







Forensic Science International: Genetics 1 (2007) 3-12

Review

DNA Commission of the International Society for Forensic Genetics (ISFG): Recommendations regarding the role of forensic genetics for disaster victim identification (DVI)

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 Received 25 September 2006; accepted 10 October 2006

Abstract

The ISFG membership consists of scientists and medical professionals specialized in using genetic testing for kinship analysis and the individualization of biological material. This expertise makes the forensic geneticist a resource of advice to international and national organizations dealing with human identifications and causes many DNA laboratories to get involved in DVI tasks. The present recommendations are meant to educate more forensic geneticists about their potential involvement in mass fatality preparedness and possible DVI efforts, as well as to provide practical guidance for each of the laboratories' individual tasks. The idea to work on DNA-specific recommendations was born after a round table discussion dealing with the 2004 Tsunami disaster in south east Asia during the 21st congress of the International Society for Forensic Genetics on the Azores, Portugal, in September 2005. The ensuing discussion between scientists and pathologists that had been involved in the International Center in Khao Lak, Thailand, revealed the need for the scientific community to be better prepared to answer the local authorities' questions by formulating generally acceptable scientific standards for the most efficient use of DNA-based victim identification methods. These recommendations, as well as the many cited references, are intended to provide guidance on establishing preparedness for the forensic genetics laboratory, on collecting and storing ante-mortem and post-mortem samples suitable for DNA analysis, on DNA extraction and genetic typing strategies, on data management, and on issues related to the biostatistical interpretation and reporting of results.

Keywords: Disaster victim identification; DNA testing; Forensic genetics

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1. Introduction

The task of having to establish the identity of victims of a mass disaster, or "disaster victim identification" (DVI) is something that every forensic pathologist, odontologist, and forensic geneticist might have to encounter during their career. Mass disasters can involve natural (e.g. earth quakes, volcanoes, avalanches, hurricanes, and tsunamis) or nonnatural catastrophes (e.g. transportation accidents, terrorist activities, wars, or political crisis). Each incident has its own characteristics and will involve a different approach. While the initial emergency response will be multidisciplinary and must be a shared task of many agencies, dealing with deceased victims in matters of body removal, victim identification and death certificates is the domain of a locally designated authority such as a coroner's or medical examiner's office. It is important to remember that the legal background and the applicable laws governing the DVI operation are based on the country where the incident has occurred.

The collection and transportation of remains by mortuary services should not interfere with any rescue operations geared towards survivors, but, nevertheless, should be part of the first response and has to be part of the mobilization plan. Even though it will be hard to avoid the chaos that is an integral part of any unexpected large scale deadly event, it is important to be prepared and have a multi-agency first responder plan [1]. Regular exercises with simulated events are needed to keep procedures up to date, contact information current, and maintain training goals. Depending on the nature of the event, the safeguarding and collection of forensic evidence will also have to be considered as part of the field response procedure. All aspects of a national or international multidisciplinary DVI approach are addressed in the Interpol Guide to Disaster Victim Identification [2], the Pan American Health Organization disaster manual [3], and a report on mass fatalities issued by the US National Institute of Justice [4]. In addition to this, the first responder field manual for the management of dead bodies after disasters covers matters concerning remains disposition [5]. This recommendation document will not repeat advice on overall strategy but focus on the specific role of the discipline of forensic genetics in the context of a DVI effort.

2. Preparedness

While forensic geneticists are often not included as first responders, DNA sample collection and a strategy for DNA-

based victim identification needs to be part of the community's preparedness plan. Following the Interpol recommendation [2] many countries will have a National DVI team that, under the appropriate circumstances, will be activated to manage the identification effort. Each local laboratory should know about the role of its National team and be clear on what type of support and resources they would still have to provide. For countries without a National DVI team, smaller agencies are encouraged to form a network of qualified laboratories and communicate a plan about a possible collaborative effort and which facilities would be able to receive samples, to the appropriate authorities. This network of qualified response laboratories needs to be kept up to date and should require continuing assurance of competency, e.g. through proficiency testing, accreditation, or ongoing demonstrated success on directly relevant case material.

