Methods Developed to Identify Victims of the World Trade Center Disaster

by Elaine Marchi

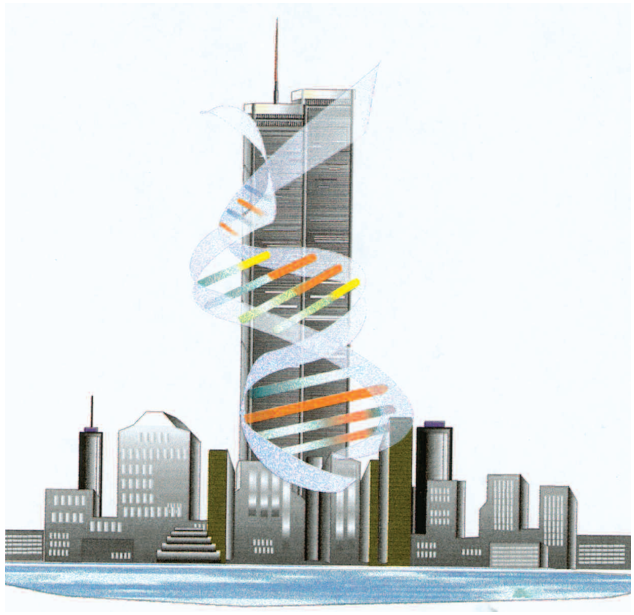
Two-and-a-half years have passed since terrorists used two commercial airliners as projectiles of destruction to bring down the twin 110-story towers of the World Trade Center in New York City. The nation pauses to remember the estimated 2819 people who lost their lives on that day. While history enters the events of September 11, 2001, as a tragedy and loss that will be ever etched in our common psyche, we recognize that forensic scientists have made an unprecedented effort to identify remains. They have used creative thinking and technical competence to develop new strategies to identify the remains of the victims, helping those surviving friends and family members still desperately seeking closure from this tragedy.

The destructive force unleashed by the collapse of the both WTC towers was so great that many victims' bodies were charred and pulverized, making visual identification impossible. The sheer nature of identification of the mass fatalities was an extraordinary challenge. Forensic efforts of the World Trade Center disaster were tested to the limits because debris consisting of crushed and broken building components fell onto surrounding buildings, igniting fires and causing additional collapses. Debris from the collapsing WTC towers caused structural damage to surrounding buildings and set off a cascade of events with the start of new fires. The collapse of each tower sent out air pressure of dust clouds consisting of building contents and occupants, who could not evacuate the buildings before the towers collapsed. Contents of the towers were both vaporized and pulverized.[1] Fires continued to burn for 99 days.

In response, forensic scientists assembled and agreed to accelerate efforts to develop new strategies to identify the remains of the victims. Their work is a testimony to American creativity and to scientific creative thinking, technical competence, and the American spirit.

Bringing together the best of the public and private sectors

Dr. Robert Shaler is Director of Forensic Biology in New York City's Office of the Chief Medical Examiner and



was responsible for identifying the victims. A week after the attacks, New York State Governor George E. Pataki issued an executive order expanding the number of laboratories to be included with the DNA analysis connected with the World Trade Center victim identification. From the first day, rescue and recovery workers struggled to quickly recover specimens under the most dangerous of working conditions, which included shifting twisted metal debris and fire flaring up underfoot. As hours and days turned into weeks, they found themselves fighting the elements of microbe, enzyme, insect, and insult from other environmental factors that accelerate decomposition and degrade DNA analysis. Initiatives were immediately implemented to identify the dead in the World Trade Center. The twofold goal remained to provide evidence for investigating the attacks and identification for families and friends whose lives are suspended in unresolved grief. Traditional forensic identification based on fingerprints, dental records, or visual identification of face and body was of limited value in this case. Identification of victims would rest heavily on DNA analysis.[2]

