.php-URL\_ID=3479&URL\_DO=D0\_TOPIC&URL\_SECTION=201.html [accessed 10 July, 2009].

protecting genetic data. The 2003 declaration emphasizes that any practice involving the collection, processing, use and storage of human genetic data should be consistent with both domestic legislation and international human rights law.

The preamble to the declaration contains some particularly far-sighted observations:

"... human genetic data have a special status on account of their sensitive nature since they can be predictive of genetic predispositions concerning individuals and (...) the power of predictability [*sic*] can be stronger than assessed at the time of deriving the data; they may have a significant impact on the family, including offspring, extending over generations, and in some instances on the whole group; they may contain information the significance of which is not necessarily known at the time of the collection of biological samples; and they may have cultural significance for persons or groups ..."

#### 6.1 Protection of personal and genetic information: Commonly accepted principles

In most countries, legislation that deals specifically with the issues arising from technological advances has not kept up with the rapid advance of DNA analysis in both forensic and medical applications. One ICRC workshop in 2002 examined international agreements and recommendations together with national legislation and compiled a set of legal principles concerning the protection of personal and genetic data that is to be respected in all circumstances.<sup>3</sup> These principles have been developed as a result of further legal research and are summarized below.

<sup>3</sup> See: ICRC,"The Missing: Action to resolve the problem of people unaccounted for as a result of armed conflict or internal violence and to assist their families, The legal protection of personal data and human remains," Geneva, 2003. (Available at: http://www.icrc.org/Web/eng/siteeng0.nsf/ htmlall/SCALLJ/\$File/ICRC\_TheMissing\_072002\_EN\_1.pdf [accessed 10 July 2009]).

The following relate to the protection of *all* personal data, including genetic data:

- biological samples left by the missing person, either as medical samples or other biological artefacts. "personal data" means any information relating to an identified or identifiable individual;
- personal data shall be collected and processed fairly and lawfully;
- the consent of the individual is required for the collection and use of personal data, except if required by a substantial public interest or for the protection of the vital interests of the person concerned;
- the collection and processing of personal data shall be limited to that which is necessary for the purpose identified at the time of collection, or beforehand;
- sensitive data should only be collected and processed with appropriate safeguards;
- personal data should be accurate, complete and updated as is necessary for the purpose for which they are used;
- security safeguards, appropriate to the sensitivity of the information, should protect personal data;
- personal data may not be used, disclosed or transferred for purposes other than those for which they were collected without the consent of the person concerned, except if required by a substantial public interest or for the protection of the vital interests of the person concerned;
- personal data may be transferred only to third parties who respect the principles of personal data protection;
- personal data should be deleted as soon as the purpose of their collection has been fulfilled, or when no longer necessary. They may, however, be retained for a definite period if required for the benefit of the individual to whom they relate or if essential for the performance of the humanitarian tasks of the organization that collected the data; and
- access to personal data should be granted to the individual to whom the data relate. Allowances should also be made for the right to challenge the accuracy and completeness of the data and to have them amended as appropriate.

The following set of principles relates specifically to the use of biological samples and the resulting DNA profiles:

- the collection, use and disclosure of DNA profiles are subject to the rules relative to the protection of personal data;
- DNA samples may be collected and analysed only for a clearly identified and specific purpose;
- identification of human remains through DNA typing should be undertaken when other investigative techniques of identification are not adequate;
- DNA samples may be taken and analysed only with the informed consent of the individual (see Section 6.2), except where an overriding public interest dictates otherwise. An overriding public interest should be limited to criminal investigations or public security and, in case of death, to the identification of remains. The specific purpose should only be direct individual identification;
- only appropriately trained persons shall take DNA samples;
- DNA information that is collected may be used and disclosed only for the purpose identified at the time of collection, or beforehand;
- DNA samples and profiles should be destroyed or deleted after they have served the purpose for which they were collected, unless required for related purposes;
- only laboratories with appropriate technical capacity and quality assurance/control measures should perform DNA analysis;
- DNA samples, profiles and records should be adequately protected from unauthorized access and use; and
- DNA profiles or samples should be disclosed, transferred or compared in the context of international cooperation only for the purpose identified at the time of collection, or beforehand, and only with the consent of the persons concerned, except in cases defined by law.

#### 6.2 Informed consent

When relatives are asked to donate reference samples, the reason for collecting the biological material should be explained, as far as possible in layman's terms, to the consenting individual. He or she should be able to understand how the collection will affect him or her. Individuals maybe said to have provided informed consent if they have understood:

- why the samples are being collected and how the identification programme will work;
- the practicalities of participating in the programme and the benefits that they are likely to receive from participation;
- how data relating to them will be managed and used, and that the principles of data protection will be respected (see Section 6.1);
- the details of the consent form that they are completing;
- how they will receive information during the identification programme; and
- that participation is voluntary; that they can withdraw from the programme if they later change their minds, and that they do not need to provide any reason for their withdrawal.

Participants should be given contact details in case they want to ask questions or withdraw from the programme.

The requirement of informed consent should prevent individuals from being coerced into providing a sample against their wishes.