Depending on the local organizational structure, the closest or most appropriate DNA laboratory might not be associated with the responsible legal medicine institution, or medical examiner or coroner's office, but should, nevertheless, form an understanding with the forensic pathologist about their potential involvement. The DNA laboratory should be consulted for guidance on appropriate ante-mortem and postmortem sample types, sample collection form requirements, kinship sample prioritization, and should be part of any plans for a center that interacts and communicates with the victims' families (family assistance center). This could be made part of preparedness by having the forensic geneticists participate in the training of the forensic pathologists and vice versa. Aside from a communication plan on how to activate the appropriate responders, the agencies in charge need a mechanism to decide early on what the goal of the identification effort will be. Is it sufficient to identify all victims, or will it be necessary to attempt a complete re-association of all recovered body parts? The families need to be informed about their options as early as possible. For multinational disasters an overall issue that needs to be addressed is a policy of how to treat deceased foreign citizens and the possible involvement of foreign DVI teams. Although the decision on this matter is in the hands of the local authority in charge of the operation, the forensic geneticist should be ready to assist in such decision-making.

Recommendation #1. Every forensic DNA laboratory should make an effort to contact the relevant authority dealing with emergency response and establish involvement in a possible mass fatality preparedness plan. Policy decisions about sample collection, scope and final goals of the effort will affect the

victims' families and the work stream and should be decided as early as possible.

If collaboration has been established, the DNA laboratory needs to have an internal response plan that includes a realistic assessment of the in house capacity and technical skills. Based on the nature of the disaster and the number of fatalities, the laboratory might not be able to perform all testing in house and should have names of qualified laboratories available. Another decision that has to be made and can be part of the preparedness plan is sample tracking. How will case and sample numbers be assigned to a mass fatality as opposed to routine casework; will the standard laboratory information management system (LIMS) be available or is dedicated software required? If no standardized solution is offered by the National DVI team, the laboratory needs to assess the software requirements for direct matches and kinship associations and make sure that reliable programs are available, and that the staff is trained to use them. The most important part for efficient preparedness within the laboratory is to identify specific individuals and team members that will be responsible for each segment of a possible DVI effort. These individuals should then be trained, and the team leaders should be allowed to participate in response drills or tabletop exercises. This list of names needs to be updated on a regular basis, and the contact information should be shared with the appropriate local or national agencies. The "lessons learned" document issued after the World Trade Center attack [6] provides guidance on many of the decisions that have to be made by the laboratory director.

Recommendation #2. The internal response plan needs to address throughput capacity, sample tracking, and must have names of supervisors responsible for different tasks that are updated as personnel changes.

3. Mass fatality sample collection

3.1. Post-mortem samples

The speed of the recovery and the preservation of the samples will impact DNA typing success rates. Sample collection at the site should follow forensic evidence collection principles and include documentation, proper labels, and a chain of custody. During the autopsy or external examination, the forensic geneticist, or a pathologist with background in forensic genetics, should ideally be available for consultation during DNA sample collection. Depending on the state of preservation, different tissue types should be collected (see Table 1), and often the forensic pathologist will need specific advice. For collection activities spanning weeks or more, or in adverse environmental conditions, bone and/or teeth samples will emerge as the most reliable sources of DNA. Spongy or cancellous bone can be rich in DNA, but preservation is not reliable, so dense cortical bone should always be the first choice, preferably from weight bearing long bones of the legs. When collecting bone samples anthropological landmarks,