According to the Department of Justice's definition of terrorism, "terrorism is the force against persons or prop-

erty in violation of the criminal laws of the United States for the purposes of intimidation, coercion or ransom and create fear among the public."[3] The National Institute of Justice is the research and development agency for the U.S. Department of Justice. The strategic direction of the National Institute of Justice includes creating the tools and technologies to meet the forensic and investigative demands of society. Thus, Shaler contacted Dr. Lisa Foreman, Director of the Investigative and Forensic Division for the U.S. Department of Justice's National Institute for Justice, because he realized that traditional DNA identification required long, intact segments of sample to type. The samples recovered from the World Trade Center site were typically fragmented and degraded. At the same time of the request, a project was already underway and funded at the National Institute for Standards and Technology (Gaithersburg, MD). Dr. John Butler has dedicated his career to the development of new forensic approaches to quantitate DNA at crime scenes and at scenes of mass destruction. For the past several years, Butler has been working with Dr. Bruce McCord, a former FBI scientist who is now an associate professor of chemistry and biochemistry at Ohio University (Athens, OH), to develop several new DNA assays they termed "miniSTRs" or "miniplexes." Dr. Yin Shen in McCord's laboratory also worked on the project.

In response to the immediate need for organization to coordinate and systematize efforts, a group was formed that became known as the WTC Kinship and Data Analysis Panel (KADAP). Members of the group included scientists from the Medical Examiner's Laboratory, the New York State Police Laboratory, and the New York State Department of Health. At the federal level, the U.S. Department of Defense (Armed Forces DNA Laboratory), Health and Human Services of the National Institutes of Health (Bethesda, MD), Commerce (National Institute of Standards and Technology), and the U.S. Department of Justice sent members to be part of the KADAP team. Researchers from several universities, including Harvard Medical School (Cambridge, MA), Yale University (New Haven, CT), and Johns Hopkins (Baltimore, MD) also contributed. A boost for KADAP came when a number of private laboratories responded to relieve some of the pressure on the system and to provide specialized technology that already existed in the private sector. For the past two years, the KADAP group has met monthly to develop new strategies and technologies to analyze the remains recovered from the disaster site.

The cooperation of the public and private sectors made this task as successful as possible. The private companies that assisted the government in this effort included **Myriad Genetics Inc.** (Salt Lake City, UT), which ran family reference samples and samples obtained from the personal effects brought in by the families; **Gene Codes Forensics** (Ann Arbor, MI) and **DNA-VIEW** (Oakland, CA), which wrote new software programs to assist in matching the samples that were submitted from family members to be matched to genetic data obtained from the WTC victims; and **Celera Genomics** (Rockville, MD), which used the instrumentation to sequence the blueprint of the human genome performed mitochondrial DNA sequencing. At its peak, **Celera** reported analyzing specimens that numbered upward of 4000 tissue samples per day. The **Bode Technology Group** (Springfield, VA) signed on early and continued to test bone samples by using several techniques including conventional short tandem repeat (STR), miniSTR, and mtDNA typing, while **Orchid Genescreen** (Dallas, TX) was involved in performing single-nucleotide polymorphism (SNP) analysis using smaller fragments of DNA.

The role of the forensic scientist

The forensic scientist contributes a vital function to the justice and regulatory system in the United States. The legal system is based on the belief that it results in justice being served. The process begins when law enforcement authorities collect samples at a crime scene. The samples are transferred to the laboratory, where scientists chemically break down the matter and isolate the DNA. The forensic scientist looks for the presence or absence of certain DNA markers on the chromosomes, including repeated DNA sequences. Environmental insult to the sample can include exposure to fire and fragmentation from explosion and exposure to the elements.[4] This is a challenge, because the DNA within the sample breaks into small fragments that do not hold up in the standard DNA typing test. As with the World Trade Center disaster, the forensic scientist must often provide analyses of heterogeneous mixtures, identifying each individual constituent. A precise analysis is made from very small samples while still preserving adequate samples for future analysis.[5]

New technologies: From STRs to MiniSTRs

In the identification of nuclear DNA, a standard technique is STR, which identifies the sequence of the four nucleotides that form DNA. The STR nuclear technique focuses on 13 specific loci spread over the 23 pairs of chromosomes that are standard in the FBI's national database. The DNA from the 13 loci is in the nonfunctioning areas of the genome. One of each of the 13 loci regions of DNA repeats itself, and the number of repetitions tends to vary among people. The number of repeats at all 13 yields a probability to a match among unrelated individuals in less than one

in a trillion.[6] Early in his career, Butler began to develop this new technology for rapid-analysis STRs with time-of-flight mass spectrometry by designing primers as close to the STR repeat regions as possible to retain the variability of the STR alleles while reducing the size of the polymerase chain reaction (PCR) product.[7] The original primers could be used with capillary electrophoresis (CE).

