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

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Preface

On September 11, 2001, 2,792 people were killed in terrorist attacks on the World Trade Center (WTC) in New York City. The number of victims, the condition of their remains, and the duration of the recovery effort made the identification of the victims the most difficult ever undertaken by the forensic community in this country.

In response to this need, the National Institute of Justice (NIJ), the research, development, and evaluation agency of the U.S. Department of Justice, brought together a group of experts to provide advice and support throughout the identification effort. Called the Kinship and Data Analysis Panel (KADAP), the group made recommendations on new forensic technologies, tools, policies, and procedures to help identify those who perished in the WTC attack.

This report contains the KADAP's "lessons learned," particularly regarding DNA protocols, laboratory techniques, and statistical approaches, in the DNA identification of WTC victims. It is written primarily for the Nation's forensic laboratory directors and other officials who may be responsible for organizing and managing the DNA identification response to a mass fatality incident.

Although New York City's mass disaster plan on 9/11 contained lessons learned from the 1993 terrorist bombing of the WTC, it did not contain policies or procedures for identifying mass disaster victims through DNA analysis. Had this been part of the city's plan in 2001, many of the issues that arose after the attacks could have been more quickly resolved.

This report discusses the incorporation of DNA identification into a mass fatality disaster plan, including how to:

- Establish laboratory policies and procedures, including the creation of sample collection documents.
- Assess the magnitude of an identification effort, and identify and acquire resources to respond.
- Identify reference and kinship samples.
- Create a comprehensive laboratory management plan, including technology management and quality assurance.
- Establish lines of communication between agencies, departments, victims' families, and the press.

Although this report does not address every aspect of a mass fatality DNA identification effort, it does stress intentional testing redundancy as a way to monitor a system's effectiveness. The report also discusses how decisions made in the first 48 hours after a mass fatality event shape the scope of the identification effort.

Designed to augment another NIJ publication, *Mass Fatality Incidents: A Guide for Human Forensic Identification* (http://www.ojp.usdoj.gov/nij/pubs-sum/199758.htm), this guide will help the Nation's forensic laboratories—whether called upon to identify victims of a major natural disaster, transportation accident, or terrorist attack—prepare for a mass fatality incident.

Message From the Director

Nothing in the history of mass fatality events prepared America's forensic community for the task of identifying those who perished when terrorists attacked the World Trade Center (WTC) in New York City on September 11, 2001. For the Nation's forensic laboratories, the primary lesson of this monumental identification effort is clear: every jurisdiction—large and small, urban and rural—should have a plan for identifying mass disaster victims through DNA analysis.

This report was prepared by the Kinship and Data Analysis Panel (KADAP), a multidisciplinary group of outstanding scientists that the National Institute of Justice (NIJ) assembled to offer advice in the identification of those who died in the WTC. The report contains guidance for forensic laboratory directors on developing a mass fatality DNA identification plan. It should become an essential resource used by forensic laboratory directors and senior public safety officials at all levels of government.

Even before this report was published, NIJ used the work of the KADAP to assist officials involved in identifying the victims of the Southeast Asia tsunami (December 2004) and of Hurricane Katrina (August 2005), a disaster that revealed how any State or municipality can be overwhelmed by the operational requirements of responding to a mass fatality event. All of these events demonstrate that it is only through planning, training, and assessment of the capabilities of our public forensic laboratories that laboratory directors—and the policymakers who support them—can prepare for a mass fatality event. But such preparation is essential to ensure that our public resources are used as efficiently as possible.

The importance of such preparedness was again demonstrated only 2 months after the 9/11 attacks when, on November 12, 2001, American Airlines flight 587 crashed in Queens, New York, killing 265 people. Because of the administrative and analytical processes that were in place for the WTC identification effort, New York City's Office of the Chief Medical Examiner (OCME) did not have to restructure its laboratory to handle the identifications of the airline crash victims. Historically, such a major identification response would have taken at least 3 months and even that would have been considered rapid. But because the OCME was prepared, all of the victims of the American Airlines crash—the second largest single-event transportation mass fatality in U.S. history—were identified within 1 month.

The challenges that accompany any large-scale DNA identification effort are substantial. Therefore, I encourage every jurisdiction to carefully consider the guidance in this report. The families of the victims of the next mass fatality disaster—indeed, the entire Nation—will need their public officials to be prepared. This guide will help us accomplish that mission.

Glenn R. Schmitt
Acting Director, National Institute of Justice

Acknowledgments

The National Institute of Justice (NIJ) is grateful for the support of the many people who were involved in this project, from the creation and empanelment of the Kinship and Data Analysis Panel (KADAP) through the development of this report.

The idea of a panel of experts to advise New York City officials in the identification of victims of the September 11, 2001, terrorist attacks on the World Trade Center (WTC) originated with Inspector W. Mark Dale, Director of Forensic Services for the New York State Police Department. Realizing that the number of victims and the condition of their remains would require his laboratory to enter uncharted territory, Inspector Dale asked NIJ to create a "brain trust" of independent scientists to offer guidance in this monumental effort.

NIJ thanks the organizations that supported their designee's participation on the KADAP. The National Institutes of Health's (NIH's) Human Genome Research Institute and the National Center for Biotechnology Informatics provided several KADAP members whose expertise became the bedrock of the Panel's deliberations. We especially thank Dr. Francis Collins and Dr. David Lipman for identifying potential KADAP members, and for their steadfast support throughout the victim identification process. We also thank Dr. Celia Hooper Kozlowski, from NIH's Office of the Director, for her guidance on NIH programs that became extraordinary resources throughout the identification effort.

NIJ also thanks the Armed Forces Institute of Pathology DNA Identification Laboratory (AFDIL), Brigham and Women's Hospital, Carleton University, the Federal Bureau of Investigation, Indiana University School of Medicine, Johns Hopkins University Center for Inherited Disease Research, the National Institute of Standards and Technology, New York State Department of Health, the University of Central Florida, the University of North Texas Health Science Center, and Yale University School of Medicine for supporting the investment of time and energy by their Panel representatives. We appreciate, too, that colleagues of KADAP members in those institutions often took on the responsibility of additional work so that the KADAP members could provide guidance on the identification of WTC victims.

Early in the process, the Panel's understanding about collection procedures and specific "Ground Zero" issues was greatly aided by then-Deputy Commissioner of the New York City Police Department, Maureen Casey, who attended KADAP meetings on behalf of Commissioner Howard Safir.

The New York State Police Forensic Services Division made the initial KADAP meetings a priority, despite many new responsibilities that the Division had for organizing victim reference samples.

Many people—from the private and public sectors—provided testimony to the Panel that resulted in the KADAP recommendations. Early demonstrations of matching software, developed for other mass fatality situations, were an important contribution. We thank, in particular, Ed Huffine, Fuad Suljetovic, and Adnan Rizvic, with the International Commission on Missing Persons in Bosnia; Jodi Irwin, with AFDIL; and Benoît Leclair, with Myriad Genetics, who received permission from the Royal Canadian Mounted Police to share a special computer program that was used in the WTC identification effort. [Note: All affiliations mentioned in this report reflect the person's affiliation at the time that the WTC identification effort was ongoing.]

The KADAP also received presentations on genetic systems that were considered for the WTC identification effort. New York City's Office of the Chief Medical Examiner (OCME) and the New York State Police (NYSP) received assistance from some of these companies through funding by the

Federal Emergency Management Agency. As the identification of victims progressed, the KADAP received peer-review assistance from many experts, including Joyce deJong (Disaster Mortuary Operational Response Teams), Terri Melton (Mitotyping Technologies), Kevin Miller (Federal Bureau of Investigation), Walther Parson (Institute for Legal Medicine in Austria), Alan Scott (Johns Hopkins University's Institute of Genetic Medicine), George Sensabaugh, Jr. (University of California, Berkeley, School of Public Health), Brion Smith and James Ross (AFDIL), and Matthew Thomas (DNAPrint).

Finally, NIJ is grateful to the OCME and NYSP scientists and, most importantly, to the KADAP members themselves. From the beginning of this extraordinary effort, these individuals worked as a seamless unit, identifying, analyzing, and crafting never-before-attempted approaches to victim identification through DNA analysis. The Panel's rigorous, thoughtful, and creative work—including important recommendations on mini-STR testing, statistical approaches to composite or "virtual" DNA profiles, and the creation of quality assurance safeguards—will have a profound impact far into the future on the identification of human remains.

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CHAPTER 1

Introduction

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.

Special attention is required for:

- Sample collection, preservation, shipping, and storage.
- Tracking and chain of custody issues.
- Clean, secure laboratory facilities.
- Quality assurance and quality control practices.
- Managing the work.
- DNA extraction and typing.
- Interpretation of results.
- Automation.
- Use of software for sample tracking and data management.
- Use of an advisory panel of experts.
- Public education and communication.
- Privacy issues.

Developing strategies that address these features of DNA identification will facilitate the identification process. As many of the features described in this report for DNA typing of mass disaster human remains are the same as those for missing persons cases, it may be possible to invest in and coordinate with missing persons identification efforts. Thus, the infrastructure for a mass disaster identification process could be in place and only surge capacity would need to be addressed in the event of a mass disaster.

Although this report deals with the identification of human remains through DNA analysis, other methods—including anthropology, dental records, tattoos—should be used in a mass fatality identification effort whenever possible. In fact, some of these identification modalities are so uniquely identifying that they may eliminate the need for the more labor-intensive DNA analysis, or at least minimize the need for reanalysis. (An extensive overview of forensic identification beyond DNA analysis can be found in *Mass Fatality Incidents: A Guide for Human Forensic Identification,* U.S. Department of Justice, National Institute of Justice, June 2005, NCJ 199758. The report is available at http://www.ojp.usdoj.gov/nij/pubs-sum/199758.htm.)

Lessons Learned From 9/11: DNA Identification in Mass Fatality Incidents is not a substitute for a comprehensive mass fatality response plan. Although the concepts explored in this report should be considered in a laboratory's mass disaster plan, the document provides only a general framework. The recommendations do not represent the only correct course of action and may not be feasible in all circumstances; details regarding implementation should be based on a laboratory's need, culture, and resources. In no case should this report be considered a legal mandate or policy directive.

After a mass fatality event, it is the job of the medical examiner to identify the victims so that death certificates can be issued. When DNA analysis is part of the identification process, the laboratory must ensure that:

- Victim, reference, and kinship samples are accessioned into the laboratory system and documented by proper chain of custody.

- DNA is extracted and genotyped, and that analysis of the genotype data, including matching and statistics, is performed.
- Samples are reaccessioned and accounted for, if they have been outsourced.
- Final administrative review—comparing the DNA results to non-DNA metadata—is conducted and, if necessary, reconciled. [Note: Metadata for a kinship sample, for example, include the kin's name, biological relationship to the victim, and when and where the sample was collected.]

This report addresses all these phases of a mass fatality DNA identification effort. It is organized by specific areas of management responsibility for the laboratory manager or director. During the World Trade Center DNA identification effort, many of the most critical management decisions were made within the first 48 hours following the terrorist attacks. This report examines many of these issues, and contains several sample forms that may be helpful. The report can be downloaded at http://www.massfatality.dna.gov. To order a hard copy or CD–ROM of the report, call 1-800–851–3420 or visit http://www.massfatality.dna.gov.

Throughout the report, members of the Kinship and Data Analysis Panel share some of their personal thoughts; please note that they are speaking for themselves, not on behalf of their employer.

The following self-assessment may help a laboratory consider whether it is ready to handle the identification of victims in a mass fatality incident. It may be helpful to complete the checklist using various numbers of samples.

Is the Laboratory Prepared to Handle a Mass Fatality?

Number of victims _____

Number of victim samples _____

Number of personal items _____

Number of kin _____

Whom will the laboratory be reporting to?

Who is responsible for funding the DNA identification effort?

How will the victim samples be collected and tracked?

How will the samples get to the laboratory?

How many family reference collection kits are immediately available? What modifications to the kits may need to be made?

Are there written instructions for kin reference sample collection?

How will the collection of reference samples be coordinated locally, nationally, and internationally?

How will the personal reference samples and elimination samples be scheduled and collected?

Is there an adequate accessioning area to receive all samples?

Are there procedures to handle incomplete or missing data?

Is there a laboratory information management system (LIMS) in place to track cases, including victim and reference samples?

Can cases be combined or separated in the LIMS?

How will a victim be defined (as a case)?

Is there adequate staffing for each of the following?

- Collection
- Accessioning
- Extraction
- Amplification
- Analysis
- Interpretation
- Reporting
- Quality control
- Family relations
- Media relations
- New personnel

Is there sufficient space for the victim and reference samples? Are the areas separate?

Will the testing be done in-house or will some of the samples be outsourced?

If samples will be outsourced, are contracts in place that can be modified?

What modifications need to be made specific to the mass fatality? For example, how will the data be reported?

Will an advisory group be needed to provide technical support and to assist the laboratory in making major decisions?

Are there adequate extraction procedures and robotics to handle the volume? Do the parameters need to be changed for victim samples?

Can additional reagents be purchased from the same lot number already used by the laboratory?

Can the mass fatality identification effort be handled without purchasing additional equipment? Does the laboratory have the capacity?

If the lab does not have the capacity, are there procedures and policies in place to acquire equipment and consumables rapidly?

How will the generated profiles be stored?

How will the matching take place?

Is there a mechanism to review the supporting metadata for accuracy?

Is there a checklist in place?

How will reports be generated?

How will reports be issued?

How will remains and personal items be returned to the families? How will this be documented?

Does the laboratory have the financial resources to handle the identification effort?

Can the laboratory handle a backlog of its normal casework while it works on the identification effort? If so, how big can the backlog get?

Does the laboratory have kinship analysis software?

Is there a policy to handle the situation in which the genetic relationship is not consistent with the biological relationship reported by the family?

Does the laboratory have a relationship with a bioethicist?

Other_____

CHAPTER 2

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 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.

Having preserved bloodstains from some of the firefighters' medical files led to several identifications. In an apparent mixup at the blood bank, however, one of the exemplars was not from any of the firefighters. We discovered this while analyzing DNA from firefighters' wives and children. In this case, it was certainly beneficial to perform kinship analysis on the exemplars before trying to match them to victims.

Robert Shaler

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

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.

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.

It is also important to plan for the preparation and storage of a high volume of reference samples, including samples from family members and other sources, such as personal items (if sufficient quantities of DNA can be recovered from these items and their sole use by the victim is assured). Cellular material for use as a potential reference sample may be derived from items such as hair, stamps, envelopes, toothbrushes, and razors. However, the use of personal items for reference samples can be problematic because the quantity of DNA that can be isolated is often minimal. In addition, ensuring before testing that only the victim used the item can be difficult. If personal items are used, it is best to collect several samples because some will undoubtedly be better suited for analysis than others. Also, some samples may need to be divided for separate analysis and quality control.

While a step-by-step discussion of DNA analysis is likely to be too rudimentary for most laboratory directors, it may offer useful information for families, family assistance coordinators, policymakers, reporters, and others who require a basic explanation of the issues faced by a laboratory that is responding to a mass fatality incident. Therefore, such an overview—at the mid-technical level—has been included as appendix H to this report, including a discussion of the following areas and how these were handled in the WTC DNA identification response:

- DNA extraction.
- DNA amplification and analysis, including short tandem repeat (STR) analysis and alternative testing methods, such as mitochondrial DNA (mtDNA) analysis, repositioning primers, and single nucleotide polymorphism (SNP) analysis.
- Making the identification.

Some laboratory directors also may be interested in further reading on the statistical issues involved in making identifications through DNA analysis; additional references have been included as appendix I.

I knew we were facing enormous management challenges. The notion that we were to reassociate potentially hundreds of thousands of remains—let alone identify them by comparing their profiles to perhaps tens of thousands of kin and effects profiles—was beyond daunting. For it to work, we needed to build a national scientific consensus for the methods that would be developed to identify the World Trade Center victims. We needed human geneticists, statisticians, bioethicists, forensic DNA scientists/managers, genetic researchers, information technologists, database managers, and program managers—and we needed them *fast*. Developing the KADAP early in the process helped in the management of this enormous undertaking.

W. Mark Dale

CHAPTER 3

Before the Incident

Decisions made by the laboratory director during first 48 hours following a mass fatality incident are crucial to the efficiency and overall success of the DNA identification effort. Asking—and answering—the questions posed in this chapter will guide a laboratory director in preparing a response plan.

The hours and days immediately following a mass fatality incident are inevitably chaotic. The laboratory director must simultaneously address a number of issues, including responding to the diverse requests from elected officials, government agencies, the media, the victims' families, and the laboratory staff. Despite these competing pressures, the laboratory director must recognize that the decisions made during the first 48 hours will largely determine the efficiency and efficacy of the DNA identification effort.

This point cannot be overemphasized. In fact, some hasty or reactive decisions made during the initial hours after the 9/11 attacks caused management obstacles that spanned the life of the project. The best strategy for avoiding reactive management decisions is to prepare a DNA identification response plan before an incident occurs, and the best mass fatality response plans—which anticipate a potential forensic DNA identification effort—consider the humanitarian, scientific, information technology, and political factors, as well as staffing and resource requirements that will be necessary to mount a response. The laboratory's mass fatality response plan should dovetail with the plans of other agencies and departments, especially the ME's office.

Several useful processes and procedures may already exist in a forensic laboratory. For example, process mapping can be useful in improving and expanding a laboratory's capabilities, and this management tool would also benefit the implementation of a mass fatality response plan.

The following questions should be considered when formulating a mass fatality response plan for a forensic laboratory:

- Are there sufficient people, resources, equipment, and funding to support the effort?
- How will the DNA identification effort be funded?
- What agencies/departments will interact with the laboratory? Who are the points of contact?
- Which agencies/departments will be in charge of specific activities—for example, collecting reference samples, collecting disaster samples, administration of funding?
- What activities will the laboratory director be responsible for, and what activities can other agencies or departments assume responsibility for?
- How and when will the laboratory director assess the degree to which samples are compromised (e.g., fragmentation, commingling, degradation)? What metrics will be used to make the assessment?

Nothing in the history of mass fatality events prepared the forensic community for the complexity of the World Trade Center identification effort. The number of victims, the extent of remains fragmentation and deterioration, and the challenge of matching victims to relatives—the demands were tremendous. These circumstances drove forward forensic technological development that was aimed at extracting maximal genotypic information from highly compromised samples and matching the extracted data to genotypes derived from references. Without this concerted effort, the number of identified victims would have been much lower.

Benoît Leclair

One of the early decisions in the World Trade Center identification effort was to try to identify every sample. Because of the extensive fragmentation of the remains, this gave us the best chance of identifying as many missing as possible.

Robert Shaler

- How, when, and by whom will reference samples be collected?
- How will additional equipment and supplies be made available in a timely manner?
- How will staff and resources be reorganized to handle the ongoing casework and the increased casework due to the mass fatality incident?
- Who will be the point of contact with the media?
- Will the laboratory outsource DNA testing? Which testing? To whom?
- What metrics will be used to describe progress in the DNA identification effort to family members, elected officials, and the media?
- What are the information technology ("informatics") needs for hardware, software, and technical support? How will those needs be met?

In addition to having a mass fatality response plan, laboratories can mitigate the impact of increased demands on capacity and, often, capabilities by creating tools in advance. Appendixes B through G are samples of such tools—sample collection forms, sample biological collection kit specifications, issues to consider when outsourcing to another laboratory, and a DNA information brochure for the families of victims—that may be helpful to laboratory directors. These resources are discussed in detail in other chapters of the report.

Laboratory directors who are responding to a mass fatality incident may need to consider using human resources from other agencies. The Armed Forces DNA Identification Laboratory (AFDIL), Disaster Mortuary Operational Response Teams (DMORT), and Federal Emergency Management Agency (FEMA) are three Federal agencies that often assist Federal, State, and local jurisdictions in the initial phases of a mass fatality incident response. Exhibit 2 describes these agencies and their roles. It is important that the laboratory maintain an updated chain of command and contact information for these Federal resources.

Exhibit 2: Federal Agency Roles in Responding to a Mass Fatality Incident

Organization	Role
AFDIL (Armed Forces DNA Identification Laboratory)	AFDIL frequently is called on to support Federal, State, and local jurisdictions in mass fatality incidents. Title 10 of the U.S. Code authorizes AFDIL to participate in mass fatality incidents determined to be under Federal jurisdiction. The Armed Forces Medical Examiner is directed to conduct investigations to determine cause and manner of death for the Department of Defense (DoD) and other Government agencies. The ME can direct AFDIL to provide DNA identification services. Upon approval, AFDIL also can provide DNA identification services in non-Federal incidents. The National Transportation Safety Board and the Federal Bureau of Investigation have standing memorandums of agreement with DoD, stating that AFDIL will provide DNA identification services for their agencies in mass fatality incidents.
DMORT (Disaster Mortuary Operational Response Teams)	DMORT provides assistance at the disaster site for incidents that exceed the capabilities of State and local agencies. DMORT is part of the U.S. Department of Health and Human Services. Requests for DMORT support are made through the State/local department requesting the Federal assistance. From www.dmort.org: "[DMORT] is a Federal Level Response team designed to provide mortuary assistance in the case of a mass fatality incident or cemetery-related incident. We work under the local jurisdictional authorities such as Coroner/Medical Examiners, Law Enforcement, and Emergency Managers." DMORT does not conduct DNA analysis, but it will collect DNA specimens from human remains.
FEMA (Federal Emergency Management Agency)	FEMA provides funding for the DNA identification effort, provided the incident meets its criteria for a disaster. From www.fema.gov: "The Stafford Act . . . requires that 'All requests for a declaration by the President that a major disaster or emergency exists shall be made by the Governor [chief executive] of the affected State' . . . As part of the request, the Governor must note that the State's emergency plan has been implemented and the situation is of such severity and magnitude that the response is beyond State and local capability and Stafford Act assistance is necessary. The Governor shall furnish information on the nature and amount of State and local resources that have been or will be committed to alleviating the results of the disaster, provide an estimate of the amount and severity of damage and the impact on the private and public sector, and provide an estimate of the type and amount of assistance needed under the Stafford Act."

CHAPTER 4

Major Decisions

Once the parameters of a mass fatality DNA identification program are set by the policymakers, the laboratory director will be responsible for determining the nature and extent of the laboratory's response. It is important for the laboratory director to answer (with input from all agencies and departments that are likely to be involved) the questions presented in this chapter—and to keep in mind that these issues are interrelated. For example, the duration of the recovery effort can affect the quality and type of samples, which in turn may affect the number of DNA tests that may be needed to generate a profile.

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.

There were two reasons that we were able to complete the 265 victim identifications from American Airlines flight 587 in just 6 weeks. First, the infrastructure was already in place because of the World Trade Center identification effort and there was no extra ramp-up time needed in the laboratory. Second, our goal with respect to the airline crash was to identify all the victims rather than all the remains, so we knew when our job was over.

Howard Baum

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.

DNA analysis can be the most reliable and robust of the identification modalities. Although it may be a second choice to dental and fingerprint analysis when such evidence exists, DNA evidence still should be collected in case dental and fingerprint records are not available.

Robert Shaler

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 forensic

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 (see chapter 12, *Statistical and Other Issues.* 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.

How long will the recovery effort last?

In addition to policies defining minimum fragment sizes and the number of retestings to obtain data that meets statistical thresholds, the location and size of a mass fatality incident largely determines the duration of the recovery effort and the DNA identification of victims. Remains from an airline crash on land generally are collected in about 2 weeks. In contrast, remains from the WTC were collected over a 10-month period. The end of remains recovery may prompt a decision about how much longer the laboratory will continue to perform the DNA analysis.

The degree of fragmentation should determine the minimum fragment size. Because of the extreme fragmentation of remains of World Trade Center victims, the minimum was approximately the size of a thumb.

Robert Shaler

Generally, remains are processed as they are accessioned into the morgue. In cases with a large number of victims and/or fragmented remains, it usually is not possible to collect all the remains before the identification process begins—although waiting until all samples have been collected and coordinated may be better (more effective and efficient) from the DNA analyst's perspective. However, this likely will not be an acceptable approach, because the public, including the victims' families and public officials, may expect the identification effort to begin at once and proceed rapidly.

Two basic metrics for estimating a laboratory's workload are the number of samples received per month, and the number of months in the recovery effort. In addition, the public, the press, policymakers, and victim advocacy groups may have expectations of the duration of the recovery and identification processes. In airline disasters, for example, people may expect the entire process—from collecting samples at the disaster site to making identifications in the laboratory—to be completed within 1 to 3 months. The public also may expect the laboratory to complete its work within 1 month of receiving the last sample from the incident site. Chapters 7 and 8 of this report provide tools for understanding and responding to these expectations.

In addition to considering the human remains, the laboratory also must consider the reference samples. People concerned with finding their loved ones want to respond quickly, so personal items and biological references may begin arriving at the laboratory very shortly after the incident. If no plan exists prior to a mass fatality incident for collecting

You have no control over the condition of the remains, so setting criteria about what you will and won't test becomes an important framework that allows the identification process to move forward.

Jack Ballantyne

reference samples—and sending them in "batches" to the laboratory—Federal, State, or local first-response agencies may help set up family assistance centers. The number of batches and how frequently they are sent to the laboratory will depend on the efficiency and duration of the reference collection process. In turn, the accuracy and completeness of information associated with the reference samples depend on the collection plan.

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. To assess this, several key variables—described in exhibit 3—should be considered.

Exhibit 3: Key Variables in Assessing Laboratory Workload

Variable	Description
Number of victims	Generally, this is a straightforward estimate in the case of airline disasters that do not involve populated areas because the laboratory has access to the passenger manifest (although babies may not be included on the manifest). This estimate is more difficult for incidents that take place in office buildings, stadiums, etc. because the number and identity of victims are not known until long after the incident occurs.
Number of recoverable fragments	It is important to distinguish between the degree of fragmentation and the number of recoverable fragments. In the World Trade Center incident, there was an incredible degree of fragmentation, with an average of only seven recovered fragments for each victim. Based on historical data, there are approximately five to eight fragments recovered per victim in airline disasters. Therefore, for general planning purposes, 10 would be a good estimate to use.
Percentage of samples to be reworked	Some percentage of samples will need to be reanalyzed before they yield usable DNA profiles; 20 percent is a conservative estimate.
Number of personal items per victim	An estimate of the number of personal items will be provided for each victim. Historically, this is between five and eight. Note that usually not all of the items collected are analyzed; there should be a process to identify those items most likely to yield useful results.
Percentage of personal items to be reworked	Some percentage of personal items will not yield usable DNA profiles. Historically, 20 percent is a good estimate. When this occurs, the items are either reanalyzed or one of the other personal items is analyzed.
Personal items quality control samples	The laboratory should be prepared to reanalyze some percentage of all personal items samples as a quality control mechanism; 5 percent is common practice.
Number of kinship samples	Historically, three or four relatives per victim is a reliable estimate of the number of kinship samples. Note that buccal swabs nearly always produce complete DNA profiles, so it is not necessary to estimate rework.

Exhibit 4, an Estimated DNA Analysis Workload Worksheet, can be used to help predict the labor and material resources required for the DNA analysis.

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

Exhibit 4: Estimated DNA Analysis Workload Worksheet

Human Remains

1. Enter the estimated number of victims. _____
2. Enter the estimated average fragmentation per victim. *(For airline disasters, this value usually ranges between five and eight; ten is a conservative estimate.)* _____
3. **Expected number of human remains to analyze.** Multiply lines 1 and 2. _____
4. **Total number of human remains to analyze, including rework.** Multiply line 3 by the number 1.2. _____

Personal Items

5. Enter the estimated number of personal items collected per victim (typically between five and eight). _____
6. **Expected number of personal items to collect, store, and track.** Multiply lines 1 and 5. _____
7. Enter the estimated number of personal items to be analyzed per victim (typically between two and four). _____
8. **Expected number of personal items to analyze.** Multiply lines 1 and 7. _____
9. **Total number of personal items to analyze, including rework and quality control.** Multiply line 8 by the number 1.25. _____

Kinship Samples

10. Enter the estimated number of biological relatives per victim (typically between three and four). _____
11. **Expected number of kinship swabs to analyze.** Multiply lines 1 and 10. _____
12. **Expected number of kinship swabs to collect, store, and track.** Multiply line 11 by the number of swabs collected (between two and six). _____

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.

One of the important decisions that must be made within the first 48 hours of a mass disaster event concerns the establishment of family assistance centers. This is extremely important, because the manner in which personal effects and kin samples are collected affects the efficacy of the entire identification effort.

Robert Shaler

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 mixups. 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.

Culture and practices can vary among forensic and nonforensic laboratories. If they are not addressed at the beginning of a mass fatality DNA identification effort, these differences can lead to communication problems. A laboratory director also should keep in mind that some terms—"acceptable molecular ladder," "acceptable positive and negative controls," and "standard reaction volume," for example—may need to be fully defined when a nonforensic vendor laboratory is used.

In addition to the actual DNA analysis, the laboratory may also be responsible for some of the following activities:

- Sample accessioning and tracking.
- Making identifications and resolving metadata problems.
- Quality control.

- Interacting with families and the media.
- Long-term sample storage.

If these activities are overlooked during the development of a mass fatality plan, resource shortfalls likely will occur.

Generally, after a DNA profile is generated, it should take about the same time to evaluate the data for an identification as it takes in a paternity/biological relationship case analysis. [Note: Although more than a quarter of a million parentage tests are performed annually in the United States, biological relationship testing, such as paternity analysis, is rarely performed in forensic laboratories. Because many of the laboratories that perform such tests use some of the same STR loci that are used by U.S. crime laboratories, it may be prudent to consult with experts in parentage testing when preparing a mass fatality response plan. The American Association of Blood Banks is responsible for accrediting the Nation's parentage-testing laboratories.]

The laboratory director must consider the impact of a mass fatality incident response on the laboratory's primary mission. Capacity issues must be addressed in the context of routine, crime scene casework or, in the case of a databasing laboratory, convicted offender analyses. As resources are redirected to a mass fatality identification effort, backlog and turnaround times are likely to increase for regular casework. Even though local police and officers of the court may support the laboratory's role in the mass fatality incident response, they may still expect their cases to be completed in a timely manner. Plans for managing both a mass fatality incident response and routine casework should be developed before the need arises.

The duration of the recovery effort also has major implications for a laboratory's capacity. A rapid recovery effort (1 to 3 months) creates a spike in the laboratory's workload; however, because of the short duration of such a response, the laboratory may be able to quickly recover. Also, local law enforcement professionals and officers of the courts may be more tolerant of delays if they occur for only a short period of time.

With respect to more lengthy recovery efforts, the arrival of samples may be uneven, and the laboratory may be able to absorb the additional workload without affecting turnaround time on routine casework. However, a prolonged DNA identification effort may drain people and resources—and good planning can help mitigate disruption if a laboratory receives a large number of samples over an extended period of time.

What is the funding source?