Table 1 Post-mortem sample collection

Condition of body	Sample to be collected
Not decomposed, whole body	Blood (on FTA card or swab) and buccal (mouth) swabs
Not decomposed, fragmented	If available, blood And Deep red muscle tissue (~1.0 g)
Decomposed, whole bodies and fragmented remains	Long compact bone samples (cut 4–6 cm, using window cut without separating the shaft) And/or Healthy teeth without fillings (molars preferable) And/or Any available bone (~10 g, if possible; dense cortical bone preferable)
Severely burnt bodies	Any of the samples above Or Swab from inside the urinary bladder (see Ref. [32])

articular margins, and fresh broken margins should be avoided [4]. When working with more degraded remains it can be worthwhile to consider if easier to collect samples (such as swabs) should be taken in addition to bone. Even if the success rate is lower, the ease of extraction may justify a certain percentage of failed attempts. However, this should be carefully balanced against the additional sample tracking burden and the need to flag failed extractions for re-processing. In any case, multiple samples should be collected from the beginning to avoid laborious re-sampling and re-labeling efforts. For prolonged DVI efforts the preservation of the remains during storage will become an issue, and it is important to have good quality initial samples. In all cases, except for intact and well-preserved bodies, bone or teeth samples must be taken.

Proper documentation of each DNA sub-sample and the body part where it came from is also crucial for quality assurance of the subsequent association of remains. It is advised to supply the morgue operations with post-mortem sampling kits or sample collection containers. Preservative solutions [7] can be used to store soft tissue samples at room temperature and are recommended for temporary morgue facilities with limited freezer space. Do not store samples in formalin, as it would lead to DNA degradation. Even if a victim has already been identified by other means, a DNA sample should be taken for body part association or exclusion purposes, as well as for the identification of other missing relatives [2].

The post-mortem sample numbering scheme could follow standard in house procedures. The Interpol DVI guide [2] suggests a numbering scheme that differentiates between whole bodies, partial remains, and samples collected not on the site but in the morgue. In either scenario the number must be unique and traceable. For mass fatalities involving body fragmentati on, the forensic pathologist is expected to formulate criteria for the testing strategy; for example, will only anatomically

recognizable remains be tested and/or is there a minimum size requirement for recovered soft tissue fragments [4]. It is important that fragmented remains are collected separately and receive individual numbers without presumption of association.

For fragmented remains, commingling or mixing of body parts might compromise the integrity of the samples. Commingling refers to the transfer of blood or tissue from one body part to another set of remains in a high impact disaster, or due to other human or animal activity, and can lead to false DNA-based associations. Jensen [8] mentions this risk in his field guide to mass fatality and casualty incidents, and this is one reason why it is recommended to employ multiple modalities for each identification. Potential cross contamination between sets of remains has to be taken into account in the field and in the autopsy room, and a separate number should be assigned to each piece of remains. No set of remains should be associated and pooled based on their appearance. The sample for DNA testing should be collected from a body part definitely connected to the remains. Do not collect loose tissue or bone fragments as a representative sample. Another issue connected to fragmented remains is the possibility of mistakenly including animal remains. The pre-screening and weeding out of nonhuman samples is a task for a physical anthropologist or an adequately trained forensic pathologist.

Recommendation #3. Several sample types (see Table 1) for DNA testing should be taken at the earliest possible stage of the investigation provided traceability is guaranteed. Samples must be collected from each body or recognizable body part, even if identity is already established. Proper storage must be assured.

3.2. Ante-mortem sample collection: family liaison and data collection unit

A family assistance center (FAC) needs to be established as soon as possible and will need to be staffed by members of different agencies such as the local police department, the Red Cross, or other aid agencies, other parts of local government and possibly airline or train company representatives. The main task of the FAC is to receive phone calls and record individuals that are reported as missing. Clear decisions must be made as to the type and format of information that will be collected; it can be beneficial to have a single form that captures ante-mortem and personal information of the missing person, data on reference family members, and a DNA collection consent form. It is important that sufficient staff is available from the beginning and that all volunteers or staff are extremely diligent when recording the information. FAC staff will have to verify that an individual is truly missing and assign a case number to the name of each missing person. For disasters without a defined victim list, it will take many days, if not weeks, to finalize the list of the deceased individuals. In the meantime multiple numbers might have been assigned to the same individual under variations of their name or based on other misleading information. In many cases the potential victim will later be found alive. A single accurate reported missing list is of crucial importance for streamlining the DNA identification

Table 2 Preferred family reference samples

Both parents
One parent, spouse and children
Children and spouse
One parent and sibling
Siblings (two or more)
Known identical twin

process. Any submissions of personal effects or family swabs need to be subsumed under a single case number. If multiple agencies or companies share sample collection and/or testing, the case number should remain constant.

