

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 allelic 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.