It would be rare for a State or local forensic laboratory to have sufficient funding to cover the expenses associated with DNA testing in a mass fatality incident response. The Federal Emergency Management Agency (FEMA) is the primary source of Federal funding for mass fatality incidents; see chapter 3, *Before the Incident,* for a discussion of FEMA assistance.

Usually, FEMA is prepared to support new equipment purchases. Laboratory directors may already have equipment lists as part of their normal budgetary responsibilities—and it saves time to have those lists scaled-up and updated for presentation to FEMA as quickly as possible.

If the response is to be funded out of State or local budgets (or both), without additional Federal support, there may be more stringent limitations on equipment purchases or resources to enhance DNA analysis capabilities. In this situation, decisions about minimum fragment size and retesting policies also will be influenced by fiscal restraints. Laboratory managers will need to make sure that the ME is aware of the fiscal impact on the ability to make identifications.

The agency responsible for an identification effort (for example, the National Transportation Safety Board (NTSB) in an airline crash, the ME, or the laboratory director) may—after evaluating the issues of capacity, capability, mission, and funding—decide that the project is not feasible for the State or local laboratory. In that case, other resources may be sought; for example, the NTSB may request assistance from the Armed Forces DNA Identification Laboratory (see chapter 3, *Before the Incident*).

CHAPTER 5

Managing Expectations

A laboratory director is likely to encounter unique management challenges in a mass fatality incident. Uncertainty, ambiguity, and stress are the hallmarks of the early stages of a mass fatality incident response. Also, a laboratory director will encounter new constituents: the victims' families, public officials, the media, and the general public all will have expectations about the technology of DNA analysis and the timeline for DNA-based identifications.

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; exhibit 5 describes the constituents that a laboratory director may serve during a mass fatality incident response.

Although these constituencies seek the same outcome—the maximum number of identifications and the maximum quantity of remains accurately returned to the family—their priorities may not be the same as the laboratory's. For example, elected officials may focus on the speed of the identification process, whereas the laboratory's primary focus may be on the quality of the collection and analysis processes. Although these goals are not mutually exclusive, they may occasionally clash.

The media, which play an important role in keeping the public informed, can place additional demands on the laboratory director. During the World Trade Center (WTC) identification project, the laboratory was able to decrease media demands by widely disseminating routine information. The laboratory director's challenge is to strike a balance among the constituencies and be prepared for the high-pressure environment that is spawned by a mass fatality event.

The laboratory director must lead the staff through these challenges while continuing to ensure that the laboratory meets its charge of traditional casework and databasing. Because it is impossible to predict all the challenges of a mass fatality response, flexibility is a critical quality for the laboratory director.

As discussed in chapter 3, the first hours after a mass fatality incident are critical. If requested to do so by the ME, the laboratory director must be prepared to provide realistic timelines and information about the DNA identification effort to the families, public officials, and the media. This important contribution may require a higher level of assertiveness and exposure than is customary for a laboratory director, requiring conversations with government officials on strategic planning of the disaster response. However, no matter how unfamiliar or uncomfortable this role may be, only the laboratory director can accurately explain what is needed to ensure the most successful DNA identification effort possible.

A number of variables affect the identifications that can be made in any mass disaster event. For example, it may not be possible to obtain family reference samples or a victim's personal effects, there may be no biological offspring, or the condition of the remains may preclude successful DNA typing.

Robert Shaler

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

Exhibit 5: Laboratory Director's Constituents

Constituency	Constituency's Goals	Laboratory Director's Goals
Victims' families and friends	Receive rapid and accurate identification of loved one. Support during the grieving process.	Be available as an information source to explain the DNA identification process. Provide data and statistics to demonstrate the progress of the identification effort.
Public officials	Restore order as quickly as possible. Reassure citizens by being responsive and sympathetic. Promptly and accurately respond to questions from the public and the media.	Be available as an information source to explain the DNA identification process. Provide data and statistics to demonstrate the progress of the identification effort. Manage expectations regarding the speed and accuracy of identifications.
Media	Rapidly report on the status of all aspects of the event, including the DNA identification process.	Be available as an information source to explain the DNA identification process. Provide data and statistics to demonstrate the progress of the identification effort. Manage expectations regarding the speed and number of identifications.
Law enforcement	Secure the incident site. Support the investigation of the mass fatality incident (if applicable) while continuing to support the investigation of routine cases.	Impart clear information about sample collection and preservation. Delineate responsibilities and roles of laboratory staff and law enforcement officers for maximum efficiency and integrity of sample collection.
Laboratory staff	Support the identification effort while continuing routine casework.	Educate and orient the staff to the challenges unique to a mass fatality incident. Avoid burnout and long-term emotional effects on staff.

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. Exhibit 6 lists the types of information that were provided during the WTC response.

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

Exhibit 6: Information Provided to the Public

Metric	Description
Number of victim samples received	The number of human remains samples collected at the incident site and submitted to the DNA laboratory in a specified timeframe (e.g., twice daily, daily, weekly).
Number of samples analyzed from victims	The number of human remains samples that have been analyzed. Combined with the number of samples received, this metric provides transparency into the laboratory's backlog and shows how well the laboratory is keeping pace with the recovery effort. The public should be aware that there are several analytical steps involved in the identification of a victim. This metric could be divided into several steps—extraction, quantitation (if used), etc.— to highlight the laboratory's workloads.
Number of samples analyzed from reference samples	This metric shows that, in addition to often-damaged samples from the disaster site, the laboratory has many other samples to analyze before a reliable identification can be made.
Number of victims identified	The number of victims that have been identified by any modality.
Number of victims identified by DNA only	The number of victims that have been identified exclusively by DNA.
Number of remains reassociated with victims	Eventually, the number of fragmented human remains associated with specific victims may become an important metric. Such a metric can be used to estimate the longitudinal efficacy of the effort and help determine when the DNA identification effort ends.

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).

Mass fatality events are all about people. If the public and the families are not kept informed of the identification effort, they will lose faith in and respect for the agency that is performing the work.

Robert Shaler

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

Speed versus accuracy will always be a tightrope in the identification of victims of a mass fatality event. Striking the balance was one of the greatest challenges in the World Trade Center effort. Pressure to establish working guidelines for the rapid reporting of results, while maintaining a high threshold to reduce the probability of misidentifications, was a constant concern—a concern that should be paramount throughout an identification effort.

Thomas Parsons

of 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 mixups. 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.

CHAPTER 6

Project Management

After a laboratory has considered the issues discussed in chapter 4 (Major Decisions) *and is prepared to assume responsibility for the identification effort, significant personnel issues must be resolved. For a variety of reasons—including staff morale, public expectations, and economic demands—the response to a mass fatality incident should be handled as a separate project rather than as a part of the laboratory's standard operations.*

Most laboratory directors come up from the "bench," rather than from a management background. Skills in technical troubleshooting, case management, molecular biology, and population statistics are important in the day-to-day running of a forensic laboratory. Managing a mass fatality identification effort, however, requires these skills and more. *A Guide to the Project Management Book of Knowledge* (Newton Square, PA: Project Management Institute, 2004) offers this important guidance for a laboratory director who must respond to a mass fatality incident:

> Organizations perform work. Work generally involves either operations or projects, although the two may overlap. Operations and projects share many characteristics; for example they are:
>
> - Performed by people.
> - Constrained by limited resources.
> - Planned, executed, and controlled.
>
> Operations and projects differ primarily in that operations are ongoing and repetitive while projects are temporary and unique. *A project can thus be defined in terms of its distinctive characteristics—a project is a temporary endeavor undertaken to create a unique product or service. Temporary* means that every project has a definite beginning and definite end. *Unique* means that the product or service is different in some distinguishing way from all similar products or services.

These definitions of "projects" versus "operations" suggest an important principle: a mass fatality incident DNA identification requires constant and diligent project management. The laboratory director (or designee; see *Project Manager*, below) must assess what controls are needed in project planning and project execution. For example, the areas of communications, risk management, and integration with non-DNA disciplines are often overlooked.

Project Functions

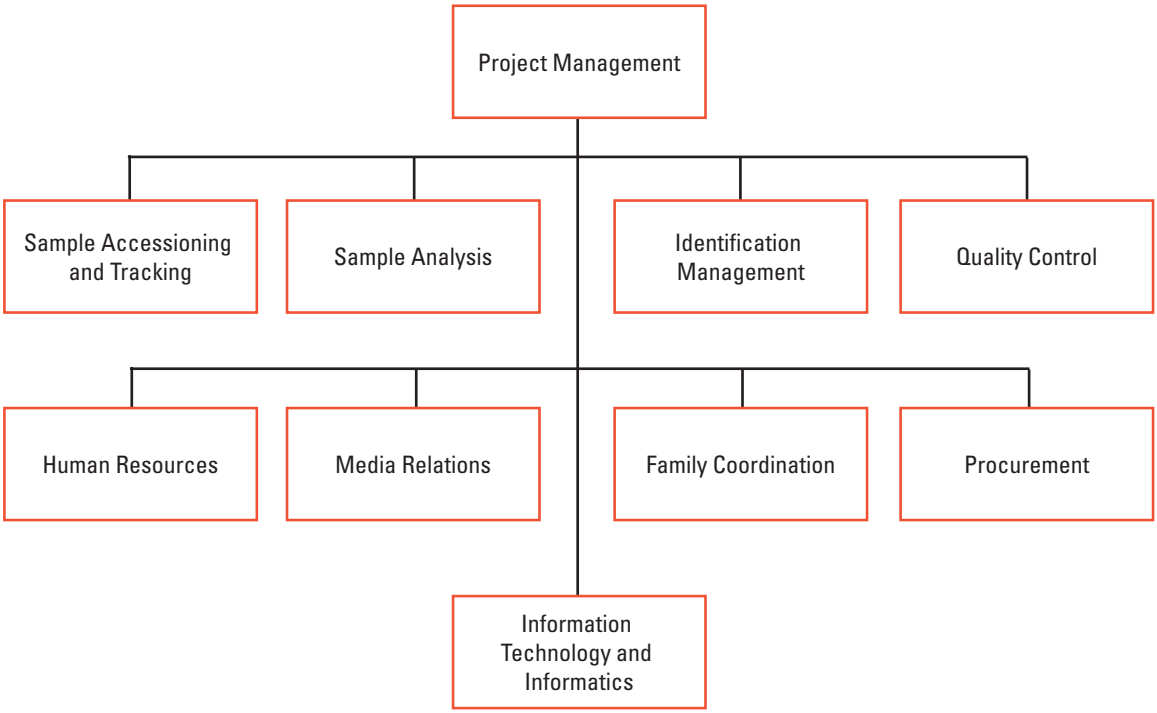
Exhibit 7 depicts the major functions—or disciplines—associated with a mass fatality incident response. In a large response, each function may require a full-time resource; in the response to a smaller incident, one person may be able to fill multiple roles. Regardless of the size of the incident, however, each of these functions should be considered during development of a project management plan. [Note: Many of these functions are discussed in other chapters of this report.]

Project functions can be defined as follows:

Project Management ensures that all functions work in concert to provide accurate identifications as rapidly as possible within budgetary constraints.

Sample Accessioning and Tracking consists of accessioning remains and reference samples, ensuring chain-of-custody documentation, and managing the flow of samples and data within the laboratory and among outsourced laboratories.

Exhibit 7: Major Project Functions



Sample Analysis means performing DNA tests on remains and reference samples.

Identification Management has two parts: (1) making identifications by matching remains and reference samples and (2) reviewing the metadata—information from all sources linked to a particular sample—associated with the reference samples to ensure they were correctly associated with the putative victim.

Quality Control refers to the processes and procedures that a laboratory uses to detect and avoid mistakes. Quality control also ensures that there are no discrepancies between DNA analysis and other modalities (i.e., that the metadata can be reconciled when a conflict occurs).

Information Technology (IT) and Informatics includes the software and hardware that supports the identification effort.

Human Resources focuses on meeting the needs of the staff, volunteers, and consultants who are working on the response effort.

Media Relations involves interacting with the press and establishing how and when information is released to the media.

Family Coordination encompasses educating families, collecting the reference samples and family data necessary to identify victims, and providing information to the families.

Procurement involves ensuring that the correct equipment, supplies, and services are available to the response in a timely manner.

Project Structure: Centralized vs. Decentralized

A centralized project structure, where all samples are accessioned and analyzed by a single laboratory, is the most common paradigm for sample receipt and analysis. The term "centralized" does not necessarily imply a specific physical location or software/hardware architecture.

In a decentralized project structure, more than one laboratory is involved. The laboratory that is ultimately responsible for the mass fatality incident response is called the managing laboratory, and other participating laboratories are referred to as partner laboratories.

Since a mass fatality DNA identification effort most likely would be added to a laboratory's casework, a decentralized structure can be more efficient if good information technology support exists. For example, in a decentralized structure, the human remains samples might be analyzed by one laboratory and the reference samples by another. Both laboratories would analyze samples independently, leveraging their respective strengths, and the overall response undoubtedly would be faster because the laboratories would be working simultaneously.

However, for a decentralized model to work well, there must be a mechanism for centralized data management so that the managing laboratory and the partner laboratory/laboratories can view information and communicate about data, regardless of where they are collected or analyzed. It is especially critical that the managing laboratory have as close to real-time access as possible to all data—including DNA profiles, chain-of-custody documentation, and metadata—that is associated with the mass fatality incident response, because the managing laboratory has the ultimate responsibility for making comprehensive and frequent updates to the families, public officials, the media, and the public. [Note: For example, metadata for a victim's toothbrush include the name of the victim, and when and by whom it was provided.]

Sample accessioning and sample storage can be decentralized as long as each partner laboratory ensures that all of the metadata are accessible by the managing laboratory. The physical samples can be stored at partner laboratories, as well. However, if a partner laboratory disengages from the response effort, all of its samples must be shipped to the managing laboratory under appropriate chain-of-custody procedures.

It is important to consider the administrative review portion of the identification process when deciding between a centralized or decentralized project structure. In a decentralized plan, if the managing laboratory needs to examine the physical item (e.g., toothbrush, hairbrush), the partner laboratory must be prepared to pull the physical item from evidence storage and send it to the managing laboratory or be prepared to provide digital images that can be accessed electronically.

Sample analysis readily lends itself to a decentralized structure. A managing laboratory can divide the workload in several different ways: by DNA technology, by sample type, or some by combination thereof. For example, bone fragments might be shipped to one laboratory for STR and mtDNA analyses, whereas tissue samples would be analyzed in-house. Or partner laboratories might receive entire samples or extracted DNA from the managing laboratory.

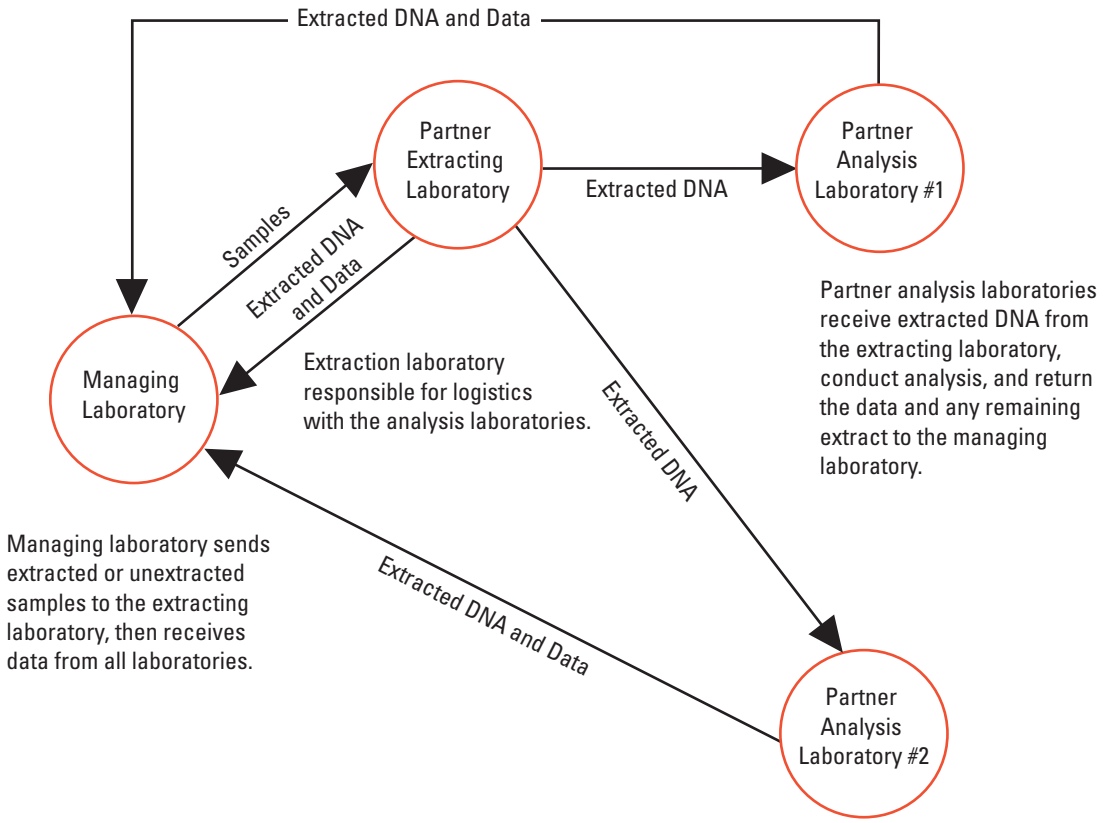
Because the managing laboratory has the ultimate responsibility for maintaining the chain of custody for samples, extracts, and data, it is important to recognize the management challenges presented by these aspects of a mass fatality identification effort. Samples, extracts, and data may be shipped to and from the managing laboratory individually or collectively, at different times and in different batches. Multiple extracts and multiple DNA profiles (data) might be derived from a single sample, and the laboratory's sample tracking system must be able to document and certify the chain of custody for each one. The sample tracking system must collect data associated with all physical transfers, including what was sent, where it was sent, when it was sent, when it was received, and by whom. The managing laboratory uses this information to document the chain of custody and to provide status updates to the public.

The Office of the Chief Medical Examiner (OCME) partnered with other laboratories in the World Trade Center identification effort. The OCME was in the management role—with ultimate responsibility for making the victim identifications—and also performed retesting of the remains and secondary testing of family samples and personal effects. However, the primary testing of bones and tissues and the initial testing of family and reference samples was contracted to outside laboratories with specialized experience.

Robert Shaler

In a decentralized structure with multiple partner laboratories, the managing laboratory must decide how samples, extracts, and data will move among the partners. There are two basic approaches: the daisy-chain model and the

Exhibit 8: Modified Daisy-Chain Workflow in a Decentralized Laboratory Structure



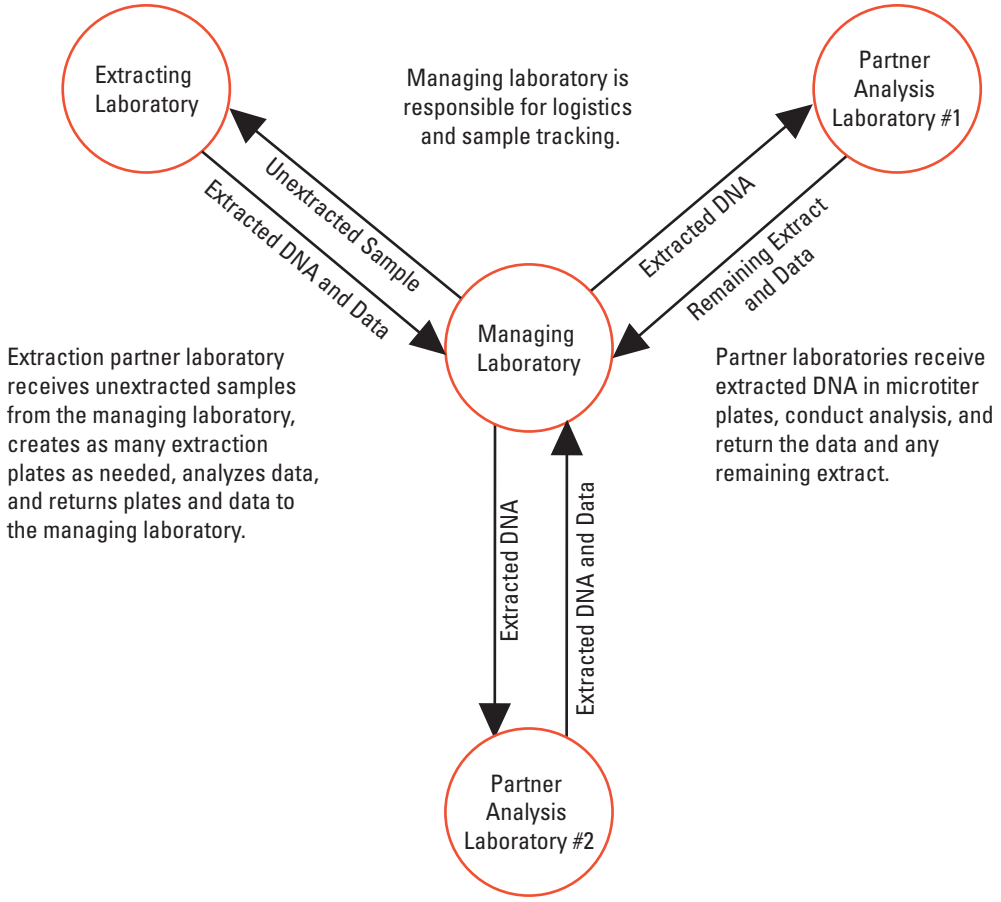
hub-and-spoke model. Both were used in different aspects of the World Trade Center (WTC) DNA identification project.

In the daisy-chain model, samples (or extracts) are shipped to the first partner laboratory, which ships extracts to the second partner laboratory, which ships extracts to the third partner laboratory, and so on. A major drawback to daisy-chaining is that the managing laboratory does not physically control the flow of samples and extracts among partner laboratories, which can make it difficult to locate missing samples and ensure proper chain-of-custody documentation.

When the managing laboratory has a partner laboratory perform DNA extraction, the daisy-chain model can become very convoluted. Exhibit 8 depicts the flow of samples, extracts, and data in a modified daisy-chain structure that would occur when the managing laboratory sends samples to the first partner laboratory for extraction and analysis. In this example, the first laboratory extracts a sufficient quantity of DNA for the two other partner laboratories and ships the extracts to them. Partner laboratories return leftover extracts and data to the managing laboratory. It is important to note that the daisy-chain model requires compatible informatics and hardware systems and shared data transfer protocols so that all parties are sharing information.

In the hub-and-spoke model (see exhibit 9), the managing laboratory centralizes the control and movement of samples, extracts, and data among partner laboratories. Although it is a simpler model than the daisy-chain for tracking chain of custody, locating missing samples, and identifying missing data, there are some limitations. The major disadvantage of a hub-and-spoke structure (in addition to time delays) is that samples or extracts must be packaged and shipped multiple times, which could result in repeated freezing and thawing, potentially decreasing the quality of the

Exhibit 9: Hub-and-Spoke Workflow in a Decentralized Laboratory Structure



DNA in the samples. For example, the managing laboratory sends a batch of samples to the partner laboratory for extraction. When the managing laboratory receives the extracts back from the partner laboratory, it must open the package and verify the contents, then repackage the contents and ship them to the second partner laboratory. This doubles the work as compared with a daisy-chain structure. However, in a hub-and-spoke structure, the managing laboratory has a higher level of control and the possibility of miscommunication between partner laboratories is reduced.

Because of the types of samples in a mass fatality incident (e.g., bone fragments, tissue, personal items, kinship swabs) and the numerous DNA technologies (short tandem repeats, mitochondrial DNA, single nucleotide polymorphisms, etc.), a decentralized structure is often necessary. Moreover, it may be prudent to create different workflow mechanisms for different types of samples. For example, kinship samples may be processed using a daisy-chain model, whereas disaster samples may be better handled using a hub-and-spoke system. Regardless of which project structure is used, however, it is safe to assume that the greater the number of partners, the greater the management complexity.

Identification management is one function that should never be decentralized. The managing laboratory is responsible for setting the parameters for DNA identifications and resolving conflicts with other identification modalities. The managing laboratory also acts as the single point of contact for the victims' families, public officials, and the media on identification-related matters. Thus, it is critical that all data—metadata and DNA profiles—be provided to the managing laboratory.

In the World Trade Center identification effort, both the fire and the police departments frequently asked the Office of the Chief Medical Examiner (OCME) to reprioritize testing of victims' remains. Although it was important to keep the testing queue intact to disrupt the work flow as little as possible, the OCME honored a number of these requests. When this happened, the testing process, including the assignment of personnel, was affected.

Robert Shaler

Special Requests

Although requests for special sample handling may disrupt efficient sample processing, such interruptions are inevitable in the aftermath of a mass fatality event. The laboratory director should plan for these contingencies and construct a separate process to handle expedited requests. The first 72 hours after a major incident will be emotionally charged, with the possibility of many urgent requests that the laboratory perform immediate DNA analyses. Requests for expedited analyses may also occur later in the identification effort, if new remains are recovered or more useful personal items or kinship references become available. In the WTC DNA identification project, the laboratory frequently received instructions to collect and analyze reference samples and search the DNA profiles against the accumulated profiles of tested victim remains within 24 hours or less. Without a process in place for handling such expedited requests, interruptions will affect efficient and orderly sample throughput.

Project Manager

One of the most serious misjudgments a laboratory director can make is failing to recognize the importance of project management. Experience gained during the WTC DNA identification effort, followed shortly thereafter by the crash of American Airlines flight 587 in Queens, New York, showed how crucial it is to avoid the natural tendency to manage a mass fatality incident response as simply another operational activity.

To meet the challenge of maintaining ongoing forensic casework while also responding to a mass fatality incident, a laboratory director should consider appointing a separate project manager to ensure that the response is appropriately executed. If the laboratory director assumes the project manager role, other responsibilities may have to be delegated. This can be an effective solution if the day-to-day operational duties associated with casework or offender analyses can be transferred to other staff. If the laboratory director is unable to delegate some of his or her other responsibilities, a dedicated project manager should be appointed for the mass fatality incident project.

The ideal project manager is someone who understands all facets of a mass fatality incident response. It often is difficult, however, to find someone with this exact skill set. At the least, the project manager should be familiar with all of the disciplines that will be brought to bear, including sample collection, DNA analysis, and information technology. In addition, the project manager should have experience planning and monitoring work and should be comfortable in a team-oriented environment.

The project manager should work with the laboratory director to formulate a strategy for the specific mass fatality incident response. With the laboratory director's consent, the project manager should implement the necessary policies and procedures. The project manager also is responsible for keeping the laboratory director apprised of the project's status and for meeting regularly to discuss progress, risks, schedules, and resources.

Even if the laboratory elects to outsource some of the response activities, a large project management role still exists. For example, the laboratory may choose to outsource the DNA analysis to one or more laboratories and make identifications in-house. This structure requires a project manager to coordinate the movement of samples and data, monitor contract compliance, and ensure that sufficient resources (people, databases, computers, etc.) are available for the identification effort.

Exhibit 10 describes some of the important duties of the project manager.

The project manager can expect the identification process to have two distinct phases. The first phase is characterized by a large number of identifications made in a relatively short period of time. The second phase is characterized by fewer, more difficult, identifications made over a relatively long period of time.

External Relationships

In addition to managing communication within the laboratory, the project manager should manage relationships with external organizations. The laboratory represents just one component of a mass fatality incident response. By working closely with other response participants, the project manager can improve the laboratory's effectiveness and efficiency. (See *Mass Fatality Incidents: A Guide for Human Forensic Identification* online at http://www.ojp.usdoj.gov/nij/pubs-sum/199758.htm.)

Needless to say, every organization has its own mission, goals, and way of conducting business. The project manager should work to understand the cultures of the various agencies and departments with which the laboratory will be working in the identification effort. In addition, the project manager should establish formal and informal channels for receiving and sharing information. For example, by building a relationship with the site recovery team, the project manager can gain insight into the volume and type of samples that will enter the laboratory during a particular timeframe. Exhibit 11 shows some of the organizations that may be involved in a mass fatality response.

Exhibit 10: Project Manager Duties

Duty	Description
Define and manage the project schedule.	The project manager is responsible for creating a schedule, assigning resources, and monitoring progress. Because there are so many unknowns at the beginning of a mass fatality incident response, it may be impossible to create a project schedule with a definite end date. However, the project manager should identify major tasks, create a precedence diagram describing the interrelationships of tasks, and establish work schedules for the project team.
Facilitate communication within the project team.	The project manager is responsible for ensuring that employees in each functional area have the information they need to plan and execute their portion of the response. The project manager should chair frequent, periodic (daily or weekly) meetings with functional-area managers and facilitate a free flow of information. Current and future challenges should be discussed. Decisions should be made only after considering the impact on each function. A classic mistake is excluding the issue of information technology from decisionmaking.
Identify and manage risks.	The project manager is responsible for identifying the major risks to the project's success. Some examples of risks include not having a particular task completed by a deadline, the laboratory information management system failing to support the mass fatality incident response, and sample mixups during accessioning. Risks will be unique to each response and undoubtedly will change as the project unfolds. One proven risk-management technique is to have the management team brainstorm the top 10 project risks, order them from highest to lowest, then identify avoidance and mitigation strategies for each. Ideally, each functional-area manager also manages his or her own top 10 risks.
Optimize the overall project, not one function or discipline.	Management theory says that the only way to optimize a large entity is to suboptimize its various components. Thus, the project manager should shift resources among functions, as necessary, to mitigate risk and ensure success. Even though the functional-area managers may object to losing resources, the project manager is responsible for the overall success of the project. This is another reason why the project manager should understand all of the functional areas.

Because we were asked to identify every fragment of human tissue, the smallest, most damaged samples were repeatedly tested. Each time—particularly, as we optimized protocols—we hoped to reveal a few more loci. The more compromised the sample, the more attempts we made to coax out the data.