A forensic geneticist should be staffing the FAC victim information and sample collection section, or at least be available for consultations or data review. A decision has to be made whether the information and the reference samples should be collected immediately or only after the individual's presence at the disaster site has been confirmed [2]. Previous experiences have shown that affected families will not wait and will bring in samples without being asked to. If adequate traceable sample collection kits are available, samples could be collected on site. For a disaster with a defined victim list it will be easier to control the intake of samples and the investigating agency should set up appointments with the affected families to collect ante-mortem data and samples. The person interviewing the family needs to be able to draw a family tree representing the biological relationships and request samples from the most appropriate relatives (see Table 2). If no first-degree relatives are available, samples from more distant relatives need to be taken (here, multiple individuals increase the statistical strength). Table 3 provides some guidance on the theoretical strength of the identification that can be achieved by different combinations of relatives

Table 3 Effectiveness of various combinations of relatives based on kinship index simulation (adopted from Ref. [9])

Family references	Probability of identity (mean posterior probability at 10% prior) (%)
One full sibling	92.1
Sibling and aunt (or uncle)	94.4
Sibling and two aunts (or uncles)	97.8
from same side of the family	
Sibling, aunt, uncle from different	99.8
sides of the family	
Sibling and half sibling	98
Sibling and two half siblings	99.4
(all sharing the mother)	
Two siblings	99.91
One parent	99.9
Sibling and parent	99.996
Father and one maternal half sibling	99.95
Father and two maternal half siblings	99.996
Father and maternal aunt	99.993
Three grandparents	96.7
Four grandparents	99.99
Three grandparents and sibling	99.994

Table 4 Direct reference classification

DNA quality	Commonly available	Might be available
Good sources of DNA	Tooth brushes	Samples from a bone marrow donor program,
		blood cards from PKU newborn screening,
	Electric and manual razors	National biobanks, criminal databases, ^a
		paternity testing labs, a reference samples
		from military personnel ^a
	Hair brushes and combs	Other clinical blood or serum samples
		Sperm bank samples
		Dried umbilical cord
		Paraffin embedded pathology specimen
Fair sources of DNA	Combs	Cervical smears
	Lipsticks, deodorant sticks	Fingernail clippings
	Pillowcases	Cigarette butts
	Used cups, drinking glasses	Pipe
	Used underwear	Mouth piece, mouth guard
		Motorcycle and other sport helmets—caps and hats
		Inner clothing items (bra, t-shirt, socks)
		Ear plugs, ear phones
		Eye glasses
		Pen with teeth marks
		Mailed envelopes or postcards
Poor sources of DNA	Jewelry	Baby hair
	Wrist watches	Dentures
	Outer clothing	Hair rollers
	Towels	Trimmers, scissors, nail files
	Shoes	
	Hair bands or ear muffs	

^a Compatible genetic profiles may be available.

[9]. The interviewers will also need to advise the families on possible direct references samples (see Table 4). In many cases the collecting individual will encounter unusual circumstances where a forensic geneticist can supply scientific guidance. Genetic counselors are another resource prepared to help interview a family about their biological pedigree [10]. It is important to keep in mind that a relationship as understood by the family might not be biological (e.g. an adopted child or an excluded paternity).

