Lessons Learned From 9/11: DNA Identification in Mass Fatality Incidents[†]

DNA analysis is the gold standard for identification of human remains from mass disasters. Particularly in the absence of traditional anthropological and other physical characteristics, forensic DNA typing allows for identification of any biological sample and the association of body parts, as long as sufficient DNA can be recovered from the samples. This is true even when the victim's remains are fragmented and the DNA is degraded. While many effective laboratory protocols are available for DNA analysis, the analytical portion is only one part of the identification process.

HOW DNA IS USED TO MAKE IDENTIFICATIONS

DNA analysis has a number of advantages over other identification methods and is a critical tool in associating severely fragmented remains, such as those that resulted from the World Trade Center (WTC) attacks, with victims. It is important for a laboratory to have a plan in place for using this forensic technique in a high volume situation.

In the United States, the medical examiner or coroner generally has the statutory responsibility and authority to identify the deceased and issue a death certificate. (Future references in this report to "ME" include medical examiners and coroners.) The ME must decide whether the forensic information available—based on judgments about a variety of data—justifies declaring an identification and signing a death

[†] This Article has been adapted and modified, with permission, from chapters one, two, four, five, eight, ten, eleven, and fourteen of LESSONS LEARNED FROM 9/11: DNA IDENTIFICATION IN MASS FATALITY INCIDENTS, U.S. Department of Justice, Office of Justice Programs, (2006). The full report is available at www.massfatality.dna.gov; print copies or CD-ROM's also may be ordered.

certificate. The consequences of a misidentification can have emotional and legal ramifications well beyond a specific case.

DNA is the newest of several methods or techniques used to identify victims of a mass fatality incident. Other methods of identification include recognition and comparison of distinguishable physical attributes (e.g., birthmarks, tattoos, medical implants, clothing and jewelry), forensic anthropology, fingerprints, odontology, and radiology. Ideally, all of the data, which may include DNA analysis, are considered before the ME issues a death certificate.

DNA profiling has advantages over traditional identification methods in some mass fatality situations. When sufficient quantities of typable DNA and informative reference samples exist, DNA profiling can be uniquely identifying. DNA analysis can be used even when recovered human remains are quite small. Often, DNA analysis is the only technique for reassociating severely fragmented remains with victims. However, DNA identification testing requires more time, effort, and specialized, skilled personnel than some of the traditional identification tools. Mass fatalities with intact bodies may not need DNA to make most of the identifications.

DNA identifications are made by comparing DNA profiles from human remains to DNA profiles from reference samples. There are several potential sources of reference samples: (1) personal items used by the victim (e.g., toothbrush, hairbrush, razor) and banked samples from the victim (e.g., banked sperm or archival biopsy tissues stored in a medical facility); (2) biological relatives of the victim (i.e., "blood kin"); and (3) human remains previously identified through other modalities or other fragmented remains already typed by DNA. Exhibit 1 describes potential sources of reference samples for DNA comparisons.

Exhibit 1: Potential Sources of DNA Reference Samples

Source	Description	Comments
Personal items (also known as direct references)	Biological samples include blood stain cards, blood stored for elective surgery, pathology samples, semen samples, and extracted or "lost" (adult or baby) teeth. Personal use items include hairbrushes, toothbrushes, razors, unwashed undergarments, and used personal hygiene items (e.g., sanitary napkins).	Personal items are the most precious of all samples (including human remains) because they are so scarce. Personal items allow for the simplest type of DNA matching: direct comparison. However, sole use by only the victim can be difficult to ensure. Before reporting an identification, the lab must verify that the DNA from the personal item belongs to the victim. This is done either administratively or through DNA interpretation. Personal items require forensic analysis conditions (extraction, quantitation, etc.).
Biological relatives (kin)	Samples are collected from biological relatives. Kinship samples are typically collected using buccal swabs.	The relatives' biological relationship to the victim largely determines the utility of the sample (e.g., parents provide better reference samples than cousins). Distant relatives can be useful if there are many of these types of relatives in kinship analysis, but the analysis of the pedigree can become very difficult. Although biologically unrelated to the victim, the surviving parent of a missing person's biological child can assist in determining an identification. Sometimes the relative does not know his or her true relationship (if any) to the victim. The lab must verify (administratively or through DNA interpretation) the relationship before reporting an identification. If collected properly, kinship samples provide an abundant quantity of DNA.
Previously identified human remains	Human remains identified using other modalities. For example, DNA from a torso identified through a medical examination or a unique tattoo may be used as a reference sample to identify other remains fragments; or well-characterized DNA profiles from other fragments may be useful to associate samples.	Like personal items, previously identified remains can be directly matched to unknown samples. Single teeth have proven to be unreliable reference samples because they are easily misidentified through non-DNA modalities.

The number of identifications that can be made using DNA analysis depends on the availability (number) and quality of the human remains and reference samples.

Often, there are severe limitations with remains or reference samples. For example, environmentally harsh conditions at the incident site may limit the quantity of typable DNA recoverable from human remains. There may be a paucity of personal items. For example, airline passengers often travel with their toothbrushes and hairbrushes, and these items may be lost or destroyed in an

airline disaster. Kinship samples may be unavailable or scarce because the victim had few living biological relatives or because the relatives are unable or choose not to participate in the identification effort. In the case of airline disasters, families often travel together, further limiting the availability of known kinship samples. Finally, public perception and expectation may play a role in deciding whether DNA testing will be used to make identifications. All of these factors must be considered when assessing the usefulness of DNA analysis for a particular incident.

Before a mass fatality incident occurs, laboratories should develop a plan for extraction procedures, alternate analytical methods for challenging samples, automation for handling high-volume analyses, and expert system software to interpret results. One of the critical steps in this process is the creation of a chain-of-custody documentation system for all materials collected at the scene. This is important not only for scene reconstruction and quality control, but also in the event of any subsequent legal proceeding; as in any situation with potential criminal implications, the proper collection and preservation of samples—using the best forensic practices—is important. In addition, improper preservation methods can lead to the loss of typable DNA, compromising the ability to make an identification.

MAJOR DECISIONS

The medical examiner's primary goal in most situations will be to identify the victims and issue death certificates. In a natural disaster, the effort is largely humanitarian, including identifying the victims so that their remains (and necessary documentation) can be returned to their families. However, when a mass fatality results from criminal activity, the identification effort has humanitarian and investigative components. In a criminal matter, the ME may expand the goals to include identifying the perpetrators and assisting with the law enforcement investigation.

HOW IMPORTANT IS DNA TO THE IDENTIFICATION EFFORT?