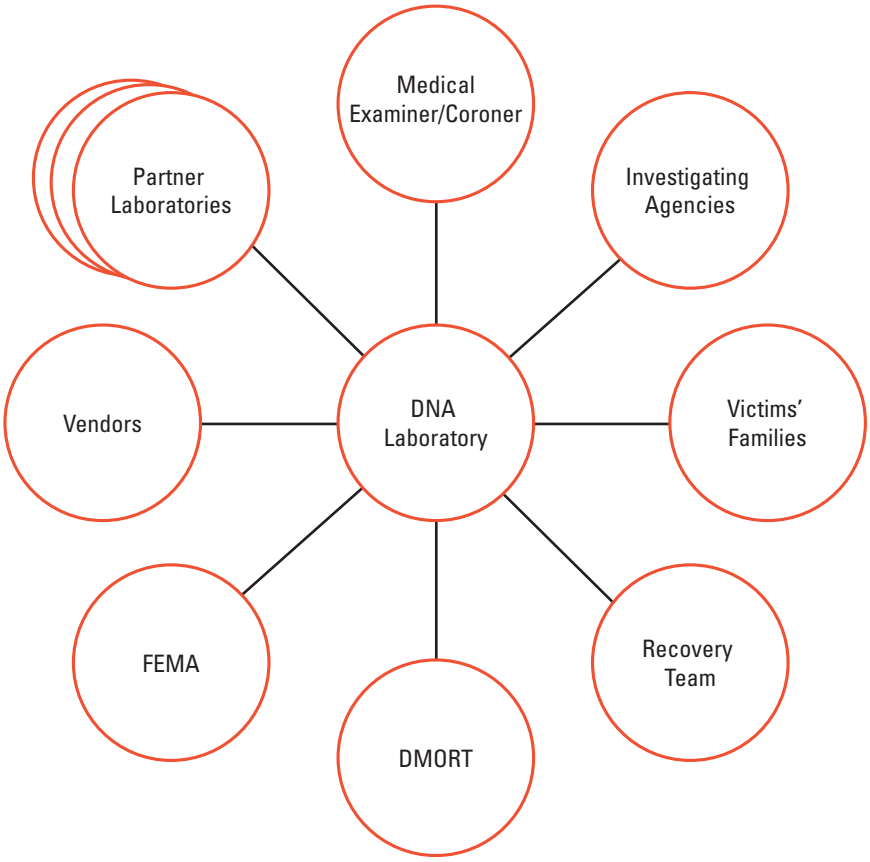
Robert Shaler

Integration of the DNA effort in the overall response to a mass fatality disaster requires extensive communication between the laboratory and all other units with responsibility. One crucial lesson learned during the WTC DNA identification project is that it is impossible to over-communicate during a mass fatality incident response. Aspects of DNA identification may not be understood by other groups that are involved in a mass disaster response; for example, sending a communication about collecting remains in clean paper—rather than plastic—bags could make the difference between obtaining a full 13-locus STR profile and a partial or failed profile. It is the project manager's responsibility to ensure that the laboratory's needs are understood by other response agencies.

As individuals and groups become preoccupied with their own obligations during a mass fatality response effort, it is possible to forget that introducing a seemingly minor change in the DNA identification process can affect the entire effort. To minimize miscommunication, the project manager should establish a single point of contact in each group involved. Regular meetings among key participants should be held. And, although the DNA project manager may initially have to guide the discussion to reduce digressions, these formal lines of communication are crucial.

The project manager should meet with the ME at least once a week. The meeting agenda should include:

Exhibit 11: Organizations Involved in a Mass Fatality DNA Identification Response



- Overall project status from the perspective of the ME and the DNA laboratory.
- Sample collection, storage, and tracking issues.
- Identification issues across modalities.
- Information technology requirements and problems.
- Information to be presented to the media.
- Anticipated workload and possible constraints.

Representatives of the laboratory, designated by the project manager, also should plan to meet with partner laboratories at least once a week. The agenda for these meetings should include:

- Overall project status, including issues regarding the transfer and tracking of samples or extracts.
- Problems and solutions regarding sample analyses and data.
- Anticipated workload and possible constraints.

These meetings may be more effective if they are conducted one on one with representatives of the partner laboratories or with the entire partner laboratory staff. Meetings with other agencies (e.g., Federal Emergency Management Agency, investigating agencies) can be less frequent, but also should occur regularly and have written agendas.

Human Resources

Following a mass fatality incident, consultants and volunteers may be called upon to supplement the capabilities of the laboratory. The project manager is responsible for ensuring the coordination of these resources.

It is fair to assume that the DNA response to a mass fatality incident will require a rapid ramp-up of staff to support the collection, accessioning, and information technology processes and beginning sample analyses. Staffing requirements are likely to peak at the time that multiple processes—for example, sample collection, analyses, identification—occur simultaneously. After the bulk of the samples have been profiled, staffing needs should begin to taper off, with the identification analyses and quality control processes assuming the bulk of the requirements for the remainder of the project.

The staffing requirements for the World Trade Center DNA identification effort followed a skewed bell-shaped curve (see exhibit 12).

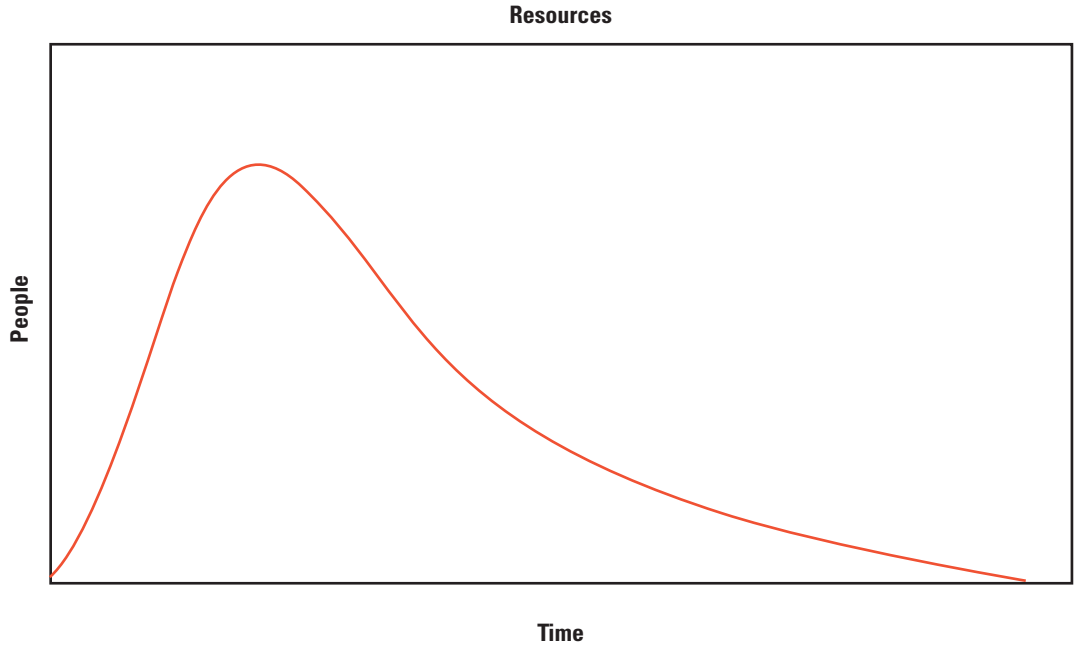
A laboratory responding to a mass fatality disaster may not have sufficient staff onboard for the peak times and may have to seek outside resources for part or all of the project. Typically, consultants are hired for a specific purpose or a specialized task. For example, the laboratory may augment its identification capability by hiring a specialist in genetic and kinship analysis to scrutinize complex pedigrees. Or, the laboratory may contract for specialized information technology expertise. Because most crime laboratories are part of the public sector, it usually is easier to hire consultants for short-term engagements. This allows contract amounts to be kept below procurement ceilings and can expedite the procurement process.

It was virtually impossible to manage both routine casework and a mass fatality event the size of the World Trade Center without help. We immediately established disaster teams in the laboratory and appointed a liaison between the New York State Police and the Office of the Chief Medical Examiner laboratories. I kept managerial control of the WTC work and charged the deputy director with the daily operation of the laboratory.

Robert Shaler

Volunteers who assist in the DNA identification after a mass fatality incident can be professional or nonprofessional. Professional volunteers are already trained in some facet of the mass fatality incident response and are able to assume some of laboratory staff's duties. Examples include Disaster Mortuary Operational Response Teams (DMORT) personnel, medical students who assist in the morgue by accessioning samples and cutting tissue, former employees (e.g., retirees), and volunteers from other laboratories. Nonprofessional volunteers—those without specialized training in sample collection or analysis—may be used to relieve laboratory personnel from some administrative and clerical duties. For example, nonprofessional volunteers might perform data entry or routine paperwork. Before assigning duties to a volunteer, the project manager should understand the scope of the volunteer's commitment. Because volunteers work free of charge and may leave the laboratory on short notice, the project manager should avoid assigning mission-critical tasks to volunteers.

Exhibit 12: Staffing Requirements Over Time



Laboratory directors should be aware of liability issues that might arise if consultants or volunteers are used. For example, confidentiality agreements should be signed by consultants and volunteers, stating that no data or information related to the DNA identification effort may be published or conveyed to the media without prior written consent of the laboratory director or a person designated by the laboratory director. The agreement also should state that no personal information should be disclosed regarding the victims, the state of the remains, or any other aspect of the incident that the consultant or volunteer learns as a result of working on the DNA identification effort. A comprehensive confidentiality agreement can help protect the laboratory from premature, unconfirmed reports and the victims' loved ones from suffering the insensitive divulging of gruesome details.

The project manager also shoulders the burden of being alert to the staff's mental health. When issues concerning staff health and well-being arise, the project manager must immediately involve the laboratory director, who may request professional advice. One way to help maintain morale is to keep the staff as fully informed as possible. Open communication between management and staff is essential to establishing and maintaining high morale. In the rush to respond to a mass fatality incident, information-sharing with staff can be neglected. But, a fully informed staff that understands upcoming challenges and goals can help management anticipate problems and overcome obstacles.

Laboratory workers are likely to experience a range of emotions throughout the DNA identification response, and, in fact, there can be a long-term emotional impact on those working on a mass fatality response. Laboratory personnel who worked on the WTC response reported experiencing extremely high stress levels. Laboratory directors or project managers must be alert to signs of burnout, depression, and other psychological reactions; they must recognize the need for—and be able to implement—stress release mechanisms. The laboratory director and the project manager should also make employees, consultants, and volunteers aware of available mental-health or other stress-relieving assistance programs.

One important difference between a mass fatality response and routine forensic casework is that, over time, laboratory staff may become intimately familiar with the lives of the victims. By the end of the response, laboratory staff is likely to know the name, gender, date of birth, family structure, and next of kin of many of the victims. Staff may learn if relatives are not aware that they were not biologically related to the victim or that some family members are estranged. Because of the nature of a mass fatality—where the suffering of many is shared by the community as a whole—laboratory staff may find themselves empathizing with the victims' families, sharing their bereavement.

There also will be additional stress on laboratory staff who are *not* assigned to the mass fatality response. If a laboratory must also continue to meet its casework and offender-processing commitments, some staff will likely need to assume the workload and responsibilities of colleagues who are assigned to the mass fatality response. Over time, this can lead to resentment. Some staff members may be unhappy about not being assigned to the mass fatality incident response; or the priority of the mass fatality incident over traditional casework may make them question their value to the laboratory. A team environment fostered by the laboratory director or project manager will help staff members support each other throughout the DNA identification effort.

The laboratory director and the project manager should continually assess stress levels within the laboratory, bringing in experts, if necessary, to help with the assessment. Because of the demands of the work, it may not be easy to spot behavioral or attitudinal changes in staff members. However, the laboratory director and project manager should watch for stress-related symptoms such as crying, a haggard appearance, a normally calm individual becoming argumentative, or a normally extroverted individual becoming quiet and withdrawn.

Every staff member worked tirelessly, in the hope that perhaps their effort would bring a modicum of comfort to the victims' despairing friends and relatives. The task was huge, and small miracles were performed daily. The emotional toll on New York State Police personnel was obvious, showing in their faces, weighing on them, but they never gave up.

Barry Duceman

In the WTC response, for example, there was an employee assistance program available to laboratory personnel, in addition to the following assistance for all employees, consultants, and volunteers who were working on the recovery effort:

- Sal's Café: The Salvation Army provided free breakfast, lunch, and dinner to anyone working on the WTC project.
- A national massage therapy association provided massages, including reflexology, through an arrangement with the city of New York.
- Religious ceremonies were regularly conducted, and religious leaders of many faiths were available in the mortuary for families and workers.
- Project Liberty, a group of mental health professionals, provided free counseling.

CHAPTER 7

Media Relations

Because DNA technology is of such interest to the public, there are likely to be many DNA-related questions from the media. To minimize the potential for misunderstandings, there should be a single point of contact between the laboratory and the press, and laboratory staff should be instructed on how to respond if contacted directly by the media. Through press briefings, the laboratory director can help educate the public and manage expectations by providing a realistic picture of what DNA analysis can—and cannot—do.

One of the roles of the media is to inform the public about all aspects of a mass fatality incident response. Although media attention is likely to be focused on the ME, who generally has final responsibility for victim identification, the laboratory staff may also receive inquiries from the media. Therefore, staff members should know the laboratory's policy—and the policy of the authorities to which the laboratory reports—regarding contact with the press.

The ME may ask the laboratory director (or another person) to interact with the media on DNA-related matters, or the ME may choose to personally handle contact with the press. However, regardless of whether media contact occurs through the ME or a designated media relations person, the laboratory must be prepared to support responses with accurate, consistent information.

If the ME asks the laboratory director to respond to DNA-related press inquiries, the laboratory must establish policies for media access. It is important that all information released by the laboratory come from a single source, usually the laboratory director. When there is more than one point of contact with the media, information can appear to be contradictory or conflicting. Such misunderstandings can result in the loss of valuable time, as misinformation is corrected.

The media may also request laboratory visits to observe, photograph, or film the analytical processes. Such exposure may benefit the public or the DNA effort itself, but the laboratory director should expect that granting such requests will disrupt workflow significantly. If filming in the laboratory is authorized, staff should be given ample notice.

The following are examples of Web sites that contain information on DNA analysis and human identification. Such background information could be provided to the media to reduce the need for time-consuming on-location filming at the laboratory:

- **The President's DNA Initiative**
  http://www.dna.gov/

  Information on funding, training and assistance provided through the President's DNA Initiative. Tutorials for law enforcement, lawyers, and judges on the use of DNA evidence in trial. Information on case studies and many other resources, organized by "audience," including forensic scientists, officers and investigators, officers of the court, researchers, victim advocates, policymakers, and lawmakers.

- **Short Tandem Repeat DNA Internet DataBase**
  http://www.cstl.nist.gov/div831/strbase/

  Tutorials and PowerPoint presentations on forensic DNA analysis and the technologies used to create profiles.

- **101 science.com's DNA Tutorial**
  http://www.101science.com/dna.html

  Basics of DNA analysis, including video clips; links to other useful sites.

- **DNA Interactive**
  http://www.dnai.org/d/index.html

  Background information on DNA profiling, DNA kinship testing, and other information including video clips.

- **DNA From the Beginning**
  http://www.dnaftb.org/dnaftb/

  Interactive site that provides background tutorials, video clips, and photos.

In addition, these books contain information on DNA profiling and statistics:

- *Forensic DNA Typing Biology, Technology, and Genetics of STR Markers,* John M. Butler, Amsterdam: Elsevier Academic Press, 2005.
- *Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists,* Ian Evett and B.S. Weir, Massachusetts: Sinauer Associates, 1998.

The laboratory director may have access to a public relations specialist who can coordinate contact with the media. Public relations specialists can screen media requests, supply basic information, and schedule interviews. However, in the absence of such assistance, and at the request of the ME, the laboratory director should plan on devoting significant time to media relations.

The Office of the Chief Medical Examiner had one spokesperson, the DNA laboratory director, who dealt with the press during the World Trade Center victim identification effort. We learned, early on, that because the same information can be presented in different ways—which can lead to different interpretations—it was critical to have one spokesperson.

Robert Shaler

The laboratory director should approach each media interaction with the goal of providing accurate, consistent information. Questions should be answered honestly and completely, without releasing sensitive or unconfirmed information. It is important to remember that information given to the media also goes to the victims' families, so care must be taken to be respectful of sensitive family-related issues. The performance and activity metrics described in chapter 5, *Managing Expectations*, should be part of status updates to the media.

Briefing the media allows the laboratory director to educate the public and manage expectations by providing a realistic picture of what DNA analysis can—and cannot—do. For example, an explanation of the relationship between DNA results and the condition of human remains must be given in a way that respects grieving families. Through briefings with the media, the laboratory director can explain the limitations of DNA analysis as an identification method and can provide a realistic timetable for completing the DNA identification effort. The laboratory director may also want to raise the issue that there may be unidentified remains at the close of the effort.

A laboratory director should also be prepared to answer questions such as:

- How many victims have been identified?
- Have you identified the terrorists?
- How much time until the work is finished? Why is it taking so long?
- Will you be able to identify everyone? How many victims will you be able to identify? Why can't you identify all of them?
- What is the condition of the remains?
- Tell us about your emotional response. What is the mood like in the laboratory? How is your staff holding up under the pressure?

Commingled remains, while a confounding issue for DNA testing, may be a particularly sensitive issue for families. Expect that the media will focus on new or unusual technologies, seeking information on their reliability, when they will be brought online, and how many new identifications they will yield. In addition, some reporters may want to "scoop" their competition and, because of this and the pressure on them to meet short deadlines, there often is insufficient time for a story to be vetted as fully as the scientific community would like. Unfortunately, some of what gets printed or broadcast may contain errors. If this happens, the gulf between perception and reality can create anxiety and confusion among the victims' families and the general public.

The laboratory director can do a number of things to ensure that the media receive accurate information. Always state first whether the information being presented is for background or for attribution. Providing information "for background only" lets the laboratory director give more detailed explanations, but avoids having sentences quoted out of context. Interviewees also may request to review quotes that are going to be attributed to them, although this is not part of the journalistic process, and a laboratory director should not count on being able to review quotes.

In addition to supplying press releases through normal channels, the laboratory director or public relations liaison may want to contact news organizations that serve the geographic regions where victims lived or worked, in order to "speak" more directly to victims' families. Laboratory directors should also keep in mind that it may not be useful to grant an interview to every reporter who asks.

The most efficient way for the laboratory director to ensure that the public has the information it needs—and still have time to oversee the project—may be to issue daily press releases (see chapter 5, *Managing Expectations,* and exhibit 6). The consistent and timely release of information also is likely to reduce requests by the media. It may, for example, be useful for the laboratory director to issue a press release when the following events occur:

- The first remains arrive at the forensic laboratory.
- The first DNA identification is made (which, to the general public, likely will signify the beginning of the DNA identification process).
- The last of the remains arrive at the laboratory.
- The final remains are analyzed.
- New technologies are brought online.
- The laboratory makes its final DNA-based identification.
- The laboratory response effort ends.

CHAPTER 8

Family Coordination and Liaison

Working with the families of victims of a mass fatality incident is likely to be foreign to most DNA laboratories. This chapter discusses the formation of a family assistance center and a family hotline and discusses a number of helpful forms, including the most recent version (in English and in Spanish; see appendix G) of the brochure on the DNA identification process that was distributed to victims' families shortly after the 9/11 attacks.

A laboratory's response to a mass fatality incident is a departure from normal criminal casework in which DNA testing generally is conducted on behalf of the State (or the defense)—with a law enforcement agency acting as the buffer between the laboratory and the victim or the victim's family. In a mass disaster DNA identification effort, however, the laboratory becomes a gateway, rather than a buffer—with the laboratory working directly with families to collect information about victims and reference samples.

Consequently, the laboratory and victims' families often share a close, albeit short-term, relationship. Families become temporary stakeholders in the laboratory's performance because many decisions made during a mass fatality incident response affect them profoundly. For example, the minimum fragment size and "when are we finished" decisions (see chapter 4, *Major Decisions*) determine how much of, and in what condition, a loved one's remains will be received by the family.

Depending on the extent to which the victims' families are organized, they may have a strong voice in shaping nonscientific decisions. The laboratory also may receive, via elected officials, complaints from victims' families. The best advice in these situations is that common sense should prevail. The needs of the families of victims of a mass fatality incident are, first and foremost, to have their loved ones identified and buried. Each family needs an official death certificate to settle their loved one's estate and collect any life insurance benefits.

Finally, families want information. Most laypeople do not understand forensic identification modalities, and DNA can seem especially mysterious. Often, families do not know why they are being asked to provide their loved one's personal items or why the laboratory is requesting DNA samples from relatives. They may not understand the difference between a biological relative and someone who is called "aunt," for example, but is not actually related. Laboratory directors would be well advised to develop a policy for dealing with a nonrelative who wants to provide a kinship sample. Being able to "do something" is a natural part of the grieving process, and the laboratory can always discard the sample. However, since this may raise false hopes, it may be best to consult with a bioethicist before developing a policy.

Some families may be concerned at what they perceive as the government asking questions about their DNA or their relationship to a mass fatality incident victim. Also, once DNA samples are provided, families may not hear anything for days, weeks, or even months, which can cause additional anxiety about the government's use of their DNA. The entire process can be bewildering and frustrating to the families of victims, which is even more reason for a laboratory's policies regarding sample disposition, privacy, and other personal information concerns to be communicated clearly and respectfully.

The Family-Laboratory Relationship

The relationship between the laboratory and the victims' families can be greatly affected by the duration of the identification effort. Lengthy mass fatality incident responses require greater interaction between the laboratory and the families. Families will look to the laboratory to provide regular updates and explain why the process may seem as if it is taking so long. In DNA identification efforts that last an appreciable time, as was the case after the World Trade Center (WTC) attacks, families may organize to more effectively convey their needs to policymakers and other decisionmakers.

There were several family groups that formed after the 9/11 attacks. The largest, Give Your Voice, was started by a single family; the firefighters had a large family group; there was a family group in Boston and one on Staten Island. We made it a point to present the DNA story to these groups, and to answer their questions when they arose.

Robert Shaler

When a single business entity is involved in a mass fatality incident (e.g., an airline company in the case of an airline disaster), the company usually establishes a family assistance center, which serves as a bridge between the ME and the victims' families. Where there is no single corporate affiliation, however, as in the case of the WTC attacks, the government may establish an entity equivalent to a family assistance center to serve as an intermediary. Family assistance centers may coordinate the collection of victim and kinship information and reference samples. In cases involving victims from foreign countries, the laboratory may have to work directly with foreign consulates.

Family assistance centers also play a role in communicating the status of the identification effort to the families. Confusion can be reduced when a family assistance representative is brought directly into the ME's or laboratory's organizational structure and receives the same briefings as the laboratory staff, as well as additional tutorials on how DNA testing works. The family assistance representative can then coordinate with other family assistance personnel to better aid the families, thus allowing the laboratory to focus on analyses and identification. This type of collaboration between the family assistance center and the laboratory can improve and expedite the identification process and is most feasible when the family assistance center is established by an official agency rather than an ad hoc emergency group. Exhibit 13 shows the relationships between the laboratory and the victims' families.

Collecting Reference Samples

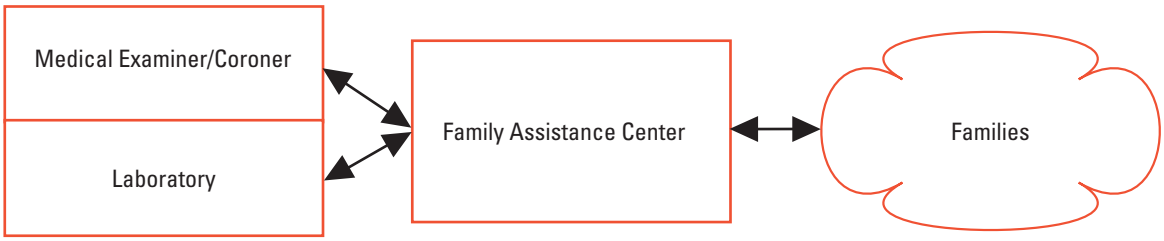
The Victim Identification Program (VIP) is software developed by the Disaster Mortuary Operational Response Teams (DMORT), a program of the U.S. Department of Homeland Security, to collect victim information. VIP contains approximately seven pages of victim-related data, tailored for making mass fatality 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:

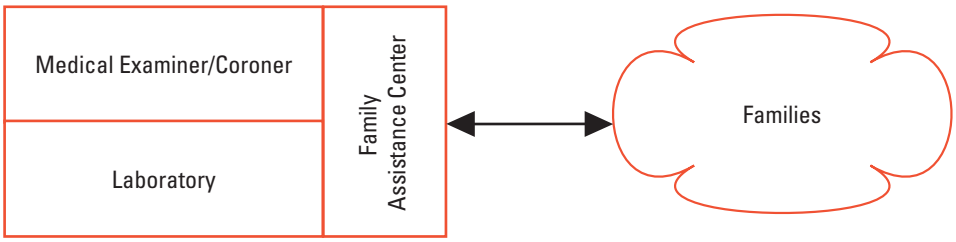
- Family members are under extreme stress in the days following a mass fatality incident, and their minds may be elsewhere during the collection process, causing them to inadvertently provide incorrect information. To avoid such mistakes, collection forms should be as simple as possible.
- Every reference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

Exhibit 13: Relationship Between Laboratory and Victims' Families

Typical Relationship Between Laboratory and Families



Preferred Relationship Between Laboratory and Families



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

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).

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.

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. (Note: Each victim should be assigned a unique case number. See chapter 9, *Information Technology*.)
- 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 mixup, allows the entire family to validate the pedigree, and uses laboratory staff time more efficiently.

Regardless of the collection approach, there invariably are some family members who—due to poor health or distance, for example—are unable to visit the collection center. In these cases, the collection center must make special arrangements to visit their homes, have other agencies (such as law enforcement agencies or phlebotomists) collect samples, or mail collection kits directly to family members. (Note: This last method circumvents appropriate chain-of-custody procedures and should not be used if strict forensic protocols are in force.)

As discussed in chapter 2, *How DNA Is Used to Make Identifications,* the number of possible

identifications depends on the condition of the human remains and the reference samples. After the laboratory develops DNA profiles for all of the personal items and kinship swabs, it will assess whether the reference samples provide sufficient information to identify the victim. In some cases, the laboratory may need additional information from biological relatives or personal items.

Family-Laboratory Communications

If directed to do so by the ME, the laboratory director may have to keep family members apprised of the identification effort, including any challenges that might hinder making identifications. The relationship between the victims' families and the laboratory is a delicate one, and the laboratory should be prepared to clarify any incomplete or incorrect information and to do everything possible to educate the families.

One way to educate the victims' families—and the public, in general—is to provide basic information on how DNA is used in mass fatality incidents. In an effort to educate families of the WTC victims, for example, the National Human Genome Research Institute at the National Institutes of Health and the National Institute of Justice at the U.S. Department of Justice created a brochure, that describes the DNA identification process, including why reference samples are collected and how they are used. Appendix G to this report is the most recent version—in English and Spanish—of the brochure.

The WTC response also had a toll-free "family hotline" to supplement the work of the family assistance center. The hotline was staffed primarily by medical and legal investigators who were fluent in English and Spanish. DNA laboratory personnel also were brought in to offer advice on kinship samples and pedigrees. The hotline became the primary way that family members were able to find out if their samples produced usable DNA profiles and, if necessary, to schedule an appointment to bring in additional reference samples.

The families of WTC victims relied on the hotline to ensure they had done everything they could to help their loved one be identified. If a hotline is established, it would be important to have appropriate multilingual responders. DNA personnel should also be available to provide guidance on questions such as whether it would be helpful for a certain family member to provide a kinship sample.

Individuals staffing the hotline should have online access to:

- A log of contacts for each family, including who provided a reference or kinship sample and the date thereof.
- The victim's information (e.g., the data collected in the Victim Identification Program (VIP)).
- Chain-of-custody information for reference samples, including the type of sample, when it was received, who donated it, etc.
- The status of each reference sample submitted by the family, including whether its analysis yielded a useful DNA profile or when it is scheduled to be analyzed.
- Whether the amount of reference material is sufficient to make an identification.

It may be useful to assign particular individuals to work with particular families. Limiting the number of people with whom a victim's family has to deal may facilitate communication, build trust, reduce stress on the family, and limit unrealistic expectations. Of course, the feasibility of this approach will depend on the size of the mass fatality incident and whether staff is available to support a hotline.

A lesson we learned in the World Trade Center identification effort was that collection of the kin reference sample had to be appropriately placed in their genetic context at the time they were collected or there could be great difficulty later on in the analyses of identification probabilities. Therefore, geneticists and genetic counselors should assist with collected family relationship data from those contributing reference samples in the aftermath of a mass casualty disaster.

Joan Bailey-Wilson

The family brochure made it possible for the families of 9/11 victims to receive reliable information about the DNA testing process, including what they could expect and the meaning of results. The document also has been used to help families of missing persons, including after Hurricane Katrina.

Lisa Forman

Depending on the duration of the response, families may form their own groups. The laboratory or a designee may be asked to participate in family group meetings. This is an opportunity to provide information and dispel rumors or misconceptions about the DNA testing processes and results. For example, phrases used by a DNA professional may be incorrectly interpreted by a layperson. The term "intact body" is likely to mean one thing to a professional and another to a victim's loved one. The laboratory director also should be aware that several groups representing the families may exist and should not assume that all family members receive information that is imparted at these meetings.

For the families of missing persons, including the victims of the 9/11 terrorist attacks, there is no such thing as 'closure' or 'moving on.' Families first must do the hard work of grieving to get to a place where they intellectually accept that their loved one is gone; then they can learn how to forever hold them in their hearts. For many, the identification of remains helps them to get through the first part of this process so that they can do the harder work of the second part.

Leslie Beisecker

Sometimes, it may be helpful for family members to tour the laboratory and ME facilities to more fully understand the identification processes. With respect to DNA analysis, for example, family members are likely to inquire about the status of samples they provided, whether those samples provided usable DNA profiles, and whether they can do anything else to assist the effort. The laboratory should be prepared to answer these questions.

Finally, here are some additional lessons learned during the 9/11 DNA identification effort:

- Some people hold negative perceptions of civil servants, leading them to believe, for example, that the laboratory is not working hard enough or does not have the expertise to perform the work.
- Obtaining reference samples from a family member who was estranged from a victim can be difficult.
- DNA analysis may uncover situations in which biological relationships are not as reported. In such cases, the laboratory must have a policy. It may be advisable to consult with a bioethicist (see http://www.bioethics.net).
- If the mass fatality resulted from criminal or terrorist activity, family members may resist a mass burial that includes the remains of the perpetrator(s); they may not want any unidentified remains of their loved one commingled with the remains of the person or people who killed them.
- One of the most painful experiences for the family of a victim is learning that a misidentification requires exhumation. It also can be difficult for a family to receive additional remains after they have buried a loved one. A laboratory director should be prepared to encounter a wide range of wishes from the victims' families if such situations occur.

CHAPTER 9

Information Technology

Information technology (IT) can be one of the most overlooked aspects of a mass fatality incident response. This is understandable—after all, most senior laboratory managers are forensic scientists, not computer scientists. However, advance planning on integrating IT throughout a mass fatality DNA identification effort saves time, speeds identification, and improves the reliability of the testing. It is crucial that the project manager include IT personnel in decisions regarding sample tracking and other business processes.

The archiving and management of the vast amount of data involved in a DNA-based identification of mass disaster victims is an enormous challenge. Because data must be retrieved, compared, and integrated reliably and efficiently, it is crucial to have sophisticated software.

In June 2005, the National Institute of Justice published *Mass Fatality Incidents: A Guide for Human Forensic Identification* (NCJ 199758; www.ojp.usdoj.gov/nij/pubs-sum/199758.htm). The study that resulted in the guide found that:

> The process of accumulating, reviewing, and interpreting DNA data is the most challenging step when employing DNA technology to identify mass fatality victims.

Ideally, an experienced IT laboratory staff member should be involved in the management of a mass fatality DNA identification effort. IT should be the cornerstone of quality control, and the IT department should continually be searching for ways to improve work processes and turnaround time.