All sample intake forms and family information should immediately be checked for obvious data entry errors so that issues can be remediated at once. All family swabs and direct reference samples need to be collected with proper documentation and a complete chain of custody and should be transferred to the laboratory as soon as possible. The laboratory should take great care when storing these samples and be prepared to release the personal effects to the submitting police agency or the families after the DVI effort has been completed. It is recommended to perform a thorough interview to understand the family structure and collect as many samples as possible from each family. Some samples might not yield the expected results, and it will reduce stress for the already traumatized family if the responsible agency does not have to approach them multiple times for additional samples [11]. At a minimum, samples from all first-degree relatives and from the appropriate spouses should be collected, as well as at least the contact information for all other family members.

Recommendation #4. Multiple direct references and samples from first-degree relatives should be collected for each missing person. Scientists with a background in genetics should be available for training or for consultations in the family liaison group.

4. Technical procedures

4.1. DNA extraction

Laboratories performing forensic casework will be properly equipped to extract a wide range of samples from minute bloodstains and tissue fragments, to semen stains and saliva swabs. These laboratories will be able to use their own established extraction protocols, and there is no need for changing procedures, unless the sample number requires a new high throughput method. The situation may be different for clinical or paternity testing laboratories where the extraction methods are not designed for small compromised samples. The extraction step is critical for human remains and personal effects. Both sample types can be limited, compromised, and might yield low amounts of DNA. It is essential that the laboratory has experience with compromised samples and that the selected extraction method has been optimized for maximum yield and typing success. DNA extraction from degraded bone samples is one of the most important capabilities in DVI cases where recovery extends beyond a short period of time, and specific experience is required. To properly extract bone samples, specialized equipment such as cutting and sanding tools, as well as drills and drill bits, or a freezer mill or another bone crushing device is needed [12,13]. To avoid contamination and for health and safety reasons, it is also necessary to effectively capture the generated bone dust and separate the bone processing from other functions.

Recommendation #5. DVI DNA testing should only be performed by laboratories with demonstrated successful capabilities and continuous experience with these specified sample types.

4.2. STR typing

Many commercially available multiplexes have been employed in the identification of human remains in mass graves or after mass disasters [14–17]. A large multiplex will preserve sample extract and yield more immediate information on the specimen. Especially if multiple laboratories/countries are participating in the testing, the decision which set of markers to use has to be made as early as possible. Even in international collaborations it is required that all laboratories test the same loci. The markers must be well established in the forensic community and the countries involved, and the reagents must be accessible to all participants. It is recommended that a *minimum* set of 12 markers (plus Amelogenin) should be attempted on all samples, but DVI efforts relying substantially on kinship statistics will be greatly abetted by multiple amplifications or larger multiplexes targeting 16 or more loci.

One way to improve the success rate for degraded DNA is to utilize redesigned STR primers that generate shorter amplicons [18–24]. Several STR systems with size-reduced fragments were used in collaborative European exercises on artificially degraded DNA and gave complete and correct results [25,26]. It is anticipated that multiplex kits combining several size-reduced STRs will become commercially available in the near future. As with other multiplex kits, the laboratories have to be aware of possible concordance issues between alternate primer pairs [22]. For mass disaster identification a laboratory might encounter discrepancies if, for example, the remains are tested using STR systems designed for degraded DNA while the reference samples are typed with a standard set of commercially available primers.

Recommendation #6. The set of loci to be analyzed has to be identified as soon as possible in concordance with the scientific community in the countries mostly involved. A minimum of 12 independent loci should be selected as standard set, but an even greater number of loci is preferred.

Depending on the state of decomposition, DNA from disaster victims can be severely degraded, and the laboratory will need to adjust their interpretation guidelines to address the regular occurrence of partial profiles and allelic drop out. An incorrect homozygote attribution can prevent correct sample matching and, if the software cannot accommodate this, any potentially affected loci should be removed while searching, but need to be reviewed for concordance after a candidate

match has been found. Low stringency search options can help identify potential matches for partial data.

Often a single sample is being retested multiple times with varying amounts of DNA or using different multiplexes. If multiple amplifications or injections have been performed for the same sample, the detected alleles may be combined in a composite profile if all results are scientifically valid within the laboratory's established interpretation guidelines and all overlapping loci are consistent.