In the summer months just prior to September 11, 2001, a collaborative project on developing the miniSTR technique was established between McCord and Butler and was funded by the Department of Justice. After the terrorist attacks, the researchers were requested to accelerate their efforts to develop various miniSTR markers in various combinations. The miniplexes, as they were termed, covered all 13 of the core STR loci. Their ingenuity paid off, since they were able to reduce allelic ladders and develop new primers for the New York City Medical Examiner's Office by January of 2002. The materials became known as "the Big Mini" assay. With a memorable name, the assay sounds like a jazz session more than the SNP technique that can turn up in runs as short as 50 DNA letters. Following the team's new approach, the Medical Examiner's Office asked the **Bode Technology Group** to scale up McCord and Butler's efforts. **Bode** responded by developing two miniSTR assays of its own known as "BodePlexes." These assays were based on the foundations established by McCord and Butler.

The role of MtDNA and CE

Forensic DNA identification is used in criminal cases for the association or exclusion of individuals from a crime scene.[8] Capillary electrophoresis has been used to identify DNA in bone, blood, semen, saliva, and hair, with a shorter analysis time than the traditional technique of slab-gel electrophoresis.[9] The attributes of CE—improved resolution, cost reduction, and high reproducibility that yields a small rate of error—have made it a reliable and flexible tool of choice as additional methodologies and analytical applications are developed.[10,11] CE is an analytical technique that can be used for the high-resolution analysis of DNA fragments produced by PCR.[9] It offers the advantages of rapid analysis time, minute sample size requirements, high efficiency, and economical operating costs. Drs. Bruce McCord and David Northrop of the Washington State Patrol Crime Lab (Kennewick, WA), along with others, have demonstrated the versatility of CE for the past decade and a half for the analysis of forensic sam-

ples exhibiting a wide range of chemical compositions, including gunshot residues, explosive residues, drugs, and DNA identification.[11] Its robust nature, small sample requirements, and high throughput were tested and proved triumphant with the determination of the human genome.

The ability to quantitate nanogram amounts of mtDNA extracted from outside the nucleus using PCR has made it possible to establish the genetic typing of a range of biological materials found at a crime scene investigation. The protocols developed and used by the special agents of the FBI's DNA II Laboratory have been adapted for the purposes of obtaining identifying specimens for evidence acquired from a crime scene.[4]

The forensic applications for mtDNA developed as part of the Mitochondrial DNA Research Project at the FBI, which began in 1991, were validated and published in 1995. The technique was carried out based on the Technical Working Group on DNA Analytical Methods (TWGDAM) Guidelines.[12,13] The technique involves the extraction of DNA with organic solvent and purification by filtration. The filtrant provides the mtDNA template for the PCR. The two hypervariable segments of the mtDNA control region are amplified into four separate reactions. After a purification step to remove unincorporated PCR primers, amplified products are quantified by CE and subjected to cycle sequencing.[10] mtDNA sample will then be compared for a match to specimens obtained from missing individuals.[14,15] CE is the robust separation technique that became the workhorse in the separations sciences to provide information for forensic scientists who analyze evidence and provide expert testimony to the criminal justice system.[16,17]

The collection process: Family members come forward to help

The families of victims were contacted shortly after September 11, 2001, and asked to provide biological reference samples. Within the first 24 hr, the New York City Police Department (NYPD) posted collection points in the city to take missing persons' reports for collecting materials to be used for reference DNA. Throughout the tristate area, police departments established reception points and forwarded the collections to the NYPD. A consolidation site was established at Pier 94 on Manhattan's Upper West Side to consolidate the information on those missing. The NYPD and the Office of the Medical Examiner established Web sites and provided toll-free numbers, available in English and Spanish, to offer guidance to the families who volunteered their participation in providing reference samples. The reference samples from close relatives and personal effects were typed for DNA, and the resulting profiles were searched against profiles generated from the victims recovered from the World Trade Center site. Swabs from the mouths of consenting relatives are also being submitted for comparison. All laboratory teams working on this project have received software from the FBI as new software was developed for the project. The personal effects and swabs, along with a DNA cover sheet, were then forwarded to the New York State Police Forensic Center in Albany. The victim's name, date of birth, and other information were included. The New York State Police entered all specimens received into a crime laboratory tracking system developed by the **Porter Lee Corp.** (Schaumburg, IL). Information obtained from cheek swabs sent to **Myriad Genetics** was returned to the State Police via the CODIS network. The profiles were loaded into a system developed by **Gene Codes Forensics** for Mass Fatalities Identification System software (M-FISys, pronounced "emphasis"). M-FISys also handled the quality control samples for all of the technologies used, including STR, mtDNA, and SNPs. The volume of victim samples and reference samples was staggering.