For example, one way to increase productivity in a mass fatality identification effort is to have the Laboratory Information Management System (LIMS) produce a daily progress report for the media and elected officials. It is preferable to develop this capability before an incident occurs, because it is extremely difficult to achieve this level of IT sophistication in the midst of a mass fatality response.

Exhibit 14 shows different functions that an IT system can support in a DNA laboratory. The arrows follow the basic flow of samples and data. In highly automated laboratories, these procedures will be monitored or controlled through the LIMS.

Regardless of their level of automation, all laboratories employ these systems, in some form or another, during routine casework and offender processing. To effectively support a mass fatality incident response, however, some of these systems require special features.

Our chief lesson learned in this arena is that, without validated, well-documented software programs to associate profiles from tens of thousands of remains with scores of direct and indirect reference samples, the matching process is untenable. The midst of a victim identification project is a difficult time to be beta-testing new versions of software.

Stephen Sherry

Prior to 9/11, mass disasters in the United States were relatively small in scale, allowing simple spreadsheet approaches to be sufficient for data management. However, even small-scale disasters require scrupulous data management. Although software programs exist for data management, access, and statistical analyses, the magnitude of the World Trade Center (WTC) disaster demanded enhanced capabilities.

In the early stages of the World Trade Center identification effort—when the results of analyses were just beginning to arrive—we had to get information about the origins of any sample by querying several different computer systems; we often had to review paper records and ask for help from the New York State Police. The problem could only get worse, and we knew there had to be a better way to include functions in the LIMS beyond just tracking laboratory reagents and samples.

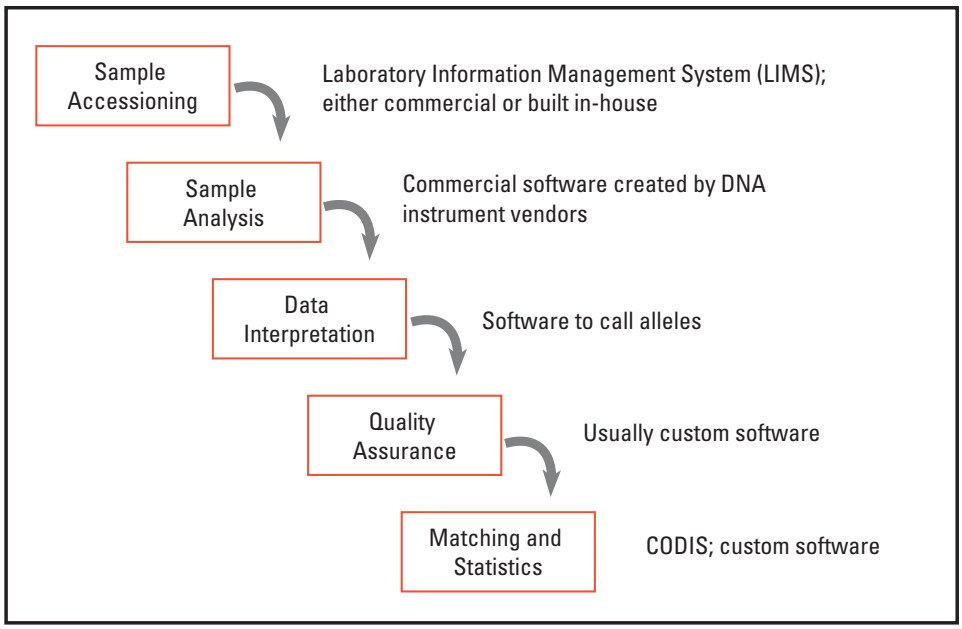
Elizabeth Pugh

Data-handling systems are needed to integrate any customized software as well as to provide a middleware system for connection and integration of different software components. Computer software must be able to assist with many functions. It must:

- Organize, store, and retrieve diverse data.
- Integrate different software systems.
- Allow technical and administrative review of data.
- Allow for annotation and recording of problems and resolutions.
- Report metrics.
- Track samples among partner laboratories.
- Prioritize sample selection and review.
- Generate family pedigrees and calculate likelihood ratios for hypothesized kinships.
- Combine remains with the same profile to facilitate searching.
- Enable profile comparisons and statistical calculations.
- Allow for users to interact with the interpretation and evaluation of ambiguities.
- Be reasonably user-friendly.

In the WTC identification effort, the Office of the Chief Medical Examiner (OCME) contracted with a private vendor that developed software with the above listed requirements in mind. However, because the software was developed in the midst of the identification effort, and was not previously documented and validated "shelfware," the majority of the kinship analyses and (initially) the remains matching had to be conducted using several commercially available programs. These programs were supplemented with customized patches developed by Kinship and Data Analysis

Exhibit 14: Information Technology in a DNA Laboratory



Panel (KADAP) members who were deployed to the OCME. Without such software, the success of the WTC identification effort—nearly 1,600 identifications made and nearly 20,000 remains profiled—would not have been possible.

Another software tool used in the WTC identification project was from the FBI's Combined DNA Index System (CODIS). Two of CODIS' four files—the Missing Persons and Unidentified Human Remains Index (CODISmp) and the Reference Samples from Personal Items and Family Index—allow the search of DNA profiles. The use of mtDNA profiles as a screening system is facilitated by the introduction of the CODISmp system. Although designed for missing persons, the system may be used to search for DNA profiles of mass disaster victims.

Sample Accessioning/LIMS Requirements

All LIMS products have a sample accessioning capability, usually centered on a case number. Each case number has multiple items, or submissions, associated with it. Some LIMS may print a barcode that assists in chain-of-custody documentation and the creation of management or status reports.

In a mass fatality incident response, human remains, personal items, and kinship samples must be accessioned. Laboratories usually retrofit these special requirements into their existing LIMS. There are a number of benefits to this approach, including that:

- Laboratory personnel are familiar with the sample accessioning process and can avoid the learning curve associated with new software.
- Chain of custody is documented and controlled using tried-and-true processes already in place.
- There is no need to purchase additional software.

The typical strategy for accessioning human remains is to assign all fragment submissions to a single case number. This is relatively straightforward if the LIMS allows a single case to have thousands of submissions (one for each remains sample). If the laboratory's LIMS does not allow for a large number of samples to be associated with a case, the laboratory will want to consider developing a system to link the cases so that all samples can be associated to each other and to the identification effort.

The commingling of remains presents another problem. For example, after a remains sample is accessioned and analyzed, the laboratory may discover that it belongs to two or more individuals. The DNA may show that the bone and tissue come from different donors, as happened in the WTC attacks, where remains were severely compacted.

From the moment commingling is discovered, the laboratory will have to assign a new submission number to one of the items, then track both items separately. This principle would apply even if there are more than two profiles from a single sample—each profile would require a new submission number. Some LIMS systems may allow a sample to have multiple DNA profiles; regardless, both samples will have to share the initial chain-of-custody and accessioning information.

Several other scenarios may further confound sample tracking. For example, there may be multiple victims from the same family, in which case the situation is complicated by partial profiles with overlapping genotypes or by full or nearly full DNA profiles from remains that are needed as reference samples for a related victim. Such difficult situations can occur and must be accommodated.

The typical strategy for accessioning reference samples is to assign each victim a case number and add reference samples as submissions under the case. The case number is important because it represents the victim's family and is used to group personal items and kinship samples for kinship matching.

Assigning case numbers is not a complicated issue in a "closed" incident—for example, when a flight manifest contains names and addresses that can be tracked. During reference sample collection in a closed event, the family assistance center can review the list of victims and assign reference samples to the correct case number. In instances where victims have similar names, the family assistance center can ask family members for clarification during the collection process.

However, assigning case numbers in an "open" incident is much more complicated, and may tax the capabilities of the laboratory's LIMS. Because there is no definitive list of victims in an open incident situation, the family assistance center—not knowing, for example, if there is more than one victim named John Smith—cannot simply assign case numbers to victims. This problem is exacerbated when reference samples are collected in an open-house forum, where members of the same family visit the collection center at different times. This also can lead to errors in the collection process, including variations of a victim's name and perhaps even date of birth.

Therefore, during accessioning, staff entering data should avoid the temptation to reconcile name variations. Rather, data should be entered exactly as specified on the collection form. Any necessary case number or victim reconciliation should occur after the final list of victims is established. This approach to accessioning will generate more case numbers than victims, but it will preserve all the information provided by the donors.

We found that instituting quality checks throughout the identification process ultimately saved time and effort. By continually validating the accuracy of the data and results at each step in the analysis, we could identify potential issues before they became impediments to an identification.

Amanda Sozer

Unless care is taken when identifying and assigning case numbers to the potential victims, the laboratory will be forced to reconcile originally assigned case numbers with a later, more refined list of case numbers. Some of the originally assigned case numbers might have to be divided, and others might have to be consolidated. The most important part of a process that requires a regrouping of reference samples is preserving the original case number so that:

- Samples do not have to be barcoded again.
- New case numbers do not have to be issued to families.
- The chain of custody is maintained.

Exhibit 15 presents different scenarios of reconciling case numbers with victims. It is important to keep in mind that some LIMS products may not allow reconciliation of case numbers with victims.

Exhibit 16 presents some additional capabilities that require LIMS support.

If possible, the software used by the family assistance center to collect reference samples should interface with the laboratory's LIMS. This avoids duplicate data entry and eliminates the potential for data-entry errors. At a minimum, the two systems should have compatible barcodes so that the samples do not have to be barcoded again during accessioning.

Ideally, the laboratory's LIMS will be able to:

- Store the data included in the reference sample collection forms (see appendixes B, C and D to this report).
- Capture photographs of remains samples and personal items and digital images of handwritten collection forms.
- Store family pedigrees and allow a victim sample to be used as a reference sample for another victim, if necessary.
- Allow cases to be divided and combined.
- Track samples to and from multiple laboratories.
- Track multiple testing of the same sample.
- Alert the end user to discrepancies in data.
- Prioritize sample testing and data analysis.

Quality Control Software

Software is not only a case-tracking tool. It is also a critical component of a DNA laboratory's quality assurance and quality control programs.

Quality metrics collected and tracked through software are used to refine and improve the laboratory's quality assurance plan, and software tools often are employed as quality control mechanisms. Mass fatality incident responses have several, specific quality control needs:

- **Identify conflicting STR results.** Remains samples and personal items may not yield usable DNA profiles on the first analysis attempt. The laboratory may choose to reanalyze these samples under altered conditions in the hope of producing a complete—or a more complete—profile. The laboratory will need to compare the results from each analysis to identify and resolve conflicts.

Exhibit 15: Reconciling Case Numbers With Victims

Case 1: An originally assigned case number maps directly to a victim.	Original Case Number — Final Victim Case Number	No additional work required.
Case 2: An originally assigned case number contains reference samples for multiple victims.	Original Case Number → Final Victim Case Number; Final Victim Case Number; Final Victim Case Number	This can occur when multiple victims have the same name or reconciliation is attempted during accessioning. More difficult to resolve from an IT perspective than Case 3.
Case 3: References for several originally assigned cases actually belong to one victim.	Original Case Number; Original Case Number; Original Case Number → Final Victim Case Number	This can occur when family members report multiple variations of the victim's name. Relatively simple to resolve from an IT perspective.
Case 4: Combination of Cases 2 and 3.	Original Case Number; Original Case Number; Original Case Number × Final Victim Case Number; Final Victim Case Number; Final Victim Case Number	This can occur when victims have the same name and family members report variations of the name (e.g., mixing up Jr.'s and Sr.'s). Most difficult to resolve from an IT perspective.
Case 5: Orphaned original case number.	Original Case Number — ?	Reference samples were collected for a nonexistent victim. This can happen in "open" incidents.
Case 6: Orphaned victim.	? —	The victim has no reference samples. Identification can be made only in a "closed" incident.

Exhibit 16: Additional Capabilities Requiring Laboratory Information Management System Support

LIMS Feature	Description
Support multiple DNA technologies.	Some LIMS products actually store the DNA profile associated with the sample. If the response employs several DNA technologies, the LIMS must support the various profile types (e.g., STRs, mtDNA, single nucleotide polymorphisms (SNPs)).
Allow samples to be tracked on multiple microtiter plates.	One sample may have numerous extracts. Depending on the types of DNA technology conducted, a sample may appear on multiple microtiter plates (e.g., STR plate, mtDNA plate, SNP plate, various reextraction plates). This feature is particularly important if extracts are shipped to partner laboratories.
Allow one sample to have multiple sample numbers.	Each sample in a mass fatality response will have several different sample numbers, each assigned during a particular business process. These sample numbers are actually references into other databases. For example, the software used at the family assistance center will assign each sample a number and the laboratory's accessioning program will assign a different number. Or, partner laboratories may assign their own number (and barcode) as they accession samples.
Support shipping and receiving samples and data from partner laboratories.	The LIMS should be able to: ■ Build shipping manifests that contain samples or DNA extracts. ■ Track the sample (or microtiter plate) as it moves among partner laboratories. ■ Track when the DNA results and physical samples are returned.
Segregate mass fatality data from regular casework.	The laboratory most likely will want to segregate the mass-fatality data from regular casework data so management reports and metrics are not merged and can be analyzed independently.

- **Identify conflicting results from different DNA technologies.** When multiple DNA technologies are used, the laboratory will need to review previously reported identifications to ensure that results from the new technologies are consistent. For example, a remains sample and a personal item may match with STRs but not with mitochondrial DNA (mtDNA).
- **Identify fortuitous matches.** Partial profiles resulting from sample degradation are a common occurrence in mass fatality incidents. A partial profile may match several reference samples fortuitously, particularly if the matching algorithm allows for the possibility of allelic dropout. The DNA analyst must review all of the candidate matches and choose an appropriate course of action. The software should produce a work list that allows the DNA analyst to record free-text comments about each discrepancy.

If the laboratory chooses to outsource samples to partner laboratories, these additional quality control tools should be considered:

- **Data file validation.** The managing laboratory may want to validate the format and content of data files that are provided by partner laboratories. Fields that may be validated include sample names (to ensure the appropriate naming scheme was applied) and loci and allele values.
- **Blind-control verification.** One method of monitoring quality in partner laboratories is to

institute a blind-control program (see chapter 14, *Quality Control*). To partner laboratories, blind controls appear to be normal samples; however, their profiles have already been determined by the managing laboratory. The managing laboratory randomly places blind controls into the batches of samples (or microtiter plates) that are shipped to partner labs. The blind controls usually are renamed so that they are indistinguishable to the partner laboratories from normal samples. Then, the managing laboratory checks the data files that are produced by partner laboratories for blind controls and verifies them against the known DNA profiles.

Matching and Statistics Software

There are two basic approaches to DNA matching: (1) direct matching, and (2) kinship matching. Direct matching compares two DNA profiles to determine whether they come from the same source ("individual"). Sophisticated direct matching algorithms consider allelic dropout for nuclear DNA and heteroplasmy for mtDNA. Kinship matching, on the other hand, uses DNA profiles to identify biological relationships among individuals. Kinship matching should consider both allelic dropout (nuclear DNA) and mutations (nuclear and mtDNA).

Exhibit 17 shows how mass fatality incident data may be searched.

One of the primary tools for making DNA identifications is "matching software." Currently, the most widely used forensic DNA matching software in the United States is the FBI's Combined DNA Index System (CODIS). However, an underlying design principle of CODIS is that matches are rare and independent events—and, in mass fatality incidents, matches are neither rare nor independent of one another. Therefore, a laboratory director should be aware of CODIS limitations in a mass fatality incident response.

CODIS is designed to rapidly search crime-scene DNA profiles against each other and against DNA profiles of known individuals. One assumption built into CODIS is that each profile will match only a tiny fraction (usually one or none) of the profiles in the database. In a criminal case, which CODIS is primarily designed to handle, the DNA profile obtained from a piece of evidence might not match any of the million-plus convicted-offender DNA profiles in the database, simply because that person has not previously been convicted of a crime that mandated collection of a DNA sample.

In a mass fatality incident, however, every human remain likely will match several samples, including other remains or personal items. Although CODIS can properly identify all of the matches in a mass fatality incident (through pairwise comparisons), it does not aggregate similar matches, and, therefore, is less useful in a situation where

Exhibit 17: Searching Mass Fatality Data

Versus (compared with)	Human Remains	Personal Items	Kinship Samples
Human remains	Use direct matching to identify multiple fragments of the same individual.	Identify human remains by direct matching to personal items.	Identify human remains by kinship matching.
Personal items	Identify human remains by direct matching to personal items.	Use direct matching to verify that multiple personal items submitted on behalf of a single victim have the same DNA profiles.	Use kinship matching to verify that the personal items belong to the victim.
Kinship samples	Identify human remains by kinship matching.	Use kinship matching to verify that the personal items belong to the victim.	Not usually performed.

the goal is to assemble all potential matches across time and space. That said, CODIS has a standard data file format that is used to report STR data, and this common ".cmf" format was used in the WTC identification analyses.

Once a potential direct or kinship match is identified, the laboratory must determine its statistical significance using a likelihood ratio for kinship matching. To declare a match as an identification, the computed estimates must exceed threshold values that are predefined for direct and kinship matches. The identification thresholds are determined based on the number of victims, the biological relationships of the victims, and the nature of the incident. This was a major focus of the KADAP and is addressed in chapter 12.

Finally, the laboratory may elect to factor nongenetic data into the identification process. For example, human remains recovered from the WTC were catalogued based on their physical location within a two-dimensional grid superimposed on the disaster site. These data are useful when likelihood-ratio thresholds cannot be met due to incomplete DNA profiles.

Sharing Information

Information technology (IT) should be considered both in the context of the identification work and as a tool to foster communication. Many laboratories have come to appreciate the value of a LIMS and sophisticated DNA matching/kinship software; however, IT also can be used to enhance interorganizational communication. The DNA laboratory will need IT solutions to exchange data with other government or vendor laboratories that are participating in the mass disaster response. For example, the ME most likely will have a system that tracks the status of all identification modalities, and the DNA laboratory's system(s) should be fully integrated so data entry is not duplicated.

Mass Fatality Incidents: A Guide for Human Forensic Identification (www.ojp.usdoj.gov/nij/pubs-sum/199758.htm), recognizes some of these challenges:

> The difficulty of [data management] is compounded when more than one laboratory is involved in providing DNA results. Participating laboratories should affirm their mutual commitment, coordinate and track sample flow, and agree to use compatible software applications for data acquisition and interpretation.

When multiple organizations participate in a mass fatality response, several types of data may need to be exchanged, depending on the relationship between the organizations. External organizations and systems that typically require IT interfaces include:

- Government (partner) laboratories.
- Vendor laboratories.
- Sample collection agencies (e.g., first responders that collect and catalogue remains or collect personal items and kinship samples).
- CODIS.
- Mass-fatality-specific software programs (e.g., the Victim Identification Program (VIP), which is provided free of charge by the Disaster Mortuary Operational Response Teams).
- LIMS.

Exhibit 18 depicts business processes that will have to be integrated among laboratories if more than one laboratory is involved in the DNA identification analysis.

To successfully build interfaces across these business processes, the IT manager should consider:

- Physical connectivity between systems (e.g., Internet, private network, CD–ROM).
- Security requirements (e.g., encryption, firewalls, access controls).
- Data archiving.
- Communication mechanisms (e.g., file transfers, enterprise messaging, database interaction); most of this type of integration in mass fatality identification responses has been accomplished through file transfers.
- Data exchange formats (e.g., fields, field lengths, data types, relationships); data exchange formats are particularly challenging due to differences among some LIMS systems.
- Compatible barcodes that allow a barcode applied in one laboratory to be scanned and read in other laboratories.

Exhibit 18: Integrating Processes in Multiple DNA Laboratories

Business Process	When Integration May Be Necessary
Sample accessioning	Multiple, independent computer systems are used to accession samples. All of the data must be accessible by the laboratory(ies) responsible for making identifications. The managing laboratory's LIMS may also need to communicate with DMORT's Victim Identification Program (VIP).
Sample analysis	One or more laboratories use the raw data produced by other laboratories to make its/their own allele calls. An example would be using an expert system to reanalyze severely degraded DNA.
Data interpretation	One or more laboratories review the allele calls made by other laboratories; participating laboratories produce CODIS-compatible import files for use by the managing laboratory.
Quality assurance	Multiple laboratories are participating in a single, unified quality assurance program.
Matching and statistics	Laboratories share their final results with one another.

Infrastructure

Mass fatality incident responses are high-profile events that are scrutinized by the public, elected officials, and the press. In addition, the response can have an aggressive timetable for completing victim identifications, and DNA is often the primary means of identification. The systems that support the DNA identification effort should be considered "mission critical." System downtime should be minimized, and robust backup and restore procedures should be among the first processes implemented.

Volunteers or members of external organizations may participate in the laboratory's disaster response, which means that the IT manager may need to provide computers and other temporary services (e.g., printing and e-mail) to those entities. The IT manager most likely will need a security policy that restricts access to certain aspects of the data by unauthorized volunteers.

The demands placed on the IT infrastructure will last for the duration of the response. Because the DNA analysis process generates large amounts of data, the laboratory will need sufficient storage capacity to absorb the additional data produced during the disaster response. Dedicated IT staff (in-house or outsourced) may need to be provided as well.

Conclusion

Seasoned IT managers agree that building IT infrastructure (hardware and software) in the midst of a crisis is extremely difficult. Ideally, the laboratory will have in place the policies, processes, and network infrastructure to support a mass fatality incident response.

Prior to a mass fatality identification effort, the IT manager's strategic plan should:

- Identify agencies and systems that may require electronic interfaces and prepare a way to implement those interfaces.
- Create a procurement list of additional hardware and software to be purchased in the event of a mass fatality incident response, allowing for the fact that operating systems and hardware are continually changing.
- Ensure laboratory personnel know how to use software packages before a response is necessary.

- Ensure that the network infrastructure can be expanded quickly by adding new servers or desktop workstations.
- Develop a plan for adding additional, temporary IT staff.

The IT manager's strategic objectives during a mass fatality incident response should be to:

- Identify and control the design of interfaces to external systems.
- Ensure that adequate access controls are in place for external users.
- Provide reliable services.

Finally, if the laboratory cannot avoid writing custom software code during a mass fatality incident response, the IT manager should attempt to limit such software, because the introduction of new programming languages, platforms, etc., during this time increases the complexity of management, drives up costs, and can result in unexpected consequences affecting already functional programs.

CHAPTER 10

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 chapter 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 mixups. 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 (see appendix G).

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.

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. (See chapter 9 of this report for a discussion of LIMS systems.) 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

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.

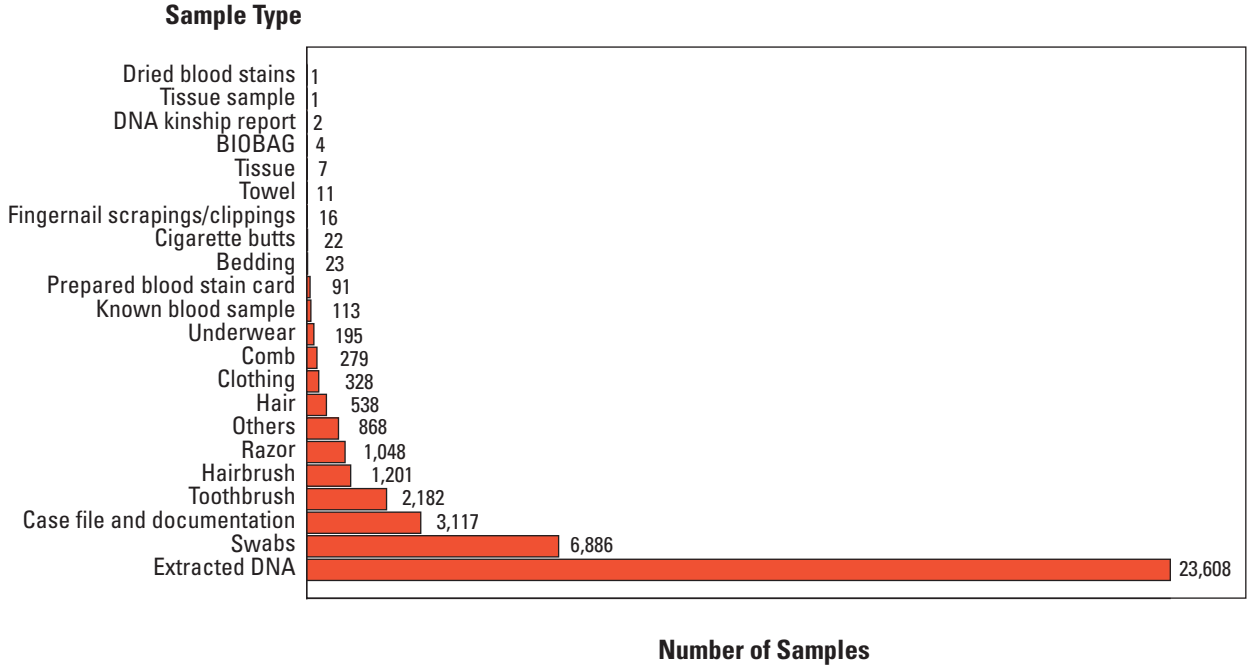
numbers. (Mass fatality incident samples often have several identifying numbers, analogous to case numbers assigned to an agency's casework samples. In addition, when multiple laboratories assist with analysis or interpretation, samples likely will receive multiple identifying numbers, one for each laboratory. 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.

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

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.

In a mass disaster event like the terrorist attacks on the World Trade Center, the commingling of remains is a real possibility. Tissue samples often yielded multiple profiles—seemingly conflicting results—and we soon learned that the most reliable results came from the analysis of bone.

Robert Shaler

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.

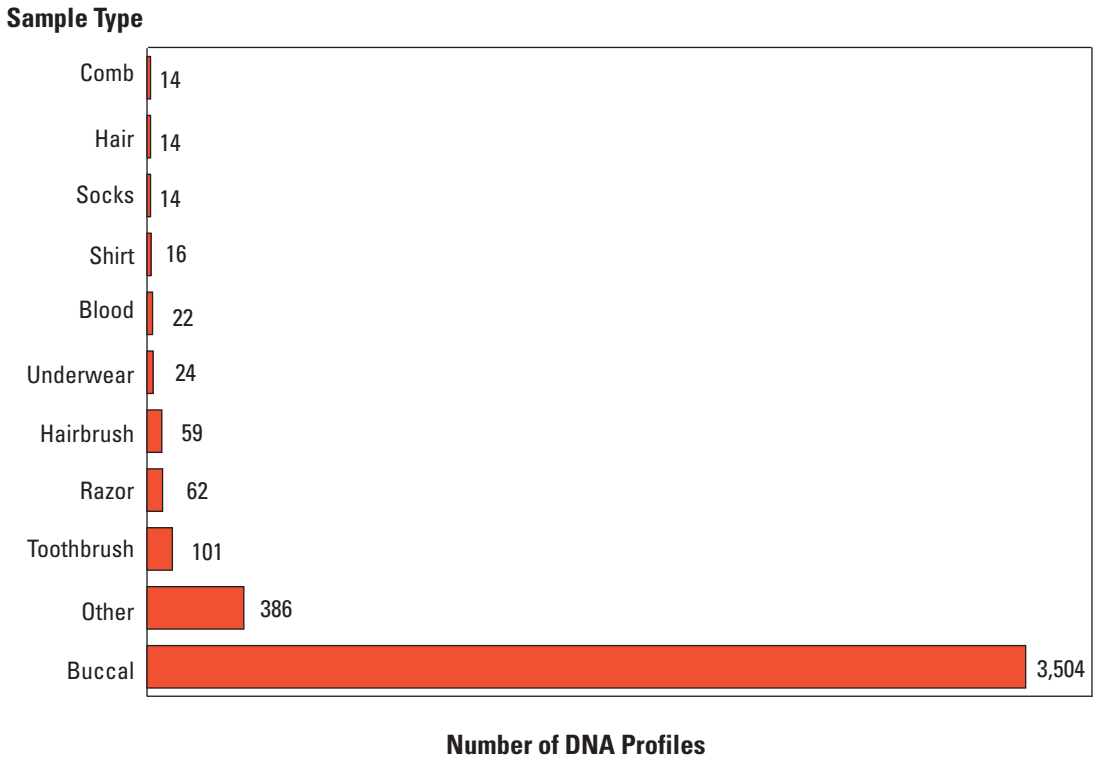
The laboratory is likely to receive and analyze disaster samples before personal effect items or kinship samples. Depending on the duration of the recovery effort, the laboratory may not be able to examine all of the remains and choose only the samples most likely to yield DNA profiles. In an extended 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.

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.

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.

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.

Without work lists, our efforts in the World Trade Center identification effort were redundant. Work lists helped to keep the nonintentional redundancy to a minimum.

Robert Shaler

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.

CHAPTER 11

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 chapter 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

We knew immediately that hundreds of environmentally challenged samples would not yield full 13-locus STR profiles and would therefore require extra laboratory effort. So, early on, we explored alternate, novel technologies to help turn samples with only partial profiles into those that would allow solid victim identifications. In doing so, we carefully considered issues regarding genetic linkage of markers, and also agreed that only methods meeting NYS validation standards would be used.

Frederick Bieber

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 chapters 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 (appendix B), Family and/or Donor Reference Collection Form (appendix C), and the Family Tree Form (appendix D).

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 (see chapter 14, *Quality Control*).

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.

The KADAP served as a model for scientific collaboration and peer review of method validation under the challenging and stressful conditions of responding to a mass fatality disaster.

Anne Walsh

Mitochondrial DNA Analysis. 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.

Repositioning Primers. 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.

Single Nucleotide Polymorphism Analysis. 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.

CHAPTER 12

Statistical and Other Issues

This chapter provides an overview of some of the statistical issues involved in making DNA-based identifications of victims of a mass fatality incident. Because both mathematics and policy should be considered when determining statistical thresholds for making an identification, a single statistical approach may not be sufficient for every mass fatality disaster. Issues to consider when setting policy for a mass fatality response would include, for example, the condition of the remains, and the existence and reliability of samples. Appendix I contains an extensive reference list that may assist laboratory managers, policymakers, or public officials who desire a more indepth understanding of the use of statistics in making DNA-based identifications.

When dealing with statistical issues—including the statistical threshold necessary to make a DNA-based identification of a victim's remains—it is important that the identification policy for a particular mass fatality response effort be consistent with the goals of the effort. Decisions about the number of and specific loci to type, the statistical thresholds, and the use of outside laboratories and consultants should be made quickly (see chapter 4, *Major Decisions*). For example, in the World Trade Center (WTC) identification effort, the Kinship and Data Analysis Panel (KADAP) endorsed the decision of the New York City Office of the Chief Medical Examiner (OCME) to use the standard Combined DNA Index System (CODIS) core 13 short tandem repeat (STR) loci, as well as the Amelogenin sex-typing locus used in forensic laboratories throughout the United States (see chapter 11 for more on "mini-STRs," single nucleotide polymorphisms, and mitochondrial DNA sequencing, which also were used in the WTC identification effort).