## Annex A: Publications relating to the preservation and extraction of DNA from human tissue

#### Guidelines for the use of forensic genetics for human identification

Prinz, M. *et al.*, DNA commission of the International Society for Forensic Genetics, "Recommendations regarding the role of forensic genetics for disaster victim identification," *Forensic Science International: Genetics*, March 2007, Vol.1, No.1, pp. 3-12.

Budowle, B., Bieber, F.R., Eisenberg, A.J., "Forensic aspects of mass disasters: strategic considerations for DNA-based human identification," *Legal Medicine*, July 2005, Vol.7, No.4, pp. 230-243.

International Criminal Police Organization, *Disaster Victim Identification Guide*, 2009. Available at: http://www.interpol.int/Public/DisasterVictim/Guide.asp [Accessed 10 July 2009].

National Institute of Justice, *Mass Fatality Incidents: A Guide for Human Forensic Identification*, 2005. Available at: http://www.ojp.usdoj.gov/nij/pubs-sum/199758.htm [Accessed 10 July 2009].

National Institute of Justice, *Lessons Learned from 9/11: DNA Identification in Mass Fatality Incidents*, 2006. Available at: http://www.massfatality.dna.gov [Accessed 10 July 2009].

AABB Guidelines for Mass Fatality DNA Identification Operations (2009). Available at: http://www.aabb.org

#### **Preservation of soft tissues**

Graham, E.A.M., Turk, E.E., Rutty, G.N., "Room temperature DNA preservation of soft tissue for rapid DNA extraction: An addition to the disaster victim identification investigators toolkit?" *Forensic Science International: Genetics*, January 2008, Vol.2, No.1, pp. 29-34.

Kilpatrick, C.W., "Non-cryogenic preservation of mammalian tissue for DNA extraction: An assessment of storage methods," *Biochemical Genetics*, No. 40, 2002, pp. 53-62.

#### **Extraction of DNA from skeletal material**

Edson, S.M. *et al.*, "Naming the Dead: Confronting the realities of rapid identification of degraded skeletal remains," *Forensic Science Review*, Vol.16, No.1, 2004, pp. 64-89.

Loreille, O.M. *et al.*, "High efficiency DNA extraction from bone by total demineralization," *Forensic Science International: Genetics*, June 2007, Vol.1, No.2, pp. 191-195.

Davoren, J. *et al.*, "Highly effective DNA extraction method for nuclear short tandem repeat testing of skeletal remains from mass graves," *Croatian Medical Journal*, August 2007, Vol.48, No.4, pp. 478-485.

## Annex B: The statistical value of biological relatives for identifying human remains

It is advisable to collect reference samples from immediate biological relatives (parents/ children), as they share half of the missing person's DNA. The usefulness of different combinations of relatives is illustrated in the chart below. The higher the percentage value, the more useful the relatives' sample in obtaining an identification. Even though samples from direct relatives offer the highest probability of determining identity, it is still advisable to collect reference samples from more than one relative to minimize the possibility of false (coincidental) matches between relatives and human remains.

Family reference	Probability of identity*
One full sibling (brother or sister)	92.1%
Sibling and aunt (or uncle)	94.4%
Sibling and two aunts or uncles from the same side of the family	97.8%
Sibling and an aunt and an uncle from different sides of the family	99.8%
Sibling and half-sibling	98%
Sibling and two half-siblings (same mother, different fathers)	99.4%
Two siblings	99.91%
One parent/child	99.9%
Sibling and parent	99.996%
Father and one maternal half-sibling	99.95%
Father and two maternal half-siblings	99.996%
Father and maternal aunt	99.993%
Three grandparents	96.7%
Four grandparents	99.99%
Three grandparents and sibling	99.994%

Table 1 The average probability of identity, \*given a 10% prior probability of identity (i.e. prior to DNA analysis, there is a 10% probability that the deceased is related to the family that is being tested). The results were reached using 15 STR loci, contained in the Identifiler PCR Amplification Kit (Applied Biosystems, Foster City, CA, USA).

Data from: Brenner, C.H., "Reuniting El Salvador families." Available at: http://dna-view.com/ProBusqueda.htm [Accessed 10 July 2009].

#### Annex C: An example of a family tree that could be incorporated into a record sheet for collecting biological material from relatives of missing individuals

Based on the form provided in *Lessons Learned from 9/11: DNA Identifications in Mass Fatality Incidents*, published by the National Institute of Justice, USA. Available at: www.DNA.gov [Accessed 10 July 2009]. Donors are invited to circle their place on the family tree to minimize errors in recording their relationship to the missing individual.



#### MISSION

The International Committee of the Red Cross (ICRC) is an impartial, neutral and independent organization whose exclusively humanitarian mission is to protect the lives and dignity of victims of armed conflict and other situations of violence and to provide them with assistance.

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Established in 1863, the ICRC is at the origin of the Geneva Conventions and the International Red Cross and Red Crescent Movement. It directs and coordinates the international activities conducted by the Movement in armed conflicts and other situations of violence.