The degree to which human remains are fragmented or degraded determines the value of DNA analysis in the identification process. Intact, large body parts lend themselves to identification by less costly methods, such as X-ray, dental examination, and fingerprints. However, DNA analysis is the only viable method for identifying severely fragmented or degraded remains. Even when whole bodies are recovered, DNA analysis still may be the best approach when materials that are necessary for other modalities—for example, dental records or verified body identification by friends or relatives—are unavailable. Remains often are identified by multiple methods, which may or may not include DNA. For example, only approximately 25 percent of the identifications of airline crash victims are generally made by DNA exclusively.

WILL EVERY PERSON OR EVERY FRAGMENT BE IDENTIFIED?

The answer to the question of whether every victim or every fragment of remains will be identified frames the scope of the DNA identification effort. Obviously, intact bodies will require fewer DNA tests than fragmented remains, although decomposing bodies may not easily yield full profiles.

For example, in an airplane crash with 50 victims, in which each victim's remains are fragmented into 100 pieces, the identification effort undoubtedly would end sooner if the goal is to identify each victim, rather than each fragment of human remains. Everyone—the public, the policymakers, and the laboratory personnel—needs to understand the answer to the important question: "When are we finished?" If the policy is to identify all of the victims, DNA analysis would stop as soon as the last victim is identified—which means that some human remains may never be analyzed or returned to the families. However, when the goal of the effort is the

attempted identification of all fragments, the work of the laboratory likely will be greater.

It is important to consider that, if a mass fatality incident is so large and devastating that it affects the psyche of a community, a country, or the world, the scope of the identification effort may be broadened to help acknowledge the breadth of the emotional ramifications. After the 9/11 attacks, for example, the Mayor of New York City directed the Office of the Chief Medical Examiner to do everything humanly possible to identify every fragment of human remains. This policy resulted in new DNA analysis techniques and approaches; any biological fragments that could not be identified were preserved for potential analysis with future technologies.

The absence of policies guiding the number of DNA tests that will be attempted on severely compromised samples can have enormous consequences.

In planning for a future mass fatality, policymakers should consider the impact on the public if technologies at the time are insufficient to obtain DNA profiles on all remains. Lessons learned from the World Trade Center (WTC) identification effort suggest that policymakers need to understand that the broadest testing scale can add years to a DNA identification effort.

WHAT IS THE MINIMUM FRAGMENT SIZE THAT WILL BE IDENTIFIED?

Policies also need to be established at the beginning of the effort that define "minimum fragment size" for DNA testing. A policy that has as a goal "all remains tested" may mean that many fragments may fail to yield results. In this situation, the DNA effort would take longer and be more costly—and, although families would be more likely to receive more of their loved one's remains, they may be unprepared for the fragmentary condition of the remains or the length of time it takes to identify them.

Decisions must be made regarding the minimum fragment size on which identifications will be attempted, the number of attempts that will be made to identify each fragment, and the statistical threshold that must be met before results are conveyed to the ME. These decisions are fundamental to a laboratory's strategic planning. Planning—including preliminary meetings between the laboratory director, the forensic anthropology staff, and the ME—is critical, because it allows each entity to understand the perspective of the others in the emotionally charged environment following a mass fatality incident.

From the laboratory director's perspective, the minimum fragment size—typically, 1 to 10 centimeters—should be based on three criteria:

(1) maximizing the probability that all victims are identified;
(2) recognizing the emotional needs of the victims' families and friends; and
(3) providing forensically relevant information.

Defining the acceptable minimum fragment size affects every aspect of the identification effort: how remains are collected at the incident site, how they are processed in the morgue, the number of samples that ultimately appear on the DNA analyst's workbench, and the likelihood of a successful DNA profile.

HOW DIFFICULT WILL IT BE TO IDENTIFY EVERYONE?

The laboratory must make a preliminary decision regarding the DNA technologies that will be used. For example, can all identifications be made with standard forensic Short Tandem Repeat (STR) markers? Will mitochondrial DNA (mtDNA) play a role and, if so, to what degree will the ME rely on mtDNA results to make an identification? Longer recovery efforts usually result in more DNA degradation, and this, in turn, affects marker choices.

Also, the decision to expand marker sets beyond those typically used by the laboratory will be driven by environmental conditions at the incident site and the resulting DNA degradation, and by the scope and duration of the DNA effort.

Whether an incident is "closed" or "open" has a significant impact on the statistical options for making DNA identifications. In a "closed" incident, the laboratory director should determine whether a list of victims is available—for example, in an airline disaster, the passenger manifest. Although it is important to keep in mind that the manifest might be incomplete or incorrect, the majority of the victims would still be known.

An "open" incident is one in which the number of victims—or their identities—is largely unknown. After the WTC attacks, for example, the final list of victims was not determined until months later, and even then, officials believed that there were up to 20 additional, unknown victims. It should also be kept in mind that open incidents are prime candidates for insurance fraud. There are people who may try to file fraudulent life insurance claims. In the WTC attacks, for example, a police investigation was performed with respect to every reported victim, and cases of fraud were still being uncovered more than 6 months after September 11, 2001.

It is possible for a closed incident to become open. If a plane crashes into a neighborhood, for example, the victims on the ground would change a typical "closed" event to "open," because it would not be known who was on the ground.

ASSUMING FUNDING, CAN THE LABORATORY DO THE WORK?

After considering the role that DNA will play in an identification effort, the type(s) of DNA analysis needed, and the duration of the recovery effort, the laboratory must determine the analytical processes. Ultimately, it must be

decided whether a laboratory has sufficient capability and capacity to do the work.

Currently, most forensic DNA laboratories are proficient in STR analysis, proven to be a powerful tool in many mass fatality incidents since the 1990s. For example, DNA identifications in three airline disasters—Swiss International Air Lines flight 111 (September 2, 1998), Alaska Airlines flight 261 (January 31, 2000), and American Airlines flight 587 (November 12, 2001)—were made exclusively with STRs; no other technologies were needed to identify every victim.

STRs are particularly informative on well-preserved soft tissue and bone samples. Analysis of the compromised remains after the WTC attacks demonstrated that STRs also work with degraded tissue and bone fragments if the DNA extraction process is optimized. However, STRs alone are often not sufficient for identification when samples are severely compromised. In those situations, additional methods—such as mtDNA sequencing or Single Nucleotide Polymorphisms (SNP)—are likely to be necessary to generate sufficient genetic markers to reach a statistical threshold.

The DNA identification response to a mass fatality incident demands forensic casework skills and high-throughput genotyping or databasing, whether from the public and/or private sectors. Because there are differences between STR genotyping for medical or research purposes, laboratories that can perform high-quality clinical or research STR genotyping should be used only after careful consideration.