It is important to note that the identification of WTC victims did not require the creation of any new statistical approach. In fact, the statistical approach recommended by the KADAP and used by the OCME was based on two well-established methods.

The first method, known as "direct matching," assesses the probability or likelihood that a DNA profile from a victim's remains and a profile developed from a personal item known to belong to a missing individual would share—by chance—the same DNA profile. The direct matching method is similar to that used in forensic genetic testing, in which there is an estimate of the strength of a match between a DNA profile from biological evidence and a profile obtained from a known reference sample. Direct matching was used in approximately two-thirds of the WTC DNA identifications.

In the field of human genetic research, genotypes of relatives have been used to reconstruct a partial or total genotype for the purpose of gene mapping. Experience gained in this area proved to be invaluable in helping to identify WTC victims.

Michael Conneally

A second statistical method, called "indirect matching," uses methods of formal genetic kinship analysis, in which a comparison is made of the DNA profile from a victim's remains and those of biological relatives in a known kindred (i.e., a "family tree" or pedigree). Also called "kinship analysis," this approach is similar to that used for parentage assignment in paternity testing, nursery mixup resolution, immigration, and probate disputes. Kinship analysis was necessary in about one-third of the DNA-based identifications of WTC victims.

The KADAP spent time considering and discussing the population makeup of the World Trade Center victims, including the population substructure that might influence the posterior probability of an identification. In the end, our consensus was to be conservative in all calculations to guard against false identifications.

George Carmody

The theories and practices of statistical analyses in making DNA-based identifications are well developed and well documented. Before any DNA testing is performed, the "posterior probability"—that is, the level of confidence needed to make an identification—should be established. The posterior probability is based on the product (multiplication) of two components: a "prior probability" and a "likelihood ratio."

Prior probability is the chance that any remains sample belongs to a particular individual; typically, it is based only on the estimated number of reported missing persons (RM), which can change over the course of the identification process. In the WTC identification effort, for example, the number of RMs was originally much higher—as many as 5,000—than the final estimate of approximately 2,750, after multiple reports, multiple nicknames, and other victim data were reconciled.

Likelihood ratio is the strength of the DNA evidence favoring identification.

Statistical Threshold

In the WTC identification effort, the statistical threshold (i.e., posterior probability) that was established before a DNA-based identification could be made was high: 99.9%. To attain this high level of confidence, the DNA laboratory targeted a likelihood ratio that, combined with the prior probability, met the statistical threshold. This likelihood ratio (**L**) was based on the following formula:

$$1 - (1-1/L)^{N} \leq p$$

In this formula, **N** = the estimated number of RMs (5,000 in the WTC); and **p** = the defined acceptable chance of error (1/1,000,000). Solving for the likelihood ratio:

$$L \geq 10{,}000{,}000{,}000$$

Open vs. Closed Incidents

Mass fatality incidents have been described as either an "open" system or a "closed" system. Open systems are those in which the number and identity of victims is unknown. Closed systems are those in which the number and identity of victims is more certain, such as in a small car or airplane crash.

As noted in chapter 4, a closed system can become an open system. Likewise, an open system can approach a closed system—or even become a closed system—as a larger proportion of RMs are identified. This concept proved useful in the WTC identification effort. As the number of victims was reduced from the initial estimate of 5,000 to approximately 2,750, the prior probability was accordingly increased, resulting in the KADAP's recommendation that the likelihood ratio necessary to make a DNA-based identification be reduced. [Note: Gender was another factor used to modify the prior probability.]

On the other hand, in a closed system, prior probability is increased as victims are identified, reducing the likelihood ratio necessary to meet the statistical threshold for identification. In rare instances (for example, in a small closed system in which the DNA profiles of all of the victims are known), it may not be necessary to establish a statistical threshold in order to make a DNA identification. Needless to say, DNA-based identifications are more straightforward in a closed system than in an open system.

Although open-system incidents may require collection of family reference samples on a much wider scale, the general principles for DNA identification are the same. (See chapters 10 and 11 for a more detailed discussion of sample issues.)

Non-DNA Data ("Metadata")

Data, other than DNA profiles, that are collected about missing persons and reference samples are often referred to as "metadata." Metadata about family reference samples—including family-member relationships (i.e., pedigree), data about the missing person, and autopsy data—are compiled in case folders. Metadata, combined with DNA test results, are used in the identification process. When these two data sets (metadata

and DNA test results) fail to reconcile, the cause of the inconsistency must be resolved. This leads to delays in an ultimate identification, stress for loved ones, and increased work for analysts.

It is essential, therefore, that metadata management be given a high priority (see chapter 10, *Sample Tracking and Management*) and that a laboratory have in place a process for comparing data from different aspects of a DNA-based identification process.

In rare instances, metadata can appear to contradict the results of DNA testing, even when the DNA results from both the remains and reference samples are scientifically and statistically sound. Of course, metadata are more subjective than DNA-testing results, and the process of proving that a personal-item or kinship sample comes from a specific person or family can be cumbersome. But, ensuring the accuracy of the original metadata obtained from victims' families—including the chain of custody of personal effects—is crucial.

Fragmented Remains

The remains of mass disaster victims may be commingled, which can result in mixed DNA profiles. In a situation of scattered, degraded, or fragmented remains, statistical sampling models should be used that increase the confidence of the DNA-based identification. In the WTC identification effort, for example, OCME scientists, attempting to find a portion of a sample that was not mixed, often returned to the original sample (if it was available) and attempted to reextract and reanalyze the DNA. It is important to keep in mind, however, that although well-established statistical analyses for mixtures can be applied, these result in reduced likelihood ratios that make meeting the identification threshold more difficult. Therefore, additional testing with alternative markers may be needed.

In theory, fragmented samples yielding partial DNA profiles could be associated based on their location at the disaster site. Grid coordinates, if available, might allow samples with overlapping partial profiles to be identified, particularly if the goal is to identify every human remain rather than every victim. However, even if the goal is to identify every victim, grid-coordinate methods may prove useful. Moreover, with appropriately conservative statistical approaches, partial DNA profiles from different remains may be combined to generate a composite "virtual" profile derived from a single victim.

Kinship Analysis

Kinship analysis is necessary when known reference samples are not available for the reported missing. In kinship analysis—well-established in the field of human genetics—close relatives provide DNA samples. Assignment of remains to a particular family is made by assessing the relative likelihood ratios.

Because the World Trade Center identification effort presented challenges not found in ordinary forensic work, it was not self-evident how to best evaluate the DNA data statistically. The KADAP discussions and recommendations were, I think, essential to reaching the final set of criteria.

Kenneth Kidd

"Indirect" DNA-based identification, or kinship analysis, involves two steps. The first is a screening process, in which DNA profiles from the remains are compared with family reference samples. The process allows families to be eliminated and "candidate families" to be isolated. The second step determines the likelihood that a particular remains sample fits into a particular candidate family. [Note: When only more distant relatives are available to provide reference samples, kinship analysis cannot provide robust estimates of relationships.]

Kinship analysis is not always successful for making unambiguous identifications of victim remains. Its limitations include difficulty in identifying remains belonging to same-sex full-siblings, particularly monozygotic twins. Also, close relatives are sometimes victims of the same disaster, and differentiating such relationships in the absence of very complete pedigrees may not be possible. It is helpful to be aware of the number of related victims prior to assigning identity, but this may not be possible in an open-system mass fatality event. In fact, this may be difficult even in a well-documented closed disaster, in which a high level of statistical confidence may not be reached without a sufficient number of close relatives to provide reference samples.

Generally, the more close relatives available as references, the better the chances for attaining the identification threshold. For example, when standard paternity trios (mother, father, child) are available and numerous polymorphic loci are typed, sufficiently high likelihood ratios typically result. However, if only one sibling is available to provide a family reference sample, it is unlikely that a statistical threshold for a DNA-based identification would be met.

Because a personal reference item does not always contain sole-source DNA (or contain the DNA of the person to whom it is attributed), it is advisable to confirm direct-comparison DNA by indirect kinship analysis. Although it may seem like additional work to first examine the profile from the personal effect against the profiles from relatives to establish the fit in the pedigree, verifying the source of the DNA from the personal effect actually reduces the time needed to finalize DNA-based victim identification. When verifying the origin of the personal effect within the kindred of reference samples, the kinship analysis by itself does not need to meet the threshold requirement for identification, as it serves only to confirm the direct comparison to personal effects.

When multiple family members—or, particularly, an entire family—are victims of an incident, identifying at least one of them with high confidence improves the ability to achieve certainty for the others; the caveat, here, is that same-gender siblings may not be distinguishable by kinship analysis unless direct reference samples are used. Although fragmented remains of same-sex siblings usually can be distinguished from each with DNA analysis, families would not be able to be told which remains came from which sibling unless direct reference samples (from one or the other) are used to differentiate the remains. Without direct references from a known personal effect, same-gender siblings usually cannot be distinguished when remains are fragmented or otherwise lacking in traditional anthropological and other phenotypic characteristics.

Administrative and Technical Reviews

Once a potential match is made between a remains profile and the profile from a personal item or a family member, a technical and administrative review of the case folder is conducted.

The administrative review helps ensure that the personal reference, kinship, and remains samples have been identified and labeled correctly. For example, stress may cause family members to provide inaccurate information regarding reference samples. Therefore, it is important to check key data such as birth dates; for example, a son should not have a birth date before that of his father. Personal effects submitted to the laboratory can be verified by comparing the DNA profile to DNA profiles obtained from the victim's relatives. It may not be unusual, for example, to discover that a male victim's razor actually contains the DNA profile of his wife, who forgot that she used the razor the morning of the disaster.

The administrative review helps ensure that each victim is registered in only one "case" folder. Partitioning reference samples across multiple "cases" for a single victim slows the ability of the laboratory to make identifications via kinship analysis.

The technical review should verify all data, interpretations, and calculations. If multiple testing systems were used, a comparison of the results should be made, and any inconsistencies—including the possibility of contamination—should be resolved.

The KADAP prepared three sample forms that may assist laboratory directors in preparing to respond to a mass fatality incident. Appendix B is a Personal Items Submission Form, appendix C is a Family and/or Donor Reference Collection Form, and appendix D is a Family Tree Form.

CHAPTER 13

Procurement and Vendor Management

This chapter considers the procurement of commodities and services and the management of those services in a mass fatality DNA identification effort. Adequate storage for consumables and adequate space and utilities to support the operation of new equipment also must be ensured.

A laboratory that is faced with responding to a mass fatality incident may need to rapidly procure laboratory reagents, supplies, equipment, testing services, and consultants. The laboratory also will have to decide how to handle and prioritize the samples. (See chapter 4 for a discussion on determining whether an outside vendor is needed to help provide testing services.)

Ordering Supplies and Equipment

If some or all of the testing is to be performed in-house, consumables and new equipment may need to be purchased. It is important to review current contracts and standing orders, because procuring the same lot number of reagents and model of equipment currently being used may be helpful.

Waiting to consider whether new equipment or test systems would be needed in the event of a mass fatality incident can impact a laboratory's personnel during a difficult time. Implementing new protocols, procedures, and equipment—unless absolutely necessary for making identifications—is best not done during a mass fatality response. If the laboratory has a validated method that is adequate for processing mass fatality samples, it may save time to use the established procedure. On the other hand, advance planning may lead to a new method or piece of equipment that will help the laboratory—or another laboratory—should a mass fatality incident occur.

The laboratory's purchasing department can help ensure that procurement rules and regulations are followed. For example, is there a cap on what can be purchased without going out on bid? Will new contracts need to be established? It also may be advisable to consult with the Federal Emergency Management Agency (FEMA) regarding procurement rules.

Assigning someone to be responsible for placing orders associated with a mass fatality incident will help ensure receipt of the correct consumables and equipment. Adequate storage for consumables and adequate space and utilities to support the operation of new equipment also must be ensured.

Outsourcing Sample Testing

The best time to establish a good relationship with a vendor is during the planning phase for a mass fatality incident. Although outsourcing testing can be expensive—from $30–$60 for a reference buccal sample to hundreds of dollars for a disaster sample—it may be necessary or more effective to have another laboratory test some or all of the samples. For example, an outside laboratory may test certain sample types—family reference, personal items, or disaster samples—or a portion of the samples for quality control or conformation testing. An outside laboratory may be used for certain types of testing technologies—mitochondrial, single nucleotide polymorphism, or new technologies, for example—or for extraction and data analysis only. On the other hand, the entire testing process, from accessioning to data analysis, may be outsourced. Even in this situation, however, the managing laboratory is ultimately responsible for the quality and accuracy of the data.

The laboratory's contracting office can ensure that contracting regulations are followed, and

discussing this issue with a vendor in advance may prevent later problems. In an emergency situation, an agency may not be required to follow the typical, lengthy contracting procedures to obtain the best value but, rather, may be able to initiate contracts without competition. If a managing laboratory's normal contracting process is not followed, however, it is very important to document the new process to reduce the potential for future problems.

To assist in the response to a mass fatality incident, a laboratory may contract with a current or new vendor, or seek help from another government agency. If the managing laboratory is already contracting with a vendor whose quality is satisfactory, it may be advantageous to use that vendor to process mass fatality samples, assuming the vendor's capabilities and capacities can support the laboratory's needs. For example, does the vendor have the capacity (e.g., equipment and staffing) to meet throughput and turn-around-time requirements, even while working on other contracts? If not, is the vendor able and willing to interrupt its regular work to take on the testing of mass fatality incident samples?

Does the vendor have experience in successfully typing samples from a mass fatality incident? The managing laboratory director needs to keep in mind that the volume may be larger and the samples more challenging than the vendor laboratory has previously experienced.

Before any new technology is brought to bear on precious and irreplaceable samples like the victim remains in the World Trade Center disaster, validation testing must be performed to verify that it is capable of producing reliable results. Beyond the core issue of test reliability, we also assessed the results of new methods to determine their power to raise a profile to the level of an identification and for issues of compatibility—linkage—with other markers.

John Butler

Meeting turnaround requirements in the face of expectations from victims' families, the media, and policymakers likely will pose other challenges—and the laboratory director should not be afraid to ask for what is needed. For example, if a laboratory director is relatively inexperienced in contracting for testing services, he or she should enlist the support of laboratories that have extensive outsourcing experience. See appendix F for a discussion of issues that a laboratory director may want to consider when outsourcing sample testing to a vendor laboratory.

Government forensic laboratories may be able to provide assistance in a mass fatality incident identification response. Each agency that is helping in a mass fatality identification effort must understand its own and others' roles and responsibilities, the scope of tasks, and the duration of expected services. It may be helpful to prepare a detailed Memorandum of Understanding (MOU), including a project point of contact for each agency.

Whether a laboratory director obtains the assistance of a private vendor laboratory or another government agency, it is important to review the testing procedures to be used. If more than one testing laboratory is used, for example, testing systems and results systems must be compatible with each other.

It is also critical to address how the samples will be numbered and how the data will be returned to the managing laboratory. The software package that evaluates the data is vital to managing this data exchange, and an MOU or vendor contract should specify how these issues will be handled.

Consultants

Consultants can provide critical support to a mass fatality incident DNA identification response. For example, consultants may write or customize computer programs to tabulate and review data or to perform complex kinship analysis.

It may save time to ask prospective consultants to submit a proposal in response to an RFI (request for information), as this may allow the winning proposal to be incorporated into a contract. Proposals should define the consultant's roles, responsibilities, tasks, acceptance criteria for deliverables, timeframes, and hours and fees. Consultants should provide a list of references, and the laboratory director should ask references such questions as:

- What did the consultant do for you?
- Was the consultant responsible and of value? Why or why not?

- What are the consultant's strengths and limitations?
- Would you hire the consultant again? Why or why not?

Consultants typically charge by the hour, and they should be able to provide an estimate of fees. Any tasks beyond the scope of the contract would be reflected in invoices. The laboratory's contracting office should ensure that contracting rules and regulations are followed when hiring a consultant, and an experienced consultant should be able to provide the necessary proposals and paperwork to make this a straightforward task.

Vendor Management

Working simultaneously with vendors, government agencies, and consultants can be challenging under the best of circumstances, but it becomes even more demanding when the laboratory is handling a mass fatality incident response. It is important to maintain open lines of communication with vendors. Regular written updates and status meetings are good tools. A meeting agenda—that is adhered to—helps keep everyone on track and serves as a paper trail of the project's progress.

It is important to retain correspondence with vendors and to maintain documentation of decisions affecting vendors. For example, saving e-mail messages is an efficient way to document decisions.

It is very important for the laboratory director to consult with the laboratory's contracting officer if the scope of work changes during the project because modifications to the contract (e.g., scope of work and fees) may be required. Working closely with the contracting officer during all stages of contract development may help to minimize future problems. The managing laboratory director can best control how tasks are performed when a contract with a vendor or consultant specifies needs and expectations. Although most vendors and consultants want to serve their clients to the best of their abilities, it is important to remember that vendor processes and approaches may conflict with the laboratory's protocols. For example, a vendor laboratory may be most comfortable and experienced with a certain DNA testing procedure that is different from the method of analysis used by the managing laboratory.

In the middle of a massive forensic and humanitarian effort, it's easy to expect that suppliers and contractors will be on the same page as the managing laboratory. But that is a sure path to misunderstanding and disappointment on both sides. Having explicit contracts can help clarify expectations and set the basis for accountability that can curb cost overruns.

Steve Niezgoda

A computer consultant, for example, may want to add a software feature that will delay making identifications, even though the feature may improve efficiencies in the long run. To the extent possible, it is best to avoid becoming a beta-test site—having to validate a new software program or piece of equipment—in the middle of a mass fatality incident response. When working with outside vendors, laboratory directors would be well advised to remember that they are the "customers" and they are ultimately responsible for the project's success.

CHAPTER 14

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 chapter 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.

We developed the KADAP kinship data set to test new versions of the software. This quality control of "evolving software" allowed us to find "bugs" and correct them, if we didn't get the results that we expected.

Robert Shaler

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. (See chapter 9, *Information Technology*, "Sample Accessioning/LIMS Requirements" for more discussion on the commingling of remains.)

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.

Appendixes

APPENDIX A

Recommendations of the Kinship and Data Analysis Panel (KADAP) to the Office of the Chief Medical Examiner of New York City During the World Trade Center DNA Identification Effort

The Kinship and Data Analysis Panel (KADAP), assembled to assist the Office of the Chief Medical Examiner of the city of New York (OCME) during the World Trade Center (WTC) DNA identification effort, prepared the following recommendations to help the OCME laboratory create policies and procedures specific to the WTC mass fatality incident. These recommendations provided a roadmap when it was necessary to depart from the laboratory's usual forensic casework protocols. The KADAP's recommendations also offered guidance for securing additional resources and provided assurance that sufficient peer review and expertise were available to support these new endeavors.

These recommendations appear here in their original form, without editing. *The annotations in italics offer an after-the-fact context for particular recommendations to the OCME. The KADAP's recommendations are included as appendix A to this report because of their historical significance, and because they may be helpful to laboratories that are developing a mass fatality incident DNA identification response plan. The recommendations and opinions represent a consensus of the KADAP members (referred to in the recommendations as "the Panel") who were present on the date indicated; not all members were present at every meeting.*

1st KADAP (October 18–20, 2001)

The following recommendation sought to inform officials beyond the OCME, New York City Police Department (NYPD), and New York State Police (NYSP) that deviations from protocols would be ongoing, that the local scientists were respected experts in their fields, and that the KADAP was involved in reviewing new protocol developments.

- The Panel recognizes the unprecedented complexity of identifying the victims from the World Trade Center attacks. They also recognize the expertise of the OCME, the New York City Police Department (NYPD), and the NYSP. Given the evolving nature of this task, the Panel stresses that these are their initial recommendations, and they may be modified by OCME, NYPD, or NYSP, as they deem necessary. The panel remains available to them for consultation upon request.

The use of multiple software programs presented numerous difficulties that had to be overcome in the face of the informatics needs of the WTC DNA identification effort and the absence of existing software programs to address the issues. The following recommendations were developed after the KADAP considered the features of all available software programs.

- No single program currently exists that meets all of the analytical needs for resolution of the WTC victims. Therefore, we recommend for the short term:
  - WTC CODIS [Combined DNA Index System] be used:
    - At high stringency for direct matches. Likelihood ratio of 1 X 1010 is sufficient to report identity. A 13-locus match using the core CODIS loci is sufficient to report identity.
    - At low stringency to screen for potential first-degree relatives (parent/offspring and some sibs) in order to manually search case-specific data for cases with additional potential relatives.

- DNA•VIEW be used to assess the putative relationship. A minimum Probability of Relationship of 99.9% is sufficient to report identity by kinship analysis. The minimum prior probability is 1/5000, which can be increased to reflect case-specific issues (e.g., members of service).

- Commercially available pedigree programs should be incorporated for kinship review.
- Middleware should immediately be developed to facilitate use of existing programs.
- A customized program, developed in a modular manner following the proposed process flow, is needed. This package should be designed to analyze complex relationships in a way that integrates validated systems when possible. By October 26, 2001, the mechanism needed to commit resources to this program will be identified and established by NIJ [and reported back] to Inspector Mark Dale.

Because mitochondrial DNA mtDNA testing had received significant public attention in several forensic cases, stakeholder expectations for its use in the WTC response were high, and the OCME laboratory received many inquiries from officials regarding its use on the WTC samples. The KADAP was concerned that this early focus on mtDNA would dilute the effort to yield sufficient short tandem repeat (STR) loci in what were likely to be difficult samples. The Panel was concerned that this might hinder the identification process by adding less powerful methods of identification before all efforts to reveal unique identities had been exhausted.

- Mitochondrial DNA typing of victim samples should be used only as a last resort after additional test reanalysis and/or the use of additional forensically validated STR, Y-chromosome, or other nuclear markers have been used.
- If forensically validated systems, including mitochondrial data, are insufficient to resolve identity, research grade systems should be explored on a case-by-case basis.
- Mitochondrial DNA typing should be performed on all maternal lineage relative's appropriate samples (e.g., buccal swabs, blood) using a suitable validated system on the extracts as provided by NYSP, Myriad Genetics, or any other authorized agency.
- Mitochondrial DNA typing should not be performed on personal effect samples until other appropriate approaches have been considered.

These consensus recommendations represent a major step towards evaluating the complex data that will be generated from the World Trade Center terrorist attacks.

2nd KADAP (November 20, 2001)

With many competing agencies involved in the WTC effort, the KADAP offered recommendations about DNA-specific resource needs to reinforce their urgency with officials in charge of prioritization.

- This Panel determines that it is critical to the success of the WTC identification project that the OCME and NYSP share rapid access to the same data sets via immediate installation of a T1 line.
- The Panel recognizes that requests for prioritization of analyses of particular samples have significant implications for the overall process. Such requests will impede the overall progress of identification, increase the chances of analytical or interpretive errors, and increase costs. The Panel strongly urges those who make such requests to take all of these factors into account and minimize requests for prioritization.

The confirmation of identification by DNA was relied upon by the Chief Medical Examiner. The following recommendation aided in establishing baseline identity estimates.

- The Panel has recommended that likelihood ratios equal to or in excess of 1010 can be adopted as sufficient evidence of identity. However, this value should not be considered as a necessary criterion for identification in all cases, and that final recommendation of identification can properly be based on lower values depending on all available information, as determined by the Chief Medical Examiner.

3rd KADAP (February 21–22, 2002)

The following recommendations considered and addressed sample processing issues. The complexity of the process is shown in the graph

that appears on the last page of this appendix, "WTC Disaster Manhattan (DM) Identification Process."

Production:

- The Panel believes that collaboration and information sharing between the different groups and agencies involved in the DNA identification of the WTC victims is a critical component to maximum identification throughput.
- Numerous production choke points exist as obstacles in meeting the goal of maximum identification throughput. Information management and software integration are major issues that need to be supported to avoid obstacles. The existing software programs should continue to be supported and effective software integration should be developed with appropriate priorities. This requires additional resources, including but not limited to hardware, software, expert systems, and personnel.
- In order to eliminate the most immediate choke points, the Panel recommends that:
  - OCME and NYSP each hire/contract two (2) additional information technology FTEs so that present staff experienced in the current process can be solely dedicated to the WTC effort.
  - OCME and NYSP each hire/contract five (5) additional forensic analyst FTEs to be solely dedicated to the WTC effort.

Validation and Quality Control:

- Documented validation protocols should be developed and implemented for software programs and interfaces.
- Dedicated personnel and equipment should be made available for validation.
- Objective unbiased peer review is a useful process to implement valid systems.
- Appropriate test genetic data should be integrated into the WTC CODIS for efficient validation of all software.
- The current procedures to confirm matches (see attached flow chart) used by OCME and NYSP are appropriate.
- The probability of miscalling alleles that would lead to false inclusions is so small that it is not necessary to review electropherograms previously reviewed by vendor laboratories for uncomplicated STR cases that meet previous recommendations for likelihood ratios.

Continued Testing:

- Successful DNA typing of all samples will not be possible due to conditions of the remains. The Panel recommends that testing of individual samples should be finite. Criteria for determining cessation of testing should be established. Development of a probative test should be investigated.

4th KADAP (April 24–25, 2002)

As the scope of the WTC effort evolved, and the complexities of data management and the number of partnerships increased, the KADAP recommended and implemented a mechanism to facilitate secure, rapid transfer of data and provided additional development of statistical approaches to kinship analyses.

Recommendations:

- In order to facilitate data flow, the Panel recommends that a mechanism of data synchronization should be created. NCBI [National Center for Biotechnology Information] should host the secure FTP resource. The Forensic Biology Unit of the OCME needs Internet access with adequate bandwidth and tools for secure access.
- Cases involving difficult kin interpretations, including such things as mutations, should be reviewed by members of the AABB Parentage Testing Community to recommend disposition to OCME.
- Kinship used to confirm a personal effect match should be accepted at a Probability of Relationship of 99.9% using a Prior Probability of 0.5.

In addition to making recommendations, the KADAP offered several statements to support the work of the OCME and the NYSP.

Statements:

- KADAP recognizes the desire of victims' relatives, public officials, and the concerned public for complete and accurate use of validated forensic methods for identification of those lost in the WTC attack.

- KADAP recognizes that elected officials and the public must balance the above goals with desire for expeditious reporting of results. These are competing goals which must be considered carefully.
- KADAP recognizes that ongoing scientific and administrative review of all data will be needed to assure the accuracy of victim identifications. KADAP has concerns that imposed time deadlines are not in the best interest of making accurate or complete identifications.
- KADAP fully supports and endorses the efforts to date of the NYC OCME and NYSP in the processing of DNA from victims, personal effects and family members. To date, over 900 identifications have been accomplished using a combination of traditional methods and modern DNA technology.
- KADAP also recognizes that many victims may not be identified despite great effort by all concerned. Similarly, incomplete DNA results on highly degraded samples are likely to preclude positive identification of many of the 19,000 remains from victims recovered to date.
- KADAP is fully committed to ongoing efforts to assist New York agencies in identification of victims and remains. KADAP recognizes that successful DNA typing of all samples will not be possible due to the condition of the remains.
- KADAP recommends that DNA testing of individual samples cannot continue indefinitely (i.e., beyond the limits of sample integrity and available technology).
- Statistical criteria should be reviewed and revised as appropriate for use in assignment of identity of remains yielding incomplete DNA profiles.

The following recommendation was made because results were obtained from fewer loci from later samples recovered from Ground Zero. At the same time, the estimate of the number of victims became more firm, allowing statistical approaches similar to that of a "closed "system to be considered.

Identification Rules:

- Compromised DM samples can be considered associated with samples that were previously matched through DNA if the LR of shared loci [is] >108. This is equivalent to one divided by the random match probability of the shared loci between the two profiles.

5th KADAP (July 15–16, 2002)

As data from fewer loci were recovered from more compromised samples, experimental methods were evaluated for application in the WTC effort. The following recommendations considered parameters for using single nucleotide polymorphism (SNP) methodology in this environment.

Commentary and Recommendations on Use of Linked SNPs for Forensic Kinship Analysis of WTC Samples:

1) Use of the CODIS STR loci is a well-established method for estimation of random match probability and for kinship studies.
2) Unlike the 13 CODIS STR loci, which are unlinked, the 70 SNP loci studied in the KADAP pilot project consist of multiple haplogroups. Many of these SNPs are closely linked with each other and with the CODIS STR loci.
3) While linkage of genetic markers, per se, may have no untoward effect on their use in match probability estimates, linkage between SNPs will alter the calculations used in certain kinship estimates.
4) Use of inherited SNPs is very promising as an adjunct or substitute for STR profiling. A KADAP subcommittee on SNPs met on 12 July 2002 in Washington, D.C. This subcommittee recognized the potential of the technique pending additional studies.
5) KADAP recommends that the OCME of NYC proceed with the pilot use of the ORCHID/Genescreen (Dallas, TX) SNP panels on WTC samples in appropriate situations.
6) Sample consumption issues must be appropriately addressed before SNP analysis proceeds.
7) KADAP also recommends the KADAP SNP subcommittee pursue further statistical analysis of existing SNP data.

6th KADAP (September 9–10, 2002)

As time passed, a more precise list of victims was established. The KADAP reassessed the character of the WTC site a year after the attack and the statistical approaches that could be used.

KADAP Recommendations Regarding Identification of WTC Victims Based on DNA Profiling:

1) For purposes of statistical analysis of genetic data, KADAP recommends that the OCME consider the WTC as a closed population at this time.
2) The size of the closed population is considered to be the number of persons reported missing (currently 2,802).
3) Therefore, KADAP recommends that prior probabilities used in match estimates be based on either the number of:
   (a) RM [reported missing] and the gender ratio, OR
   (b) nongenetically identified RM individuals (of appropriate gender) plus the number of genetically identified individuals who cannot be excluded from the DNA profile in question.

Operationally, KADAP recommends that the OCME use 3(a) above until such time as 3(b) is necessary to refine statistical estimates.

Based on the assumption of a closed population of WTC victims and on the reduced estimate of the number of missing persons (from 5,000 to 2,802), KADAP recommends reducing the threshold for direct matching of remains from a likelihood of 1x1010 to 4x109.

Based on the gender ratio of the Reported Missing WTC victims (as of 9/10/02), the appropriate thresholds for direct matching of remains of known gender are 2x108 for females and 2x109 for males.

MtDNA Recommendations:

- KADAP recommends use of an mtDNA database that reflects, as closely as possible, the population mix of the WTC victims. The mtDNA from one maternal relative or positively identified personal item can serve as the reference sample for the RM. Certain relatives, including spouses, can be used to constitute the mtDNA database. Thus, when multiple relatives of a victim are available, mtDNA profiles from different maternal lineages can be included.
- KADAP recommends that the upper bound of the frequency estimate of an observed mtDNA sequence in a population should, at this time, be reported as:

  $X/N + 1.96 \div (p(1-p)/N)$,

  where $p = X/N$, and where

  X = # of "matching" mtDNA sequences in a database of size N.