Recovery effort at Fresh Kill Landfill revisited

It was the responsibility of the forensic community to unravel the circumstances that claimed at least 2819 individuals and to establish DNA identity for those who perished in the midst of mass destruction that created more than 1.5 million tons of rubble. Staten Island lies five miles across the bay from Manhattan. For the past two years, the Fresh Kill Landfill was cordoned off with a series of 15-ft fences that encircled the hillside. The site was illuminated 24 hr a day with large stadium lights. The acreage resembled a lunar surface, for it appeared dusty white, barren, remote, and strewn with mounds of debris. The gray vastness of the area was contrasted by the colors of a single American flag that was hoisted onto the mast of a 150-ft crane at the edge of the site. That is now gone, too. By evening, the area is once again shrouded in darkness, since the site officially closed earlier last year. The conveyer belts, sifting machines, and grapplers used to separate the tons of iron from fragile and limited organic matter were removed in the spring of last year. For the first 18 months, the steady drone of transport trucks battened down with heavy canvas could be heard every day, along with the sound of docking flatbed barges that transported the material for the initial sifting process to begin.

On any given day, 300 detectives worked 12-hr shifts. In addition to body parts recovered, more than 1700 personal items were found, ranging from pieces of jewelry to charred

credit cards. The two 12,000-ft^2 shelters that were erected by AmeriCares to help the project proceed throughout the two past winters have been removed. Before September 11, 2001, the Fresh Kill Landfill was the most hated tract of land on Staten Island and, after a brief ceremony that was planned by the NYPD and held on July 16, 2002, the landfill was officially reclosed. The U.S. Army Corps of Engineers climbed the hill at Fresh Kill and dismantled the conveyor belts, screening equipment, storage containers, and worker decontamination facilities.

The remains of the charred emergency vehicles, including all of the 98 New York City Fire Department fire trucks destroyed that day, are no longer visible from the Staten Island Expressway, as native grasses and wildflowers now fill the hillside. This task required the cooperative efforts of many city, state, and federal agencies whose first task was to establish standard protocols and maintain chain-of-custody of specimens. As the debris was delivered, large pieces of concrete and steel were removed and set aside, while all of the materials underneath were gathered and run along a conveyor belt. Any material that did not run through the belt was carefully raked and examined by a troop of 15 pairs of investigators, each facing one another. If nothing was found, sanitation workers then cleared the field and the process was repeated. According to New York City Police Lieutenant Bruce Bonvino, who was assigned to the Fresh Kill site, the template was established and a standard procedure was followed for all investigators who made a discovery of what appeared to be a human organ, tissue, bone, or tooth. First, notification was made to a shift supervisor who carried the specimen to an on-site crime-scene trailer where it was verified as human remains. If the specimen was human, it was then photographed, labeled, logged, and refrigerated. A police escort took the samples from the landfill to the city morgue located in lower Manhattan. The Office of the Medical Examiner issued a form for family members to fill out with details reflecting the families' wishes for notification: either initial notification of identity or families could elect to be contacted every subsequent time a DNA match was made of a victim. Few selected the second option because with a disaster of this magnitude, one individual's DNA could appear in hundreds of retrieved specimens. If the family decided to be notified once, the City of New York assumed the respectful burial of additional specimens of that individual.[2]

The present forensic numbers

To date, the New York City Medical Examiner's Office has catalogued 2600 samples from families. The current figure of the missing stands at 4339, of which 478 victims' bodies have been recovered and 425 have been identified. The New York City Medical Examiner's Office coordinated the

24-hr/day operation. The remains of an estimated 1000 people may never be identified, according to the New York City Medical Examiner's Office. Originally, the goal was for 2000 identifications. In a recent interview, Dr. Shaler said that he no longer considered the goal realistic. He hopes for 1700–1800 identifications, and that identification rests largely on DNA analysis of some 15,000 body parts.