DNA from human remains in a mass fatality incident—and personal reference sample items—are collected from many different sources, each requiring chain-of-custody protocols not typically used by clinical or research laboratories. To increase the probability of obtaining full profiles from the personal effects samples, DNA should be extracted using forensic casework extraction protocols. Likewise, full polymerase chain reaction (PCR) volumes usually are necessary to develop complete profiles from the victim samples.

On the other hand, kinship samples are more uniform and lend themselves to standardized high-throughput processes that are used (although perhaps with different protocols) by forensic databasing laboratories and some nonforensic genotyping laboratories. Forensic databasing laboratories often have sophisticated information technologies for tracking samples and avoiding mix-ups. In addition, forensic databasing laboratories often are more experienced than forensic casework laboratories with outsourcing work to private laboratories.

Depending on the mass fatality event, kinship samples, for example, might be analyzed by high-throughput clinical laboratories that are willing to implement appropriate protocols (assuming that the kin are those of the victims, not kin of those suspected of being perpetrators of the mass disaster). This procedure focuses the most rigorous forensic protocols on the limited and compromised victim samples. And, although mass fatalities from natural disasters may fall outside the parameters of a forensic investigation, laboratory directors and MEs should weigh all potential issues before departing from chain-of-custody and other forensic procedures.

However, most mass fatality events likely will require a forensic approach for at least some of the samples. In these instances, as previously noted, laboratories that can perform high-quality clinical or research STR genotyping will have to modify their protocols and analysis methods. For example, clinical and research laboratories may not typically use the same (or any) molecular ladders as size standards for allelic interpretation. It is important to ensure that all laboratories involved in the DNA analyses use protocols that permit standardized evaluations of victim profiles. Standard STR forensic DNA marker analysis is based on well-established and comprehensive procedures that enable profile frequencies to be calculated from existing and well-validated databases.

MANAGING EXPECTATIONS

A laboratory director who is faced with responding to a mass fatality incident will encounter a host of new constituents, in addition to the laboratory's traditional constituents.

The laboratory director should assume that the public, including public officials and the media, knows little about the realities of DNA identification analysis, popular television shows notwithstanding. The public will have to be educated in order to develop realistic expectations about the speed and power of DNA testing. The public must be encouraged to understand that the nature and scope of a mass fatality disaster can affect the laboratory's ability to make DNA identifications, including the fact that some of the victims and some of the remains may not be identified. In mass fatality incidents, fragments may be collected and analyzed, but never identified. A laboratory director's effort to frame realistic expectations and candidly discuss issues such as the limitations of the technologies can limit disappointments in the future.

The laboratory director can help officials and the public understand the identification process by collecting, monitoring, and reporting key facts and metrics. Frequent status updates to stakeholders can save the laboratory time by reducing the need to respond to ad hoc requests for information.

The public's ultimate measure of the laboratory's performance is the number of victims identified. The importance of educating constituencies about the many steps in the analytical process is critical to reducing unrealistic expectations. Raising awareness that DNA testing takes longer—sometimes much longer—than depicted in television dramas is an important message. Using metrics such as the number of samples received and the number of samples analyzed, the laboratory director can help convey the complexity and time requirements of DNA analysis. Activity metrics can demonstrate that the laboratory is working hard and that seemingly low numbers of identifications may be

attributable to factors such as the quality of the DNA from the remains or the availability of appropriate reference samples.

The laboratory director should initiate discussions with those responsible for disseminating information on what metrics will be used to describe the laboratory's progress. Without this direction, people unfamiliar with forensic DNA identification testing will use their own perceptions to measure progress and success. This could result in the laboratory being unjustly criticized about the speed and number of identifications—and this, in turn, can create a credibility gap when laboratory directors and their supervisors are asked to explain seeming "delays" or "deficiencies" in results and reports. Therefore, it is incumbent on the laboratory director to educate the various constituencies regarding what DNA information can and cannot reasonably be provided and why. To the extent possible, the laboratory director also should determine the frequency and duration of progress reports. Ideally, periodic status reports will be automatically generated by the Laboratory Information Management System (LIMS).

Although the vast majority of victim identifications will be properly made and reported, a prudent laboratory director will be mindful of the potential for civil action—over issues such as misidentification, release of information, control remains, intellectual property—against a laboratory that is responding to a mass fatality incident. It would be prudent for the laboratory director to work closely with the agency's contracting officers and attorneys on issues such as contracts, intellectual property rights, and privacy issues, including the creation of a next-of-kin release policy.

Advance planning allows the laboratory director to design safeguards, like ensuring appropriate sample collection processes and preparing an informatics framework that can avoid sample mix-ups. And, since a mass fatality incident response may have a measurable impact on a laboratory's capabilities and capacity, the response plan should contain a

procedure for informing—and updating—superiors on this issue.

Faced with the reality that backlogs and turnaround times may suffer during a mass fatality incident response, a laboratory director should be prepared to: (1) request additional resources (including people and equipment) early and often, and (2) justify requests with estimations of time delays should additional resources not be forthcoming.

The laboratory director will need to use numerous skills to organize and manage a mass fatality incident response. Flexibility, innovation, and creativity likely will be demanded. Mass fatality incidents intensify the routine pressures faced by laboratories and often expose the laboratory to heightened scrutiny.

COLLECTING REFERENCE SAMPLES

The Victim Identification Program (VIP) is software developed by the Disaster Mortuary Operational Response Teams (DMORT), a program of the U.S. Department of Homeland Security, to collect victim information. VIP contains approximately seven pages of victim-related data, tailored for making mass fatality incident identifications. This information (primarily non-DNA-related) is gathered by DMORT personnel or collection center officials through interviews with the victims' families. Although the families generally complete the printed VIP forms with the aid of family assistance centers, it is possible for the process—if well organized and well financed—to be done via computers.

Currently, there are no standards that govern the collection of reference samples (i.e., personal items and kinship samples) from families. Historically, DNA laboratories have designed forms used in the collection process on an ad hoc basis—and, in some situations, forms have been designed on-the-fly, hours before they have been put into use. Appendixes B and C to this report (a sample Personal Items Submission Form and a sample Family and/or Donor Reference Collection Form) may be helpful. It may be important to also keep in mind:

- Family members are under extreme stress in the days following a mass fatality incident, and their minds may be elsewhere during the collection process, causing them to inadvertently provide incorrect information. To avoid such mistakes, collection forms should be as simple as possible.