  If $X = 0$, then the upper bound of the frequency estimate = $1 - \text{alpha}(1/N)$, where alpha = 0.05

Additional recommendations were made as the SNP technology was assessed.

SNP Recommendation (December, 2002)

Based on the UHT [ultre-high throughput] SNP validation data provided by Orchid Biosciences in Dallas, Texas, the KADAP recommends that this technology may be used by the OCME for WTC specimens as a potentially useful, but research grade, identification technology. The KADAP recommends going forward with limited testing of WTC specimens for investigational purposes, proceeding in a staged approach, with continuous evaluation of the utility and validity of this technology.

7th KADAP (January 21–22, 2003)

As the identification effort progressed, review of collection issues highlighted the need to adopt new methods of data collection for future mass fatality situations. The following recommendations were made after dialogues with those responsible for data collection from the Disaster Mortuary Operational Response Teams (DMORT).

KADAP Recommendations to DMORT

The KADAP recognizes the importance of the Victim Identification Program (VIP) as a vehicle for collecting the critical data relied upon for making precise identifications in mass fatality incidents. The VIP can be made more useful to DNA Laboratories by including additional genetic information. Therefore, KADAP respectfully offers the following recommendations:

1) Amend the VIP form to include more comprehensive fields to assist in DNA-based identifications. The KADAP would be pleased to assist the DMORT committee in revision of existing forms.

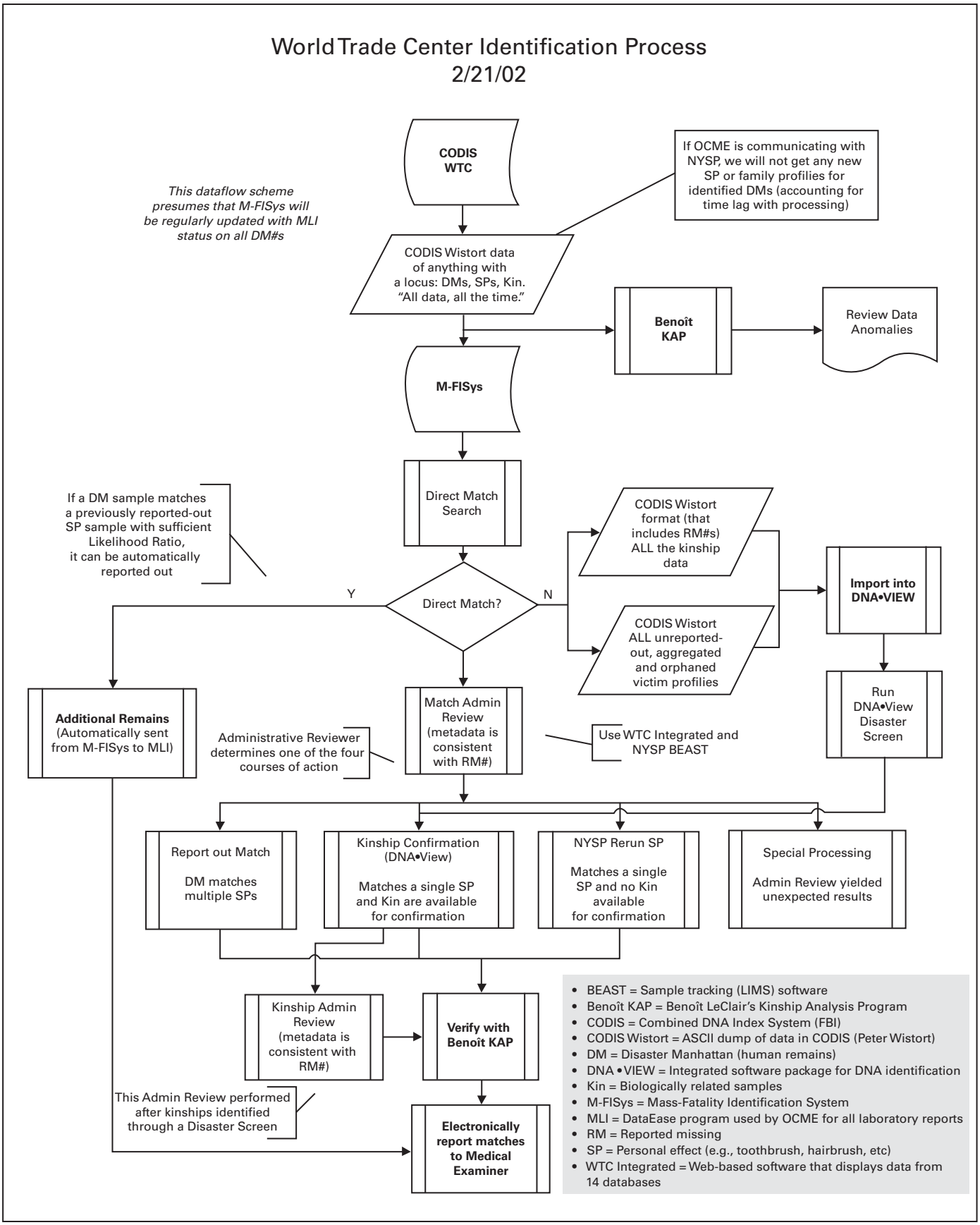
2) DMORT should consider adding one or more DNA identification specialists to the Family Assistance Center (FAC) teams to allow for timely onsite collection of kinship data and personal effects needed for DNA extraction/profiling.

8th KADAP (July 7–8, 2003)

Nearly 2 years after the attack, the KADAP assessed the capabilities of existing technologies for the remaining and most challenging samples. This recommendation was made to help families and other stakeholders understand the limitations of existing technologies for identifying these remains.

KADAP recognizes that DNA testing will not be successful for many samples and therefore some of the WTC victims will not be positively identified by STR, mtDNA or SNP testing.

KADAP further recognizes that OCME has exhausted appropriate contemporary methods of DNA extraction and genotyping on recovered WTC biological samples. While it cannot be ruled out that future scientific advances may reopen promise for additional testing, KADAP recommends that completion of ongoing work with current technologies be viewed as a stopping point in the identification process.


World Trade Center Identification Process
2/21/02
This dataflow scheme presumes that M-FISys will be regularly updated with MLI status on all DM#s
CODIS WTC
If OCME is communicating with NYSP, we will not get any new SP or family profiles for identified DMs (accounting for time lag with processing)
CODIS Wistort data of anything with a locus: DMs, SPs, Kin. "All data, all the time."
Benoît KAP
Review Data Anomalies
M-FISys
Direct Match Search
If a DM sample matches a previously reported-out SP sample with sufficient Likelihood Ratio, it can be automatically reported out
CODIS Wistort format (that includes RM#s) ALL the kinship data
Import into DNA•VIEW
Y
Direct Match?
N
CODIS Wistort ALL unreported-out, aggregated and orphaned victim profiles
Additional Remains (Automatically sent from M-FISys to MLI)
Administrative Reviewer determines one of the four courses of action
Match Admin Review (metadata is consistent with RM#)
Use WTC Integrated and NYSP BEAST
Run DNA•View Disaster Screen
Report out Match
DM matches multiple SPs
Kinship Confirmation (DNA•View)
Matches a single SP and Kin are available for confirmation
NYSP Rerun SP
Matches a single SP and no Kin available for confirmation
Special Processing
Admin Review yielded unexpected results
Kinship Admin Review (metadata is consistent with RM#)
Verify with Benoît KAP
This Admin Review performed after kinships identified through a Disaster Screen
Electronically report matches to Medical Examiner
• BEAST = Sample tracking (LIMS) software
• Benoît KAP = Benoît LeClair's Kinship Analysis Program
• CODIS = Combined DNA Index System (FBI)
• CODIS Wistort = ASCII dump of data in CODIS (Peter Wistort)
• DM = Disaster Manhattan (human remains)
• DNA •VIEW = Integrated software package for DNA identification
• Kin = Biologically related samples
• M-FISys = Mass-Fatality Identification System
• MLI = DataEase program used by OCME for all laboratory reports
• RM = Reported missing
• SP = Personal effect (e.g., toothbrush, hairbrush, etc)
• WTC Integrated = Web-based software that displays data from 14 databases

APPENDIX B

Sample Personal Items Submission Form

After a mass fatality incident, friends and family members will provide authorities with personal items that may contain a missing person's DNA. The DNA profile obtained from the personal item(s) will be searched against the profiles obtained from the remains samples. To efficiently and effectively use DNA analysis to identify human remains, it is important that personal items be correctly identified.

The purpose of this sample form is to help a laboratory:

- Determine who is missing.
- Provide information on the types of personal items that loved ones should submit.
- Identify the submitter and the items being submitted.
- Clarify what other DNA might be on the item; for example, if personal items of the missing individual are submitted, a reference sample from a spouse, domestic partner, or full-time roommate may be useful, even if no biological relationship exists.
- Begin chain-of-custody documentation for the items.
- Obtain permission from the submitter to test the items.
- Provide notification that the articles may be damaged or destroyed during testing.

Personal Items Submission Form

Missing Individual Information

Last Name	Suffix (Jr., Sr.)	First Name:	Middle Name	Sex (circle) M F

The missing person is/has been known by the following additional names (include maiden name)	Date of Birth Year: _ _ _ _ Month: _ _ Day : _ _	Social Security Number _ _ _ -_ _-_ _ _ _

Submitter Information

Last Name	Suffix (Jr., Sr.)	First Name	Middle Name

Telephone numbers (in order of preference)

1st : ( ) 2nd: ( ) 3rd: ( )

Home Street Address	City	State

Country	ZIP Code	E-mail address

I am providing a reference sample from the missing individual.
I am the missing individual's_____.
(e.g., mother, father, sister, son, roommate)

Please list the personal items below:

Item Number	Item Description	Other Possible DNA Sources on Item. Please Explain.
0	*Example: Pink toothbrush with white handle*	*My husband and I may have used the same toothbrush*
1		
2		
3		
4		
5		
6		

Name of Missing Individual:_____
(Last, First, Middle, Suffix)

Please note:

- *If there is a possibility that there may be someone else's DNA on a personal item, it is helpful to submit a biological sample from the person(s) who might have also used the item (reference sample). Please refer to the* ***Sample Family and/or Donor Reference Collection Form****.*
- *Items submitted should be directly attributable to the missing individual.*
  - *Biological samples suitable for testing include:*
    - *Bloodstain cards (e.g., newborn screening cards [Guthrie cards] or cards obtained from other repositories).*
    - *Oral swabs (e.g., from home DNA identification kits).*
    - *Blood stored for elective surgery.*
    - *Pathology samples (e.g., biopsy samples, PAP smears).*
    - *Extracted teeth (baby/wisdom).*
    - *Hair samples.*
  - *Personal items that might contain the missing individual's DNA include:*
    - *Used toothbrushes.*
    - *Used shavers/razors.*
    - *Unwashed undergarments and other suitable clothing items.*
    - *Used personal hygiene items (e.g., feminine sanitary napkins).*
    - *Other personally handled or used items (consult the testing laboratory for specific criteria).*

I, _____ hereby grant permission to
(Please print or type name of submitter)
extract and type DNA from the items listed on page 1 for the purpose of assisting in the identification of a missing person. I understand that in the testing process the item may become damaged or destroyed and may not be returned.

_____ _____
(Signature of submitter) *(Date)*

The items were received on _____at _____
(Date) *(Collection location)*

_____
(Collection address)

Sample(s) received by _____ _____.
(For testing agency use only)

APPENDIX C

Sample Family and/or Donor Reference Collection Form

After a mass fatality incident, a missing person's friends and family members provide identification information to officials who are handling the recovery and identification efforts. Complicated family structures—for example, multiple marriages, adoptions, same-sex partners—present challenges in collecting family relationship information. Obtaining an accurate family structure helps minimize gaps in information.

The information requested in this sample form is quite comprehensive, including a description of the jewelry worn by the missing individual, dental history, and a list of family members who may be able to provide DNA samples for the kinship identification process. This information typically is stored in the Victim Identification Program (VIP), a database supplied by the Federal Emergency Management Agency (FEMA). VIP is the central repository of all missing individual identification information, which can be accessed by pathologists, laboratory personnel, and medical examiners who are involved in the identification process.

Once family members have been identified and documented in the VIP, DNA samples need to be collected. Collection kits—used to collect the family and donor reference samples to determine biological relationships—should be available at family assistance centers and can be sent to family members all over the world.

The purpose of this sample form is to assist the laboratory in:

- Determining the identity of the missing individual.
- Identifying the donor of the reference sample.
- Clarifying the biological relationship between the missing individual and the donor; for example, if personal items from the missing individual are being submitted for analysis, a reference sample from a spouse, domestic partner, or full-time roommate is useful even if no biological relationship exists.
- Obtaining chain-of-custody information for the family reference sample.
- Obtaining permission to test the sample.
- Providing information on the best types of family reference samples to collect.

Family and/or Donor Reference Collection Form

(Each donor needs to fill in a separate form and submit a separate sample for each missing person.)

Missing Individual Information

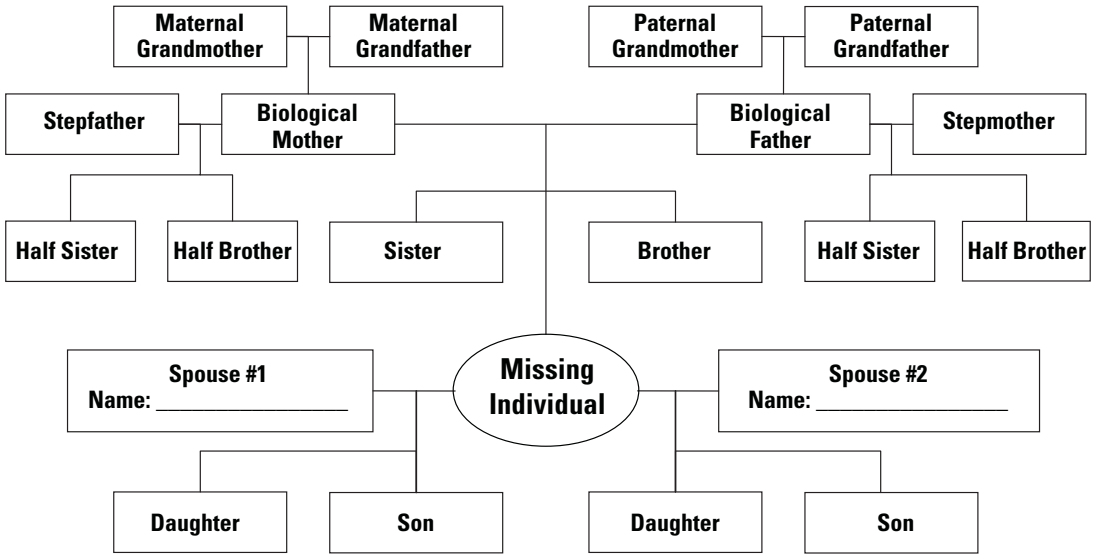
Last Name	Suffix (Jr., Sr.)	First Name	Middle Name	Sex (circle) M F
The missing person has been known by the following additional names (include maiden name)		Date of Birth Year __ __ __ __ Month __ __ Day __ __		Social Security Number or citizenship (if not a U.S. citizen) __ __ __ -__ __-__ __ __ __

Donor Information

Last Name	Suffix (Jr., Sr.)	First Name	Middle Name
Telephone numbers (in order of preference) 1st :( ) 2nd :( ) 3rd :( )			
Home Street Address			
City	State	ZIP	Country
Date of Birth Year __ __ __ __ Month __ __ Day __ __	Sex (circle) M F	E-mail address (please print)	

I am providing a family reference sample, as I am the missing individual's _____
(e.g., mother, father, sister, son)

Please circle your relationship to the missing individual:



Other: *(please specify)* _____ *(e.g., grandchild, friend, roommate)*

Name of Missing Individual:_____
(Last, First, Middle, Suffix)

Please note:

- *If personal items of the missing individual are being submitted for analysis, a biological reference sample from the spouse, domestic partner, or full-time roommate is useful even if no biological relationship exists. Please refer to the* ***Personal Items Submission Form*** *when submitting personal items.*

- *The biological parents and biological children are the best comparison samples for identification through kinship. If these samples are unavailable, samples from other biological relatives may be submitted.*

- *If a child provides a sample for parental identification, the child's other biological parent should also provide a sample.*

- *For identification through kinship analysis:*
  - *Full siblings are preferable over half siblings.*
  - *Grandparents should provide a sample only if the mother or father cannot provide a sample.*
  - *Grandchildren should provide a sample only if their parent, who is related to the missing individual (as a son or daughter), is unavailable.*
- *The laboratory will assess the samples provided. The most appropriate sample(s) will be used to identify the missing individual. The family may be contacted if additional samples are needed.*

I am also a relative of the following other missing individuals:_____

I, _____ hereby grant permission to extract and type
(Please print or type name of donor)

my DNA for the purpose of assisting in the identification of a missing person.

_____ _____
(Signature of donor or guardian if donor is a minor) *(Date)*

The sample was collected on _____ at _____
(Date) *(Collection location)*

_____
(Collection address)

Sample was collected by *(if self-collected indicate "self")*_____

APPENDIX D

Sample Family Tree Form

The complexity of modern family structures (e.g., multiple marriages, adoptions, same-sex partners) can challenge the collection of family relationship information. The purpose of this Sample Family Tree Form is to help a laboratory:

- Determine who is missing.
- Identify the individual providing the information.
- Provide family relationship information.

This type of form should be completed each time someone provides information about a missing individual and/or donates a sample. Because of the complexity of determining biological relationships, it generally is advisable to have a trained interviewer—such as a geneticist or genetic counselor—complete the form.

FAMILY RELATIONSHIP TREE

Victim's Name:_____
(Last, First, Middle, Suffix)

It is important for the DNA laboratory to correctly understand the victim's family structure when using DNA to establish biological relationships and identify victims. In order to obtain an accurate family structure, please complete the contact information for the interviewee (the person providing the information on the family relationships) as well as your contact information as the interviewer. This form may be completed each time a new individual provides information about a potential victim and/or donates a sample using the family reference collection form. Using the directions below, please describe the family of the victim, including the interviewee, the victim, and all other close relatives.

Interviewee Contact information:

Last Name:	Suffix:	First Name:	Middle Name:
Telephone numbers (in order of preference): 1st: ( ) 2nd: ( ) 3rd: ( )			
The interviewee is the missing individual's_____ *(e.g., mother, sister, son, roommate)*			

Interviewer Information:

Name:	Date:
Affiliation and address:	
Telephone numbers (in order of preference): 1st: ( ) 2nd: ( ) 3rd: ( )	

Directions:

- Use the box on the other side of the page to draw the family tree.
- A picture of the family should be drawn by placing the interviewee in the center, providing he or she is biologically related to the missing individual.
- Use circles for women and squares for men.
- Put each person's name in the circle or square.
- In the circle or square, indicate whether the individual is living, deceased, or missing.
- Draw a line between parents and place children below the line.
- Include wives and husbands.
- Provide a narrative if you think it will be helpful.
- If the interviewee is not biologically related to the victim, indicate his or her relationship to the victim and draw the victim's family structure as outlined above.
- Add comments below the box to clarify relationships as needed.

Victim's Name:_____
(Last, First, Middle, Suffix)

Family Tree:

Comments:_____

_____

_____

_____

_____

_____

_____

_____

APPENDIX E

Guidelines for Family and/or Donor Reference Collection Kit Components and Oral Swab Collection Instructions

To obtain a properly collected and labeled sample, it is preferable to use a tamper-evident, presealed oral swab collection kit. Some laboratories may prefer to have the swabs air-dry for 15 minutes to an hour prior to placing the oral swab in the swab envelope. Although the process of air-drying the swabs may lead to a more pristine sample, the process of air-drying is risky and may inadvertently lead to a sample mixup if more than one person's sample is collected at a time. The laboratory may also want to incorporate some type of notification system in which the collection location calls or faxes the DNA laboratory when the sample has been collected, alerting the DNA laboratory that the sample is on the way. A tamper-evident, presealed oral swab collection kit may contain:

Collection instructions (See sample Oral Swab Collection Instructions below)

Collection form for family reference sample (See Sample Family and/or Donor Reference Collection Form, appendix C)

Form describing the family relationship (See Sample Family Tree form, appendix D)

Pair of gloves (preferably one-size-fits-all Nitrile gloves)

Sterile, cotton-tipped swabs (2–6)

If the collection is performed correctly on a healthy individual, two swabs are sufficient to get adequate amounts of DNA for a short tandem repeat (STR) analysis. If extended testing may be required, it is preferable to collect six swabs.

Fastener (optional)

A small rubber band or twist-tie may be included to bind together all of the swabs from one individual prior to placing them in the swab envelope. Alternatively, a label may be included to secure and label the swabs.

Swab envelope

Once the swabs have been collected, they should be placed in an envelope that can be uniquely identified with the donor's information.

Tamper-evident, sealable bag, containing desiccant packet

If mass collections are to be performed, inadvertent sample switches may occur if the swabs are allowed to air-dry in the open; therefore, a desiccant can be used to help keep the moist swab from molding. If a Ziploc bag is used, tamper-evident police-evidence seals can be placed on the bag.

Mailing envelope

A preprinted mailing envelope with an appropriate prepaid shipping label will help ensure that the swabs are delivered to the correct location. Make sure the shipping carrier services the area where the sample will be collected. Different air bills and customs documents may be needed if samples will be shipped from outside the United States.

Oral Swab Collection Instructions

To avoid sample mixups, identification, collection, and sample sealing should be performed for *one* individual at a time. Also, it would be advisable to:

- Have a trained individual interview the family member and complete a family tree.
- Wear gloves while collecting the sample, and change gloves before collecting from the next individual.
- Collect samples from one individual at a time.
- Verify the identity of the individual whose sample is being collected and confirm that the mouth is free of tobacco products, gum, food, etc., before collecting the oral swab. If necessary, have the individual rinse his or her mouth with water prior to collection.

- Have the donor fill out a Family and/or Donor Reference Collection form.
- Open the swab packages provided, being careful to not handle the cotton tip of the swabs.
- Remove one swab and collect the specimen by rubbing the swab vigorously and thoroughly on the inside surfaces of the cheeks and gums. Rub the swab up and down and back and forth about 10 times, while slowly turning the swab, so that all sides of the swab are in contact with the side of the cheek.
- Place the swab in the envelope provided. Do not place the swab back into the original packaging. Repeat the process with the remaining swabs.
- Identify the swab envelope with the date, the donor's name, and the collector's name. Have the donor sign the envelope to verify the information.
- Complete the collection information on a Family and/or Donor Reference Collection form, and verify that the donor completed the requested information.
- Seal the swab envelope. Place the swab envelope and completed Family and/or Donor Reference Collection form in the plastic bag with the desiccant, and place in shipping envelope. Maintain the sample in a cool, dry environment until shipment. Do not store under extreme hot or cold conditions.

APPENDIX F

Issues to Consider When Outsourcing Reference Samples

There are many issues a laboratory director must consider when making the decision to send mass fatality samples to an outside vendor for short tandem repeat (STR) analysis testing. This list of issues is not meant to be inclusive; rather, it is offered as a starting point to aid in considering the use of a vendor laboratory to test personal items, reference samples, or remains samples.

Tasks and Requirements

- What standards of quality assurance are to be met.
- What certification will be provided that testing is performed in accordance with quality assurance standards.
- Specific tasks (for example: "The Vendor shall analyze all samples for the 13 CODIS core STR loci plus Amelogenin—FGA, vWA, D3S1358, CSF1PO, TPOX, THO1, D18S51, D21S11, D8S1179, D7S820, D13S317, D5S818, and D16S539—in accordance with the Federal Bureau of Investigation's NDIS [National Data Index System] Standards for Acceptance of DNA Data and the Contracting Agency/Vendor Testing and Reporting Guide.")
- Accreditations/certifications that the vendor laboratory should maintain, and penalties if accreditation/certification is not maintained.
- Timeframe for analysis and reporting turnaround (for example, "x" kinship samples per week, etc.).
- External proficiency testing program(s) that the vendor must complete during a specific timeframe, along with terms for submitting a certified statement of compliance and documentation of any failed proficiency tests and the remediation that was done to resolve the issue(s).
- Terms regarding the individual DNA analyst's compliance with a semiannual external proficiency testing program.
- Requirements that changes in the vendor's key personnel (specific personnel) be approved.
- Protocols and procedures for making analysis of the samples, quality control documents, and validation documentation available for review, inspection, and monitoring, including onsite reviews of the vendor's facility and records.
- Standard operating procedures and quality assurance procedures (including any changes made during the process) with respect to the receipt and analysis of samples.
- Terms regarding the vendor's ability to subcontract (or prohibition against subcontracting) any portion of the testing or analysis of the samples to any other laboratory without prior written authorization.
- Format for processing samples (for example, "Whole blood in tubes that the vendor shall be required to stain onto cotton fabric, 903 S&S paper, FTA paper," etc.; buccal swabs on a swab or placed on 903 S&S paper or FTA paper; extracted DNA; personal items (toothbrushes, hair brushes, clothing); victim bone and tissue, etc.)
- Preprinted shipping labels and shipping containers, and requirements regarding notification of when a shipping container is received, including notification upon discovery of any damage to the shipping container that would compromise the integrity of a sample.
- Chain-of-custody documentation, including, for example, a unique identifier on the overnight shipping label, sample receipt (and verification of seal integrity), sample transfers during processing, analysis and reporting, and return of the samples and resulting data.
- Storage of samples.
- Use of automated transfers (for example, use of a "plate fingerprinting" system to uniquely identify a 96-well plate, including the strategic placement of known controls on a 96-well

plate in a manner that allows any plate mixup to be detected).

- Use of NDIS-approved STR analysis kits specified in the NDIS Standards for Acceptance of DNA Data; if applicable, use of NDIS-approved STR analysis platforms and expert systems.
- Analytical procedures (for example, using appropriate controls and standards on each gel/run/batch; each sample used in reporting having an acceptable extraction positive, extraction negative, amplification positive, amplification negative, and ladder associated with each locus, and, if a sample is rerun, all controls to be rerun).
- The manner in which data are to be reported (for example, genotypes to be compiled in the common message format for insertion into the FBI's Combined DNA Index System (CODIS) and transmitted in electronic form (floppy disk, CD–ROM, a ZIP disk, secure Web site, or other method); cost of CD–ROM or ZIP disks and shipping to be included in the proposed cost per sample of completed analysis).
- Return of extraction, amplification, gel data sheets (including spreadsheets, original gel scans, and the final gray-scale/color-corrected gel images), and electropherogram data; return of instrument data collection files and files generated in the analysis of the samples in a prescribed form (CD–ROM, ZIP disk, posted to a secure Web site, etc.); return of samples, DNA extracts, amplified product, etc.
- Determination of when the analysis of a specimen is considered complete (for example, not until genotypes for all 13 CODIS core STR loci (plus Amelogenin) have been generated and accepted; requirements for when a sample does not yield a complete profile (for example, retest the sample a minimum of two times, altering conditions within the boundaries of the laboratory's written standard operating procedures, as necessary, to produce a complete profile, etc.).
- Terms for analysis failure (requests for additional samples, etc.).
- Sample shipping responsibilities (method, chain-of-custody safeguards, timeliness, tracking, etc.).
- Confidentiality of samples and the results of testing, including handling outside inquiries.
- Ownership of data, materials, and documentation.
- Procedures for notification regarding problems in testing.
- Contamination quality assurance checks.
- Retention of testing and quality control records.
- Written weekly reports, including changes to management and key personnel; assessment of technical risks and analytical and quality control processes; description of analytical errors detected during processing and corrective action taken; customer service logs; and performance metrics by sample type (reference, disaster, personal items), including, for example:
  - Number of samples received.
  - Running total for samples received.
  - Number of samples reported.
  - Number of failed samples (for example, those in which no profile or an incomplete profile—not all 13 CODIS core loci + Amelogenin—was generated.
  - Number of samples received more than 30 days ago, but not yet tested, analyzed, and reported.
  - Biweekly briefings.

Deliverables and Delivery Schedule

- Testing, analysis, and reporting services, including shipping; DNA profile; quality control results and records; testing and chain-of-custody documentation; data generated during the receipt, testing, analysis, and reporting; and unused samples.

Suspension and Termination

- Terms for suspension or termination for poor performance, including quality issues, customer service complaints, and inability to meet sample throughput commitments.

Equipment and Materials

- Who will furnish equipment and materials.

Security, Place of Performance, and Period of Performance

Here is a sample vendor testing and reporting guide that may contain components that laboratory directors may consider when contracting with an outside vendor.

(One form for each sample type: family reference, disaster, personal item)

Sample Type_____

1. Samples will be provided to the vendor in the following manner:

2. Samples will come from the following agencies/locations:

3. Samples will be provided to the vendor at the rate of:

4. Samples will be provided with the following identification, which shall be reported with the profile:

5. Samples will be rejected by the vendor for testing for the following reasons, with the following course of action:

6. No more than ___ percent of a sample shall be consumed by the vendor without permission.

7. DNA shall be extracted to a final volume of _____ at a concentration of _____.

8. The following DNA aliquots shall be made for additional testing:

9. The vendor shall use only the following testing and analysis systems:

   Extraction method:

   Amplification conditions (including kit and amplification volume):

   Analysis platform:

   Conditions for retesting if a complete profile is not initially obtained:

10. Procedural changes affecting sample processing must be approved ___ days prior to the processing of samples.

11. Manual transfer shall be allowed only during the following steps:

12. Spiking or enriching a sample is acceptable ___yes ___no.

    Comments:

13. Vendor controls:

    a. Amplification positive

       Name:
       When introduced:
       Considered acceptable when:
       Location on analysis:
       Location in data files:
       Acceptable results:

    b. Amplification negative

       Name:
       When introduced:
       Considered acceptable when:
       Location on analysis:
       Location in data files:
       Acceptable results:

    c. Extraction positive

       Name:
       When introduced:
       Considered acceptable when:
       Location on analysis:
       Location in data files:
       Acceptable results:

    d. Extraction negative

       Name:
       When introduced:
       Considered acceptable when:
       Location on analysis:
       Location in data files:
       Acceptable results:

    Other:

       Name:
       When introduced:
       Considered acceptable when:
       Location on analysis:
       Location in data files:
       Acceptable results:

A data file is defined as
_____.