In the two years since the collapse of the towers, the Medical Examiner's Office has worked to match the 19,858 remains recovered with the people listed as missing. Many of the new DNA matches made this year have yielded additional remains for those already identified. The highest number of matches to one person is more than 200. As of September 11, 2003, the remains of 63%, or 12,471 remains, and 1271 victims, or 46%, have not been identified. Issues in the DNA identification process for such a large population of victims required the correlation references with correct victims while avoiding spurious assignments. The ratio of men to women who died is 3:1. The median range of ages of those who died is between 35 and 39. There were 289 bodies found intact and 19,858 other body parts recovered. In the past two years, the Federal Emergency Management Administration (FEMA) allocated $970 million to the emergency in New York City.

Conclusion

Forensic science provided a threefold service in the recent World Trade Center terrorist attack. First, it developed new strategies to analyze the remains recovered from the World Trade Center. Second, it supplied evidence for those investigating the attacks. Finally, it provided the families of many individuals lost in the World Trade Center with identification of the victims for the beginning of emotional closure. The degraded nature of the DNA specimens recovered required the innovative and cooperative effort of the public and private sector to succeed.

The collapse of the World Trade Center structures was the worst building disaster in history—the fires generated after the collapse continued to burn for 99 days. Fellow fire, police, and emergency personnel have been recognized for their heroic efforts in the rescue and recovery of the World Trade Center disaster. We in the science community recognize Bruce McCord, Ph.D., and his colleagues at Ohio University, and John Butler, Ph.D., and his colleagues at the National Institute of Standards and Technology, as the quiet heroes they are, and for their extraordinary demonstration of the American spirit in forensic discovery. As we recently observed the second anniversary of the World Trade Center attacks, the forensic community has helped to reduce the rawness and pain of loss for many.

References

1. Marchi E, Chastain TZ. Am Lab 2002; 34(24):13–17.
2. Marchi E. Am Lab 2001; 33(24):13–16.
3. National Institute of Justice. Omnibus Crime Control and Safe Streets Act of 1968 as amended (42 USC §§ 3721–3722).
4. Kuffner C, Marchi E. Anal Chem 1996; 68(7):241A–6A.
5. Marchi E, Pascareta R. J Cap Elec 1997; 044(4):145–56.
6. Isenberg A, McCord B, Koons G, Budowle B, Allen R. Electrophoresis 1996; 17(2321):1–7.
7. Northrop DM, McCord B, Butler J. J Cap Elec 1994; 1(2):158–68.
8. Tagliaro F, Smith FP. Trends Anal Chem 1996; 15(10):513–25.
9. Kline MC, Duewer DL, Redman JK, Butler JM. Anal Chem 2003; 75(10):2463–9.
10. Butler JM, McCord BR, Jung JM, Allen RO. Biotechniques 1994; 17(6):1062–4.
11. Northrop DM, Martire DE, MacCrehan WA. Anal Chem 1991; 63:1038–42.
12. Wilson M, Polanskey D, Butler J, DiZinno JA, Replogle J, Budowle B. Biotechniques 1995; 18(4):662–9.
13. Butler J, McCord B, Jung J, Wilson M, Budowle B, Allen R. J Chromatogr 1994; 658:271–80.
14. Northrop DM. In: Guzman NA, ed. Capillary electrophoresis technology. New York, NY: Marcel Dekker, 1993.
15. Itiguchi RG, von Beroldigen CH, Sensabaugh GF, Ehrlich HA. Nature 1998; 332:543–6.
16. Wilson MR, Stoneking M, Holland MK, DiZinno JA, Budowle B. Crime Lab Dig 1993; 20:68–77.
17. McCord B, Haragon K, Hall K, Burmeister SG. Anal Chim Acta 1994; 288:43–56.

Ms. Marchi is a Research Scientist, NYS Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Rd., Staten Island, NY 10314, U.S.A.; tel.: 718-494-5361; fax: 718-494-4806. The author wishes to thank Mr. Michael McDermott; Orlando L. Manfredi, M.D., Deputy Director of Technology Transfer for NYS Institute for Basic Research; and the Office of the Mayor of the City of New York for their helpful discussions. Mr. Stephen C. Jauntz, a retired FDNY graphic artist, created the illustration accompanying this article. The author and illustrator each live on Staten Island, where 241 neighbors perished in the World Trade Center disaster.