- Every reference sample form should contain the following information about the victim:

  - Full name, including whether they are a Junior, Senior, etc.

  - Date of birth.

  - Social Security number (if known).

It is not uncommon for several victims in a large disaster to share the same name but be unrelated. Similarly, related individuals with the same names—cousins, for example—may be victims in a single event. Consistent use of the following guidelines will ensure that the proper reference samples are assigned to each victim:

- Always collect the donor's full name and date of birth. During times of grief, relatives may not realize that they are using nicknames or that a father's "Bob" may be a mother's "Robby."

- Europeans and Americans write dates differently (the standard European notation is DD/MM/YY). Ensure that month and day fields are unambiguous on collection forms.

- Family members frequently transpose their relationship to the victim. In most cases, this is a result of a poorly worded question such as, "What is

your relationship to the victim?" It is better to ask questions from the perspective of the donor. For example, "The victim is my _____." or "I am the victim's _____." Also, the dates of birth of the donor and the victim can be used to help correct these mistakes.

- Collect as much information as possible about the relevant family structure; the sample form found in appendix C may be a helpful guide. The laboratory can compare purported pedigrees from members of the same family, then use dates of birth and genotypes to help discern the true relationships.

- Collect as much information and as many samples as possible. There may not be another opportunity.

Generally, collection centers are staffed by members of the family assistance center, DMORT, and ME personnel. It is critical that the laboratory staff participate in the reference sample collection process, and it is advisable for the laboratory to define and control the process. Non-DNA laboratory personnel usually do not have the expertise to assess how kinship samples or personal items will contribute to the DNA identification effort. For example, a family member might ask, "I have a second cousin living overseas; should we contact her for a sample?" Individuals trained in DNA analysis and genetics must be available to respond to such questions and ensure that the most valuable samples (from a DNA identification perspective) are collected and analyzed.

During the World Trade Center (WTC) DNA identification project, a software program that estimates whether a specific kinship sample will benefit the identification was explored. For example, suppose buccal swabs have been collected from a victim's father and sister. Will collecting DNA from the victim's grandson help meet the statistical threshold for making an identification? Charles H. Brenner, Ph.D., developed such a program to assist in the

WTC identification efforts (see http://dna-view.com/simulate.htm).

Traditionally, the metadata associated with a reference sample are collected on paper, then transferred to computer. Ideally, however, all information is entered directly into a database during the collection process. This helps reduce transcription and other data entry errors, such as those resulting from illegible handwriting. It would helpful, for example, if a specialized collection workstation could be constructed to streamline the collection procedure and guarantee greater accuracy. Features of a specialized collection workstation—many which are included in the software that the Armed Forces DNA Identification Laboratory (AFDIL) uses to collect reference samples—might include:

- Two monitors, one oriented toward the individual performing the data entry, the other oriented toward the family member (allowing the family member to validate information as it is entered).

- A device that electronically captures the donor's signature; these devices are already in use in some retail stores.

- A printer for creating copies of forms to be given to the donor at the end of the interview.

- A barcode printer; for example, buccal swabs and personal items could be immediately barcoded for the laboratory's sample tracking system.

- A digital camera to photograph personal items.

Two approaches may be used to collect reference samples from families: an "open house" (family members visit the collection center without an appointment during the day) and,

the preferred approach, scheduled appointments when all family members are able to attend.

The primary advantage of the open house approach is that family members can come and go according to their own schedules. However, an open house has drawbacks, including:

- The collection site must be staffed, even when there is low or no demand.
- It can become chaotic if many people arrive at the same time (e.g., lunch hour, after work).
- Because members of the same family may arrive at different times, it can be difficult to ensure that specific personal items and kinship samples are assigned to the proper victim. This can occur, for example, if one family is mistakenly assigned more than one case number.
- There is a greater probability that family members will provide conflicting pedigree information.

The preferred approach to collecting reference samples, however, is to schedule an appointment with an entire family unit. The primary advantage with this approach is that all the reference samples for a victim are collected at one time.

Although each collection will take more time when an entire family is present, this approach decreases the chance of a sample mix-up, allows the entire family to validate the pedigree, and uses laboratory staff time more efficiently.

SAMPLE TRACKING AND MANAGEMENT

The laboratory must be prepared for an influx of samples following a mass fatality event. The physical location of each sample—and all other data associated with it—must be tracked through the DNA analysis processes. This section discusses important considerations in sample accessioning,

naming and numbering schemes, handling the possibility that remains may be commingled, and work lists that can be generated by the LIMS to facilitate DNA identifications.

The size and quality of the DNA from victims' remains greatly affects the ability to obtain DNA profiles for identification purposes. Similarly, the availability of reference samples from close biological relatives or from personal effects can impact the ability to identify victim remains. In addition, the often chaotic environment at a mass disaster site can lead to sample mix-ups. Even when the sample collections are conducted by another agency, the laboratory manager should be directly involved in establishing guidelines for collection, handling, and preservation of all samples to ensure quality and accuracy throughout the process.

Chain of custody and the origin ("provenance") of collected remains are important aspects of the identification management process. They are also critical to the collection of reference samples for comparison with victim remains. Chain-of-custody practices are necessary for reference-sample attribution, even when there is no criminal investigation component to the identification effort (e.g. in a natural disaster), since death certificates based on DNA identification will always include forensic elements.

Establishing the source of personal effects that are used as reference samples—for example, toothbrushes, razors, medical biopsy samples, clothing—can be problematic. The Kinship and Data Analysis Panel (KADAP) developed an informational brochure to help victims' families understand what types of samples are helpful in making an identification based on DNA analysis

It is important to keep in mind that other sample issues can complicate the identification process. These include inconsistencies that may arise from data in the Victim Identification Program (VIP) forms. For example, there may be inadvertent reference-sample switching by bereft loved ones.

Or, there may be name misspellings or unlinked nicknames (for example, Bobby vs. Bobbi vs. Bob vs. Rob vs. Robert) associated with the same last name. Inconsistent case numbering during field collections can also occur. These issues can reduce the efficiency and accuracy of the identification process.

Family members may state with certainty that their missing relative was the only one to have contact with a personal effect that is brought in for DNA testing. However, mixed DNA profiles from toothbrushes or other personal effects may eliminate that reference sample as a single-source reference. If one of the profiles on a personal effect can be attributed to another family member, the remaining profile may be inferred as the victim's, but this situation adds uncertainty concerning source and missed or shared alleles and makes for a more complex analysis.

Other complications—including assumed, but incorrect, parentage—may come to light after DNA testing. In some mass fatalities, such as a tidal wave, personal effects belonging to victims can be lost or contaminated at the site itself. Managing sample collection and tracking in a controlled, documented fashion is essential to the DNA identification process.