Prior to commencing testing, the forensic DNA laboratory needs to decide on a duplication policy for the disaster samples. This is important because a specimen that was mislabeled at the collection stage, a mislabeled or switched sample at any of the subsequent analysis steps, as well as an extract-to-extract contamination event can lead to a wrong identification, when based on a single extraction. Depending on the circumstances, such mistakes might not be discovered until the final evaluation of all the data and circumstances associated with the case. Without duplicated sample analysis errors might go undetected for disasters with a very uniform victim pool or for severely fragmented remains where no additional information is available.

Although it is desirable to have 100% duplication for all samples, in some instances this might not be necessary or practical. For example, personal effects can often be verified through comparison with family reference swabs, or concordance can be established for multiple personal effects if available. Similarly, a family tree can be checked for consistency instead of duplicating each sample. Once a problem has been observed more testing has to be conducted. For post-mortem remains two extractions, or concordant results from two specimens collected from the same body or body part fulfill the duplication policy.

Recommendation #7. All allele calls and all candidate matches have to be reviewed thoroughly. Composite DNA profiles can be generated if derived from the same specimen and consistent for overlapping loci. The duplication policy should consider the logistics and circumstances of the mass fatality incident.

4.3. Other DNA typing methods

For extremely degraded samples mitochondrial (mt) DNA typing is the only option [27], and even then it might still be necessary to use special primer sets [28]. While under most circumstances (except in a closed population) a mitochondrial DNA match cannot yield an absolute identification, the test might be the only applicable choice for extremely small and compromised samples or if the only available references are far removed, but maternally related relatives. Mitochondrial testing might also provide additional information on unidentified remains, for example a hypothesis for the geographic origin [29]. Y-chromosomal STR analysis is well established and can be very useful for matching to male relatives [30].

Nuclear DNA single nucleotide polymorphism (SNP) analysis has the potential to provide results from highly degraded DNA. SNPs are characterized by short PCR

amplicons, the availability of thousands of markers, and the potential for high through put testing [31–33]. SNP analysis is a valid choice for single source samples but can only be used for DVI purposes if the loci can be shown to be sensitive, unlinked and if appropriate population databases are available [11].

Recommendation #8. If the standard autosomal STR typing fails to give sufficient information, additional typing system such as mtDNA, Y-chromosomal STRs, or SNP markers may be used in selected cases.

5. Data management and linking of samples

Depending on the number of fatalities, a DVI effort will amass a large amount of data that needs to be maintained in an organized and searchable fashion. Ante-mortem data include victim physical characteristics, family pedigree, and other information collected from the families. Post-mortem data are recorded at the site and in the autopsy room during examination and sampling. All identification modalities share the need to match ante-mortem data to results obtained on the remains. For the forensic geneticist the results will consist of three sets of DNA profiles—direct references, kinship references and remains. It is important that all DNA data reside in a single database and are not divided into subsets. Even if the testing has been distributed over a group of participating laboratories, a single centralized agency must be put in charge of the data management and the search for matches. Manual data entry is prone to transcription errors. The host of the database must define a data transfer protocol as soon as possible, and all laboratories must use compatible software for sample tracking, data generation and uploading.

Efforts should be made to generate full STR DNA profiles. However, partial profiles need to be uploaded as well and it is up to the central agency how to use this information. It is not necessary to have all amplification attempts in the central database if a properly reviewed consensus profile is present and previous data is available for review.

Recommendation #9. A centralized database is required for all data comparison. Electronic upload is recommended to avoid transcription errors.

