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.

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

<table>
<thead>
<tr>
<th>Source</th>
<th>Description</th>
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| 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.