One of the most important decisions that a laboratory responding to a mass fatality event will have to make is whether to treat the incident as a humanitarian effort, civil incident, or criminal matter. This decision will drive chain-of-custody requirements. Exhibit 19 describes some of these issues.

Exhibit 19: How the Event Is Treated

Treat Incident As	Implication
Humanitarian effort	Although it is important to correctly identify a sample, strict chain-of-custody procedures and documentation may not be required. This can simplify and streamline processes—particularly among multiple laboratories—but this scenario may require new sample tracking processes.
Civil matter	Most mass fatality incidents have a civil component—i.e., the need to issue death certificates. Chain-of-custody procedures and documentation are required, but they are less stringent than for incidents considered as criminal matters. This scenario may allow simplification/streamlining of the sample handling processes and may (or may not) require new processes.
Criminal matter	Some mass fatality incidents (e.g., acts of terrorism) are criminal matters, and therefore, they require rigorous chain-of-custody procedures and documentation. Public forensic DNA laboratories currently have established chain-of-custody systems that can be used.

Most public forensic laboratories have a chain-of-custody system in place, and generally it makes sense to use the existing system as a foundation in a mass fatality incident response, modifying the processes as necessary (particularly if the movement of samples must be tracked to and from multiple laboratories). It is also important to keep in mind when establishing documentation processes for tracking the provenance of samples that personal effects provided as reference samples can be incorrectly characterized by loved ones as having been used solely by the victim. It is not unusual for mixed DNA profiles to be found on shared intimate items, such as toothbrushes. As previously mentioned, these types of mixed profiles can also reveal that family members may have had incorrect assumptions about biological relationships, so it is helpful to have a policy in place to deal with such discoveries.

In a transportation mass fatality event, for example, collecting samples can be complicated because people who are traveling usually have their personal effects with them, and these can be lost or contaminated at the scene. In this case, additional DNA testing, such as mitochondrial DNA (mtDNA), may help to resolve identifications by grouping maternally linked victims.

In planning for a mass fatality incident response, it is important to consider how samples will be accessioned into

the laboratory. Laboratories are likely to maintain higher efficiency if their existing Laboratory Information Management System (LIMS) can be used for handling mass disaster samples. When evaluating whether a forensic LIMS can be adapted to a mass fatality incident, the laboratory director should consider whether:

- The mass fatality samples can be segregated from regular casework samples. (The laboratory likely will want to track casework and mass fatality samples and metrics separately.)

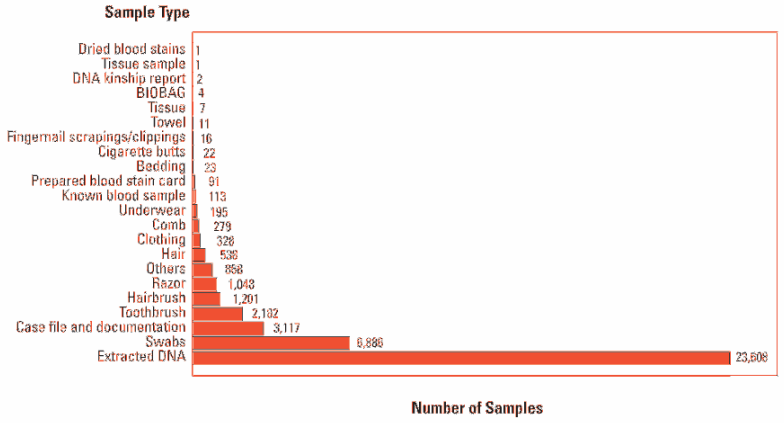
- Numbering should begin with "1" or a different numbering sequence should be established to designate mass fatality incident samples as separate from casework samples. (It is helpful for mass fatality incident samples to be numbered sequentially, not mixed with routine casework numbers.)

- The LIMS can support a single sample being given more than one sample number and can support cross-referencing multiple sample numbers. (Mass fatality incident samples often have several identifying numbers, analogous to case numbers assigned to an agency's casework samples. In addition, when multiple laboratories assist with analysis or interpretation, samples likely will receive multiple identifying numbers, one for each laboratory. The LIMS should be able to accept additional sample numbers and cross-reference them so the sample can be easily queried.)

Because of the large number of samples that may be accessioned in a mass fatality response, the laboratory may need teams of people entering data and checking each other's work if the samples are not barcoded. The laboratory also should plan on receiving many different types of samples, and, therefore, must be capable of extracting DNA from numerous substrates and analyzing samples with varying

quantities of DNA. Exhibit 20, provided by the New York City Office of the Chief Medical Examiner (OCME), shows the number of samples, by sample type, received during the World Trade Center (WTC) DNA identification effort.

Exhibit 20: Types of Samples From the World Trade Center Response



Source: Information provided by the New York City Office of the Chief Medical Examiner.

A laboratory responding to a mass fatality event must establish a sample-naming scheme that distinguishes personal items, kinship samples, and disaster samples. To limit potential sample mix-ups and ensure that different DNA technologies produce compatible results, the laboratory also will need to track the number and type of analysis performed on each sample.

Typically, DNA laboratories encode information in the sample name or identification number. Although this is not optimal from an information technology (IT) perspective, it is a common practice in forensic DNA analyses, because it allows analysts to track analysis-related information along with the sample name. For victim samples, data encoded in the sample identification number may include:

- Identity of the laboratory (in a multilab response) that performed the extraction.

- Identity of the laboratory (in a multilab response) that performed the analysis.
- Extraction attempt number.
- Type of DNA analysis performed (e.g., short tandem repeat (STR), single nucleotide polymorphism (SNP), mtDNA).
- Plate number, tube number, well number, etc.

For personal effect samples, data encoded in the sample name may include:

Victim identification number.

- Identity of the laboratory (in a multilab response) that performed the extraction.
- Identity of the laboratory (in a multilab response) that performed the analysis.
- Extraction attempt number.
- Type of DNA analysis performed (e.g., STR, SNP, mtDNA).
- Plate number, tube number, well number, etc.

For kinship samples, data encoded in the sample name may include:

- Victim identification number.
- Relationship to victim (e.g., biological mother, father).

In the WTC identification effort, forensic anthropologists triaged disaster samples and decided which ones would undergo DNA analysis. The anthropologists usually were able to separate human from non-human remains. They attempted to identify commingled remains, a seemingly single tissue that yields multiple profiles. These presented some of the greatest challenges in managing the DNA effort. Any laboratory responding to a mass fatality event must identify the extent of commingling (i.e., determine how many individuals are represented in the sample), and then create, administratively, a subsample for each.