In disasters with body fragmentation the database has the additional function of associating matching body parts to each other. This is a secondary function after the DVI process has been completed. In closed disasters with a known number of deceased victims, it is expected that typing with at least 10–13 STR markers will generate a number of unique STR profiles that corresponds to the victim list. A sorting step will find aggregates of matching genotypes that can then be considered to have come from the same individual. This step is complicated in cases with an unknown number of deceased individuals and in the presence of incomplete partial profiles; here each possible link needs to meet a statistical threshold. Brenner and Weir [34] describe the association of concordant profiles as the "collapsing stage" that can be used to estimate the likely number of victims by assigning statistical thresholds to deal with partial profiles. After

successfully "collapsing" the results to distinct sets of different genotypes this smaller set of data can be used for comparisons to personal references or kinship samples. After a unique profile has been assigned to each victim, a smaller subset of loci can be sufficient for body part re-associations.

Discovering direct matches can theoretically be achieved in a simple sort able spreadsheet format. For the Kaprun tunnel disaster 100% of the victims could be matched to comparative material, and for this incident Meyer [35] describes the use of a Filemaker client server database. For direct matches the data quality is crucial; allelic drop out and other degradation artifacts can cause mismatches through incorrect homozygote types for individual loci, and this has to be taken into account when setting the search stringency. Discrepancies between two possibly matching samples can also be caused by null alleles due to primer concordance issues or STR mutations in pathology specimen used as direct reference. An almost but not completely matching sample set might also indicate that the remains belong to a relative of the candidate victim. All staff members interpreting data and using the matching software must receive training, follow standard operating procedures, and be competent in DNA analysis.

To discover matching family member profiles, several parameters such as allele sharing, kinship index, or genetic distance between profiles can be used. Spreadsheet macros looking for allele sharing to find parent/child samples were employed, for example, during the Taoyuan air crash investigation in Taiwan and the victim identification of the Swiss air crash in Canada which both happened in 1998 [36,37]. Cowell and Mostad [38] describe an approach of first forming clusters of suspected relatives versus suspected unrelated individuals and then applying kinship software to the reduced data set of possibly related individuals only. Brenner and Weir mention a different strategy where in a sort of triangulation approach a potential victim-family association is prioritized if it is indicated by at least two members of the same family [34].

In many mass disasters, such as earthquakes or airline crashes, one of the largest risks for declaring an incorrect identification stems from the fact that multiple, or all, members of the same family may be among the victims. Without additional anthropological information about age or gender of the remains or DNA results for a personal effect, it can be possible to associate a victim to the correct family but not to determine the position in the family tree [37,38]. Relying solely on kinship reference samples when less than complete family reference sets are available can lead to a large number of coincidental chance hits [39] and false inclusions [40]. It is often necessary to add post-mortem DNA profiles from identified family members to the family reference database to be able to find other victims [34,37]. Brenner [41] describes how the presence of relatives in the victim pool can depress the likelihood ratio for individual identifications. All personnel dealing with kinship evaluations have to be properly trained and made aware of these specific mass fatality pitfalls.

Recommendation #10. Especially if multiple family members are involved, DNA-based identification should whenever pos-

sible be anchored by anthropological and/or circumstantial data, a second identification modality, or multiple DNA references.

6. Statistical evaluations and reporting of completed identifications

After a set of remains has been linked to a personal effect or a candidate family, the statistical confirmation of the match can be performed using established methods such as random match probability for direct matches or paternity/kinship index calculations [42]. It is important to use population databases that reflect the ethnic origins of the missing individuals. The following considerations are taken into account for DNA data that are interpreted in the absence of any other supporting evidence. This would, for example, be the scenario for extremely small fragments or an isolated piece of soft tissue with no other characteristics. Under those circumstances the minimal statistical threshold for DNA identification depends on the number of victims in an incident and on the error rate the DVI team is willing to tolerate. For example, in order to achieve a posterior probability of 99.9% of correctly identifying all victims in a mass fatality with 1000 victims, the threshold for a direct match would have to be 1 in 1 million [34]. For kinship cases the same authors suggest using a 99.9% confidence for each kinship, with the corresponding likelihood ratio depending on the prior odds [34]. As described by Biesecker et al. [43], the prior odds can be based on the number of victims reported missing and can be lowered as the identification progresses and the number of missing individuals decreases.