14. Samples with the following microvariants do not need to be retested:

15. Samples with trialleles shall be processed in the following manner:

16. Samples with triallelic profiles ___shall ___do not need to be retested. The following documentation shall be reported:

17. Samples with microvariants (not on an approved list) ___shall ___do not need to be retested. The following documentation shall be reported:

18. Profiles exhibiting multiple contributors shall be handled in the following manner:

19. Data analysis:

    a. General peak characteristics

       The following reporting criteria apply to:

       _____ Samples

       _____ Ladders

       _____ Controls

       _____ Internal size standard

       Minimum peak height:
       Maximum peak height:
       Shape:

       Spikes ___not allowed ___allowed under the following circumstances:

    b. Internal size standard

       The following peaks are required to be present for reported samples:

       Size of 245 peak (on 310) must be _____.

    c. Allelic Peaks

       Stutter:

       –A:

       Minimum allowable peak height ratio:

20. Data reporting

    a. Composite profiles (instances where the 13 CODIS core loci are created from more than the minimum multiplex data file[s] because one or more of the loci do not meet reporting criteria) ___shall ___ shall not be acceptable unless:

    b. Nonreported samples ___ may ___shall not be intermixed in reported data files.

    c. Data from all sample runs ___must ___need not be provided.

    d. Minimum and maximum number of reportable samples with complete profiles in a single data file is:

    e. Minimum and maximum number of samples (complete 13 locus profile) in a reported batch:

    f. The following documentation shall be provided/associated with the reported profiles:

    g. Data and data files shall be reported in the following format:

    h. Data shall be reported at a frequency of:

21. Samples shall be returned on the following date and in the following condition:

22. Other:

APPENDIX G

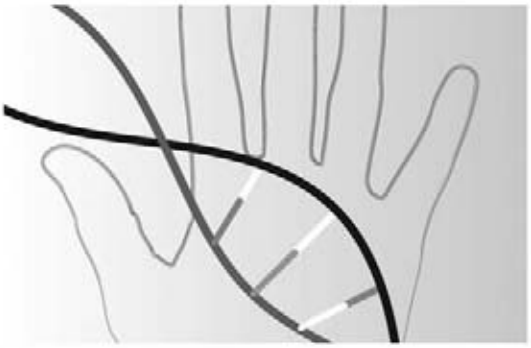
Identifying Victims Using DNA: A Guide for Families

This is a PDF file of a publication (English/Spanish) that can be downloaded at http://www.ojp.usdoj.gov/nij/pubs-sum/209493.htm; to order hard copies, call 1–800–851–3420 or order online at www.ncjrs.gov.

April 2005

Identifying Victims Using DNA: A Guide for Families

In English and Spanish



PRESIDENT'S DNA INITIATIVE

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The National Institute of Justice is the research, development, and evaluation agency of the U.S. Department of Justice. NIJ's mission is to advance scientific research, development, and evaluation to enhance the administration of justice and public safety. This and other publications and products of the U.S. Department of Justice, Office of Justice Programs, and NIJ can be found at *http://www.ojp.usdoj.gov/nij*.

The National Human Genome Research Institute (NHGRI) is one of 27 institutes and centers at the National Institutes of Health, an agency of the Department of Health and Human Services. NHGRI supports grants for research, training, and career development at sites nationwide and conducts research on its campus to develop and implement technology to understand, diagnose, and treat genomic and genetic diseases. Information about NHGRI can be found at *http://www.genome.gov*.

April 2005

Identifying Victims Using DNA: A Guide for Families

NCJ 212872

What is this brochure about?

Any circumstance in which lives are lost is a tragedy that can have immediate and lasting effects on our communities. We extend our most sincere condolences and sympathy to you at this difficult time. You have been given this brochure to help you understand the process of identifying the remains of a victim through DNA analysis.

Why go through the process of identifying remains?

The decision to pursue identification of the remains of a victim through DNA testing is very personal and may be different for each family. Some families may find comfort in knowing that the remains of their loved one have been identified and returned. These remains can be interred according to the family's traditions. This may help with the healing and adjustment to their terrible loss. For others, the testing process may interfere with their healing.

For DNA testing to work, it may be necessary to gather more information, samples, or personal items. Gathering these may cause your family further distress. If the testing does not identify your loved one's remains, it may be a disappointment, adding to your grief.

DNA testing can be provided to help those families who want it. If you choose not to, your decision will be honored. You may take time to talk about it with others who you feel are appropriate. People who can help include family, friends, religious leaders, health professionals, and victim advocates.

How is this testing done?

In many cases, DNA testing is one of the best methods to identify a victim or victims. DNA is the material in cells that stores the inherited traits that make up our bodies. In many (but not all) cases, DNA can be isolated from human remains or other samples. To identify the remains of a victim, DNA from remains must be matched to DNA known to be from the victim or the victim's relatives. Thus, it is necessary to collect DNA samples from family members and from personal items or prior medical specimens from the victim.

How long will the process take?

The process of identifying a victim might be relatively quick or it can be quite lengthy. In some instances, not every victim can be identified. When an identification is made, the next of kin will be notified and asked if they wish to be contacted if more remains are found in the future.

How can I help identify my loved one?

Accurate and complete information about the victim (unique physical characteristics, dental records, etc.) should be submitted. Sometimes this information will be sufficient to render an identification. In many cases, such information may have been provided prior to considering DNA testing. To have any success with DNA testing, samples from relatives of the victim will need to be collected to compare with the remains.

What are the sources of DNA samples that can be used?

DNA can often be obtained from the biological remains. This DNA will be compared to DNA known to be from the victim or to DNA from the victim's relatives.

What are the sources of DNA from the victim?

DNA from the victim's previously collected medical specimens or personal items can be used to make a direct match to remains. For example, if a loved one recently had surgery or blood work done, a specimen may have been stored at the hospital or clinic. You should provide any known medical specimens or ask for help in locating them. The first row of the table below provides examples of the kinds of medical specimens the laboratory can use.

DNA Sources	Examples	Degree of Usefulness
Medical specimens	Bone marrow donor sample Biopsy sample Newborn screen bloodspot	Most useful
Personal items	Toothbrush Hairbrush	Very useful
Close relatives	Biological parents of victim Children of victim Brother or sister of victim	Useful
Other relatives	Maternal relatives (aunts, uncles, cousins, half-sisters or -brothers on the victim's mother's side)	Less useful

DNA from the victim may also be found on their personal items. The second row of the table above gives some examples of these. A toothbrush or other items containing saliva are often good sources. However, it is very important that these items were used only by the victim or rarely used by anyone else. For example, a hairbrush used by the whole family would not be a good source of DNA from the victim.

How can DNA from relatives be used?

If personal items or medical specimens are not available or if the testing on them does not work, DNA testing can be done on samples from blood relatives. The DNA from adoptive parents, adopted children, stepparents, or other nonblood relatives cannot provide information on the genetic identity of a victim.

The ability to match victims to their relatives depends on how closely related they are to the victim. The most useful DNA samples are from close blood relatives such as the victim's biological mother, father, children, brothers, or sisters. This is because DNA of close relatives is more similar than the DNA of more distant relatives. The pictures on the following pages show the relatives who are most useful for identifying a victim. If DNA from the victim's children is used, it is helpful to have DNA from the children's other biological parent.

DNA from more distant relatives can be used, but this is more difficult. In some cases, samples may be requested from specific relatives. For example, DNA samples could be requested from a maternal relative of the victim such as the victim's aunt, uncle, or half-brothers or half-sisters on the mother's side of the family.

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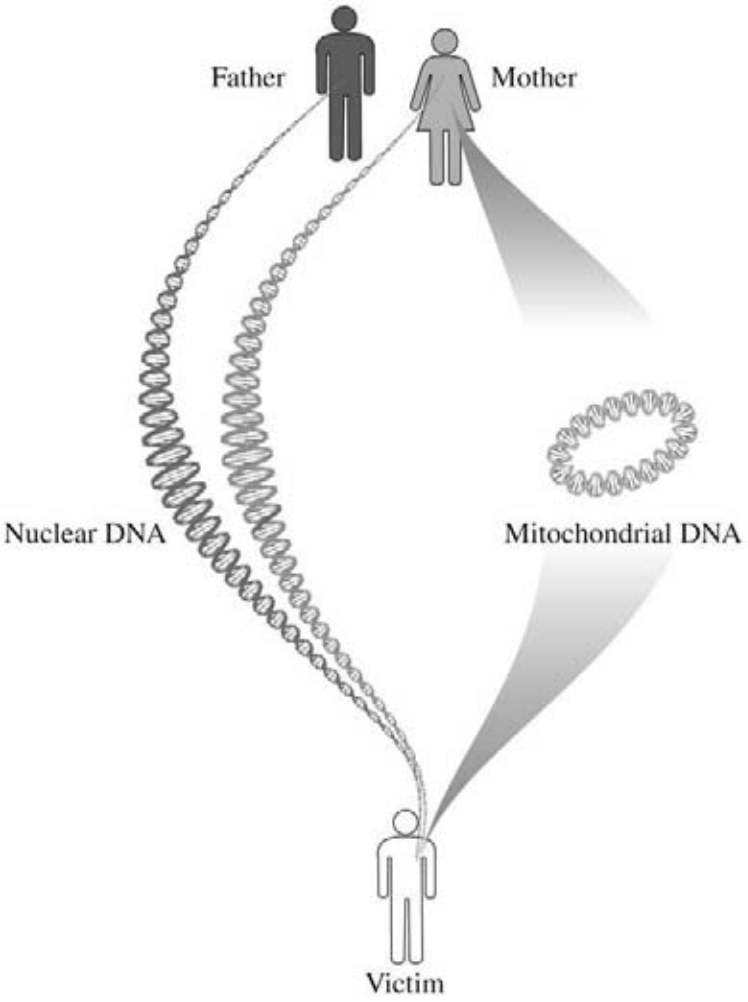
Why might DNA analysis not work?

DNA testing might not be able to identify your loved one. The most likely reason would be that there is no usable DNA in the recovered remains. Some victims' remains may not be found. Also, DNA testing may not work if no usable DNA can be found on personal items submitted.

How does DNA testing work?

DNA is the hereditary material that contains instructions to build a human being. DNA can be collected from very small amounts of blood, mouth (cheek) scrapings, hair roots, or other samples. There are two kinds of DNA in the body: nuclear DNA and mitochondrial DNA. Both kinds of DNA can be used for DNA identification.



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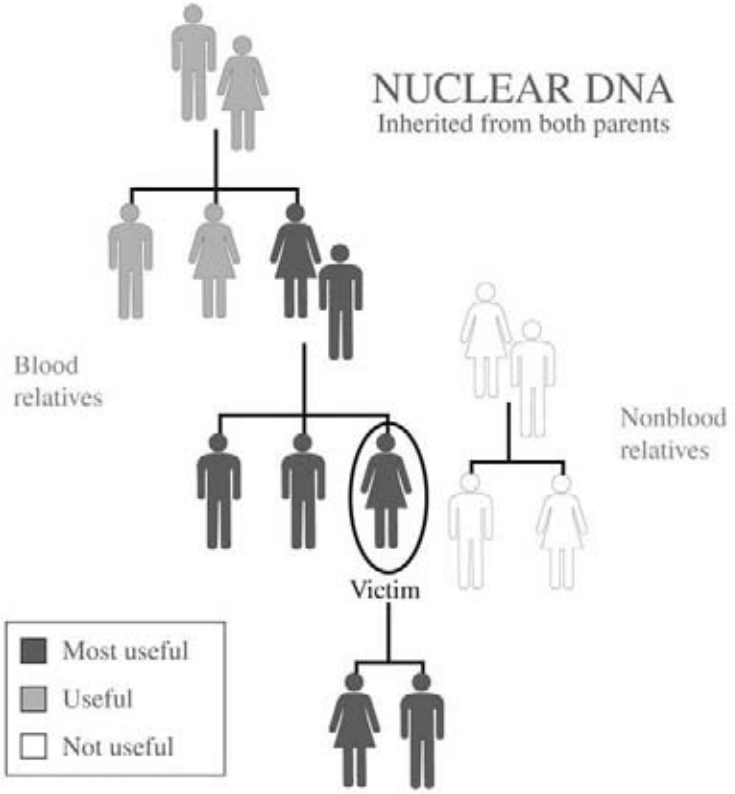
Nuclear DNA comes from the cell nucleus and is inherited from both parents, half from the mother and half from the father (see figure below). Each person's nuclear DNA is unique—except for identical twins, who have the same DNA. When a sufficient nuclear DNA profile from the victim's remains matches the nuclear DNA profile from a sample known to have come from the victim, we can be very sure of the identity of the victim.



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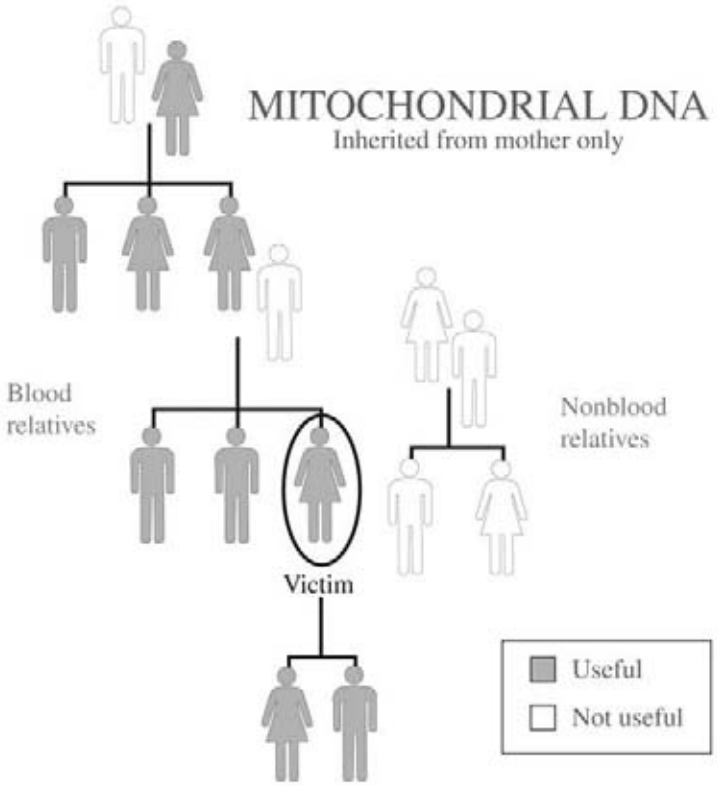
Because of the way it is inherited, DNA from blood relatives is somewhat similar. Nuclear DNA from the victim's remains can be compared to nuclear DNA from family members to identify the victim in some circumstances.

The second kind of DNA is called mitochondrial DNA (mtDNA). It is inherited only from the mother (see figure on page 7). Fathers never pass on mitochondrial DNA to

their children. However, mitochondrial DNA typically is not as powerful for making identifications as nuclear DNA. This means that in some instances two unrelated people may have similar mitochondrial DNA. Because of the way it is inherited, only maternal relatives, such as a brother, sister, or mother, can be used for mitochondrial DNA testing.



Nuclear DNA can be easily damaged by extreme heat and other conditions and therefore is not always available to be used for an identification. Mitochondrial DNA, however, can often be found in very small or damaged DNA samples. Typically, scientists test nuclear DNA first. If there are insufficient results for an identification, they will attempt mitochondrial testing. Despite best efforts, some testing may not be successful. But the scientists seeking to identify your loved one will work hard to do so and provide closure for your family.

NIJ is a component of the Office of Justice Programs, which also includes the Bureau of Justice Assistance, the Bureau of Justice Statistics, the Office of Juvenile Justice and Delinquency Prevention, and the Office for Victims of Crime.

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Findings and conclusions of the research reported here are those of the authors and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

President's DNA Initiative Partners

Office of Justice Programs

National Institute of Justice

Office on Violence Against Women

Bureau of Justice Assistance

Office of Community Oriented Policing Services

Federal Bureau of Investigation

Office for Victims of Crime

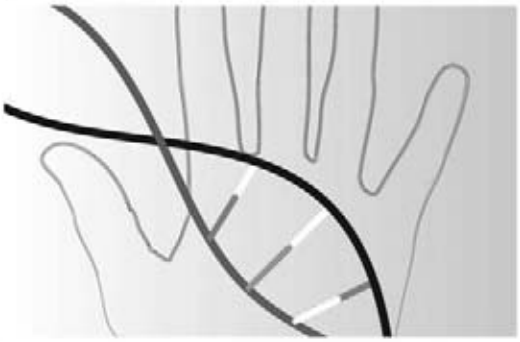
Office of Juvenile Justice and Delinquency Prevention

9

Abril de 2005

Guía para las familias sobre la identificación de víctimas mediante análisis de ADN

En inglés y español



LA INICIATIVA DEL ADN DEL PRESIDENTE

Departamento de Justicia de Estados Unidos

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El Instituto Nacional de Justicia es la agencia de investigación, desarrollo y evaluación del Departamento de Justicia de Estados Unidos. La misión del NIJ es promover la investigación, el desarrollo y la evaluación científica con el fin de fomentar la administración de justicia y la seguridad pública. Puede encontrar ésta y otras publicaciones y productos del Departamento de Justicia de Estados Unidos en *http://www.ojp.usdoj.gov/nij.*

El Instituto Nacional de Investigación del Genoma Humano (NHGRI) es uno entre 27 institutos y centros de los Institutos Nacionales de la Salud, una agencia del Departamento de Salud y Servicios Humanos. El NHGRI ofrece apoyo mediante subvenciones para las labores de investigación, capacitación y desarrollo de carreras profesionales en locales por toda la nación y realiza investigación en sus propias instalaciones para desarrollar e implantar la tecnología orientada a comprender, diagnosticar y tratar enfermedades genómicas y genéticas. Puede encontrar información sobre el NHGRI en *http://www.genome.gov.*

Abril de 2005

Guía para las familias sobre la identificación de víctimas mediante análisis de ADN

NCJ 212872

¿De qué se trata este folleto?

Toda circunstancia en la que la se pierden vidas es una tragedia que puede causar efectos inmediatos y duraderos en nuestras comunidades. Deseamos expresarle nuestro más sentido pésame durante estos momentos difíciles. Se le ha entregado este folleto para ayudarlo a comprender el proceso para identificar los restos de una víctima mediante el análisis de ADN.

¿Por qué realizar el proceso de identificar los restos de una persona?

La decisión de solicitar la identificación de los restos de una víctima mediante el análisis de ADN es muy personal y puede ser distinta para cada familia. Algunas familias pueden sentir consuelo al saber que los restos de su ser querido han sido identificados y devueltos. Estos restos pueden sepultarse según las tradiciones de la familia. Esta experiencia puede ayudar a las personas a recuperarse y adaptarse a la terrible pérdida. Para otros, el proceso de análisis podría interponerse en la recuperación.

Para que funcione el análisis de ADN, es posible que sea necesario recopilar más información, muestras u objetos personales. Reunir estos objetos podría apenar aún más a la familia. Si la prueba no identifica los restos de su ser querido, podría sentirse desalentado y afligirse aún más.

El análisis de ADN puede ofrecerse para ayudar a las familias que desean que se realice. Si usted elige no solicitarlo, se honrará su decisión. Tal vez desee conversar sobre este tema con algunas personas que considere adecuadas. Las personas que pueden ayudar son, entre otras, la familia, las amistades, los líderes religiosos, los profesionales de la salud y los defensores de víctimas.

¿Cómo se realiza esta prueba?

En muchos casos, el análisis de ADN es uno de los mejores métodos para identificar a la víctima o las víctimas. El ADN es el material en las células que contiene los rasgos hereditarios que componen nuestro cuerpo. En muchos casos (pero no en todos), el ADN puede aislarse de los restos de un ser humano o de otras muestras. Para identificar los restos de una víctima, el ADN de los restos debe ser compatible con el ADN que se sabe con certeza que proviene de la víctima o de los parientes de dicha víctima. Por lo tanto, es necesario obtener muestras de ADN de los miembros de la familia y de objetos personales, o bien, muestras médicas previas de la víctima.

¿Cuánto tiempo toma el proceso?

El proceso de identificación de una víctima puede ser relativamente rápido o sumamente prolongado. En algunos casos, no es posible identificar a las víctimas. Cuando se logra la identificación, notificarán al pariente más cercano y le pedirán si desea que se comuniquen con él o ella si se hallan más restos en el futuro.

¿Cómo puedo ayudar para identificar a mi ser querido?

2

Debe presentarse información correcta y completa sobre la víctima (características físicas especiales, registros dentales, etc.). A veces esta información será suficiente para lograr la identificación. En muchos casos, es posible que dicha información ya se haya proporcionado antes de haberse estudiado la posibilidad de realizar un análisis de ADN. Para que el análisis de ADN tenga éxito, es necesario obtener muestras de los parientes de la víctima y compararlas con los restos.

¿Qué fuentes de muestras de ADN pueden usarse?

El ADN con frecuencia puede obtenerse de restos biológicos. Este ADN se compara con el ADN que se sabe con certeza que proviene de la víctima o con el ADN de los parientes de la víctima.

¿Qué fuentes de ADN se usan de la víctima?

Es posible utilizar el ADN de muestras médicas obtenidas previamente o de objetos personales de la víctima para realizar una prueba de compatibilidad directa con los restos. Por ejemplo, si el ser querido tuvo recientemente una cirugía o análisis de sangre de laboratorio, alguna muestra podría haberse guardado en el hospital o clínica. Debe proporcionar cualquier prueba médica que provenga con seguridad de la víctima o pedir ayuda para obtenerla. El primer renglón de la tabla a continuación ofrece algunos ejemplos de los tipos de muestras médicas que puede usar el laboratorio.

Fuentes de ADN	Ejemplos	Grado de utilidad
Muestras médicas	Muestra de un donante de médula ósea Muestra de biopsia Prueba de evaluación con mancha seca de sangre del recién nacido	Más útil
Objetos personales	Cepillo dental Cepillo para el cabello	Muy útil
Parientes cercanos	Padres biológicos de la víctima Hijos de la víctima Hermano o hermana de la víctima	Útil
Otros parientes	Parientes maternos (tías, tíos, primos, hermanastras o hermanastros del lado de la madre de la víctima)	Menos útil

También es posible obtener el ADN proveniente de objetos personales de la víctima. El segundo renglón de la tabla de arriba ofrece algunos ejemplos. Un cepillo dental u otros objetos que contengan saliva a menudo son buenas fuentes de ADN. Sin embargo, es de suma importante que estos objetos los haya usado sólo la víctima o pocas veces otra persona. Por ejemplo, un cepillo para el cabello que usa toda la familia no es una buena fuente de ADN de la víctima.

¿Cómo puede usarse el ADN de los parientes?

Si no hay disponibles objetos personales ni muestras médicas, o si la prueba no funciona en ellos, el análisis de ADN puede realizarse en las muestras de sangre de los parientes. El ADN de padres adoptivos, hijos adoptados, padrastros y madrastras u otros parientes que no tengan consanguinidad con la víctima no ofrecen información sobre la identidad genética de dicha víctima.

La capacidad de emparejar a las víctimas con sus parientes depende del grado de parentesco que tengan los parientes con la víctima. Las muestras más útiles de ADN provienen de parientes consanguíneos cercanos, como la madre, el padre, los hijos, hermanos o las hermanas biológicas de la víctima. Esto se debe a que el ADN de los parientes cercanos se asemeja más que el ADN de parientes distantes. Las imágenes en las siguientes páginas ilustran los parientes que son más útiles a la hora de identificar a una víctima. Si se usa el ADN de los hijos de la víctima, es útil obtener el ADN de los hijos del otro padre biológico.

Es posible usar el ADN de parientes más distantes, pero esta tarea es más difícil. En algunos casos, se podrían solicitar muestras de parientes específicos. Por ejemplo, es posible solicitar muestras de ADN de un pariente por parte de la madre de la víctima, como la tía, el tío o los hermanastros o hermanastras de la víctima del lado de la familia de la madre.

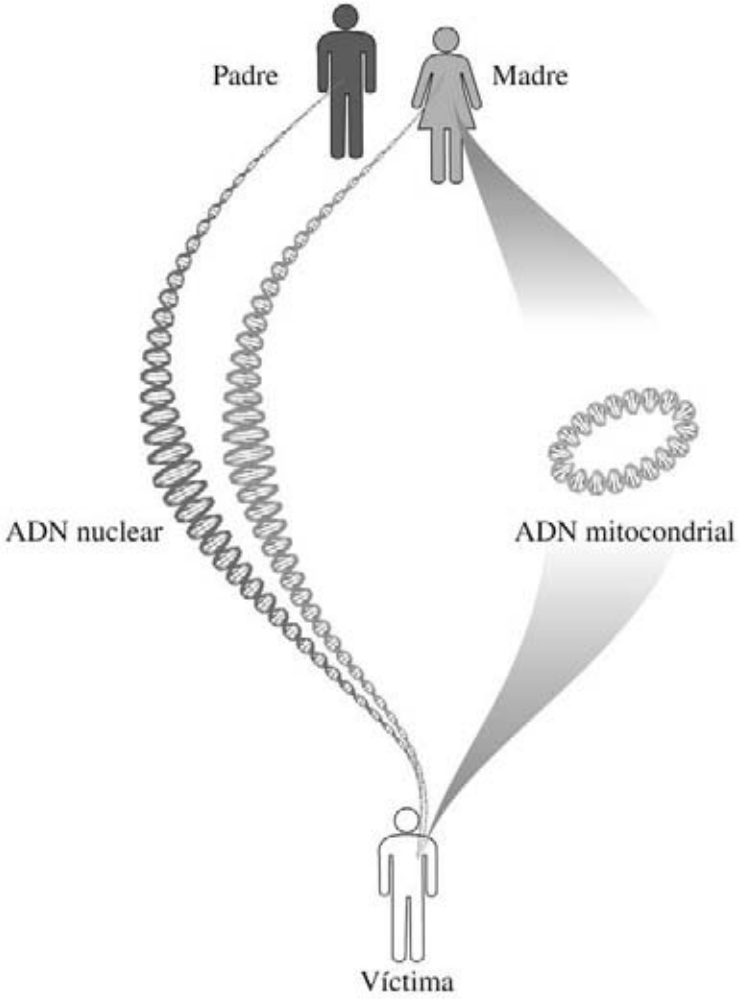
4

¿Por qué a veces no funciona el análisis de ADN?

Puede que el análisis de ADN no identifique a su ser querido. La razón más probable de ello es que no hay ADN utilizable en los restos recuperados. A veces tampoco es posible encontrar los restos de algunas víctimas. Además, el análisis de ADN no funcionará si no es posible encontrar ADN utilizable en los objetos personales entregados.

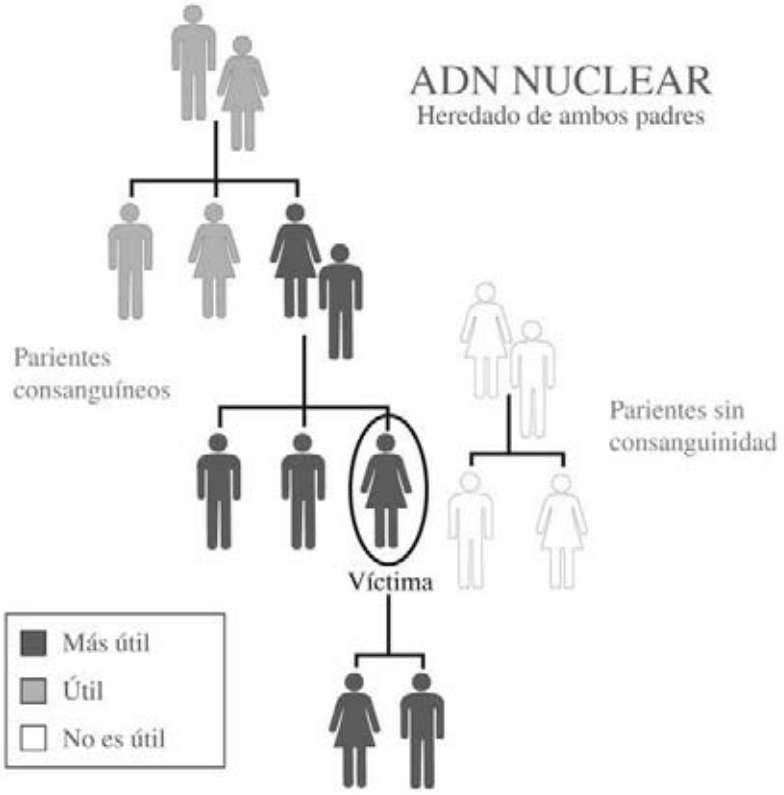
¿Cómo funciona el análisis de ADN?

El ADN es el material hereditario que contiene las instrucciones que forman el ser humano. El ADN puede obtenerse a partir de pequeñas cantidades de sangre, raspaduras de la boca (de la parte interior de las mejillas), raíces del cabello u otras muestras. Hay dos tipos de ADN en el cuerpo: ADN nuclear y ADN mitocondrial. Ambos tipos de ADN pueden usarse para el análisis de identificación por ADN.



5

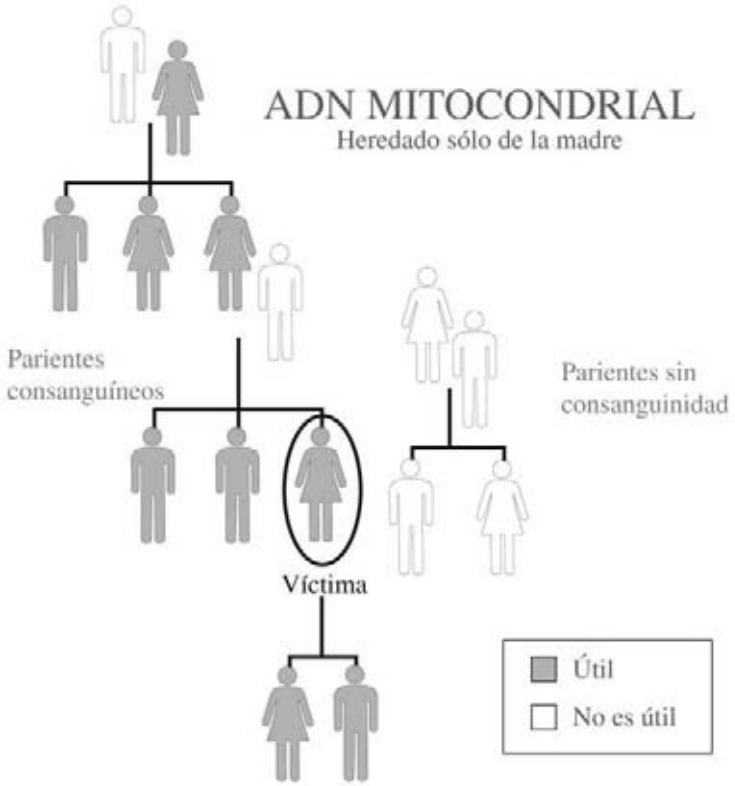
El ADN nuclear proviene del núcleo de las células y se hereda de ambos padres, la mitad de la madre y la mitad del padre (consulte la figura a continuación). Cada persona tiene un ADN nuclear único—excepto los gemelos idénticos, quienes tienen el mismo ADN. Cuando una cantidad adecuada del perfil de ADN nuclear de los restos de la víctima es compatible con el perfil de ADN nuclear de la muestra que se sabe con certeza que proviene de la víctima, podemos identificar a la víctima con mucha seguridad.



6

Debido a la forma en que se hereda, el ADN de parientes consanguíneos es algo parecido. El ADN nuclear de los restos de la víctima puede compararse con el ADN nuclear de los parientes para identificar a la víctima en algunas circunstancias.