DNA personnel should work closely with the anthropologists—or other professionals who are designated to perform the triage—to develop a decision tree for collecting DNA samples from the disaster site. Such a decision tree should consider these issues:

- Commingling of remains—although it requires a different way of thinking, in many types of mass fatality responses, it will simplify the laboratory's work to assume that remains may be commingled.

- Whenever possible, bone or deep tissue should be sampled; bones are much less likely to yield multiple profiles than tissue.

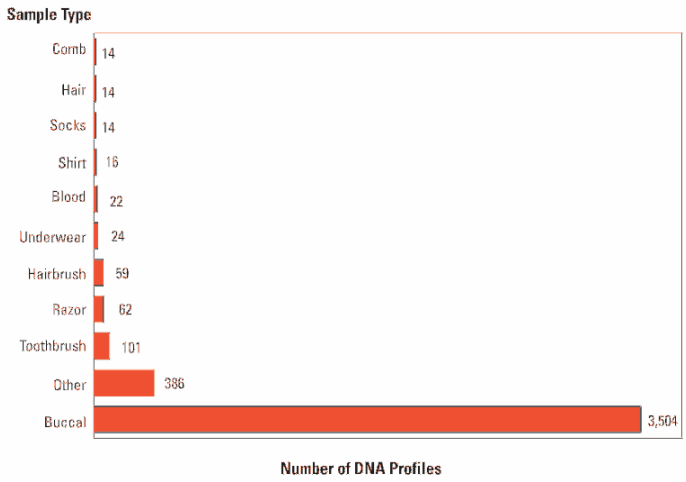
- Unless the tissue is covered by intact skin, do not assume that a tissue sample belongs to one individual. Remains that are not directly linked by tissue should be treated as belonging to separate individuals. Even when the sample is covered with skin, multiple DNA profiles can occur if the victims were in contact with each other.

- When bone is surrounded by tissue, treat the tissue and bone as separate samples, and assign them separate sample numbers.

The laboratory is likely to receive and analyze disaster samples before personal effect items or kinship samples. Depending on the duration of the recovery effort, the laboratory may not be able to examine all of the remains and choose only the samples most likely to yield DNA profiles. In an extended recovery effort, the laboratory will have to work samples as they arrive and not assume that "better" or "larger" samples will be available in the future.

Personal items and kinship samples can be collected over a long period of time. Of the three types of samples (disaster, personal effect item, and kinship), personal effect items usually are the most precious because the DNA they yield is likely to be a small quantity. The best personal items from a DNA perspective are toothbrushes, razors, and hairbrushes. Saved letters, with their original licked stamps and envelopes may also provide sufficient quantities of usable DNA for references, but those who provide such letters should be made aware that the testing process will alter the appearance of the envelope. Exhibit 21, provided by the OCME, depicts DNA profiles, by sample type, from the WTC response.

Exhibit 21: DNA Profiles by Sample Type From the World Trade Center Response



Source: Information provided by the New York City Office of the Chief Medical Examiner.

Initially, the laboratory may choose to analyze the most promising personal effect items, analyzing other items only if necessary. Kinship samples can be considered less precious, because they usually have abundant DNA and, hopefully, additional samples can be collected from victims' relatives, if necessary.

In a mass fatality incident response, the laboratory will need a strategy for managing its work. Although work lists may be unnecessary in a small laboratory for routine limited-volume testing, in a mass fatality incident, testing and verification is much more complex, requiring work lists to provide structure, accountability, and traceability in managing the data.

Work lists that are automatically generated by the LIMS greatly facilitate fast and accurate DNA identifications. Since the identification process may change in response to additional testing needs, the LIMS must be flexible. It also must support a "comments" field, where sample and match-specific information can be stored, easily identified, and viewed by laboratory personnel.

Work lists—which should contain sample numbers, dates of previous procedures, and comments—also can be used to:

(1) Notify laboratory personnel of the matching, identification, and reporting tasks that need to be performed.

(2) Minimize duplication of effort by documenting completed work.

(3) Avoid inefficient data processing that can occur when analysts must:

- Search more than one database for a potential match.

- Compare potential matches to identifications that have been established and should have been documented in the LIMS.

- Spend time deducing what new potential matches need to be processed whenever a new match is attempted.

(4) Identify work volumes, allowing the laboratory director to assess the progress of work and target bottlenecks with resources.

(5) Serve as a repository for sample information. By maintaining documentation of the case analyses, the analyst is able to identify processing history, and, by documenting each stage of matching, identification, and reporting with date and user information (in a stage field), the analyst can determine:

- The stage of each potential match/ identification.
- How long a potential match/identification has been in each stage.
- The last person responsible for creating information on the potential match/ identification.

Other work lists that may be important in a mass fatality identification effort include:

- New match between a previously untested remains fragment and an already tested remains fragment.
- New potential match made with a single personal effect and available kin.
- New potential match made with a single personal effect (no kin).
- New potential match made with kin only.
- Administrative review.

- Reference rerun.
- Administrative resolution.

SAMPLE ANALYSIS

Although the Nation's forensic laboratories generally have the policies, systems, and tools to collect, extract, amplify, and analyze many biological samples, most would not be able to handle the number of samples associated with a mass fatality event. This section offers an overview of processes involved in the DNA typing of a large number of samples in a relatively short period. See appendix H for a more rudimentary discussion of DNA analysis.

A forensic laboratory's mass fatality plan should include large-scale collection and extraction procedures, alternate analytical methods for particularly challenging samples, automation for handling high-volume analyses, and quality assessment tools for interpreting results. The plan also should consider work and storage spaces, including sample accessioning and processing areas that have sufficient bench space and biological containment hoods.

Laboratories may plan to use robotics in batch analysis in a mass fatality identification. In the World Trade Center (WTC) identification effort, robotics was essential in handling the quantity of samples. It is important for laboratory directors to note, however, that there is likely to be a steep learning curve with such new procedures. Therefore, advance planning is important.

As was the case after the 9/11 attacks, the environmental conditions to which samples are exposed can compromise the quantity or quality of extractable DNA. Of course, the quality of biological samples will be incident specific, ranging from good quality, high molecular weight to highly degraded. Therefore, DNA-typing methods need to be robust.

SAMPLE COLLECTION

Although all components of the DNA identification process are important, sample collection may be the most critical and frequently overlooked. In the urgency to identify the victims, there may be little attention paid to how the remains are collected. Planning can have a great impact on the quality and quantity of typable DNA. To standardize the collection materials—which, in turn, will simplify the extraction process—the laboratory manager should be involved in the sample collection process.