If the identification relies on a combination of autosomal, Y-chromosomal, and mtDNA results, the biostatistical evaluation of these systems should be expressed in the form of likelihood ratios based on conservative and population-specific genotype and haplotype frequency estimates to allow a reasonable combination of these LR's into a single posterior probability.

Including other data, such as anthropological characteristics and candidate matches through other modalities, can reduce the error risk, as well as the prior odds. Thus identifications below the "DNA only" statistical threshold can still be made if the DNA finding sufficiently supports presumptive ID's made by other means. In that case a forensic geneticist with a good knowledge of biostatistics should participate in the interdisciplinary reconciliation session with the other identification experts allowing for full integration of the DNA evidence.

After a DNA match is made, the local authority that will issue a death certificate and notify the families must review and reconcile all data associated with the missing person. All identifications should be considered putative until this administrative review has resolved all inconsistencies [4]. All family related data accessible to the forensic geneticist are of course confidential, and this information, as well as the genetic profiles generated for the different samples cannot be used for other purposes. In cases where an instance of non-paternity is discovered during the identification effort, this should not be disclosed to the family members.

Recommendation #11. In DVI work, DNA statistics are best represented as likelihood ratios that permit DNA results to be combined among multiple genetic systems or with other non-DNA evidence. Likelihood ratio thresholds should be determined for when DNA data alone can suffice for an identification; this will be based on the size and circumstances (e.g. closed versus open) of the event. All evidence and/or circumstances should be checked in making an identification, even if DNA provides the primary or sole evidentiary factor.

7. Sample disposition and data archive

During and after a DVI effort the laboratory will have to deal with potentially thousands of samples. For each sample type the agency needs a policy dealing with the sample disposition in accordance with local laws. All identified human remains should be released to the family for proper burial. If the victims' remains show fragmentation, the families should be given several options for the associated body parts. Do they want to be notified each time another fragment has been discovered or only at the very end? Or do they only want to be notified about the initial identification and then not anymore, thus authorizing the agency to decide on the disposition of the unclaimed body parts? In most instances a common grave will need to be planned for the unidentified or unclaimed remains, but the details will depend on local customs and laws. The arrangements for a common resting place can take a long time, and the agency should be prepared to store the remains for several years. The DNA laboratory will have to decide how to deal with the sub-samples collected for testing. In some instances this sample will have been the only fragment recovered for a certain individual and in this case the sample should be released to the family. In all other cases, it is prudent for the laboratory to retain at least one post-mortem specimen for a defined period of time for paternity or retesting requests.

Family swabs are normally a renewable sample type and should be discarded according to the institute or agency's protocol. The personal effects might be of emotional value to the family and they should have the option to have the item returned to them. Unclaimed personal effects should be discarded after the identification has been accepted. No items should be discarded on incomplete identifications.

All paperwork and data connected to the DVI effort needs to be archived in a central location. Depending on the local structure, the DNA laboratory might not be this central location but will transfer the archived material to a national identification center. For this purpose the laboratory needs to plan how to close out each individual case based on the status of the identification. The accumulated DVI documents might become evidence in civil, as well as criminal trials and need to be treated accordingly.

Recommendation #12. The preparedness plan of the laboratory needs to include policies for family notification, long-term sample disposition, and data archiving.

8. Final considerations

While it is unpleasant to think about future mass fatalities it is nonetheless important for our field to train forensic geneticists in DVI tasks and actively participate in response planning. The skills involved in DVI are closely related to both DNA testing in criminal casework and kinship investigations. Validated procedures and the adherence to good laboratory practices will minimize false negative results and increase the reliability of the identifications. DNA should not be considered the sole identification tool, as many circumstances will allow for faster naming of the victims using dental records or fingerprint characteristics [44]. Moreover, consistent results across multiple modalities will also improve the confidence level for each identification. An interdisciplinary approach is encouraged and needs to be worked out early on among the DVI team members.

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