El segundo tipo de ADN se denomina ADN mitocondrial (mtDNA). Este ADN se hereda sólo de la madre (consulte la figura). Los padres varones nunca pasan el ADN mitocondrial a sus hijos. Sin embargo, el ADN mitocondrial por lo general no es tan confiable como el ADN nuclear para la identificación. Esto quiere decir que en algunos casos el ADN mitocondrial de dos personas que no guardan parentesco alguno puede ser semejante. Debido a la forma en que se hereda, sólo los parientes maternos, como un hermano, hermana o madre, pueden usarse para analizar el ADN mitocondrial.



El ADN nuclear puede deteriorarse fácilmente en condiciones de calor extremo y bajo otras circunstancias, por lo tanto, no siempre está disponible para usarse con fines de identificación. El ADN mitocondrial, no obstante, puede encontrarse a menudo en muestras de ADN muy pequeñas o

deterioradas. Por lo general, los científicos prueban primero el ADN nuclear. Si los resultados no son lo suficientemente definidos para la identificación, procurarán entonces analizar el mitocondrial. A pesar de realizar los mejores esfuerzos, puede que algunos análisis no sean exitosos. Pero los científicos que buscan identificar a su ser querido trabajarán arduamente para lograrlo y permitirle a su familia poner fin a esta situación.

8

El NIJ forma parte de la Oficina de Programas de Justicia, que también incluye el Despacho de Asistencia Jurídica, el Despacho de Estadística Jurídica, la Oficina de Justicia Juvenil y Prevención de la Delincuencia, y la Oficina para Víctimas del Crimen.

Los hallazgos y conclusiones que se expresan en este documento corresponden al autor y no representan necesariamente la posición ni las políticas oficiales del Departamento de Justicia de Estados Unidos.

9

Colaboradores en la Iniciativa del ADN del Presidente

Oficina de Programas de Justicia

Instituto Nacional de Justicia

Oficina de Violencia Contra la Mujer

Despacho de Asistencia Jurídica

Oficina de Servicios Policiales Orientados a la Comunidad

Oficina Federal de Investigaciones

Oficina de Víctimas del Crimen

Oficina de Justicia Juvenil y Prevención de la Delincuencia

10

APPENDIX H

Sample Analysis: An Overview

While a step-by-step discussion of the processes involved in DNA typing is likely to be too rudimentary for most laboratory directors, it may offer useful information for family assistance coordinators, policymakers, reporters, and others who require a mid-level technical explanation of the issues faced by a forensic laboratory that is responding to a mass fatality 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 procedure; as in any situation with potential criminal implications, the proper collection and preservation of samples—using the best forensic practices—is critically important. In addition, improper preservation methods can lead to the loss of typable DNA, compromising the ability to make an identification.

Any information that provides reliable identification is valuable. Although this report focuses on DNA analysis, other traditional identification methods (anthropology, dental records, tattoos, etc.) should be used whenever possible, and the metadata should be used in a corroborative way. Some of these identification assays are so uniquely identifying that they may eliminate the need for the more labor-intensive DNA analysis or minimize the need for reanalysis. Furthermore, upfront anthropological screening will be beneficial for identifying the best samples for DNA analysis.

Sample Receipt Accessioning and Storage

Once samples are collected and preserved at the site, they are sent to the laboratory for analysis. The magnitude of samples delivered to the laboratory after a mass fatality incident can be overwhelming. Receiving, accessioning, and storing such samples can disrupt normal laboratory practices because most crime laboratories are not prepared to accommodate such a surge in numbers of samples. To ensure that sample identification is reliable, the laboratory should institute a quality control process to accommodate the surge in sample receipts. If an existing Laboratory Information Management System (LIMS) is not sufficient, one should be created to handle the mass casualty situation. While it is possible that existing chain-of-custody procedures will be sufficient, this issue should be evaluated before a mass fatality incident occurs.

In the event of a mass fatality incident, it is likely—as occurred after the World Trade Center (WTC) attacks—that other laboratories will offer assistance to the lead laboratory. If appropriate chain-of-custody, accessioning, and other infrastructural concerns can be addressed, some of the capacity problems can be shared or outsourced. 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.

DNA Extraction

The first step in the analytical process is extracting DNA from the reference and disaster samples. Successful DNA typing relies on isolating DNA of sufficient quantity, quality, and purity to yield an adequate DNA profile. DNA extraction protocols that overcome, remove, or dilute enzymatic inhibitors are the most desirable.

The quantity and quality of DNA yielded from a mass fatality sample can be compromised by conditions specific to the event and can range from apparently pristine to highly degraded to substantially contaminated. Disaster samples and personal effects samples may be degraded and contaminated with materials that inhibit analytical processes, particularly for enzymatic reactions such as the polymerase chain reaction (PCR), an in vitro process that increases the amount of small, specific targeted sequences.

Care should be taken to get the best quality DNA possible in order to maximize the number of loci that will be amplified. Consider an extraction procedure that will yield DNA suitable for mitochondrial testing or low copy number (LCN) testing. Also, it is important to keep in mind that it may not be apparent which test systems will be useful until a first round of testing is completed.

The process for DNA extraction is laborious and time consuming. This can be exacerbated in a mass fatality identification if a large number of bone samples—often, the only type of sample available—are sent to the laboratory. Bones can contain substances that inhibit the PCR; therefore, inhibitory substances must be removed if the DNA is to be suitable for typing. In these cases, a laboratory may need to modify its routine extraction procedures to remove PCR inhibitors.

Standard DNA extraction procedures exist for the types of materials that may be encountered. They include: (1) organic solvent, (2) column exchange, and (3) cation exchange resins, such as Chelex–100. The quality of recovered DNA will be limited by the quality of the sample. For some samples, sufficient high-molecular-weight DNA without chemical contaminants may be obtained. For others, the environmental destruction may have been so great that no usable DNA is available for typing. Thus, extraction methods that minimize the loss of DNA are the most desired.

Short Tandem Repeat (STR) Analysis

It is most expedient for laboratories already experienced in DNA casework to use well-known and well-established technologies such as short tandem repeat (STR) typing as their initial method of analysis—and, in fact, many disaster samples may be typable by STR analysis. The 13 core STR loci currently used in the United States and many other countries are composed of tandemly repeated DNA sequences, each of which is typically 4 or 5 base pairs in length. The number of alleles at the forensically employed STR loci typically ranges from 5 to 20.

Amplified STR alleles are manufactured to be somewhat larger, up to 500 bases in length. Because of this, the starting (or template) DNA must be of sufficient quality and quantity to achieve full typing of all the STR loci. When DNA of this quality and quantity is available, STRs can be typed—including with the use of commercial kits that are available to assist in typing the multiple loci (multiplexing)—with a high degree of specificity and sensitivity in a relatively short time period.

Electrophoresis, a process that separates charged molecules in an electric field, is a cornerstone in forensic DNA typing. For the standard forensic loci, the size of the PCR product for an individual is determined by comparison with a commercially available alleleic ladder. To resolve STR loci, most laboratories employ capillary electrophoresis, and the instrumentation associated with this analysis enables automation that allows a higher throughput analysis.

Alternative Testing Methods

In some mass fatality incidents, samples may be so compromised that alternate DNA analysis techniques will be needed to achieve complete identification. The best technologies will, of course, depend on the state of the art, including the ability to demonstrate the reliability of new technologies on compromised samples. Molecular biology is a dynamic field, and new analytical tools are always being developed.

In the WTC response, the Office of the Chief Medical Examiner of New York relied on the recommendations of the Kinship and Data Analysis Panel (KADAP) to help explore new methods to further the identification of compromised samples. For example, the panel looked at whether there would be sufficient extracted material to support all attempted technologies and satisfy quality control inquiries that might arise. The KADAP also considered how to handle statistical

issues using the additional technologies, including linkage and haplotype/genotype comparisons.

Making the Identification

In the WTC identification effort, when the DNA profile from a victim matched a reference sample or was included within a reference family pedigree, statistical significance was placed on the likelihood of such an occurrence. A certain threshold was required for assigning identity. (See appendix A.)

Generally, such a quantitative assessment is based on the frequency of occurrence of alleles from major population groups, such as African-Americans, Asians, Caucasians, and Hispanics. Once the individual frequencies of each independent genetic marker are determined, the frequencies are multiplied using the product rule to estimate the rarity of each of those characteristics occurring as a single profile. It is the combination of the genetic markers that enables the identification.

When personal items are the reference samples, a direct comparison of the profiles is performed, and a random match probability is calculated for those samples that are considered a potential source. For family reconstructions, DNA profiles from relatives are compared with the sample profile (e.g., a mother and a father of a missing child). A likelihood ratio is generated to evaluate whether sufficient evidence exists to support a biological relationship.

A large number of genetic markers are available for identity testing of human remains, and, by typing a sufficient number of these loci, identifications equivalent to uniqueness can be made readily for some, but not all, samples. Limitations include:

- Sample degradation or a sample that is too small to analyze, allowing only a partial DNA profile. This reduces the power to unequivocally identify the source of the sample.

- The existence of reference samples is critical to making an identification. Even if a mass disaster sample yields a complete DNA profile, an identification may not be possible if there are insufficient reference samples. For example, it may be relatively easy to identify a missing child when his or her biological parents and two siblings are typed. However, if the only relative available for comparison is a half-sibling, the genetic information will be far more limited and an identification may not be possible. Therefore, every effort should be made to obtain samples from as many close family members as possible. Personal effects enable direct comparisons of profiles, but at times the alleged source of a personal effect is questionable. Obviously, the more that is known about a personal item, the greater the confidence in using it as a reference sample.

- Because of the violent nature of many mass disasters, remains can be commingled. In such cases, a mixture of DNA profiles may be observed. The best practice is to avoid interpreting such profiles; it is better to perform a reextraction from the sample, if possible.

APPENDIX I

Additional References on Statistical Issues in DNA Identification

The following additional references discuss statistical issues that arise when making an identification through DNA analysis.

Ballantyne, J. "Mass Disaster Genetics." *Nature Genetics* 15(4) (1997): 329–331.

Bieber, F.R. "Reckoning with the Dead." *Harvard Medical Alumni Bulletin* 76(2) (2002): 34–37.

Bieber, F.R. "Science and Technology of Forensic DNA Profiling." Lazar, D. (ed.), *DNA and the Criminal Justice System*. Cambridge, MA: MIT Press, 2004: 23–62.

Biesecker, L.G., Bailey-Wilson, J.E., Ballantyne, J., Baum, H., Bieber, F.R., Brenner, C., Budowle, B., Butler, J.M., Carmody, G., Conneally, P.M., Duceman, B., Eisenberg, A., Forman, L., Kidd, K.K., Leclair, B., Niezgoda, S., Parsons, T.J., Pugh, E., Shaler, R., Sherry, S.T., Sozer, A., and Walsh, A. "DNA Identifications After the 9/11 World Trade Center Attack." *Science* 310 (2005): 1122–1123.

Bing, D.H. and Bieber, F.R. "Collecting and Handling Samples for Parentage and Forensics DNA-Based Genetic Testing." *Current Protocols in Human Genetics*. New York: John Wiley, 1997: 14.2.1–14.2.6.

Bing, D.H., Bieber, F.R., Holland, M.M., and Huffine, E.F. "Isolation of DNA from Forensic Evidence." *Current Protocols in Human Genetics*. New York: John Wiley, 2000: 14.3.1–14.3.19 (Supplement 26).

Bogenhagen, D. and Clayton, D.A. "The Number of Mitochondrial Deoxyribonucleic Acid Genomes in Mouse L and Human HeLa Cells." *Journal of Biological Chemistry* 249 (1974): 7791–7795.

Brenner, C.H. "Symbolic Kinship Program." *Genetics* 145(2) (1997): 535–542.

Brenner, C.H. and, Weir, B.S. "Issues and Strategies in the Identification of World Trade Center Victims." *Theoretical Population Biology* 63 (2003): 173–178.

Brinkmann, B., Klintschar, M., Neuhuber, F., Huhne, J., and Rolf, B. "Mutation Rate in Human Microsatellites: Influence of the Structure and Length of the Tandem Repeat." *American Journal of Human Genetics* 62 (1998): 1408–1415.

Budimlija, Z.M., Prinz, M.K., Zelson-Mundorff, A., Wiersema, J., Bartelink, E., MacKinnon, G., Nazzaruolo, B.L., Estacio, S.M., Hennessey, M.J., and Shaler, R.C. "World Trade Center Human Identification Project: Experiences with Individual Body Identification Cases." *Croatian Medical Journal* 44(3) (2003): 259–63.

Budowle, B. "SNP Typing Strategies." *Forensic Science International* 146 Suppl. (2004): S139–142.

Budowle, B., Adamowicz, M., Aranda, X.G., Barna, C., Chakraborty, R., Cheswick, D., Dafoe, B., Eisenberg, A., Frappier, R., Gross, A.M., Ladd, C., Lee, H.S., Milne, S.C., Meyers, C., Prinz, M., Richard, M.L., Saldanha, G., Tierney, A.A., Viculis, L., and Krenke, B.E. "Twelve Short Tandem Repeat Loci Y Chromosome Haplotypes: Genetic Analysis on Populations Residing in North America." *Forensic Science International* 150(1) (2005): 1–15.

Budowle, B., Baechtel, F.S., Comey, C.T., Giusti, A.M., and Klevan, L. "Simple Protocols for Typing Forensic Biological Evidence: Chemiluminescent Detection for Human DNA Quantitation and RFLP Analyses and Manual Typing of PCR Amplified Polymorphisms." *Electrophoresis* 16(9) (1995): 1559–1567.

Budowle, B., Bieber, F.R., and Eisenberg, A.J. "Forensic Aspects of Mass Disasters: Strategic Considerations for DNA-Based Human Identification." *Legal Medicine* (Tokyo) 7(4) (2005): 230–243.

Budowle, B., Chakraborty, R., Carmody, G., and Monson, K.L. "Source Attribution of a Forensic DNA Profile." *Forensic Science Communications* 2(3) (2000): http://www.fbi.gov/hq/lab/fsc/backissu/july2000/source.htm.

Budowle, B., Masibay, A., Anderson, S.J., Barna, C., Biega, L., Brenneke, S., Brown, B.L., Cramer, J., DeGroot, G.A., Douglas, D., Duceman, B., Eastman, A., Giles, R., Hamill, J., Haase, D.J., Janssen, D.W., Kupferschmid, T.D., Lawton, T., Lemire, C., Llewellyn, B., Moretti, T., Neves, J., Palaski, C., Schueler, S., Sgueglia, J., Sprecher, C., Tomsey, C., and Yet, D. "STR Primer Concordance Study." *Forensic Science International* 124 (2001): 47–54.

Budowle, B., Moretti, T.R., Niezgoda, S.J., and Brown, B.L. "CODIS and PCR-Based Short Tandem Repeat Loci: Law Enforcement Tools." *Second European Symposium on Human Identification*. Madison, WI: Promega Corporation, 1998: 73–88.

Budowle, B., Planz, J., Campbell, R., and Eisenberg, A. "SNPs and Microarray Technology in Forensic Genetics: Development and Application to Mitochondrial DNA." *Forensic Science Review* 16 (2004): 22–36.

Budowle, B., Schutzer, S.E., Einseln, A., Kelley, L.C., Walsh, A.C., Smith, J.A.L., Marrone, B.L., Robertson, J., and Campos, J. "Building Microbial Forensics as a Response to Bioterrorism." *Science* 301 (2003): 1852–1853.

Budowle, B., Sinha, S.K., Lee, H.S., and Chakraborty, R. "Utilization of Y-Chromosome Short Tandem Report Haplotype in Forensic Application." *Forensic Science Review* 15(2) (2003): 153–162.

Budowle, B., Smith, J., Moretti, T., and DiZinno, J. *DNA Typing Protocols: Molecular Biology and Forensic Analysis*. Natick, MA: Eaton Publishing, BioTechniques Books, BioForensic Sciences Series, 2000.

Budowle, B. and Sprecher, C.J. "Concordance Study on Population Database Samples Using the PowerPlex™ 16 Kit and AmpFlSTR® Profiler Plus™ Kit and AmpFlSTR® COfiler™ Kit." *Journal of Forensic Sciences,* 46(3) (2001): 637–641.

Budowle, B. and SWGMGF Members. "Quality Assurance Guidelines for Laboratories Performing Microbial Forensic Work." *Forensic Science Communications* 5(4) (2003): http://www.fbi.gov/hq/lab/fsc/backissu/oct2003/2003_10_guide01.htm.

Budowle, B., Wilson, M.R., DiZinno, J.A., Stauffer, C., Fasano, M.A., Holland, M.M., and Monson, K.L. "Mitochondrial DNA Regions HVI and HVII Population Data." *Forensic Science International* 103 (1999): 23–35.

Butler, J.M., Shen, Y., and McCord, B.R. "The Development of Reduced Size STR Amplicons as Tools for Analysis of Degraded DNA." *Journal of Forensic Sciences* 48(5) (2003): 1054–1064.

Campobasso, C.P., Falamingo, R., and Vinci, F. "Investigation of Italy's Deadliest Building Collapse: Forensic Aspects of a Mass Disaster." *Journal of Forensic Sciences* 48(3) (2003): 635–639.

Clayton, T.M., Whitaker, J.P., Fisher, D.L., Lee, D.A., Holland, M.M., Weedn, V.W., Maguire, C.N., DiZinno, J.A., Kimpton, C.P., and Gill, P. "Further Validation of a Quadruplex STR DNA Typing System: A Collaborative Effort to Identify Victims of a Mass Disaster." *Forensic Science International* 76(1) (1995): 17–25.

Clayton, T.M., Whitaker, J.P., and Maguire, C.N. "Identification of Bodies from the Scene of a Mass Disaster Using DNA Amplification and Short Tandem Repeat (STR) Loci." *Forensic Science International* 76(1) (1995): 7–15.

Collins, P.J., Hennessy, L.K., Leibelt, C.S., Roby, R.K., Reeder, D.J., and Foxall, P.A. "Developmental Validation of a Single-Tube Amplification of the 13 CODIS Loci, D2S1338, D19S433, and Amelogenin: The AmpFlSTR® Indentifiler® PCR Amplification Kit." *Journal of Forensic Sciences* 49(6) (2004): 1265–1277.

Cooper, D.N., Smith, B.A., Cooke, H.J., Niemann, S., and Schmidtke, J. "An Estimate of Unique DNA Sequence Heterozygosity in the Human Genome." *Human Genetics* 69 (1985): 201–205.

Corach, D., Sala, A., Penacino, G., and Sotelo, A. "Mass Disasters: Rapid Molecular Screening of Human Remains by Means of Short Tandem Repeats Typing." *Electrophoresis* 16(9) (1995): 1617–1623.

Cotton, E.A., Allsop, R.F., Guest, J.L., Frazier, R.R., Koumi, P., Callow, I.P., Seager, A., and Sparkes, R.L. "Validation of the AmpFlSTR SGM Plus System for Use in Forensic Casework." *Forensic Science International* 112(2–3) (2000): 151–161.

Cowell, R.G. and Mostad, P. "A Clustering Algorithm Using DNA Marker Information for Sub-Pedigree Reconstruction." *Journal of Forensic Sciences* 48(6) (2003): 1239–1248.

Daoudi, Y., Morgan, M., Diefenbach, C., Ryan, J., Johnson, T., Conklin, G., Duncan, K., Smigielski, K., Huffine, E., Rankin, D., Mann, R., Holland, T., McElfresh, K., Canik, J., Armbrustmacher, V., and Holland, M. "Identification of the Vietnam Tomb of the Unknown Soldier: The Many Roles of Mitochondrial DNA." Presented at the Ninth International Symposium on Human Identification, Madison, WI: Promega Corporation, 1998: http://www.promega.com/geneticidproc/ussymp9proc/abstracts/ab72.pdf.

DNA Advisory Board. "Statistical and Population Genetics Issues Affecting the Evaluation of the Frequency of Occurrence of DNA Profiles Calculated from Pertinent Population Database(s)." *Forensic Science Communications* 2(3) (2000): http://www.fbi.gov/hq/lab/fsc/backissu/july2000/dnastat.htm.

Egeland, T., Mostad, P.F., and Olaisen, B. "A Computerized Method for Calculating the Probability of Pedigrees from Genetic Data." *Science & Justice* 37(4) (1997): 269–274.

Egeland, T., Mostad, P.F., Mevag, B., and Stenersen, M. "Beyond Traditional Paternity and Identification Cases: Selecting the Most Probable Pedigree." *Forensic Science International* 110(1) (2000): 47–59.

Evett, I.W. and Weir, B.S. *Interpreting DNA Evidence.* Sunderland, MA: Sinauer Associates, Inc., 1998.

Gabriel, M.N., Calloway, C.D., Reynolds, R.L., and Primorac, D. "Identification of Human Remains by Immobilized Sequence-Specific Oligonucleotide Probe Analysis of mtDNA Hypervariable Regions I and II." *Croatian Medical Journal* 44(3) (2003): 293–298.

Gill, P., Ivanov, P.L., Kimpton, C., Piercy, R., Benson, N., Tully, G., Evett, I., Hagelberg, E., and Sullivan, K. "Identification of the Remains of the Romanov Family by DNA Analysis." *Nature Genetics* 6 (1994): 130–135.

Giusti, A.M. and Budowle, B. "Chemiluminescence-Based Detection System for Human DNA Quantitation and Restriction Fragment Length Polymorphism (RFLP) Analysis." *Applied and Theoretical Electrophoresis* 5 (1995): 89–98.

Hall, T.A., Budowle, B., Jiang, Y., Blyn, L., Eshoo, M., Sannes-Lowery, K.A., Sampath, R., Drader, J.J., Hannis, J.C., Harrell, P., Samant, V., White, N., Ecker, D.J., and Hofstadler, S.A. "Base Composition Analysis of Human Mitochondrial DNA Using Electrospray Ionization Mass Spectrometry: A Novel Tool for the Identification and Differentiation of Humans." *Analytical Biochemistry* 344 (1) (2005): 53–69.

Hellmann, A., Rohleder, U., Schmitter, H., and Wittig, M. "STR Typing of Human Telogen Hairs—A New Approach." *International Journal of Legal Medicine* 114 (2001): 269–273.

Hochmeister, M.N., Budowle, B., Jung, J., Borer, U.V., Comey, C.T., and Dirnhofer, R. "PCR-Based Typing of DNA Extracted from Cigarette Butts." *International Journal of Legal Medicine* 104(4) (1991): 229–233.

Holland, M.M., Cave, C.A., Holland, C.A., and Bille, T.W. "Development of a Quality, High Throughput DNA Analysis Procedure for Skeletal Samples to Assist with the Identification of Victims from the World Trade Center Attacks." *Croatian Medical Journal* 44(3) (2003): 264–272.

Holland, M.M., Fisher, D.L., Mitchell, L.G., Rodriquez, W.C., Canik, J.J., Merril, C.R., and Weedn, V.W. "Mitochondrial DNA Sequence Analysis of Human Skeletal Remains: Identification of Remains from the Vietnam War." *Journal of Forensic Sciences* 38(3) (1993): 542–553.

Huffine, E., Crews, J., Kennedy, B., Bomberger, K., and Zinbo, A. "Mass Identification of Persons Missing from the Break-Up of the Former Yugoslavia: Structure, Function, and Role of the International Commission on Missing Persons." *Croatian Medical Journal* 42(3) (2001): 271–275.

Kahana, T., Freund, M., and Hiss, J. "Suicidal Terrorist Bombings in Israel—Identification of Human Remains." *Journal of Forensic Sciences* 42(2) (1997): 260–264.

Kayser, M., Roewer, L., Hedman, M., Henke, L., Henke, J., Brauer, S., Krüger, C., Krawczak, M., Nagy, M., Dobosz, T., Szibor, R., de Knijff, P., Stoneking, M., and Sajantila, A. "Characteristics and Frequency of Germline Mutations at Microsatellite Loci from the Human Y Chromosome, as Revealed by Direct Observation in Father/Son Pairs." *American Journal of Human Genetics* 66(5) (2000): 1580–1588.

Kayser, M. and Sajantila, A. "Mutations at Y–STR Loci: Implications for Paternity Testing and Forensic Analysis." *Forensic Science International* 118(2–3) (2001): 116–121.

Krenke, B.E., Tereba, A., Anderson, S.J., Buel, E., Culhane, S., Finis, C.J., Tomsey, C.S., Zachetti, J.M., Masibay, A., Rabbach, D.R., Amiott, E.A., and Sprecher, C.J. "Validation of a 16-Locus Fluorescent Multiplex System." *Journal of Forensic Sciences* 47(4) (2002): 773–785.

Kruglyak, L. and Nickerson, D.A. "Variation is the Spice of Life." *Nature Genetics* 27(3) (2001): 234–236.

Ladd ,C., Lee, H., Yang, N. and Bieber, F.R. "Interpretation of Complex Forensic DNA Mixtures." *Croatian Medical Journal* 42(3) (2001): 244–246.

Leclair, B., Fregeau, C.J., Bowen, K.L., and Fourney, R.M. "Enhanced Kinship Analysis and STR-Based DNA Typing for Human Identification in Mass Fatality Incidents: The Swissair Flight 111 Disaster." *Journal of Forensic Sciences* 49(5) (2004): 939–953.

Leclair, B., Shaler, R., Carmody, G.R., Eliason, K., Hendrickson, B.C., Judkins, T., Norton, M.J., Sears, C., and Scholl, T. "Bioinformatics and Human Identification in Mass Fatality Incidents: The World Trade Center Disaster." Submitted to *Journal of Forensic Sciences*, 2006.

Leibelt, C., Budowle, B., Collins, P., Daoudi, Y. Moretti, T., Nunn, G., Reeder, D., and Roby, R. "Identification of a D8S1179 Primer Binding Site Mutation and the Validation of a Primer Designed to Recover Null Alleles." *Forensic Science International* 133(3) (2003): 220–227.

Li, C.C. and Sacks, L. "The Derivation of Joint Distribution and Correlation Between Relatives by the Use of Stochastic Matrices." *Biometrics* 10(3) (1954): 347–360.

Lorente, J.A., Entrala, C., Alvarez, J.C., Lorente, M., Carrasco, F., Arce, B., Heinrich, B., Budowle, B., and Villanueva, E. "Social Benefits of Non-criminal Genetic Databases: Missing Persons and Human Remains Identification." *International Journal of Legal Medicine* 116(3) (2002): 187–190.

Ludes, B., Tracqui, A., Pfitzinger, H., Kintz, P., Levy, F., Disteldorf, M., Hutt, J.M., Kaess, B., Haag, R., Memheld, B., et al. "Medico-Legal Investigations of the Airbus A230 Crash Upon Mount Ste-Odile, France." *Journal of Forensic Sciences* 39(5) (1994): 1147–1152.

Lutz, S., Weisser, H.J., Heizmann, J., and Pollak, S. "mtDNA as a Tool for Identification of Human Remains." *International Journal of Legal Medicine* 109(4) (1996): 205–209.

Mandrekar, P.V., Krenke, B.E., and Tereba, A. "DNA IQ™: The Intelligent Way to Purify DNA." *Profiles in DNA* 4(3) (2001): 16.

Mandrekar, P.V., Flanagan, L., and Tereba, A. "Forensic Extraction and Isolation of DNA from Hair, Tissue and Bone." *Profiles in DNA* 5(2) (2002): 11–13.

Meyer, H.J. "The Kaprun Cable Car Fire Disaster—Aspects of Forensic Organisation Following a Mass Fatality with 155 Victims." *Forensic Science International* 138(1–3) (2003): 1–7.

National Commission on the Future of DNA Evidence. *The Future of Forensic DNA Testing: Predictions of the Research and Development Working Group.* Washington, DC: U.S. Department of Justice, Office of Justice Programs, National Institute of Justice, 2000: http://www.ncjrs.gov/pdffiles1/nij/183697.pdf.

National Research Council Committee on DNA Forensic Science. The Evaluation of Forensic DNA Evidence. Washington, DC: National Academy Press, 1996.

Olaisen, B., Stenersen, M., and Mevag, B. "Identification by DNA Analysis of the Victims of the August 1996 Spitsbergen Civil Aircraft Disaster." *Nature Genetics* 15(4) (1997): 402–405.

Olson, K.B. "Aum Shinrikyo: Once and Future Threat." *Emerging Infectious Disease* 5(4) (1999): 513–516.

Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A., and Arnheim, N. "Enzymatic Amplification of Beta-Globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia." *Science* 230(4732) (1985): 1350-1354.

Sajantila, A., Lukka, M., and Syvanen, A.C. "Experimentally Observed Germline Mutations at Human Micro- and Minisatellite Loci." *European Journal of Human Genetics* 7(2) (1999): 263–266.

Traver, M. "Numeric Statements of the Strength of the Genetic Evidence." *Guidance for Standards for Parentage Testing Laboratories.* Bethesda, MD: American Association of Blood Banks, 2004.

Tsukada, K., Takayanagi, K., Asamura, H., Ota, H., and Fukushima, H. "Multiplex Short Tandem Repeat Typing in Degraded Samples Using Newly Designed Primers for the TH01, TPOX, CSF1PO, and vWA Loci." *Legal Medicine* (Tokyo) 4(4) (2002): 239–245.

Venter, J.C., Adams, M.D., Myers, E.W., Li, P.W., Mural, R.J., Sutton, G.G., Smith, H.O., Yandell, M., et al. "The Sequence of the Human Genome." *Science* 291(5507) (2001): 1304–1351.

Walsh, S. "Commentary on Kline, M.C., Jenkins, B., Rodgers, S. Non-amplification of a vWA Allele." *Journal of Forensic Sciences* 43(5) (1998): 1103–1104.

Wang, D.G., Fan, J.B., Siao, C.J., Berno, A., Young, P., Sapolsky, R., Ghandour, G., Perkins, N., et al. "Large Scale Identification, Mapping, and Genotyping of Single-Nucleotide Polymorphisms in the Human Genome." *Science* 280(5366) (1998): 1077–1082.

Waye, J.S., Presley, L., Budowle, B., Shutler, G.G., and Fourney, R.M. "A Simple Method for Quantifying Human Genomic DNA in Forensic Specimen Extracts." *BioTechniques* 7(8) (1989): 852–855.

Weir, B.S. *Genetic Data Analysis II.* Sunderland, MA: Sinauer Associates, Inc., 1996.

Wiegand, P. and Kleiber, M. "Less is More—Length Reduction of STR Amplicons Using Redesigned Primers." *International Journal of Legal Medicine* 114(4–5) (2001): 285–287.

Wilson, M.R., DiZinno, J.A., Polanskey, D., Replogle, J., and Budowle, B. "Validation of Mitochondrial DNA Sequencing for Forensic Casework Analysis." *International Journal of Legal Medicine* 108(2) (1995): 68–74.

Wilson, M.R., Polanskey, D., Butler, J., DiZinno, J.A., Replogle, J., and Budowle, B. "Extraction, PCR Amplification, and Sequencing of Mitochondrial DNA from Human Hair Shafts." *BioTechniques* 18(4) (1995): 662–669.

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