Protocols for chain-of-custody documentation in collecting evidence and handling samples must be a part of a laboratory's mass fatality plan. This is important not only for scene reconstruction and quality control, but also for any subsequent legal proceedings. As in any situation with potential judicial implications, it is critically important to use the best forensic practices in collecting and preserving samples. Improper preservation methods can lead to the loss of typable DNA and the potential compromise of data that is necessary for a positive identification.

A mass fatality plan should provide for the collection of personal items from family members and others. After a mass fatality event, family members will be eager to provide samples to help identify a loved one. In a smaller incident, family reference samples may be easier to collect and analyze than a victim's personal items. However, in a larger event, it may be more efficient to use personal items for identification, assuming sufficient quantities of DNA can be recovered from a personal effect and its sole use by the victim can be assured.

As noted in prior sections of this report cellular material can be derived from hair, stamps, envelopes, toothbrushes, razors, and unwashed clothing. If personal effects are used in a mass fatality identification effort, it is advisable to collect several samples, if possible, as some will be better suited for analysis than others. It can be challenging to develop instructions for submission of a victim's personal items,

including a way to ensure that only the victim used the item. Also, it is important to keep in mind that a family's emotional attachment to a loved one's personal item may be strong.

It also may be necessary to collect reference samples from around the world. In this case, it may be helpful to consult with professionals who work at paternity testing laboratories with remote sample collection experience.

Three sample forms that may provide general guidance are included with this report: Personal Items Submission Form, Family and/or Donor Reference Collection Form, and the Family Tree Form.

Needless to say, it should always be considered that a personal item may contain the DNA from someone other the victim/purported owner. That is why the Sample Personal Items Submission Form (appendix B) solicits detailed information regarding everyone who may have used the item. To prevent misidentification of remains due to the presence on the personal item of DNA from other contributors, the DNA profile recovered from the personal item should, if possible, be compared to the DNA profiles of family members to ensure that the proper biological relationship exists between the DNA on the personal item and the DNA from the family members.

SAMPLE STORAGE

Work and storage space must accommodate sample accessioning and processing, including sufficient bench space and safety hoods. An estimate of the number of potential samples should be made so that sufficient storage space can be assured (see exhibit 4). Soft tissue samples need to be stored in ultra-low-temperature freezers. In addition to securing appropriate freezer space, additional refrigerators may be needed to store samples during the extraction and analysis phases. If sample recovery at the disaster site is a long-term process, tissue decomposition will become a factor in planning for sufficient storage space.

Depending on the conditions at the disaster site, larger portions of tissue may be needed to compensate for degradation as time passes during the collection process. In the case of bone, for example, a few cubic centimeters may (under optimal conditions) be adequate for analysis, but an entire femur may be required in more compromised situations. Not only do larger samples require more storage space, but extraction procedures may require modification to accommodate larger sample sizes.

Following the WTC attacks, other laboratories offered to assist the Office of the Chief Medical Examiner (OCME). Such offers are likely to occur after any future mass fatality incident. If appropriate chain of custody, accessioning, and other infrastructure concerns are addressed, outsourcing may be considered. Obviously, however, if samples are sent to other laboratories at any stage of the analysis, the same quality control and chain-of-custody practices must be maintained.

SHORT TANDEM REPEAT (STR) DNA AMPLIFICATION AND ANALYSIS

In general, polymerase chain reaction (PCR) issues in a mass fatality identification effort are no different than in any other situation, except for the greater number of samples. Although different analytical approaches may eventually be required to make identifications, it is most expedient to use familiar and well-established technologies (i.e., short tandem repeat (STR) typing) as the method of first analysis. In fact, many disaster samples may be wholly typable by STR analysis.

It should be remembered when performing extractions, however, that additional testing may be needed; therefore, extraction techniques that will accommodate other testing methods—such as mitochondrial DNA (mtDNA) sequencing—should be considered.

After extraction, the template DNA is subjected to PCR, which is particularly useful for analyzing materials that may contain degraded DNA. A typical PCR requires three steps

and is based on specific annealing and extension of oligonucleotide primers (two per marker) that flank a defined target DNA segment. The template DNA to be amplified by the PCR is first denatured, usually by heating the sample to 95 degrees Centigrade.

After denaturation, the two primers hybridize to the separated strands at a given locus. Primer annealing is accomplished by lowering the temperature to a defined point, typically between 45–65 degrees Centigrade. The next phase in the PCR process, primer extension, is generally carried out at 72 degrees Centigrade, the temperature at which Thermus aquaticus DNA polymerase can most effectively copy the original template DNA by extending the primers and making complementary copies of the original template DNA. These three steps (denaturation, primer annealing, and primer extension) represent a single PCR cycle.

Upon repeated cycles of the PCR, an exponential accumulation of a discrete DNA fragment containing the genetic marker of interest is achieved. Thus, PCR generates large amounts of specific DNA sequences from relatively small (picogram or nanogram) quantities of genomic DNA. Amplification of target sequences of DNA is primarily a technique to prepare the sample for typing.

Only a limited template may be available, and inhibitors to PCR may further reduce the yield of PCR product. Efforts should be made to optimize the components of the PCR to overcome the vagaries of environmental contamination. Some practices used by laboratories during routine analyses—using reduced reaction volumes, for example—may not be appropriate when samples are compromised. A larger reaction volume may dilute inhibitors to the point that the PCR can be successful. Additional enhancements to reduce the impact of inhibitors, such as Bovine Serum Albumen, may be considered part of the protocol for maximizing DNA yields from compromised samples.

ALTERNATIVE TESTING METHODS

In the WTC identification effort, the OCME relied on the recommendations of the Kinship and Data Analysis Panel (KADAP) regarding new identification methods for analyzing compromised samples. In considering additional typing technologies and strategies, the KADAP considered the sufficiency of extracted material to support all attempted technologies, as well as any quality control issues that might arise. The KADAP also considered how to handle the statistical approach using other technologies, including linkage and haplotype/genotype comparisons.

STRs reside in the human cell nucleus; outside the nucleus, in the cytoplasm, are mitochondria. Mitochondria are subcellular organelles that contain an extra chromosomal genome separate and distinct from the nuclear genome. Human mitochondrial DNA differs from nuclear DNA in that it is a closed, circular (rather than linear) molecule; it is smaller, consisting of approximately 16,569 base pairs; it is maternally inherited; it does not undergo recombination; and it is present in high copy number in a cell.

The maternal inheritance and lack of recombination characteristics are particularly helpful in identifying human remains. Associations can be made or refuted where known maternal relatives are the reference sample sources, even if they are several generations removed from the victim.

The primary advantage of using mtDNA (as opposed to nuclear DNA analysis) on compromised samples is the high copy number of mtDNA molecules in a cell. When the amount of extracted DNA is very small or degraded (as can be the case in mass disaster tissue samples of bone, teeth, and hair), an identification is more likely using mtDNA analysis than using the polymorphic markers found in nuclear DNA.

In the WTC identification effort, a number of samples could not be typed sufficiently with STR loci to identify the source with a high degree of confidence. In these cases, mtDNA sequencing was attempted to increase the discrimination power. Although the extraction process for mtDNA typically requires a relatively clean environment, this

was not possible in the WTC identification effort, due to the number of samples. However, reasonable precautions were taken, including a reduction in the number of amplification cycles (28 or 29 instead of the typical 36). This reduced contamination issues, although at the expense of the sensitivity of detection.

Although not as informative as a battery of autosomal STR loci, a unique mitotype may be sufficient to make an identification, if the victims are from a closed population. The mitotype can be used to group individuals into smaller categories, narrowing the candidate pool. It may then be possible for a less informative partial STR profile to become a unique identifier within the mtDNA subcategory. Screening by mtDNA sequencing would be possible because of the availability of high-throughput analysis, coupled with software that automatically interprets mitotypes.

In the WTC identification effort, recovered DNA was often too degraded and fragmented to produce STR results with standard commercial STR kits. However, by repositioning the primers so that they resided closer to the repeat region, the amplified product (or amplicon) was made smaller than some of the fragmented DNA template molecules, thus making genetic characterization of the sample possible for more STRs than when using traditional typing. These STR miniplexes were invaluable for analyzing the more degraded samples, and, in fact, results were obtained for some samples at loci that were not typable using commercially available kits.

The general assay procedure for the miniplex test used in the identification of WTC victims was similar to that used for forensically validated STRs. After evaluating the methods, reagents, and validation data, the KADAP determined that no additional equipment and training was necessary.

The PCR amplicon size can be further reduced by amplifying regions that contain a class of genetic markers known as single nucleotide polymorphisms (SNPs). Although an abundant supply of SNPs exists for identity testing, most SNPs are biallelic and, therefore, not as informative for

identity testing as STR loci. However, because the amplicon size can be reduced 60–80 base pairs in length, DNA that is degraded beyond the limits of STR typing may be typable.

In the WTC identification effort, an SNP typing method was validated for the more difficult-to-type samples. In fact, identifications that otherwise would not have been possible were made using this technology. Combining the features of a chip array, the primer extension assay, and universal tags, the multiplex assay method was carried out in a flat-bottom microplate, in which each well contained a total of 16 individual antitag sequences for 12 SNPs and 4 controls. (Basically, each PCR primer, about 45 bases long, is comprised of a 25-base-long segment that is complementary to the area immediately adjacent to the SNP extension site and a 20-base-long sequence—that is, the tag sequence—that is complementary to an antitag sequence attached to the bottom of a well.)

Using that process, the SNP extension product was transferred after PCR and allowed to hybridize in the array of antitags. A fluorescent detection system allowed typing of the two possible alleles at the SNP site by comparing signals from fluorescent dyes used to label the two different allelic products in the PCR extension reaction. With this technology, identifications were made on some very compromised samples that otherwise would not have been possible to identify.

QUALITY CONTROL

Quality control can be one of the biggest challenges for a laboratory that must respond to a mass fatality incident. Careful monitoring is necessary to help avoid problems that can result from the increase in scope and volume of work. This section offers suggestions for monitoring quality control.

Laboratory directors understand that quality management—quality assurance and quality control—is critical to reporting data in an accurate and timely manner. Quality assurance is based on policies and procedures that provide confidence in a laboratory's ability to produce

accurate DNA profiles. Quality control focuses on gathering and analyzing process data to determine whether the results are as expected.

In order to assure quality, a laboratory responding to a mass fatality incident should make every effort to follow the relevant standards for sample testing and the analysis of DNA profiles. These standards may include the Federal Bureau of Investigation's Quality Assurance Standards for Forensic DNA Testing Laboratories and Convicted Offender DNA Data-Basing Laboratories. A laboratory also may follow the American Association of Blood Banks' Standards for Parentage Testing. However, each mass fatality incident is unique—and, after careful consideration and consultation with experts and others involved in creating standards, a laboratory may decide to modify policies to facilitate more rapid reporting of identifications. Of course, any increase in the speed of reporting must occur without compromising accuracy. And any modifications to an existing standard—whether made on a per-sample or ad hoc basis—should be fully documented and retained in a quality management record created specifically for the mass fatality incident response.

Although every individual involved in the testing process is responsible for maintaining quality, at least one laboratory employee should be given the responsibility and authority to ensure that the laboratory adheres to proper standards in processing the mass fatality incident samples. This quality control manager plays a critical role in ensuring that the entire laboratory meets the criteria of the quality program, particularly because errors left uncorrected become more difficult to resolve as time goes by.

INTENTIONAL REDUNDANCY

Although unintentional redundancy can diminish productivity, it may be an important quality control measure to use a 5–10 percent redundancy when making DNA identifications of mass fatality victims. Intentional

redundancy may take several forms, including the duplicate analysis of samples or using multiple software programs for confirming matches and kinship. Also, a second laboratory might perform a duplicate analysis. To accomplish this, two cuttings are taken—and given separate numbers—when the samples are prepared. Needless to say, care should be taken to ensure that duplicate cuttings are from the same sample, as, depending on the type of disaster incident, the commingling of remains may be a concern. In such cases, it should not be assumed, for example, that tissue samples from the same shoe are from the same victim.

MULTIPLE TEST AND SOFTWARE SYSTEMS

Another useful redundancy is running multiple test systems, either in-house or by vendors. If multiple test systems are used—including different multiplex kits—the profiles from each should be compared. Even though there is a match in one system, there may be a nonmatch in another as a result of a mutation, testing problems, or differences in the power of exclusion. Of course, all discrepancies must be resolved prior to reporting an identification.

Redundancy of software systems, such as multiple matching and kinship programs, may also be considered. In addition, the particular realities of each mass fatality incident may require new software approaches. If a program is written—or significantly modified—for a particular event, it may be advisable to run "control" data through another software system to ensure consistent results. Relying on a new version of software without testing it against a validation data set can lead to errors in identifications, especially in terms of finding and ordering partial profiles. In the World Trade Center identification effort, validation data sets were critical to ensuring that the continually evolving software programs were operating properly.